Differential impact of glomerular and tubule-interstitial histological changes on kidney outcome between non-proteinuric and proteinuric diabetic nephropathy

Fumihiro Fukata, M.D.[1], Masahiro Eriguchi, M.D., Ph.D.[1], Hiroyuki Tamaki, M.D.[1], Takayuki Uemura, M.D.[1], Hikari Tasaki, M.D.[1], Riri Furuyama, M.D.[1], Masatoshi Nishimoto, M.D., Ph.D.[1], Takaaki Kosugi, M.D., Ph.D.[1], Kaori Tanabe, M.D., Ph.D.[1], Katsuhiko Morimoto, M.D.[2], Ph.D., Keisuke Okamoto, M.D.[1], Masaru Matsui, M.D., Ph.D.[3], Ken-ichi Samejima, M.D., Ph.D.[1], Kazuhiko Tsuruya, M.D., Ph.D.[1]

1. Department of Nephrology, Nara Medical University

2. Department of Nephrology, Nara Prefecture Seiwa Medical Center, Nara, Japan

3. Department of Nephrology, Nara Prefecture General Medical Center, Nara, Japan

Corresponding author: Masahiro Eriguchi, MD, PhD

Department of Nephrology, Nara Medical University

840 Shijo–cho, Kashihara, Nara, 634-8521, Japan

Tel: +81–744–29–8865

Fax: +81–744–23–9913

E-mail: meriguci@gmail.com

Running title: Histology and kidney outcome in DKD

Word count: Abstract 243 words, manuscript 2551 words including abstract

Abstract

Background: Studies on kidney function and histological findings in diabetic nephropathy (DN) with low urinary protein (UP) are few. We examined the differential impact of histological changes on kidney outcomes between non-proteinuric and proteinuric DN.

Methods: Patients diagnosed with DN by renal biopsy during 1981–2014 were divided into non-proteinuric (UP ≤0.5 g/day) and proteinuric (UP >0.5 g/day) DN. The Cox proportional hazard model was used to examine the association of glomerular lesions (GLs) and interstitial fibrosis and tubular atrophy (IFTA) with end-stage kidney disease (ESKD) development after adjusting for relevant confounders.

Results: The non-proteinuric and proteinuric DN groups included 197 and 199 patients, respectively. During the 10.7-year median follow-up period, 16 and 83 patients developed ESKD in the non-proteinuric and proteinuric DN groups, respectively. In the multivariable Cox hazard model, hazard ratios (HRs) [95% confidence intervals (CIs)] of GL and IFTA for ESKD in proteinuric DN were 2.94 [1.67–5.36] and 3.82 [2.06–7.53], respectively. Meanwhile, HRs [95% CIs] of GL and IFTA in non-proteinuric DN were <0.01 [0–2.48] and 4.98 [1.33–18.0], respectively. IFTA was consistently associated with higher incidences of ESKD regardless of proteinuria levels (*P* for interaction = 0.49). The prognostic impact of GLs on ESKD was significantly decreased as proteinuria levels decreased (*P* for interaction <0.01).

Conclusions: IFTA is consistently a useful predictor of kidney prognosis in both non-proteinuric and proteinuric DN, while GLs are a significant predictor of kidney prognosis only in proteinuric DN.

Key words: Diabetic nephropathy, glomerular lesion, interstitial fibrosis and tubular atrophy, kidney outcome

Introduction

Diabetic nephropathy (DN), characterized by overt proteinuria and rapid chronic kidney disease (CKD) progression, is a major cause of end-stage kidney disease (ESKD) and is also a risk factor for cardiovascular disease (CVD) and mortality. A growing body of evidence indicates that diabetic kidney disease (DKD), a conceptual term for CKD caused by diabetes including DN, does not always show overt proteinuria (so-called non-proteinuric DKD) and varies in its glomerular filtration rate (GFR) decline rate.[1-3] Because kidney biopsy is rarely performed to confirm the diagnosis, most DN is clinically diagnosed with overt proteinuria, pre-existing diabetic retinopathy, or long-term diabetic history.[4] Indeed, previous studies revealed that among patients with diabetes who underwent kidney biopsy, two-thirds of patients had evidence of DN, but one-third did not.[4,5] In contrast, an autopsy study suggests DN is also underdiagnosed: 20 (18.9%) of the 106 histologically proven DN cases did not present with DN-associated clinical manifestations within their lifetime.[6] This fact was reported by another study.[7] With this evidence, DKD encompasses these heterogeneous manifestations of DN, including proteinuric/non-proteinuric DKD[8,9] and rapid/slow estimated GFR (eGFR) decliner.[2]

Generally, kidney function declines more rapidly as histological findings such as glomerular lesions (GLs) and interstitial fibrosis and tubular atrophy (IFTA) become more severe.[10-13]Proteinuria level is the best surrogate marker of kidney prognosis.[8,14] However, the long-term outcomes of proteinuric and non-proteinuric DKD associated with kidney histological changes remain uncertain.

In this study, we investigated the difference in the association of kidney histological lesions (GLs and IFTA) with kidney outcomes between non-proteinuric and proteinuric biopsy-proven DN.

Materials and Methods

Study Design and Participants

This retrospective cohort study examined patients with DN confirmed by kidney biopsy at Nara Medical University Hospital from June 1981 to December 2014. We performed kidney biopsies in diabetic patients (type 1 and type 2 diabetes) who had urinalysis abnormalities higher than microalbuminuria, or who were

determined to be at high risk of CVD during hospitalization. Patients with DN complicated with other kidney diseases and those with insufficient glomeruli for diagnosis or missing data for analyses were excluded. To examine the difference in the impact of kidney histological findings on the development of ESKD between patients with non-proteinuric and proteinuric DN, patients were divided into two groups according to proteinuria levels: urinary protein (UP) >0.5 g/day (proteinuric DN) and UP ≤0.5 g/day (non-proteinuric DN). The study protocol was approved by the Nara Medical University Ethics Committee (No. 2005-18) and registered in the University Hospital Medical Information Network clinical trial registry (UMIN000031121), as previously described.[15] The opt-in or opt-out approach was used to obtain the consent for this study.

Clinical examination

Baseline demographics and laboratory data at the time of biopsy were retrospectively obtained from medical records. The following clinical data as of renal biopsy were obtained: age, sex, body mass index (BMI), smoking habit, presence of diabetic retinopathy, systolic and diastolic blood pressure, smoking habit, hemoglobin, albumin, serum creatinine, eGFR, urinary protein, uric acid, total, HDL and LDL cholesterol, triglyceride, blood glucose, hemoglobin A1c (HbA1c), c-reactive protein, and, treatment information including anti-diabetic therapies, renin-angiotensin-aldosterone system (RAAS) inhibitors and statins. eGFR was calculated by the equation developed by a previous report.[16] Serum creatinine values measured by the Jaffe method were converted to values for the enzymatic method by subtracting 0.207 mg/dL. HbA1c levels were presented as National Glycohemoglobin Standardization Program values according to the recommendations of the Japanese Diabetic Society and the International Federation of Clinical Chemistry.[17] Urinary protein was measured by 24-h urine protein or spot urine protein creatinine ratio if 24-h urine protein was unavailable.

Kidney biopsy and histological examinations

Histological examinations were performed independently by at least two kidney pathologists, with differences resolved by consensus.

According to the guidelines of the Research Committee of the Renal Pathology Society,[18,19] GLs were classified into four categories: Classes IIa, mild mesangial expansion; IIb, severe mesangial expansion; III, nodular sclerosis with <50% global glomerulosclerosis; and IV, ≥50% global glomerulosclerosis. We did not

include patients with Class I DN, which was confirmed by the thickening of the glomerular basement membrane as detected by electron microscopy because not all the patients have undergone electron microscopic evaluation of renal tissues. The severity of IFTA was graded as follows: 0: no IFTA, 1: <25%, 2: 25–50%, 3: ≥50%. We also evaluate nine GLs, two interstitial lesions, and two vascular lesions as previously described.[19] The nine GLs consisted of a diffuse lesion (0: normal or mild mesangial expansion, 1: mesangial expansion < capillary lumen, 2: mesangial expansion = capillary lumen, 3: mesangial expansion > capillary lumen), nodular lesion (0: absent, 1: present), subendothelial space widening (0: <10%, 1: 10–25%, 2: 25–50%, 3: >50%), exudative lesion (0: absent, 1: present), microaneurysm (0: absent, 1: present), perihilar neovascularization (0: absent, 1: present), global glomerulosclerosis, segmental glomerulosclerosis, and glomerulomegaly. The two interstitial lesions were IFTA and interstitial cell infiltration (0: undetected, 1: <25%, 2: 25–50%, 3: >50%). The two vascular lesions were hyalinosis (0: no hyalinosis, 1: one or more partial arteriolar hyalinosis, 2: approximately 50% hyalinosis, 3: more than 50% hyalinosis, or penetrating hyalinosis) and intimal thickening (0: no intimal thickening, 1: intimal thickness/media thickness <1, 2: intimal thickness/media thickness ≥1).

Exposure of this study

Regarding renal histological findings as predictors, the severity of GL and IFTA were classified into two categories; the presence of GL: IIb or higher and the presence of IFTA: ≥25%.[15]

Outcome measurements

The outcome of this study was the development of ESKD. ESKD was defined as the requirement of kidney replacement therapy or death from kidney failure. Patients were followed up until October 2018 or lost to follow-up.

Statistical analysis

All the analyses were performed in each subgroup divided by proteinuria levels (non-proteinuric and proteinuric DN). Continuous and categorical variables were presented as the median and interquartile range (IQR) or total number and percentage, respectively. Kaplan-Meier methods and Cox proportional hazards regression models were used to evaluate the association between histological findings (GLs and

IFTA) and the development of ESKD. Model results were estimated using two progressive sets of potential confounders: (1) sex and age; (2) model 1 plus BMI, eGFR, and systolic blood pressure; (3) model 2 plus hyalinosis and intimal thickening. The severity of hyalinosis and intimal thickening were classified into two categories; the presence of hyalinosis: one or more partial arteriolar hyalinosis or higher and the presence of intimal thickening: intimal thickness/media thickness <1 or higher.[15] The Fine-Gray model was used to consider the impact of death as a competing risk. The interaction terms for the association of kidney histology with ESKD were assessed between low- and high-UP groups. Statistical significance was defined as P <0.05. All analyses were performed using R 4.0.4 (http://www.r-project.org/).

Results

Baseline characteristics of the cohort

Initially, there were 408 consecutive potential participants who were confirmed with DN by a kidney biopsy. After excluding 12 patients due to missing data for proteinuria level, the remaining 396 patients were included for analysis in this study. Among 197 patients with non-proteinuric DN, only 13 (6.6%) had severe GL (IIb or higher). In contrast, 118 (59.3%) patients had severe GL among 199 patients with proteinuric DN. Similarly, 24 (12.2%) and 122 (61.3%) patients had severe IFTA (≥25%) among the non-proteinuric and proteinuric DN groups, respectively.

Tables 1 and 2 show baseline characteristics stratified by the severity of GL and IFTA in non-proteinuric and proteinuric DN groups. In general, the presence of GL (IIb or higher) was associated with higher systolic blood pressure, more diabetic retinopathy, lower hemoglobin, serum albumin and eGFR, higher proteinuria and uric acid levels, and more RAAS inhibitor user in non-proteinuric and proteinuric DN groups. Although, the associations of GL with systolic blood pressure, eGFR, proteinuria, and uric acid levels were weak in the non-proteinuric DN group compared to the proteinuric DN group (Table 1). IFTA (≥25%) was associated with older age, higher BMI and systolic blood pressure, more diabetic retinopathy, lower hemoglobin, serum albumin, and eGFR, higher proteinuria level, and more RAAS inhibitor user in both groups. Similarly, the associations of IFTA with some of these parameters, including BMI, diabetic

retinopathy, hemoglobin, eGFR, and proteinuria levels, were weak in the non-proteinuric DN group compared to the proteinuric DN group (Table 2).

Tables 3 and 4 show detailed histological findings stratified by the severity of GL and IFTA in non-proteinuric and proteinuric DN groups. In this cohort, segmental sclerosis was very rare, and perihilar neovascularization was present in most patients, making it difficult to detect statistically significant differences. As expected, severe GL (IIb or higher) was associated with more prevalence of severe tubulointerstitial lesions and vascular lesions in patients with proteinuric DN. Although there were no differences in the severity of subendothelial space widening, glomerulomegaly, global sclerosis, and IFTA between mild GL (IIa) and severe GL (IIb or higher) in patients with non-proteinuric DN (Table 3), except for glomerulomegaly, the presence of IFTA (>25%) was consistently associated with more prevalence of severe GLs, tubulointerstitial lesions, and vascular lesions in non-proteinuric and proteinuric DN groups (Table 4).

Relationship of kidney histological findings with the development of ESKD in non-proteinuric and proteinuric DN

During the median [IQR] follow-up period of 10.7 [4.0–17.5] years, 99 patients developed ESKD, including 16 patients in the non-proteinuric DN group and 83 in the proteinuric DN group. Fig. 1 shows adjusted Kaplan-Meier survival curves for ESKD between the severity of GL or IFTA in the non-proteinuric and proteinuric DN groups. The presence of severe GL (IIb or higher) was associated with the development of ESKD in the proteinuric DN group (P <0.01) but not in the non-proteinuric DN group (P =0.99) (Fig. 1a and 1b). Contrastingly, the presence of severe IFTA was consistently associated with the development of ESKD in the proteinuric and non-proteinuric DN groups (P <0.01 and P =0.01, respectively) (Fig. 1c and 1d). Similar results were obtained when considering death as a competing risk with the Fine-Gray model (Fig. S1). Table 5 and Fig. 2 show the association of GL or IFTA with the development of ESKD in a multivariable Cox hazard model. Although the association of GL with ESKD was not observed in the non-proteinuric DN group, this association in the proteinuric DN group remained statistically significant even after adjusting for clinically relevant factors. In the main model (model 2), the fully adjusted hazard ratios (HRs) [95%

confidence intervals: CIs] of the presence of severe GL for ESKD in non-proteinuric and proteinuric DN groups were <0.01 [0–2.5] and 3.0 [1.7–5.3], respectively. There was a significant interaction between GL and proteinuria (Fig. 2; *P* for interaction <0.01). Meanwhile, the association of IFTA with ESKD remained statistically significant even after adjusting for clinically relevant factors in non-proteinuric and proteinuric DN groups. The fully adjusted HRs [95% CIs] of the presence of severe IFTA for ESKD in the non-proteinuric and proteinuric DN groups were 5.0 [1.4–17.8] and 4.0 [2.1–7.6], respectively. There was no interaction between IFTA and proteinuria (*P* for interaction =0.49). The similar results were obtained when considering death as a competing risk with the Fine-Gray model. The fully adjusted HRs with competitive risk analysis were shown in Table S1.

When proteinuria level was used as a continuous variable, proteinuria levels were positively associated with the risk of ESKD in the severe (IIb or higher) and mild GL (IIa) groups. However, the severity of GLs was not consistently associated with ESDK risk (Fig. 3a). Contrastingly, higher proteinuria levels and severe IFTA were consistently associated with a higher incidence of ESKD (Fig. 3b).

Discussion

We examined the differential impact of diabetic kidney histological findings on the development of ESKD between proteinuric and non-proteinuric DN. In proteinuric DN, classically considered a typical DN, the presence of GL or IFTA was consistently associated with the development of ESKD. In contrast, only IFTA was convincingly associated with the development of ESKD in non-proteinuric DN.

Because the incidence of hard endpoints in the kidneys is relatively low in patients with non-proteinuric DKD, previous reports included a deterioration of eGFR by 50% and/or, or a doubling of creatinine levels in addition to ESKD as kidney outcomes.[3,11] In this study, we exclusively examined the hard endpoint (ESKD) with relatively larger sample size (197 patients) and longer period (median follow-up: 10.7 years) as compared to previous reports. Several previous studies have reported that GLs and IFTA are associated with decreased kidney function in DN.[10-12,20] However, some previous works revealed the importance of tubule-interstitial lesions in DN.[21-23] Okada et al. reported that interstitial lesions, but not GLs, significantly

predict kidney prognosis in patients with type 2 DN and overt proteinuria.[22] Similarly, the diabetic mice model revealed that tubular injury, but not endothelial cells in the glomeruli, contributes to the development of microalbuminuria, suggesting the importance of tubular injury for non-proteinuric (microalbuminuric) DN.[23]

Currently, the established therapeutic agents for DKD are RAAS inhibitors, sodium-glucose co-transporter 2 (SGLT2) inhibitors, and newly developed non-steroidal mineralocorticoid receptor antagonists (MRAs). Classical RAAS inhibitors, including angiotensin II receptor blockers and angiotensin-converting enzyme (ACE) inhibitors, focuses on targeting GLs (glomerular hyperfiltration) to reduce proteinria.[24] Similarly, SGLT2 inhibitors inhibit sodium reabsorption in the tubules and normalize the overactivation of the tubular glomerular feedback mechanism, ameliorating glomerular hyperfiltration.[25-27] In addition to the beneficial effect on GLs, SGLT2 inhibitors also have a protective effect on IFTA by inhibiting TGF-β1-induced THBS1, TNC, and PDGF-B overexpression in human paroxysmal tubular cell lines.[28] New non-steroidal MRAs focused more on anti-fibrotic/anti-inflammatory effects than electrolyte effects compared to classical steroidal MRAs, revealing cardio-renal protections.[29-33] Altogether with our study result, tubulointerstitial lesions could be an attractive therapeutic target, especially in non-proteinuric DKD. Indeed, newly established DKD therapeutic strategies, including SGLT2i[34] and non-steroidal MRAs,[35] benefit tubulointerstitial lesions in non-proteinuric DKD/CKD of animal models. These drugs revealed an additive effect on classical RAS inhibitors, mainly affecting GLs, suggesting the residual risk for DKD progression by tubulointerstitial lesions.

Our study has several limitations. First, this study was conducted in a single facility and was limited to Japanese patients. Second, it is also a retrospective observational study. Third, although a relatively large number of non-proteinuric DN patients (about half of the patients were non-proteinuric: UP <0.5 g/day) with long-term observation (median follow-up period of 10.7 years) were included in this study, only 16 patients developed ESKD in non-proteinuric DN, suggesting the lack of statistical power. It is necessary to accumulate more cases in the future to make our conclusions more convincing. Forth, class I diabetic GLs (electron microscopy-proven glomerular basement membrane thickening) were not included, as it is difficult

to examine all patients with diabetes by electron microscopy in routine clinical practice. Despite these limitations, we are confident that this study holds significant importance and exhibits great potential for future clinical applications.

In conclusion, IFTA was convincingly associated with ESKD in non-proteinuric and proteinuric DN. In contrast, GLs were associated with ESKD only in proteinuric DN but not in non-proteinuric DN.

Acknowledgments

None.

Conflict of interest

All authors have nothing to disclose.

Funding

This work was supported by grants from the Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research 18K15984) to KS.

Author Contributions

Research idea and study design: FF and ME; Drafting the manuscript: FF; Revising the manuscript: ME, data analysis/interpretation: FF and ME; statistical analysis: FF; supervision or mentorship: HT, TU, HT, RF, MN, TK, KTa, KM, KO, MM, KS, and KTs. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions on the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

References

1. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS et al. Clinical Manifestations of Kidney Disease Among US Adults With Diabetes, 1988-2014. JAMA. 2016;316(6):602-10. doi:10.1001/jama.2016.10924.

2. Jiang G, Luk AOY, Tam CHT, Xie F, Carstensen B, Lau ESH et al. Progression of diabetic kidney disease and trajectory of kidney function decline in Chinese patients with Type 2 diabetes. Kidney Int. 2019;95(1):178-87. doi:10.1016/j.kint.2018.08.026.

3. Yamanouchi M, Furuichi K, Hoshino J, Toyama T, Hara A, Shimizu M et al. Nonproteinuric Versus Proteinuric Phenotypes in Diabetic Kidney Disease: A Propensity Score-Matched Analysis of a Nationwide, Biopsy-Based Cohort Study. Diabetes Care. 2019;42(5):891-902. doi:10.2337/dc18-1320.

4. Espinel E, Agraz I, Ibernon M, Ramos N, Fort J, Seron D. Renal Biopsy in Type 2 Diabetic Patients. J Clin Med. 2015;4(5):998-1009. doi:10.3390/jcm4050998.

5. Sharma SG, Bomback AS, Radhakrishnan J, Herlitz LC, Stokes MB, Markowitz GS et al. The Modern Spectrum of Renal Biopsy Findings in Patients with Diabetes. Clin J Am Soc Nephrol. 2013;8(10):1718-24. doi:10.2215/cjn.02510213.

6. Klessens CQ, Woutman TD, Veraar KA, Zandbergen M, Valk EJ, Rotmans JI et al. An autopsy study suggests that diabetic nephropathy is underdiagnosed. Kidney Int. 2016;90(1):149-56. doi:10.1016/j.kint.2016.01.023.

7. Ekinci EI, Jerums G, Skene A, Crammer P, Power D, Cheong KY et al. Renal structure in normoalbuminuric and albuminuric patients with type 2 diabetes and impaired renal function. Diabetes Care. 2013;36(11):3620-6. doi:10.2337/dc12-2572.

8. Yamanouchi M, Furuichi K, Hoshino J, Ubara Y, Wada T. Nonproteinuric diabetic kidney disease. Clin Exp Nephrol. 2020;24(7):573-81. doi:10.1007/s10157-020-01881-0.

9. Robles NR, Villa J, Gallego RH. Non-Proteinuric Diabetic Nephropathy. J Clin Med. 2015;4(9):1761-73. doi:10.3390/jcm4091761.

10. An Y, Xu F, Le W, Ge Y, Zhou M, Chen H et al. Renal histologic changes and the outcome in patients with diabetic nephropathy. Nephrol Dial Transplant. 2015;30(2):257-66. doi:10.1093/ndt/gfu250.

11. Shimizu M, Furuichi K, Toyama T, Kitajima S, Hara A, Kitagawa K et al. Long-term outcomes of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy. Diabetes Care. 2013;36(11):3655-62. doi:10.2337/dc13-0298.

12. Mise K, Hoshino J, Ubara Y, Sumida K, Hiramatsu R, Hasegawa E et al. Renal prognosis a long time after renal biopsy on patients with diabetic nephropathy. Nephrol Dial Transplant. 2014;29(1):109-18. doi:10.1093/ndt/gft349.

13. Shimizu M, Furuichi K, Yokoyama H, Toyama T, Iwata Y, Sakai N et al. Kidney lesions in diabetic patients with normoalbuminuric renal insufficiency. Clin Exp Nephrol. 2014;18(2):305-12. doi:10.1007/s10157-013-0870-0.

14. Gohda T, Murakoshi M, Koshida T, Ichikawa S, Li ZI, Adachi ERI et al. Concept of Diabetic Kidney Disease - Paradigm Shift from Albuminuria-Based to GFR-Based Kidney Disease. Juntendo Medical Journal. 2019;65(6):510-6. doi:10.14789/jmj.2019.65.JMJ19-R16.

15. Morimoto K, Matsui M, Samejima K, Kanki T, Nishimoto M, Tanabe K et al. Renal arteriolar hyalinosis, not intimal thickening in large arteries, is associated with cardiovascular events in people with biopsy-proven diabetic nephropathy. Diabet Med. 2020;37(12):2143-52. doi:10.1111/dme.14301.

16. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53(6):982-92. doi:10.1053/j.ajkd.2008.12.034.

17. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson JO, Little R et al. Statistical methods for monitoring the relationship between the IFCC reference measurement procedure for hemoglobin A1c and the designated comparison methods in the United States, Japan, and Sweden. Clin Chem. 2008;54(8):1379-85. doi:10.1373/clinchem.2008.103556.

18. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556-63. doi:10.1681/ASN.2010010010.

19. Furuichi K, Yuzawa Y, Shimizu M, Hara A, Toyama T, Kitamura H et al. Nationwide multicentre kidney biopsy study of Japanese patients with type 2 diabetes. Nephrol Dial Transplant. 2018;33(1):138-48. doi:10.1093/ndt/gfw417.

20. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? Kidney Int. 1999;56(5):1627-37. doi:10.1046/j.1523-1755.1999.00721.x.

21. Bonventre JV. Can we target tubular damage to prevent renal function decline in diabetes? Semin Nephrol. 2012;32(5):452-62. doi:10.1016/j.semnephrol.2012.07.008.

22. Okada T, Nagao T, Matsumoto H, Nagaoka Y, Wada T, Nakao T. Histological predictors for renal prognosis in diabetic nephropathy in diabetes mellitus type 2 patients with overt proteinuria. Nephrology (Carlton). 2012;17(1):68-75. doi:10.1111/j.1440-1797.2011.01525.x.

23. Eriguchi M, Lin M, Yamashita M, Zhao TV, Khan Z, Bernstein EA et al. Renal tubular ACE-mediated tubular injury is the major contributor to microalbuminuria in early diabetic nephropathy. Am J Physiol Renal Physiol. 2018;314(4):F531-f42. doi:10.1152/ajprenal.00523.2017.

24. Sawaf H, Thomas G, Taliercio JJ, Nakhoul G, Vachharajani TJ, Mehdi A. Therapeutic Advances in Diabetic Nephropathy. J Clin Med. 2022;11(2). doi:10.3390/jcm11020378.

25. Chao EC, Henry RR. SGLT2 inhibition--a novel strategy for diabetes treatment. Nat Rev Drug Discov. 2010;9(7):551-9. doi:10.1038/nrd3180.

26. Cherney DZ, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation. 2014;129(5):587-97. doi:10.1161/circulationaha.113.005081.

27. Anders H-J, Huber TB, Isermann B, Schiffer M. CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. Nature Reviews Nephrology. 2018;14(6):361-77. doi:10.1038/s41581-018-0001-y.

28. Pirklbauer M, Schupart R, Fuchs L, Staudinger P, Corazza U, Sallaberger S et al. Unraveling reno-protective effects of SGLT2 inhibition in human proximal tubular cells. American Journal of Physiology-Renal Physiology. 2019;316(3):F449-F62. doi:10.1152/ajprenal.00431.2018.

29. Bauersachs J, Jaisser F, Toto R. Mineralocorticoid receptor activation and mineralocorticoid receptor antagonist treatment in cardiac and renal diseases. Hypertension. 2015;65(2):257-63. doi:10.1161/HYPERTENSIONAHA.114.04488.

30. Agarwal R, Kolkhof P, Bakris G, Bauersachs J, Haller H, Wada T et al. Steroidal and non-steroidal mineralocorticoid receptor antagonists in cardiorenal medicine. Eur Heart J. 2021;42(2):152-61. doi:10.1093/eurheartj/ehaa736.

31. Haller H, Bertram A, Stahl K, Menne J. Finerenone: a New Mineralocorticoid Receptor Antagonist Without Hyperkalemia: an Opportunity in Patients with CKD? Curr Hypertens Rep. 2016;18(5):41. doi:10.1007/s11906-016-0649-2.

32. Ito S, Shikata K, Nangaku M, Okuda Y, Sawanobori T. Efficacy and Safety of Esaxerenone (CS-3150) for the Treatment of Type 2 Diabetes with Microalbuminuria: A Randomized, Double-Blind, Placebo-Controlled, Phase II Trial. Clin J Am Soc Nephrol. 2019;14(8):1161-72. doi:10.2215/cjn.14751218.

33. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P et al. Effect of Finerenone on Chronic Kidney Disease Outcomes in Type 2 Diabetes. N Engl J Med. 2020;383(23):2219-29. doi:10.1056/NEJMoa2025845.

34. Tomita I, Kume S, Sugahara S, Osawa N, Yamahara K, Yasuda-Yamahara M et al. SGLT2 Inhibition Mediates Protection from Diabetic Kidney Disease by Promoting Ketone Body-Induced mTORC1 Inhibition. Cell Metab. 2020;32(3):404-19.e6. doi:10.1016/j.cmet.2020.06.020.

35. Lattenist L, Lechner SM, Messaoudi S, Le Mercier A, El Moghrabi S, Prince S et al. Nonsteroidal Mineralocorticoid Receptor Antagonist Finerenone Protects Against Acute Kidney Injury-Mediated Chronic Kidney Disease: Role of Oxidative Stress. Hypertension. 2017;69(5):870-8. doi:10.1161/hypertensionaha.116.08526.

Tables

Table 1. Patient characteristics according to glomerular lesions in non-proteinuric and proteinuric DN

	Non-proteinuric DN			Proteinuric DN		
	GL: IIa	GL: IIb or higher	*p-value*	GL: IIa	GL: IIb or higher	*p-value*
Number of patients	184	13	-	81	118	-
Demographics						
Age, years	57 [49, 64]	63 [49, 65]	0.52	61 [50, 68]	61 [52, 67]	0.77
Sex: male, n (%)	114 (62.0)	7 (53.8)	0.78	49 (60.5)	79 (66.9)	0.43
BMI, kg/m^2	23.5 [21.4, 25.5]	21.6 [20.1, 24.0]	0.11	23.9 [22.0, 26.0]	23.9 [22.0, 27.1]	0.55
Systolic BP, mmHg	126 [112, 140]	134 [123, 144]	0.11	132 [120, 150]	140 [130, 159]	<0.01
Diastolic BP, mmHg	72 [64, 80]	74 [68, 82]	0.98	78 [64, 80]	78 [70, 88]	0.07
Diabetic retinopathy (%)	48 (26.7)	8 (61.6)	<0.01	31 (39.2)	90 (78.3)	<0.01
Smoking habit						
Never smoker, n (%)	68 (37.0)	4 (30.8)	0.59	37 (45.7)	44 (37.3)	0.49
Past smoker, n (%)	24 (13.0)	3 (23.1)		13 (16.0)	23 (19.5)	
Current smoker, n (%)	92 (50.0)	6 (46.2)		31 (38.3)	51 (43.2)	
Laboratory						
Hemoglobin, g/dL	13.8 [12.8, 15.1]	11.9 [9.9, 13.5]	<0.01	13.6 [12.0, 15.0]	11.6 [10.4, 12.8]	<0.01
Albumin, g/dL	4.2 [3.9, 4.4]	3.9 [3.5, 4.1]	<0.01	4.1 [3.6, 4.3]	3.2 [2.7, 3.8]	<0.01
Creatinine, mg/dL	0.80 [0.70, 1.09]	1.10 [0.80, 1.30]	0.06	0.90 [0.70, 1.20]	1.29 [0.95, 1.80]	<0.01
eGFR, mL/min/1.73 m^2	66.9 [49.4, 89.8]	47.6 [40.0, 64.0]	0.06	62.2 [44.7, 83.1]	40.9 [28.1, 59.7]	<0.01
Urinary protein, g/day	0.14 [0.10, 0.26]	0.20 [0.10, 0.36]	0.12	0.87 [0.70, 2.15]	3.88 [2.02, 6.36]	<0.01
Uric acid, mg/dL	5.0 [3.9, 5.9]	6.0 [5.1, 8.2]	0.1	5.8 [4.5, 6.9]	6.9 [5.6, 7.7]	<0.01
Total cholesterol, mg/dL	198 [173, 223]	198 [163, 231]	0.97	210 [182, 239]	218 [182, 258]	0.13
HDL cholesterol, mg/dL	45 [37, 51]	38 [36, 46]	0.26	44 [38, 54]	47 [38, 63]	0.11
LDL cholesterol, mg/dL	127 [105, 151]	143 [112, 144]	0.51	135 [102, 160]	134 [101, 173]	0.54
Triglyceride, mg/dL	127 [89, 173]	118 [113, 168]	0.6	142 [112, 192]	151 [111, 200]	0.4
Blood glucose, mg/dL	137 [109, 187]	144 [111, 186]	0.81	128 [104, 176]	140 [110, 197]	0.29
Hemoglobin A1c, %	8.1 [6.8, 9.4]	7.7 [7.1, 8.1]	0.67	7.6 [6.7, 8.7]	7.1 [6.4, 8.6]	0.08
C-reactive protein, mg/dL	0.2 [0.1, 0.3]	0.1 [0.1, 0.4]	0.59	0.2 [0.1, 0.3]	0.1 [0.1, 0.3]	0.18
Treatments						
Anti-diabetic therapies, n (%)	109 (59.2)	12 (92.3)	0.02	56 (69.1)	88 (74.6)	0.42
RAAS inhibitors, n (%)	18 (9.8)	4 (30.8)	0.04	30 (37.0)	71 (60.2)	<0.01
Statins, n (%)	16 (8.7)	1 (7.7)	1	14 (17.3)	29 (24.6)	0.29

Results are shown as median [interquartile range] or prevalence (percentage)

Abbreviations: DN: diabetic nephropathy; GL: glomerular lesion; BMI: body mass index; BP: blood pressure; eGFR: estimated glomerular filtration rate; LDL: low-density lipoprotein; HDL: high-density lipoprotein; RAAS: renin-angiotensin-aldosterone system.

Table 2. Patient characteristics according to IFTA in non-proteinuric and proteinuric DN.

	Non-proteinuric DN			Proteinuric DN		
	IFTA <25%	IFTA ≥25%	*p-value*	IFTA <25%	IFTA ≥25%	*p-value*
Number of patients	173	24	-	77	122	-
Demographics						
Age, years	57 [48, 64]	62 [54, 68]	0.03	58 [48, 66]	62 [54, 69]	0.01
Sex: male, n (%)	102 (59.0)	19 (79.2)	0.09	45 (58.4)	83 (68.0)	0.22
BMI, kg/m^2	23.1 [21.0, 25.2]	24.3 [22.4, 26.3]	0.12	23.2 [21.4, 25.9]	24.1 [22.5, 27.3]	0.02
Systolic BP, mmHg	126 [110, 140]	138 [122, 150]	0.02	132 [120, 150]	140 [130, 156]	<0.01
Diastolic BP, mmHg	72 [64, 80]	70 [68, 80]	0.58	78 [64, 80]	78 [70, 86]	0.23
Diabetic retinopathy, n (%)	47 (27.7)	9 (39.1)	0.46	36 (48.0)	85 (71.4)	<0.01
Smoking habit						
Never smoker, n (%)	66 (38.2)	6 (25.0)	0.06	34 (44.2)	47 (38.5)	0.5
Past smoker, n (%)	20 (11.6)	7 (29.2)		11 (14.3)	25 (20.5)	
Current smoker, n (%)	87 (50.3)	11 (45.8)		32 (41.6)	50 (41.0)	
Laboratory						
Hemoglobin, g/dL	13.8 [12.8, 15.1]	13.4 [12.0, 14.7]	0.16	13.2 [11.8, 14.8]	11.7 [10.3, 13.2]	<0.01
Albumin, g/dL	4.2 [3.9, 4.4]	4.2 [3.9, 4.3]	0.26	4.0 [3.4, 4.3]	3.3 [2.9, 3.9]	<0.01
Creatinine, mg/dL	0.8 [0.6, 1.0]	1.2 [0.9, 1.5]	<0.01	0.9 [0.7, 1.2]	1.4 [1.0, 1.9]	<0.01
eGFR, mL/min/1.73 m^2	68.9 [49.4, 90.8]	50.3 [39.0, 60.7]	<0.01	67.2 [45.3, 86.7]	40.4 [27.4, 56.4]	<0.01
Urinary protein, g/day	0.12 [0.10, 0.25]	0.22 [0.10, 0.35]	0.06	1.10 [0.70, 2.77]	3.50 [1.43, 6.38]	<0.01
Uric acid, mg/dL	5.0 [3.8, 5.8]	7.0 [5.4, 8.0]	<0.01	5.5 [4.4, 6.7]	6.9 [5.7, 7.8]	<0.01
Total cholesterol, mg/dL	198 [171, 226]	200 [185, 224]	0.77	210 [190, 244]	213 [168, 253]	0.98
HDL cholesterol, mg/dL	46 [37, 51]	39 [34, 52]	0.26	44 [37, 53]	48 [39, 62]	0.05
LDL cholesterol, mg/dL	130 [107, 152]	115 [95, 140]	0.2	135 [106, 173]	132 [95, 165]	0.34
Triglyceride, mg/dL	126 [89, 168]	129 [107, 212]	0.16	130 [104, 168]	151 [115, 202]	0.08
Blood glucose, mg/dL	137 [110, 187]	143 [109, 188]	1	143 [111, 208]	131 [106, 183]	0.12
Hemoglobin A1c, %	8.0 [6.9, 9.4]	7.7 [6.5, 9.2]	0.29	7.9 [6.6, 9.0]	7.0 [6.4, 8.5]	0.03
C-reactive protein, mg/dL	0.2 [0.0, 0.3]	0.1 [0.1, 0.3]	0.77	0.2 [0.0, 0.4]	0.1 [0.1, 0.2]	0.43
Treatments						
Anti-diabetic therapies, n (%)	104 (60.1)	17 (70.8)	0.38	53 (68.8)	91 (74.6)	0.42
RAAS inhibotors, n (%)	12 (6.9)	10 (41.7)	<0.01	23 (29.9)	78 (63.9)	<0.01
Statins, n (%)	14 (8.1)	3 (12.5)	0.44	11 (14.3)	32 (26.2)	0.05

Results are shown as median [interquartile range] or prevalence (percentage)

Abbreviations: DN: diabetic nephropathy; IFTA: interstitial fibrosis and tubular atrophy; BMI: body mass index; BP: blood pressure; eGFR: estimated glomerular filtration rate; LDL: low-density lipoprotein; HDL: high-density lipoprotein; RAAS: renin-angiotensin-aldosterone system.

Table 3. Histological findings according to glomerular lesions in non-proteinuric and proteinuric DN.

		Non-proteinuric DN			Proteinuric DN		
		GL: IIa	GL: IIb or higher	*p-value*	GL: IIa	GL: IIb or higher	*p-value*
Number of patients		184	13	-	81	118	-
Glomerular lesions							
Diffuse lesion, n (%)	0	2 (1.1)	0 (0.0)	<0.01	0 (0.0)	0 (0.0)	<0.01
	1	95 (51.6)	1 (7.7)		26 (32.1)	4 (3.4)	
	2	76 (41.3)	1 (7.7)		32 (39.5)	6 (5.1)	
	3	11 (6.0)	11 (84.6)		23 (28.4)	108 (91.5)	
Nodular lesion, n (%)		0 (0.0)	12 (92.3)	<0.01	0 (0.0)	113 (95.8)	<0.01
Subendothelial space widening, n (%)	0	70 (38.0)	3 (23.1)	0.4	20 (24.7)	11 (9.3)	<0.01
	1	84 (45.7)	6 (46.2)		40 (49.3)	58 (49.2)	
	2	26 (14.1)	4 (30.8)		4 (4.9)	28 (23.7)	
	3	4 (2.2)	0 (0.0)		1 (1.2)	21 (17.8)	
Exudative lesion, n (%)		26 (14.1)	5 (38.5)	0.05	37 (45.7)	101 (85.6)	<0.01
Microaneurysm, n (%)		17 (9.2)	4 (30.8)	0.05	17 (21.0)	77 (65.3)	<0.01
Perihilar neovascularization, n (%)		179 (97.3)	13 (100.0)	1	79 (97.5)	118 (100.0)	0.16
Glomerulomegaly, n (%)		31 (16.8)	4 (30.8)	0.37	28 (34.6)	66 (55.9)	<0.01
% global sclerosis, %		6 [0, 16]	8 [0, 10]	0.92	14 [0, 27]	23 [9, 42]	<0.01
Segmental sclerosis, n (%)		3 (1.6)	2 (15.4)	0.03	11 (13.6)	25 (21.2)	0.24
Tubulointerstitial lesions							
IFTA, n (%)	0	28 (15.2)	0 (0.0)	0.1	3 (3.7)	0 (0.0)	<0.01
	1	136 (73.9)	9 (69.2)		46 (56.8)	28 (23.7)	
	2	13 (7.1)	3 (23.1)		18 (22.2)	33 (28.0)	
	3	7 (3.8)	1 (7.7)		14 (17.3)	57 (48.3)	
Inflammatory cell infiltration, n (%)	0	97 (52.7)	6 (46.2)	<0.01	15 (18.5)	4 (3.4)	<0.01
	1	79 (42.9)	4 (30.8)		40 (49.4)	30 (25.4)	
	2	2 (1.1)	2 (15.4)		17 (21.0)	35 (29.7)	
	3	6 (3.3)	1 (7.7)		9 (11.1)	49 (41.5)	
Vascular lesions							
Hyalinosis, n (%)	0	41 (22.3)	0 (0.0)	<0.01	7 (8.6)	3 (2.5)	<0.01
	1	57 (31.0)	1 (7.7)		12 (14.8)	4 (3.4)	
	2	25 (13.6)	0 (0.0)		11 (13.6)	9 (7.6)	
	3	61 (33.2)	12 (92.3)		51 (63.0)	102 (86.4)	
Intimal thickening, n (%)	0	46 (28.2)	1 (7.7)	0.07	15 (20.0)	16 (14.0)	0.49
	1	42 (25.8)	7 (53.8)		26 (34.7)	47 (41.2)	
	2	75 (46.0)	5 (38.5)		34 (45.3)	51 (44.7)	

Glomerular lesions and IFTA were classified according to the Research Committee of the Renal Pathology Society guidelines. Other parameters were defined according to a previous report. [18]

Results are shown as median [interquartile range] or prevalence (percentage)

Abbreviations: DN: diabetic nephropathy; GL: glomerular lesion; IFTA: interstitial fibrosis and tubular atrophy.

The severity of diffuse lesions of glomeruli was graded on a scale of 0 to 3 as follows: grade 0, normal or mild mesangial expansion; grade 1, mesangial expansion < capillary lumen; grade 2, mesangial expansion = capillary lumen; and grade 3, mesangial expansion > capillary lumen. Subendothelial space widening was graded on a scale of 0 to 3 by % of double contour basement membrane (determined in a peripheral capillary of the most severe glomerulus) as follows: grade 0, <10%; grade 1, 10–25%; grade 2, 25–50%; and grade 3, 50% or higher. Inflammatory cell infiltration was graded on a scale of 0 to 3 as follows: grade 0, no cell infiltration; grade 1, <25%; grade 2, 25–50%; and grade 3, 50% or higher. Hyalinosis was graded on a scale of 0 to 3 as follows: grade 0, no hyalinosis; grade 1, one or more partial arteriolar hyalinosis; grade 2, approximately 50% hyalinosis; and grade 3, more than 50% hyalinosis, or penetrating hyalinosis. Intimal thickening was graded on a scale of 0 to 2 as follows: grade 0, no intimal thickening; grade 1, intimal thickness/media thickness <1; and grade 2, intimal thickening and intimal thickness/media thickness ≥1. The nodular lesion, exudative lesion, microaneurysm, perihilar neovascularization, glomerulomegaly, and segmental sclerosis were shown as their presence or absence.

Table 4. Histological findings according to IFTA in non-proteinuric and proteinuric DN.

		Non-proteinuric DN			Proteinuric DN		
		IFTA <25%	IFTA ≥25%	*p-value*	IFTA <25%	IFTA ≥25%	*p-value*
Number of patients		173	24	-	77	122	-
Glomerular lesions							
Diffuse lesion, n (%)	0	2 (1.2)	0 (0.0)	<0.01	0 (0.0)	0 (0.0)	<0.01
	1	93 (53.8)	3 (12.5)		22 (28.6)	8 (6.6)	
	2	63 (36.4)	14 (58.3)		24 (31.2)	14 (11.5)	
	3	15 (8.7)	7 (29.2)		31 (40.3)	100 (82.0)	
Nodular lesion, n (%)		8 (4.6)	4 (16.7)	0.05	28 (36.4)	85 (69.7)	<0.01
Subendothelial space widening, n (%)	0	68 (39.3)	5 (20.8)	0.02	20 (26.0)	11 (9.0)	<0.01
	1	80 (46.2)	10 (41.7)		38 (49.4)	60 (49.2)	
	2	21 (12.1)	9 (37.5)		13 (16.9)	32 (26.2)	
	3	4 (2.3)	0 (0.0)		6 (7.8)	19 (15.6)	
Exudative lesion, n (%)		21 (12.1)	10 (41.7)	<0.01	38 (49.4)	100 (82.0)	<0.01
Microaneurysm, n (%)		11 (6.4)	10 (41.7)	<0.01	19 (24.7)	75 (61.5)	<0.01
Perihilar neovascularization, n (%)		169 (97.7)	23 (95.8)	1	76 (98.7)	121 (99.2)	1
Glomerulomegaly, n (%)		27 (15.6)	8 (33.3)	0.07	24 (31.2)	70 (57.4)	<0.01
% global sclerosis, %		5 [0, 14]	21 [8, 31]	<0.01	8 [0, 19]	28 [17, 42]	<0.01
Segmental sclerosis, n (%)		3 (1.7)	2 (8.3)	0.22	8 (10.4)	28 (23.0)	0.04
Glomerular lesion, n (%)	IIa	94 (54.3)	3 (12.5)	<0.01	21 (27.3)	5 (4.1)	<0.01
	IIb	70 (40.5)	17 (70.8)		28 (36.4)	27 (22.1)	
	III	8 (4.6)	4 (16.7)		26 (33.8)	69 (56.6)	
	IV	1 (0.6)	0 (0.0)		2 (2.6)	21 (17.2)	
Tubulointerstitial lesions							
Inflammatory cell infiltration, n (%)	0	101 (58.4)	2 (8.3)	<0.01	19 (24.7)	0 (0.0)	<0.01
	1	72 (41.6)	11 (45.8)		58 (75.3)	12 (9.8)	
	2	0 (0.0)	4 (16.7)		0 (0.0)	52 (42.6)	
	3	0 (0.0)	7 (29.2)		0 (0.0)	58 (47.5)	
Vascular lesions							
Hyalinosis, n (%)	0	39 (22.5)	2 (8.3)	0.02	7 (9.1)	3 (2.5)	<0.01
	1	54 (31.2)	4 (16.7)		12 (15.6)	4 (3.3)	
	2	23 (13.3)	2 (8.3)		8 (10.4)	12 (9.8)	
	3	57 (32.9)	16 (66.7)		50 (64.9)	103 (84.4)	
Intimal thickening, n (%)	0	43 (27.9)	4 (18.2)	0.01	16 (22.2)	15 (12.8)	0.1
	1	37 (24.0)	12 (54.5)		30 (41.7)	43 (36.8)	
	2	74 (48.1)	6 (27.3)		26 (36.1)	59 (50.4)	

Glomerular lesions and IFTA were classified according to the Research Committee of the Renal Pathology Society guidelines.

Results are shown as median [interquartile range] or prevalence (percentage)

Abbreviations: DN: diabetic nephropathy; IFTA: interstitial fibrosis and tubular atrophy.

The severity of diffuse lesions of glomeruli was graded on a scale of 0 to 3 as follows: grade 0, normal or mild mesangial expansion; grade 1, mesangial expansion < capillary lumen; grade 2, mesangial expansion = capillary lumen; and grade 3, mesangial expansion > capillary lumen. Subendothelial space widening was graded on a scale of 0 to 3 by % of double contour basement membrane (determined in a peripheral capillary of the most severe glomerulus) as follows: grade 0, <10%; grade 1, 10–25%; grade 2, 25–50%; and grade 3, 50% or higher. Inflammatory cell infiltration was graded on a scale of 0 to 3 as follows: grade 0, no cell infiltration; grade 1, <25%; grade 2, 25–50%; and grade 3, 50% or higher. Hyalinosis was graded on a scale of 0 to 3 as follows: grade 0, no hyalinosis; grade 1, one or more partial arteriolar hyalinosis; grade 2, approximately 50% hyalinosis; and grade 3, more than 50% hyalinosis, or penetrating hyalinosis. Intimal thickening was graded on a scale of 0 to 2 as follows: grade 0, no intimal thickening; grade 1, intimal thickness/media thickness <1; and grade 2, intimal thickening and intimal thickness/media thickness 1 or higher. The nodular lesion, exudative lesion, microaneurysm, perihilar neovascularization, glomerulomegaly, and segmental sclerosis were shown as their presence or absence.

Table 5. Association of renal histology with ESKD in non-proteinuric DN and proteinuric DN.

		Crude	Model 1	Model 2	Model 3
Non-proteinuric DN	GL (IIb or higher)	<0.01 (0–2.38)	<0.01 (0–2.76)	<0.01 (0–2.48)	<0.01 (0-Inf)
	IFTA (≥25%)	7.19 (2.27–22.73)	6.37 (1.88–21.53)	4.98 (1.39–17.8)	6.63 (1.20-36.8)
Proteinuric DN	GL (IIb or higher)	3.74 (2.15–6.50)	3.85 (2.20–6.74)	2.97 (1.66–5.32)	3.05 (1.67-5.57)
	IFTA (≥25%)	4.47 (2.41–8.29)	4.42 (2.37–8.26)	4.01 (2.11–7.63)	3.76 (1.96-7.24)

Results are shown as hazard ratio (95% confidence interval) for ESKD.

N=396 patients and 99 ESKD events.

Model 1 adjusted for age and sex.

Model 2 (main model) adjusted for model 1 factors + body mass index, estimated glomerular filtration rate, and systolic blood pressure.

Model 3 adjusted for model 2 factors + hyalinosis and intimal thickening.

Abbreviations: DN: diabetic nephropathy; GL: glomerular lesion; IFTA: interstitial fibrosis and tubular atrophy; ESKD: end-stage kidney disease.

Figure Legends

Figure 1. Association of ESKD with GL or IFTA among proteinuric and non-proteinuric DN

Kaplan-Meier survival curves for ESKD between the GL categories in non-proteinuric DN (a) and proteinuric DN (b). Kaplan-Meier survival curves for ESKD between IFTA categories in non-proteinuric DN (c) and proteinuric DN (d). The severe GL or IFTA was significantly associated with a higher incidence of ESKD in the proteinuric DN. In non-proteinuric DN, severe IFTA but not GL was significantly associated with a higher incidence of ESKD.

Abbreviations: ESKD: end-stage kidney disease; GL, glomerular lesion; IFTA: interstitial fibrosis and tubular atrophy; DN: diabetic nephropathy.

Figure 2. Adjusted hazard ratio of glomerular lesion or IFTA for ESKD among proteinuric and non-proteinuric DN. Adjusted hazard ratios with 95% confidence intervals are shown. Hazard ratios were adjusted for age, sex, BMI, systolic blood pressure, and eGFR.

Abbreviations: IFTA, interstitial fibrosis and tubular atrophy; ESKD: end-stage kidney disease; DN, diabetic nephropathy; BMI: body mass index; eGFR: estimated glomerular filtration rate.

Figure 3. Restricted cubic spline analyses of the probability of ESKD with proteinuria

a. The association of ESKD with proteinuria stratified by the glomerular lesion.

The risk for ESKD in the severe IFTA group was consistently higher than that in the mild IFTA group, regardless of proteinuria levels.

b. The association of ESKD with proteinuria stratified by IFTA.

Proteinuria levels were positively associated with the risk of ESKD in both the severe and mild glomerular lesion groups. However, the severity of glomerular lesions was not consistently associated with ESDK risk.

Abbreviations: ESKD: end-stage kidney disease; IFTA: interstitial fibrosis and tubular atrophy.