Title

Renal arteriolar hyalinosis, not intimal thickening in large arteries, is associated with cardiovascular events in people with biopsy-proven diabetic nephropathy

Running Title: Pathological characteristics and outcomes of diabetic nephropathy

Authors

Katsuhiko Morimoto, M.D.[1,2], Masaru Matsui, M.D., Ph.D.[1,2], Ken-ichi Samejima, M.D., Ph.D.[1,2], Tomoko Kanki, M.D.[2], Masatoshi Nishimoto, M.D.[1,2], Kaori Tanabe, M.D.[1,2], Miho Murashima, M.D., Ph.D.[1,2], Masahiro Eriguchi, M.D., Ph.D. [1], Yasuhiro Akai, M.D.[1,2], Ph.D., Masayuki Iwano, M.D., Ph.D.[2], Hideo Shiiki, M.D., Ph.D.[2], Hiroharu Yamada, M.D., Ph.D.[2], Masao Kanauchi, M.D., Ph.D.[2], Kazuhiro Dohi, M.D., Ph.D.[2], Kazuhiko Tsuruya, M.D., Ph.D.[1], and Yoshihiko Saito, M.D., Ph.D.[2]

Affiliations

[1]Department of Nephrology, Nara Medical University, Nara, Japan

[2]First Department of Internal Medicine, Nara Medical University, Nara, Japan

Address for correspondence

Kenichi Samejima

Department of Nephrology, Nara Medical University,

840 Shijo-cho, Kashihara, Nara, 634-8522 Japan,

Phone +81-744-22-3051 Ext. 3441;

Fax +81-744-22-9726;

E-mail: ksame@naramed-u.ac.jp

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What's new?

1) In diabetic nephropathy, relationship between two histological vascular lesions in kidney tissues and future cardiovascular events is unclear. We evaluated hyalinosis in small arterioles with <150-μm diameter, and intimal thickening in double-layered large arteries with 150-300-μm diameter. Arteriolar hyalinosis was significantly associated with cardiovascular events, whereas intimal thickening in large arteries was not.
2) Systolic blood pressure was strongly related to arteriolar hyalinosis but not to intimal thickening in large arteries, suggesting that hypertensive injury of smaller arterioles of the kidneys was more strongly associated with cardiovascular events and mortality than that of larger arteries in diabetic nephropathy.

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Abstract

Aims

Diabetic nephropathy, a pathologically diagnosed microvascular complication of diabetes, is a strong risk factor for cardiovascular events, which mainly involve larger arteries than those affected in diabetic nephropathy. However, the association between diabetic nephropathy pathological findings and cardiovascular events has not been well studied. We aimed to investigate whether the pathologic findings in diabetic nephropathy are closely associated with cardiovascular event development.

Methods

This retrospective cohort study analysed 377 people with type 2 diabetes and biopsy-proven diabetic nephropathy, with a median follow-up of 5.9 years (IQR 2.0 to 13.5). We investigated how cardiovascular events were impacted by two vascular diabetic nephropathy, lesions, namely arteriolar hyalinosis and arterial intimal thickening, and by glomerular and interstitial lesions.

Results

Of the 377 people with diabetic nephropathy, 331 (88%) and 295 (79%) had arteriolar hyalinosis and arterial intimal thickening, respectively. During the entire follow-up period, those with arteriolar hyalinosis had higher cardiovascular event rates in the crude Kaplan-

Meier analysis than those without these lesions (P=0.005 by the log-rank test). When fully adjusted for clinically relevant confounders, arteriolar hyalinosis independently predicted cardiovascular events (hazard ratio [HR], 1.99; 95% confidence interval [CI], 1.12, 3.86), but we did not find any relationship between arterial intimal thickening and cardiovascular events (HR, 0.89; 95% CI, 0.60, 1.37). Additionally, neither glomerular nor interstitial lesions were independently associated with cardiovascular events in the fully adjusted model.

Conclusions

Arteriolar hyalinosis, but not intimal thickening of large arteries, was strongly associated with cardiovascular events in people with diabetic nephropathy.

Keywords: diabetic nephropathy, cardiovascular disease, kidney biopsy, arteriolar hyalinosis, arterial intimal thickness

Introduction

Diabetic nephropathy, a pathologically diagnosed microvascular complication of diabetes, is the leading cause of end-stage renal disease (ESRD) [1,2]. However, people with diabetic nephropathy are more likely to develop cardiovascular events, which are macrovascular complications of diabetes, before reaching ESRD, than those who have reached ESRD [3].

Pathological findings in diabetic nephropathy, are complex, and there is no united classification system for the condition. The Renal Pathology Society recently reported a newly developed pathologic classification scheme for diabetic nephropathy, which divides the condition into four hierarchical glomerular lesions with varying degrees of interstitial and vascular involvement [4]. Arteriolar hyalinosis and atherosclerosis characterised by intimal thickening in large arteries are individually assessed as having vascular lesions of diabetic nephropathy and graded into three categories.

Earlier studies [5-7] involving cohorts with biopsy-proven diabetic nephropathy showed a significant association between severity of glomerular lesions and ESRD development, independent of clinical parameters such as proteinuria and estimated glomerular filtration rate (eGFR). Mise et al. reported that the degree of interstitial fibrosis and tubular atrophy (IFTA) increased the risk of ESRD [8]. This is understandable because ESRD is caused

mainly by the destruction of the glomerular architecture. However, the association between diabetic nephropathy pathological findings, especially vascular lesions, and cardiovascular events, has not been well studied.

To evaluate the vascular lesions of diabetic nephropathy according to the recent criteria, we measured arterial diameters of biopsied specimens, then evaluated hyalinosis in arterioles with <150-μm diameter and intimal thickening in arteries with ≥150-μm diameter. We also investigated the relationship between vascular lesions and cardiovascular events in biopsy-proven people with diabetic nephropathy.

Participants and Methods

Participants

Inclusion criteria are people who have been clinically diagnosed with type 2 diabetes at Nara Medical University Hospital between June 1981 and December 2014 and who need to be differentiated from other renal diseases. For example, patients with high urine protein levels, with hematuria, or with a very rapid decline in renal function. Exclusion criteria were those with insufficient glomeruli for diagnosis or diagnosis other than diabetic nephropathy, and missing data for analyses.

The study protocol was approved by the Nara Medical University Ethics Committee (No. 2005-18) and registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (UMIN000031121). Informed consent was obtained from the participants.

Clinical examinations

Baseline demographics and laboratory results of the participants at the time of biopsy were obtained by chart review. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure > 90 mmHg, or current use of antihypertensive drugs. Dyslipidaemia was defined as low-density lipoprotein cholesterol (LDL-C) >3.6 mmol/L, high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L, triglyceride (TG) >1.7 mmol/L, or taking a lipid-lowering agent. Hyperuricaemia was defined as uric acid level >416.4 μmol/L or current use of anti-hyperuricaemic drugs. eGFR was calculated by the equation developed for Japanese [9]. Serum creatinine values measured by the Jaffe method were converted to values for the enzymatic method by subtracting 18.3 μmol/L [10]. HbA1c levels are presented as National Glycohemoglobin Standardization Program values according to the recommendations of the Japanese Diabetic Society [11] and International Federation of Clinical Chemistry [12].

Renal biopsy and pathological examinations

The indications for renal biopsy were one or more of the following: proteinuria >0.5 g/day, persistent haematuria, and elevated serum creatinine level with a diagnosis of diabetes. Histologic examinations were performed independently by at least two renal pathologists, with differences resolved by consensus.

In accordance with the guidelines of the Research Committee of the Renal Pathology Society [4], vascular lesions were categorised based on the severity of arteriolar hyalinosis and atherosclerosis in large arteries. We evaluated arteriolar hyalinosis in small arterioles

with <150-μm diameter [13] (**Figure 1A and 1C**). Arteriolar hyalinosis was scored 0 if no arteriolar hyalinosis was present, 1 if at least one area of arteriolar hyalinosis was present, and 2 if more than one area of arteriolar hyalinosis was present. We also evaluated atherosclerosis in double-layered large arteries with 150-299-μm diameter, corresponding to interlobular and segmental arteries (**Figure 1B and 1D**). Atherosclerosis in large arteries was scored 0 if no intimal thickening was present, 1 if intimal thickening was less than the medial thickness, and 2 if intimal thickening was greater than the medial thickness. We ultimately classified the 377 people into 2 groups based on the absence or presence of at least one area of arteriolar hyalinosis. They were also divided into 2 groups according to the absence or presence of intimal thickening in large arteries. We also quantitatively measured intimal and medial thicknesses and lumen diameter of large arteries (BZ-X710, Keyence, Osaka, Japan).

Glomerular lesions were classified as follows: Class I, characterised by thickening of the glomerular basement membrane as detected by electron microscopy; Classes IIa and IIb, mild and severe mesangial expansion, respectively; Class III, nodular sclerosis with <50% global glomerulosclerosis; and Class IV, >50% global glomerulosclerosis. We did not include Class I people, because the renal tissues were not examined by electron microscopy. Those with Class III or IV were combined, as both had Kimmelstiel-Wilson lesions. The current study

compared the risk of adverse events between people with Classes IIb-IV and Class IIa. Based on the guidelines of the Renal Pathology Society [4], severity of IFTA was graded as follows: 0, the biopsy specimen showed no IFTA; 1, <25% IFTA was present; 2 at least 25% but <50% IFTA was present; and 3, at least 50% IFTA was present.

Main outcomes

Primary endpoints were cardiovascular events consisting of the first occurrence after biopsy of any of the following: coronary re-vascularisation, fatal or non-fatal acute myocardial infarction, unexpected hospitalisation due to worsening of congestive heart failure, fatal arrhythmia, fatal and non-fatal stroke, major amputation, and sudden death. Myocardial infarction was defined as chest pain associated with ST elevation and an increase in cardiac biomarkers. Coronary re-vascularisation included percutaneous coronary intervention or coronary artery bypass grafting. Heart failure was defined according to the Framingham criteria. Stroke was defined as a new fixed neurologic deficit caused by cerebral infarction or haemorrhage.

Secondary endpoints were ESRD development and all-cause death. ESRD development was defined as the commencement of long-term dialysis due to renal failure. People were followed up until the end of 2015 or death. Most events were obtained through a chart review, but death is partly confirmed by telephone interviews with families for those

without routine visits to our hospital.

Statistical analysis

Categorical variables were compared by the Chi-square test or Fisher's exact test as appropriate, and continuous variables were compared using the unpaired t-test, Mann-Whitney U test, analysis of variance, or Kruskal-Wallis H test, as appropriate. Survival curves were obtained using the Kaplan-Meier method and compared by a log-rank test. The Cox proportional hazard model was used to calculate HRs and 95% confidence intervals (CIs).

In Cox regression analysis, model 1 was initially adjusted for age and sex. Model 2 consisted of model 1 plus risk factors including hypertension, dyslipidaemia, hyperuricaemia, smoking, and body mass index. Model 3 consisted of model 2 plus haemoglobin, fasting blood sugar, eGFR, and proteinuria. Model 4 consisted of model 3 plus renin–angiotensin system blockers and diabetes treatment. We further assessed the robustness of other results using a sensitivity analysis.

A *P*-value <0.05 was considered to indicate statistical significance. All analyses were performed using JMP 10.0.2 (SAS Institute Inc., Cary, NC).

Results

Patient profiles and vascular lesions

There were 4379 renal biopsies performed at Nara Medical University Hospital between June 1981 and December 2014. 525 biopsies related to people with type 2 diabetes; of these, 81 were excluded because of inadequate samples or absence of pathologically diagnosed diabetic nephropathy, and 67 were excluded due to missing data for analyses, leaving 377 for analysis (**Figure 1, 2**).

The baseline characteristics are shown in **Table 1** and **Supplemental Table 1**. Median age was 60 years, and 63% were men.

Arteriolar hyalinosis and arterial intimal thickening were present in 331 (88%) and 295 (79%) people, respectively. Regarding glomerular lesions, 217 people were classified as Class IIa, 34 as Class IIb, and 126 as Classes III-IV. Thirty-three people had no IFTA, 235 with <25% IFTA, 65 with 25%-50% IFTA, and 77 with >50% IFTA. Median follow-up of 5.9 years (IQR 2.0 to 13.5). Cardiovascular events and ESRD, and death developed during follow-up in 149 (58.6 events/1000 person-years) and 68 (22.4 events/1000 person-years), 15 (15.2 events/1000 person-years) people, respectively (**Table 2**).

Multivariate logistic regression analysis showed that arteriolar hyalinosis was significantly associated with systolic blood pressure and proteinuria (**Supplemental Table 2**). There was also an independent relation between intimal thickening in large arteries and both

eGFR and proteinuria.

Cardiovascular events

During the entire follow-up period, cardiovascular events occurred in 135 of 331 (57.5 events/1000 person-years) people and 14 of 46 (29.3 events/1000 person-years) people with and without arteriolar hyalinosis, respectively, and in 115 of 295 (50.1 events/1000 person-years) people and 34 of 82 (56.0 events/1000 person-years) people with and without intimal thickening in large arteries, respectively (**Table 2**). This corresponded to unadjusted HRs of 2.05 (95% CI (1.22,3.72); *P*=0.005) and 1.05 (95% CI (0.72,1.56)) in people with arteriolar hyalinosis and arterial intimal thickening, respectively (**Table 3**) (**Figure 3A and 3B**). In a multivariate Cox proportional hazard model, the fully adjusted HR for cardiovascular events in people with arteriolar hyalinosis compared to those without was 1.99 (95% CI (1.12,3.86)) (**Table 3**); however, intimal thickening in large arteries remained statistically nonsignificant.

By classification of glomerular lesions, cardiovascular events occurred in 92 of 217 (42%) people with Class IIa, 12 of 34 (35%) with Class IIb, and 45 of 126 (36%) with Class III-IV. Class IIb-IV people tended to experience more cardiovascular events than Class IIa people (*P*=0.093 by the log-rank test) (**Figure 3C**), and the fully adjusted HR for cardiovascular events in Class IIb-IV people, as compared with Class IIa people, was 1.57

(95% CI (0.88,2.79)) (**Table 3**). In the crude model, IFTA was significantly associated with cardiovascular events with HR of 1.43 (**Figure 3D**) (95% CI (1.00,2.02); *P*=0.049), but this relationship was attenuated in the fully adjusted model (HR, 0.98; 95% CI (0.61,1.55)).

The sensitivity analyses showed similar results, when we restricted the analysis to people who enrolled since 1990 and who were followed for at least 1 and 3 years after enrolment, and when C-reactive protein, glycated haemoglobin, urine protein-to-creatinine ratio, and diabetic retinopathy were included in the fully adjusted model (**Table 4**).

Development of ESRD

The co-primary endpoint of ESRD occurred in 64 of 331 (25.7 events/1000 person-years) people with arteriolar hyalinosis and 55 of 295 (23.9 events/1000 person-years) people with intimal thickening in large arteries, respectively. We found a significant association between arteriolar hyalinosis and ESRD incidence, with a crude HR of 3.04 (95% CI (1.25,10.0); *P*=0.012) (**Supplemental Figure 1A**). This relationship remained statistically significant when adjusted for Model 1 covariates but lost its significance when adjusting for additional covariates, including hypertension, fasting blood sugar, eGFR, and proteinuria (**Supplemental Table 3**). However, the presence of intimal thickening in large arteries was consistently not associated with ESRD incidence either without or with covariate adjustment

(**Supplemental Figure 1B, Supplemental Table 3**).

ESRD development occurred in 48 of 126 (38%) people with Class III–IV glomerular lesions, 9 of 34 (26%) people with Class IIb lesions, and 11 of 217 (5%) people with Class IIa lesions. Kaplan-Meier analysis revealed that people with Class IIb–IV glomerular lesions experienced a higher ESRD incidence than those with Class IIa lesions (P<0.001) (**Supplemental Figure 1C**). A multivariate Cox proportional hazard model demonstrated a fully adjusted HR of 5.50 (95% CI (2.41,13.5); P<0.001) for ESRD development in people with Class IIb and III–IV lesions, as compared to those with Class IIa lesions (**Supplemental Table 3**). A strong association between IFTA and ESRD was also observed in both crude and adjusted models (**Supplemental Figure 1D, Supplemental Table 3**). In the fully adjusted model, participants with at least 25% IFTA exhibited a 3.47-fold higher risk of ESRD than those with <25% IFTA.

All-cause mortality

Deaths from all causes occurred in 3 of 46 (5.3 events/1000 person-years) people versus 47 of 331 (17.3 events/1000 person-years) people without and with arteriolar hyalinosis, respectively (**Table 2**). As shown in **Supplemental Table 4**, arteriolar hyalinosis, but not intimal thickening in large arteries, was independently associated with all-cause

mortality. When adjusting for all covariates, however, neither IFTA nor glomerular lesions were associated with all-cause mortality.

Intimal thickness modelled as a continuous value and outcomes

Next, we assessed the relationship between intimal thickness modelled as a continuous value and outcomes. Median intimal, medial, and luminal diameters were 19 μm (IQR 7 to 38), 20 μm (IQR 14 to 28), and 65 μm (IQR 41 to 103), respectively. In the fully adjusted model, intimal diameter was not associated with cardiovascular events with HR of 0.99 (95% CI (0.99,1.00); *P*=0.618). Similar results were obtained in intima-to-media ratio (HR, 0.72; 95% CI (0.42,1.41)) and intima-to-lumen ratio (HR, 0.95; 95% CI (0.76,1.03); *P*=0.336). We also did not find any relationship of these values with ESRD and all-cause mortality.

Discussion

The present study showed that, among the diabetic nephropathy vascular lesions, arteriolar hyalinosis, but not arterial intimal thickening, was strongly associated with cardiovascular events and all-cause mortality, independent of clinical risk factors. Sensitivity analyses further strengthened the robustness of the present findings. However, we did not

find a significant relationship between these vascular lesions and ESRD development. Contrarily, the severity of both glomerular and interstitial lesions was independently associated with ESRD development, but not cardiovascular event development. Therefore, these data indicate that vascular, glomerular, and interstitial lesions differ in clinical significance, and furthermore, among the vascular lesions of diabetic nephropathy, arteriolar hyalinosis and arterial intimal thickening differ in this regard as well. Each type of diabetic nephropathy lesion, including arteriolar hyalinosis and arterial intimal thickening, may differ pathogenetically.

The present study does not clarify why people without arteriolar hyalinosis have a low risk of cardiovascular events. When comparing people with and without arteriolar hyalinosis, the latter are younger and have lower frequencies of hypertension and diabetic retinopathy, lower systolic blood pressure, higher eGFR, and a lower urine protein-to-creatinine ratio. These characteristics would seem to place them at lower risk of cardiovascular events. However, our data showed that arteriolar hyalinosis was significantly associated with cardiovascular events even after full adjustment for these clinical parameters, suggesting that arteriolar hyalinosis is a strong and distinct risk factor for cardiovascular events.

The cardiovascular event rates were approximately 58 and 29 per 1000 person-years in people with and without arteriolar hyalinosis, respectively. Thus, people with diabetic

nephropathy are at extremely high risk of cardiovascular events [14], especially those with arteriolar hyalinosis, who comprised >85% of people with diabetic nephropathy in the present study. Given that systolic blood pressure is closely correlated with the presence of arteriolar hyalinosis, strict blood pressure control might be important to prevent development and progression of these lesions and therefore also cardiovascular events, as shown in earlier investigations [15,16].

We assessed hyalinosis in small arterioles with <150-μm diameters, and intimal thickening in double-layered large arteries with 150-300-μm diameters. Hyalinosis of arterioles with <150-μm diameter is not only observed in diabetic nephropathy, but also sometimes in other glomerulonephritis such as nephrosclerosis, although the hyalinosis of efferent arterioles, whose diameters are always <150-μm, is assumed to be relatively specific to diabetic nephropathy [17]. A few prior studies have used biopsied or autopsied samples to investigate the relationship between high blood pressure and arteriolar hyalinosis [18-20]. Two of these reports showed a weak correlation between hypertension and intimal thickening in smaller arteries [19,20]. In the present study, systolic blood pressure was strongly related to arteriolar hyalinosis but not to intimal thickening in large arteries. The nature of the causal relationship between hypertension and arteriolar hyalinosis has not yet been clarified. However, possible mechanisms include the status of arterioles as so-called resistance

arteries, and also endothelial dysfunction [21.22]. Endothelial dysfunction is strongly associated with the progression of atherosclerosis and worsening of heart failure [23]. However, we found no association between intimal thickness in large arteries of the kidneys and cardiovascular events. The pathogenesis of arterial intimal thickness may vary among organs, including the heart, brain, and kidneys. There is a striking regional and segmental heterogeneity in the effect of the endothelial cell layer on the peripheral vascular tone [24]. The endothelium of large arteries has a greater nitric oxide synthase activity than that of smaller arteries [25]. Taken together, hypertensive injury of resistance arterioles <150-μm diameter is more strongly associated with future cardiovascular events and mortality than that of larger arteries in people with diabetic nephropathy.

We know of only one previous report, by Shimizu et al., that investigated the relationship between renal histological findings and cardiovascular events in people with biopsy-proven diabetic nephropathy [5]. which revealed that systolic blood pressure and arterial intimal thickening, but not arteriolar hyalinosis, were significantly associated with cardiovascular events. Although the reason for the discrepancy with our own findings is not clear, one possible explanation is the criteria used for diabetic vascular lesions. Shimizu et al. used the old criteria by Takazakura et al [26]. that did not focus on the vascular diameter of affected arteries or arterioles.

We confirmed the findings of earlier studies [5-7] that both glomerular and interstitial lesions were strong predictors of ESRD. However, arteriolar hyalinosis and arterial intimal thickening did not predict renal outcomes. An observational study showed that vascular indexes classified according to the description by Tervaert et al. [4] did not have any impact on renal outcomes, underscoring the necessity of redefining such indexes [7]. The relationship between vascular lesions and renal events remains controversial; therefore, further research is needed.

Several limitations were noted. First, this was a relatively small-sized, single-centre, retrospective study that included only Japanese people. However, this study was larger and had a longer follow-up period than other cohort studies of biopsy-proven diabetic nephropathy [5-7]. Second, this study did not include people with Class I diabetic nephropathy according to the recent classification system of the Renal Pathology Society [4], because this diagnosis, which requires electron microscopic examination, was not used at the beginning of our investigation. Third, we evaluated vascular lesions in small arterioles and large arteries, but it remains unclear whether these data were obtained from similar vascular beds in all participants. Fourth, we analysed the effect of only baseline medical treatment on clinical outcomes and did not follow medical treatment strategies for diabetic nephropathy, which changed significantly during the follow-up period. Fifth, for patients who

discontinued visits to the hospital, we confirmed their prognoses with the patients themselves or a family member by letter or telephone interview. However, we could not confirm the prognosis of every patient, which is a source of potential bias in this study. Sixth, we excluded the 36 people whose medical records were discarded and the 31 people whose renal biopsy tissue could not be evaluated because it did not contain arterioles. Selection bias could not be ruled out for the 36 people whose medical records were discarded. We compared age, sex, urinary protein, eGFR, blood pressure, arteriolar hyalinosis, glomerular lesions, and IFTA between the 31 people who were excluded because the renal biopsy tissue could not be evaluated due to a lack of arterioles and the 377 people in this study. However, age, sex, eGFR, systolic blood pressure, proteinuria dipstick, and histological change (arteriolar hyalinosis, glomerular lesions, and IFTA) between two groups were not significantly different.

The 31 people who were excluded because their renal biopsy tissue could not be evaluated due to a lack of arterioles tended to have more urinary protein, lower eGFR, and more severe arteriolar hyalinosis, glomerular lesions, and IFTA, although the differences were not significant.

In conclusion, arteriolar hyalinosis but not intimal thickening in large arteries was strongly associated with cardiovascular events in people with diabetic nephropathy.

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Table 1. Baseline characteristics by vascular lesion

Characteristic	All cases n=377	Arteriolar hyalinosis			Intimal thickening		
		Absence n=46	Presence n=331	*P* value	Absence n=82	Presence n=295	*P* value
Age, years	60 (51, 66)	54 (47, 62)	60 (52, 67)	0.005	56 (47, 63)	60 (52, 67)	0.006
Demographic and risk factors, no. (%)							
Sex, male	237 (63)	26 (57)	211 (64)	0.344	53 (65)	184 (62)	0.701
Hypertension	267 (71)	26 (57)	241 (73)	0.003	52 (63)	197 (76)	0.106
Dyslipidaemia	280 (74)	34 (74)	246 (74)	0.953	64 (78)	216 (73)	0.376
Hyperuricaemia	148 (39)	10 (22)	138 (42)	0.007	25 (30)	123 (42)	0.060
Previous cardiovascular diseases	125 (33)	14 (30)	111 (34)	0.672	26 (32)	99 (34)	0.751
Smoking, no. (%)	230 (61)	28 (61)	202 (61)	0.958	50 (61)	180 (61)	0.994
Body mass index, kg/m^2	23.6 (21.7, 26.0)	23.7 (21.7, 26.8)	23.6 (21.6, 25.9)	0.607	23.3 (21.2, 26.6)	23.7 (21.8, 26)	0.323
Blood pressure, mmHg							
Systolic	132 (120, 150)	126 (113, 137)	134 (120, 150)	0.004	130 (110, 146)	134 (120, 150)	0.065
Diastolic	76 (66, 82)	79 (64, 80)	76 (66, 82)	0.952	76 (64, 83)	76 (66, 82)	0.864
Laboratory results							
Haemoglobin, g/L	132 (117, 147)	139 (130, 149)	130 (115, 146)	0.009	138 (124, 152)	131 (115, 146)	0.020
Serum creatinine, μmol/L	88.4 (61.9, 115)	70.7 (53.0, 106)	88.4 (70.7, 115)	0.033	79.6 (53.0, 97.2)	88.4 (70.71, 124)	<0.001
eGFR, mL min^{-1} 1.73 m^{-2}	64 (42, 82)	75 (46, 89)	62 (42, 80)	0.022	75 (53, 87)	59 (40, 79)	<0.001
Serum albumin, g/L	40 (34, 43)	42 (38, 44)	40 (33, 43)	0.002	41 (35, 44)	40 (33, 43)	0.042
Fasting blood sugar, mmol/L (n=369)	7.66 (6.05, 10.4)	7.49 (5.77, 10.6)	7.66 (6.05, 10.4)	0.504	133 (6.05, 9.99)	7.66 (6.05, 10.5)	0.852
HbA1c, mmol/mol (n=323)	59 (47, 72)	62 (51, 71)	58 (47, 72)	0.458	61 (48, 75)	59 (47, 76)	0.412
[%] (n=323)	[7.6 (6.5, 8.8)]	[7.9 (6.9, 8.7)]	[7.5 (6.5, 8.8)]	[0.430]	[7.8 (6.6, 9.0)]	[7.6 (6.5, 8.7)]	[0.397]
Triglycerides, mmol/L	1.56 (1.13, 2.09)	1.52 (1.07, 1.85)	1.57 (1.13, 2.10)	0.464	1.50 (1.11, 2.15)	1.57 (1.14, 2.09)	0.816
T-cholesterol, mmol/L	5.35 (4.53, 6.18)	5.38 (4.68, 6.10)	5.35 (4.53, 6.26)	0.996	5.46 (4.63, 6.52)	5.30 (4.45, 6.15)	0.162
HDL cholesterol, mmol/L (n=319)	1.19 (0.98, 1.42)	1.11 (0.98, 1.24)	1.19 (0.98, 1.45)	0.071	1.14 (0.98, 1.37)	1.19 (0.98, 1.45)	0.583
LDL cholesterol, mmol/L (n=313)	3.36 (2.64, 4.03)	3.21 (2.74, 3.90)	3.36 (2.64, 4.06)	0.842	3.44 (2.56, 4.32)	3.34 (2.66, 3.96)	0.613
Proteinuria, No. (%)	288 (76)	25 (54)	263 (79)	<0.001	50 (61)	238 (81)	<0.001
Urine PC ratio, mg/mmol (n=319)	59 (19, 303)	13 (7, 52)	76 (24, 360)	<0.001	44 (14, 141)	69 (22, 312)	0.043

Microhaematuria, No. (%)	150 (40)	11 (24)	139 (42)	0.021	31 (38)	119 (40)	0.683
Diabetic retinopathy, No. (%), (n=362)	166 (46)	8 (18)	158 (50)	<0.001	33 (42)	133 (47)	0.487
Use of ACE inhibitor, ARB, or both, No. (%)	121 (32)	7 (15)	114 (34)	0.009	23 (28)	98 (33)	0.375

Data are expressed as medians (interquartile range). Previous cardiovascular diseases included myocardial infarction, coronary revascularisation, stroke and heart failure. Abbreviations: CRP, C-reactive protein, eGFR, estimated glomerular filtration rate (CKD-EPI); HbA1c, glycated haemoglobin; T-cholesterol, Total- cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein, PC ratio, urine protein-to-creatinine ratio. Comparison between the two groups was analyzed using the Chi-square test and the Mann-Whitney U test.

Table 2. Clinical outcomes by vascular lesion

Clinical outcomes	All		Arteriolar hyalinosis				Arterial intimal thickening			
	Total (n=377)		Absence (n=46)		Presence (n=331)		Absence (n=82)		Presence (n=295)	
	Number of events (%)	1000 person-years	Number of events (%)	1000 person-years	Number of events (%)	1000 person-years	Number of events (%)	1000 person-years	Number of events (%)	1000 person-years
Cardiovascular event	149 (40)	58.6	14 (30)	29.3	135 (41)	57.5	34 (42)	56.0	115 (39)	50.1
Stroke	28 (7)	8.3	3 (7)	5.1	25 (8)	9.0	5 (6)	6.3	23 (8)	9.0
Myocardial infarction	26 (7)	8.0	2 (4)	3.5	24 (7)	9.0	3 (4)	3.8	23 (8)	9.4
Revascularisation	51 (14)	18.1	7 (15)	13.5	44 (13)	19.2	13 (16)	20.5	38 (13)	17.5
Congestive heart failure	27 (7)	8.5	2 (4)	3.5	25 (8)	9.6	5 (6)	6.6	22 (7)	9.1
Others[a]	17 (5)	5.3	0 (0)	0	17 (5)	6.4	8 (10)	10.4	9 (3)	3.7
ESRD	68 (18)	22.4	4 (9)	7.3	64 (19)	25.7	13 (16)	17.7	55 (19)	23.9

All-cause death	50 (13)	15.2	3 (7)	5.3	47 (14)	17.3	13 (16)	16.7	37 (13)	14.8

Abbreviations; ESRD, end-stage renal disease. [a]Others included cardiac sudden death, amputation, fatal arrhythmia, and cardiac surgery.

Table 3. Risk of cardiovascular events according to renal histological findings

	Crude	Model 1	Model 2	Model 3	Model 4
Arteriolar hyalinosis	2.05 (1.22, 3.72)	1.76 (1.04, 3.21)	2.04 (1.16, 3.87)	1.97 (1.10, 3.80)	1.99 (1.12, 3.86)
Intimal thickening in large arteries	1.05 (0.72, 1.56)	0.87 (0.60, 1.30)	0.88 (0.60, 1.34)	0.88 (0.59, 1.35)	0.89 (0.60, 1.37)
Glomerular lesions	1.33 (0.95, 1.86)	1.24 (0.88, 1.75)	1.23 (0.75, 2.01)	1.58 (0.91, 2.73)	1.57 (0.88, 2.79)
IFTA	1.43 (1.00, 2.02)	1.17 (0.81, 1.66)	1.03 (0.66, 1.60)	0.99 (0.63, 1.57)	0.98 (0.61, 1.55)

Model 1 adjusted for age and sex. Model 2 adjusted for covariates in model 1 plus hypertension, fasting blood sugar, estimated glomerular filtration rate, and proteinuria. Model 3 adjusted for covariates in model 2 plus dyslipidaemia, hyperuricaemia, smoking, previous cardiovascular diseases, and body mass index, and haemoglobin. Model 4 adjusted for covariates in model 3 plus renin-angiotensin system blockers and diabetes treatment. Statistical analysis: Cox regression analysis

Table 4. Sensitivity analysis

	No. of people	No. of CV events	Arteriolar hyalinosis HR (95% CI)	Arterial intimal thickening HR (95% CI)
All participants	377	149	2.11 (1.20, 4.07)	0.80 (0.53, 1.22)
Participants since 1990	287	121	1.98 (1.10, 3.94)	0.90 (0.57, 1.46)
Follow-up of at least 1 year	319	142	2.25 (1.25, 4.44)	0.73 (0.48, 1.13)
Follow-up of at least 3 years	258	124	2.23 (1.23, 4.41)	0.79 (0.50, 1.26)
Fully adjusted model including CRP	325	131	2.02 (1.08, 4.16)	0.74 (0.48, 1.17)
Fully adjusted model including HbA1c	318	130	2.14 (1.16, 4.35)	0.77 (0.50, 1.21)
Fully adjusted model including urine PC ratio	318	127	1.96 (1.07, 3.93)	0.75 (0.48, 1.18)
Fully adjusted model including diabetic retinopathy	356	139	1.93 (1.08, 3.76)	0.77 (0.51, 1.20)

Abbreviations: HbA1c, glycated haemoglobin; PC ratio, urine protein-to-creatinine ratio. Statistical analysis: Cox regression analysis

Figure 1. Arteriolar hyalinosis and intimal thickening in large arteries

Yellow arrow shows arterioles without hyalinosis (A), Black arrow shows larger arteries without intimal thickening (B), and, Red arrow shows arterioles with hyalinosis, and blue arrow shows intimal thickening in large arteries. Green arrow shows a glomerulus (C and D).

Figure 2. Patient Flowchart

We enrolled people who underwent renal biopsy at Nara Medical University between June 1981 and December 2014. Data were finally analysed for 377 subjects. DM indicates diabetes; diabetic nephropathy, diabetic nephropathy; IgAN, IgA nephropathy; mesPGN, mesangial proliferative glomerulonephritis; MN, membranous nephropathy; BNS, benign nephrosclerosis; MPGN, membranous proliferative glomerulonephritis; FSGS, focal segmental glomerulosclerosis; MCD, minor change disease.

Figure 3. Kaplan-Meyer Curves of Cardiovascular Events

Panel shows the time to the first occurrence of cardiovascular events among people with or without pathological lesions including arteriolar hyalinosis (A), intimal thickening in large arteries (B), glomerular lesions (C) and interstitial fibrosis and tubular atrophy (D), Statistical analysis method: Kaplan-Meier method

Figure 1

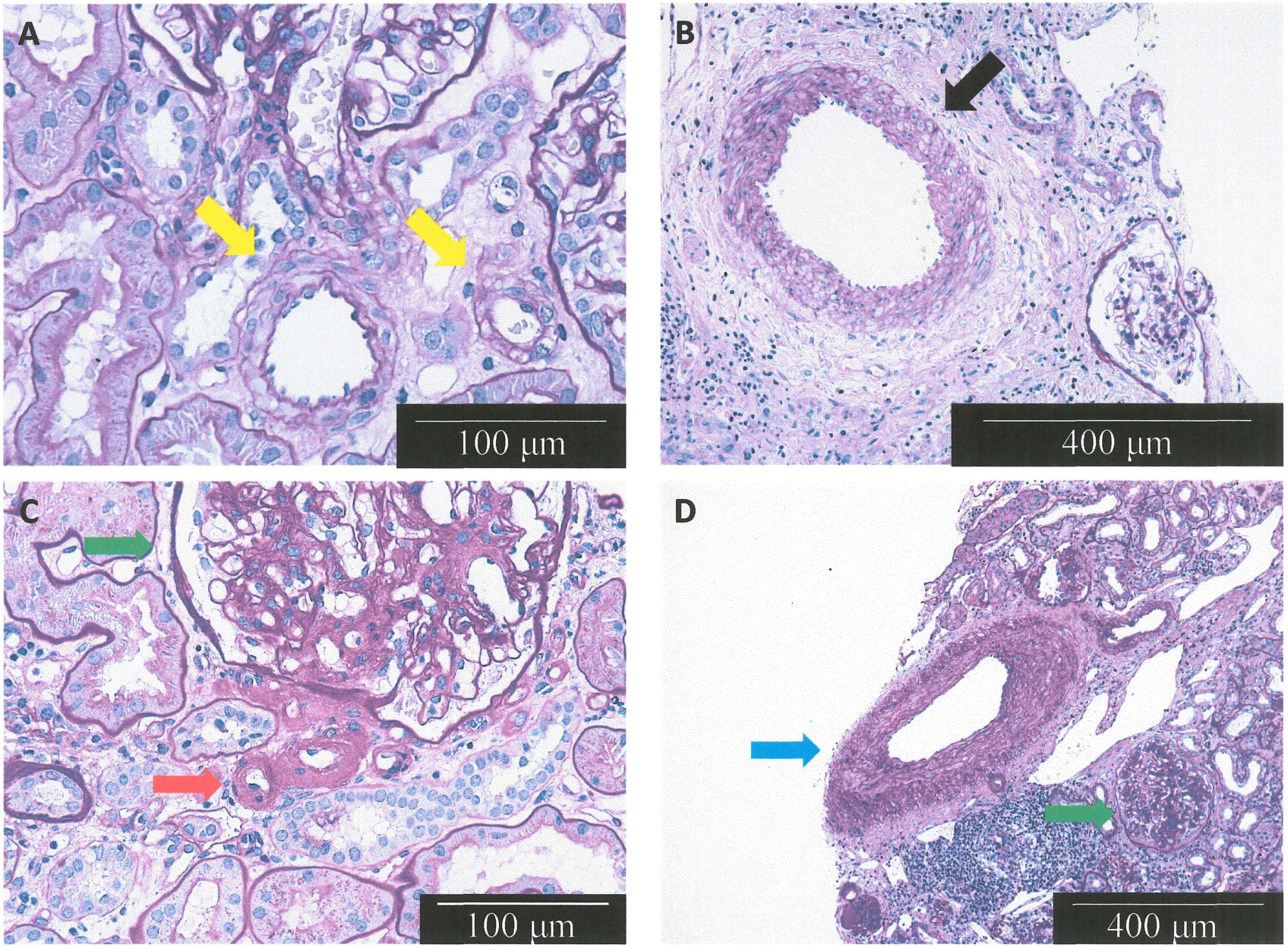


Figure 2. Flowchart

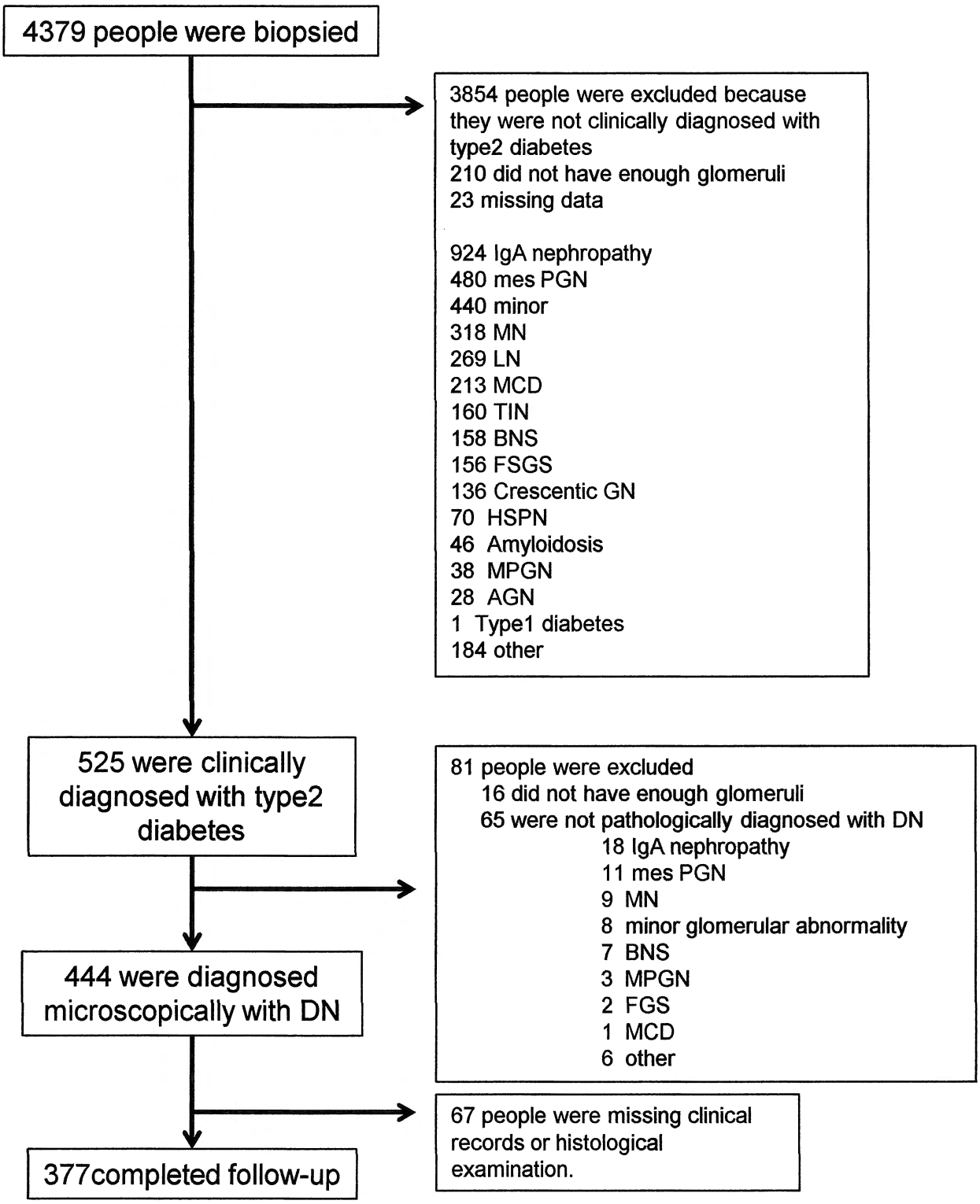
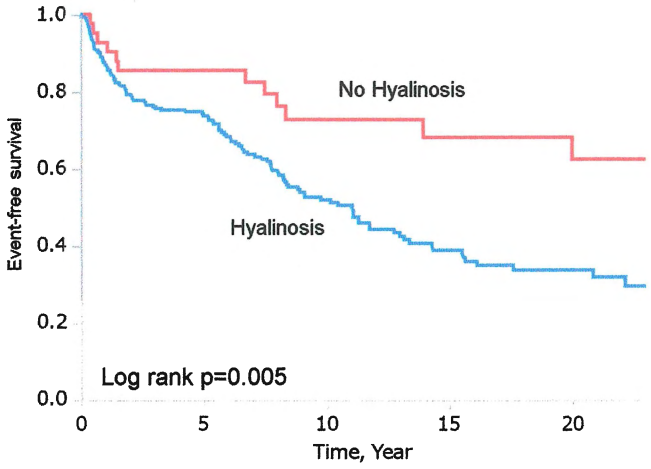


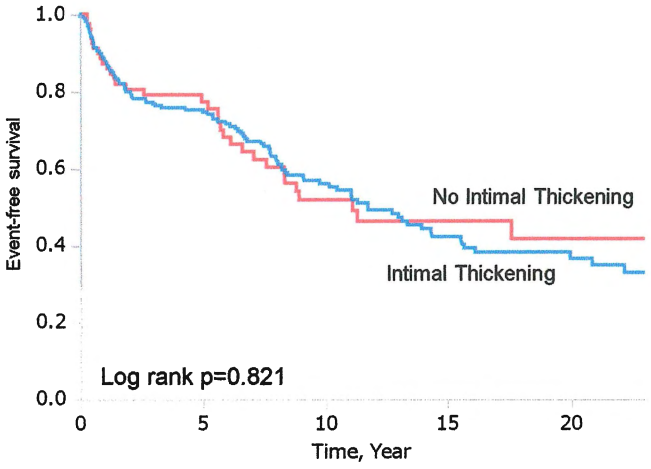
Figure 3. Kaplan-Meyer curves of the cardiovascular Events

(A) Cardiovascular Events by Arteriolar Hyalinosis



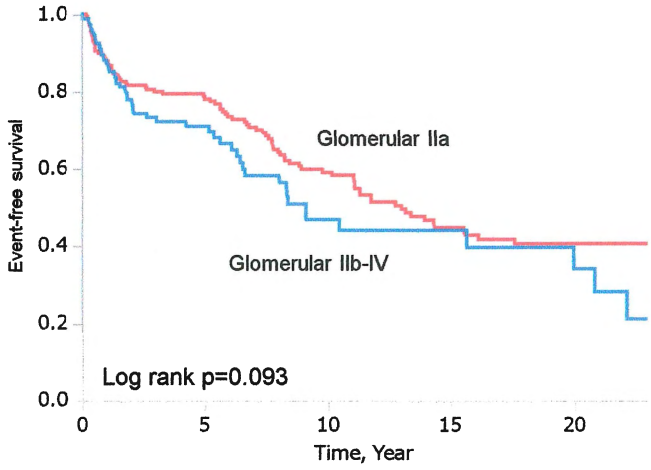
No Hyalinosis	46	31	20	16	12
Hyalinosis	331	143	75	41	19

(B) Cardiovascular Events by Intimal Thickening



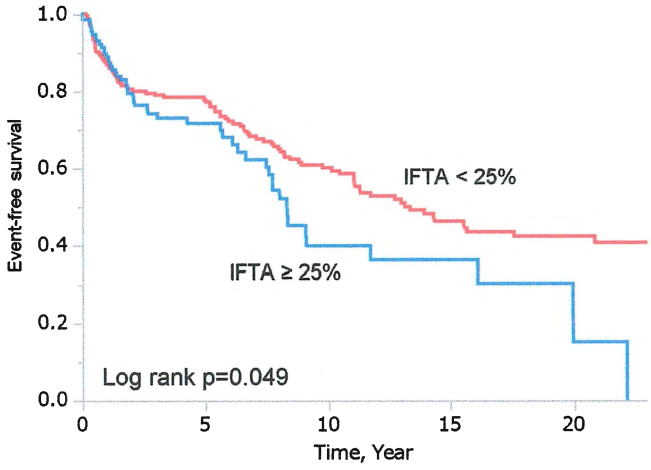
No Intimal Thickening	82	45	23	14	8
Intimal Thickening	295	128	72	43	23

(C) Cardiovascular Events by Glomerular Lesions



Glomerular IIa	217	123	76	46	24
Glomerular IIb - IV	160	51	19	11	7

(D) Cardiovascular Events by interstitial fibrosis and tubular atrophy (IFTA)



IFTA < 25%	235	129	81	50	29
IFTA ≥ 25%	142	45	14	7	2

Supplemental Figure 1 Kaplan-Meyer curves of ESRD