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ORIGINAL ARTICLE

Renal histologic changes and the outcome in patients with diabetic nephropathy

Yu An*, Feng Xu*, Weibo Le, Yongchun Ge, Minlin Zhou, Hao Chen, Caihong Zeng, Haitao Zhang and Zhihong Liu

National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

Correspondence and offprint requests to: Zhihong Liu; E-mail: liuzhihong@nju.edu.cn; zhihong–liu@hotmail.com
*Yu An and Feng Xu contributed equally to the work; both are first authors.

ABSTRACT

Background. The progression of diabetic nephropathy (DN) is frequently determined by clinical parameters; however, the predictive value of histologic lesions remains largely unknown. Our aim was to evaluate the relationship between histologic changes and renal outcome in patients with type 2 diabetes mellitus (T2DM).

Methods. A total of 396 patients with T2DM and biopsy-proven DN who received follow-up for at least 1 year were recruited. The severity of different histologic lesions was assessed using the pathologic classification established by the Renal Pathology Society. Renal outcomes were defined by progression to end-stage renal disease and doubling of serum creatinine. The influence of histologic findings on renal outcomes was assessed using univariate and multivariate Cox regression.

Results. A univariate Cox regression showed that the severity of glomerular and interstitial lesions had a significant impact on renal outcomes ($P < 0.001$). Scores of vascular lesions demonstrated no association with renal outcomes ($P > 0.05$). A

multivariate COX analysis demonstrated that the glomerular classes and scores of interstitial fibrosis and tubular atrophy were significantly associated with renal outcomes when adjusting for baseline proteinuria, mean arterial pressure and estimated glomerular filtration rate ($P < 0.05$). The glomerular and interstitial lesions correlated significantly among each other. However, in several patients, the severity of interstitial lesions did not correlate with glomerular lesions.

Conclusion. These findings indicated that the severity of glomerular and interstitial lesions were significantly associated with renal outcomes in patients with DN, whereas the vascular indexes did not have any impact on renal outcomes.

Keywords: diabetes nephropathy, renal outcome, renal pathology

INTRODUCTION

The prevalence of diabetic nephropathy (DN) has been increasing worldwide [1–3]. DN is now the primary cause of end-stage renal disease (ESRD) in developed countries and accounts for approximately 16.4% of all cases of ESRD in China [4]. The diagnosis of DN is, for the most part, based on the course of clinical manifestations, and kidney biopsy is only performed in patients with atypical presentations. However, evidence suggests that the prevalence of nondiabetic renal disease in patients with type 2 diabetes mellitus (T2DM) varies from 27 to 79% [5]. Specific histologic features of DN are required to confirm the diagnosis. Microalbuminuria has been considered to be a prognostic predictor for the progression of DN; however, recent studies suggested that several patients with microalbuminuria may demonstrate a return to normal [6–8]. Therefore, developing more sensitive and specific markers for predicting the progression of DN is urgently needed.

Recently, endeavors have targeted predictive biomarkers of DN from plasma or urine [9–12]. However, the importance of renal biopsy in DN was largely ignored. Several studies have described different types of histologic changes of DN, primarily in patients with T2DM [13–16]. The new pathologic classification established by the Renal Pathology Society provides a more detailed characterization of the pathology of DN. In this recent classification, DN is divided into four hierarchical glomerular lesions with a separate evaluation for interstitial and vascular involvement [17]. This classification is considered to be a very important first step toward the development of a clinically useful classification system for DN [18]. However, whether this pathologic classification can be used for predicting renal outcome has not been established.

Two recent studies published in 2012 by Oh *et al.* [5] and Okada *et al.* [19] attempted to establish the relationship between the pathologic classification system and clinical findings from small cohorts of 50 and 69 patients with T2DM. Unfortunately, the conclusions of these two studies were conflicting. In this study, we aimed to evaluate the relationship between histologic changes and renal outcome based on the new classification in 396 patients with T2DM and biopsy-proven DN who were followed up for >1 year after kidney biopsy.

MATERIALS AND METHODS

Inclusion criteria

All of the patients with biopsy-proven DN in the Nanjing Diabetic Nephropathy Registry from 2003 to 2011 were reviewed. The general indication for renal biopsy at our institution is persistent albuminuria or decreased serum creatinine, with a particular emphasis on sudden onset overt proteinuria, obvious hematuria or rapidly progressive glomerular nephropathy. DN was defined according to the following criteria: (i) a previous history of diabetes, usually concomitant with diabetic retinopathy, neuropathy and atherosclerotic complications; (ii) the presence of persistent albuminuria or decreased renal function; (iii) biopsy-proven kidney disease caused by diabetes and (iv) an exclusion of nondiabetic renal disease. A total of 396 patients were recruited, including 53 patients with microalbuminuria (30–300 mg/day), 173 patients with macroalbuminuria (>300 mg/day) and 170 patients with increased serum creatinine (>1.24 mg/dL). The inclusion criteria consisted of T2DM patients who had biopsy-proven DN, with a biopsy specimen comprising 10 or more total glomeruli, and who were followed up at our hospital for >12 months. Patients who progressed to ESRD within 12 months were also included in the study. Patients who had diseases concomitant with nondiabetic renal diseases, such as IgA nephropathy or membranous nephropathy, were excluded. The baseline and follow-up data of the patients were obtained from the Nanjing Diabetic Nephropathy Registry database.

Clinical and laboratory information

The baseline clinical characteristics, which were collected within 1 month of renal biopsy, included age and duration of diabetes at the time of biopsy, gender, systolic and diastolic blood pressures, weight, height, fasting blood glucose, HbA1c, 24-h urinary protein, serum creatinine (mg/dL), estimated glomerular filtration rate (e-GFR) (mL/min/1.73 m^2), serum albumin, total cholesterol and triglyceride levels. Subsequent follow-up visits of these patients were performed 2–4 times per year based on the patient's individual condition. The information for each visit was recorded. The mean arterial pressure (MAP) was defined as diastolic blood pressure plus 1/3 of pulse pressure. e-GFR was estimated using the Modification of Diet in Renal Disease equation. Renal survival was the primary end point of our study and was defined by the progression to ESRD, which is e-GFR < 15 mL/min/1.73 m^2 or the initiation of chronic renal replacement therapy. The secondary end point was the doubling of baseline serum creatinine levels. Patients who did not reach the end point were recorded using the information of their last follow-up visit.

Pathologic classification

For each biopsy specimen, light microscopy, immunofluorescence and electron microscopy were routinely performed. Sections for light microscopy were stained with hematoxylin–eosin, periodic acid–Schiff, Masson's trichrome and periodic acid methenamine silver. All of the patients were categorized based on the pathologic classification of the Renal Pathology

Society [17]. The glomerular classifications were as follows—class I: glomerular basement membrane thickening; class IIa: mild mesangial expansion; class IIb: severe mesangial expansion; class III: nodular sclerosis and class IV: global glomerulosclerosis in >50% of glomeruli. Interstitial fibrosis and tubular atrophy (IFTA) were scored as follows: 0, absent; 1, <25%; 2, 25–50% and 3, >50% of the total area. Interstitial inflammation was scored as follows: 0, absent; 1, inflammation only in relation to IFTA and 2, inflammation in areas without IFTA. Arteriolar hyalinosis was scored as follows: 0, absent; 1, at least one area of arteriolar hyalinosis and 2, more than one area of arteriolar hyalinosis. Arteriosclerosis was scored as follows: NA, absent of large vessels; 0, no intimal thickening; 1, intimal thickening less than thickness of media and 2, intimal thickening greater than thickness of media.

In addition, we also evaluated several common glomerular changes in this study, such as global and segmental sclerosis, atubular glomerulus, nodular lesions, microaneurysms, hyaline caps, capsular drops and fibrinoid exudation. All of the specimens were scored by the same pathologist (Dr Feng Xu) who was blinded to the clinical findings. In order to assess the reliability and reproducibility of the classification, 100 cases of biopsy slides were selected randomly and scored independently by another pathologist (Dr Hao Chen).

Statistical analysis

The data were analyzed using SPSS version 18. For continuous variables, data were presented as the mean ± standard deviation or the median with range, and the means were compared using one-way analysis of variance. For categorical variables, the data were presented as counts and percentages, and the differences were analyzed using the Chi-square test.

Renal outcome was assessed using Kaplan–Meier survival analysis, and the association between pathologic findings and renal outcome was calculated using Cox regression. Multivariate Cox analysis was used to determine the independent predictors of prognosis. Two-sided P values were reported, and $P < 0.05$ was considered to be statistically significant. The reproducibility of the pathology variables was evaluated using intraclass correlation coefficients (ICCs). Correlations between pathology variables were assessed using the Spearman test, and the Holm–Bonferroni method was used to minimize the risk of type 1 statistical error.

RESULTS

Clinical and pathologic characteristics

Of the 396 patients enrolled in the study, 250 were male (63.1%). At the time of biopsy, the mean age was 50.0 ± 9.1 years. The median duration of diabetes mellitus was 84 months (range, 1–336). The median follow-up period was 29 months. The mean baseline serum creatinine level was 1.29 ± 0.66 mg/dL, the mean e-GFR was 73 ± 33 mL/min/1.73 m^2 and the median 24-h proteinuria was 1.45 g/day (range, 0.1–21.6). Ninety patients (22.7%) progressed to ESRD during follow-up. Six patients reached ESRD within 12 months from the time of renal biopsy. A doubling of creatinine was observed in 102 patients (25.8%).

Based on the glomerular classification [17], 53 patients (13.4%) were in class I, 87 (22.0%) in class IIa, 32 (8.1%) in class IIb, 168 (42.4%) in class III and 56 (14.1%) in class IV. The percentage of patients who progressed to ESRD in classes I, IIa, IIb, III and IV was 0, 2.3, 18.8, 30.4 and 55.4%, respectively. Figure 1 illustrates several typical pathologic features of

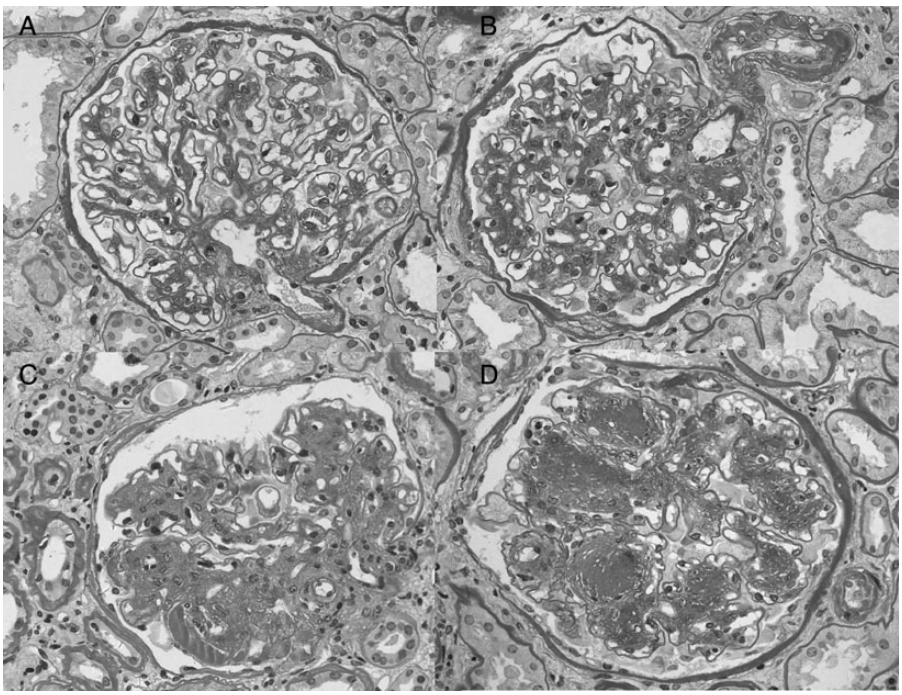

FIGURE 1: Representative examples of different glomerular classes in DN. (**A**) Mild changes by light microscopy and GBM thickening. (**B**) Mild mesangial expansion. (**C**) Severe mesangial expansion. (**D**) Kimmelstiel–Wilson lesion.

different classes. The ICC for glomerular class was 0.82. The ICCs for each pathologic variable were provided in the Supplementary Data, Table S1. Clinical features of patients with different glomerular lesions were shown in Table 1.

Glomerular lesions and renal outcome

Survival curves of the primary and secondary end points are shown in Figures 2 and 3. The Kaplan–Meier survival analysis showed an overall 5-year renal survival rate of 61.0% in these patients. Patients of different glomerular classifications had 5-year renal survival rates of 100% (class I), 90.1% (class IIa), 75.4% (class IIb), 39.0% (class III) and 15.3% (class IV). The univariate Cox analysis showed that glomerular lesions can significantly impact renal survival in these patients [hazard ratio (HR) = 2.99, P < 0.001] and doubling of creatinine (HR = 2.55, P < 0.001). Patients in class III had a poorer renal survival than those in class IIb (HR = 2.98, P = 0.013). After adjusting for baseline proteinuria, MAP and e-GFR, the severity of glomerular lesions remained an independent risk factor for both progression to ESRD and doubling of creatinine (Table 2).

Moreover, the univariate Cox analysis showed that the following lesions were significantly associated with renal outcomes (P < 0.001): nodule lesions, microaneurysms, atubular glomeruli, segmental sclerosis, hyaline caps and fibrinoid exudation, except capsular drops (Supplementary Data, Table S2). As the percentage of global sclerosis increased by 10%, the risk of progression of DN to ESRD increased by a factor of 1.5 (P < 0.001). However, when adjusted for the pathologic classification, the above-mentioned changes were not associated with renal outcomes, whereas the glomerular classes remained an independent risk factor.

Interstitial lesions and renal outcome

IFTA of scores 0, 1, 2 and 3 were observed in 29 (7.4%), 208 (52.5%), 79 (19.9%) and 80 (20.2%) patients, respectively. Interstitial inflammation of scores 0, 1 and 2 were observed in 51 (12.8%), 205 (51.8%) and 140 (35.4%) patients, respectively. As shown in Table 2, IFTA and interstitial inflammation were significantly associated with renal outcomes in a univariate Cox analysis (P < 0.001). However, in a multivariate Cox regression analysis after adjusting for baseline proteinuria, MAP and e-GFR, IFTA remained an independent risk factor for renal survival (HR = 1.51, P = 0.028) and doubling of creatinine (HR = 1.45, P = 0.031). However, interstitial inflammation failed to remain an independent predictor (P > 0.05).

When patients in classes I and IIa were excluded from the analysis, glomerular lesions were still significantly associated with renal outcomes using a univariate Cox analysis, but failed to be an independent risk factor. However, IFTA still remains an independent predictor (Supplementary Data, Table S3).

Vascular lesions and renal outcome

Among 414 patients, arteriolar hyalinosis was absent in seven patients (1.8%). More than one area of arteriolar hyalinosis (scored as 2) was found in 380 patients (96.0%). In 26 (6.6%) patients, large vessels were absent in renal biopsies. Among the remaining 370 patients, 67 (18.1%) had no intimal thickening (scored as 0) and 251 (67.8%) had severe arteriosclerosis (scored as 2). A univariate Cox analysis revealed no significant correlation between the current vascular indexes and renal outcomes (Table 2).

Correlation between glomerular and interstitial lesions

As shown in Table 3, scores of interstitial lesions increased in proportion to the severity of glomerular lesions (P < 0.001 for trend). For patients in class I, the majority of the biopsy specimen showed only mild interstitial changes with IFTA and interstitial inflammation scores of 0 (32.1 and 43.4%) and 1 (67.9 and 54.7%). However, in patients in class IV, 96.4% had severe IFTA (scores of 2 and 3) and 83.9% had severe

Table 1. Clinical features of patients with different glomerular lesions

	Class I (n = 53)	Class IIa (n = 87)	Class IIb (n = 32)	Class III (n = 168)	Class IV (n = 56)	Total (n = 396)
Age (years)	47.4 ± 9.0	48.4 ± 8.9	53.0 ± 8.9*§	50.8 ± 9.0†	51.7 ± 9.1†§	50.1 ± 9.1
Male	27 (50.9)	66 (75.9)	26 (81.3)	91 (54.2)	40 (71.4)	250 (63.1)
Duration of diabetes (m)	24 (1–192)	48 (1–226)	120 (6–288)	120 (1–336)	138 (2–336)	84 (1–336)
BMI (kg/m^2)	26.5 ± 3.6	27.1 ± 3.6	25.2 ± 2.5§	24.4 ± 3.5*‡	25.0 ± 4.4†‡	25.4 ± 3.8
SBP (mmHg)	131 ± 16	134 ± 15	142 ± 21*§	144 ± 19*‡	147 ± 17*‡	140 ± 13
DBP (mmHg)	82 ± 10	84 ± 9	84 ± 8	84 ± 10	86 ± 10	84 ± 10
MAP (mmHg)	98 ± 11	101 ± 10	103 ± 11†	104 ± 11*§	106 ± 10*‡	103 ± 11
History of hypertension	31 (58.5)	69 (79.3)	27 (84.4)	140 (83.3)	54 (96.4)	321 (81.1)
24-h proteinuria (g/day)	0.4 (0.1–10.2)	0.6 (0.1–6.4)	1.3 (0.3–9.4)	2.5 (0.1–21.6)	3.2 (0.3–10.5)	1.45 (0.1–21.6)
Serum creatinine (mg/dL)	0.79 ± 0.39	1.02 ± 0.49†	1.23 ± 0.62*	1.36 ± 0.56*‡	1.99 ± 0.74*‡¶#	1.29 ± 0.66
e-GFR (mL/min/1.73 m^2)	105 ± 25	90 ± 30†	76 ± 29*	64 ± 28*‡	41 ± 18*‡¶#	73 ± 33
Serum albumin (g/L)	42.7 ± 6.5	43.9 ± 5.08	39.5 ± 5.0†‡	34.3 ± 5.9*‡#	35.1 ± 5.8*‡#	38.1 ± 7.1
Cholesterol (mmol/L)	5.07 ± 1.95	4.49 ± 1.53	4.63 ± 0.88	5.52 ± 1.64‡#	5.62 ± 1.75‡#	5.18 ± 1.68
Triglyceride (mmol/L)	2.22 ± 1.26	2.15 ± 1.08	1.77 ± 0.94	1.87 ± 1.00	2.06 ± 1.32	2.00 ± 1.10
FBS (mmol/L)	6.95 ± 2.04	6.51 ± 1.60	7.15 ± 2.02	6.72 ± 2.56	5.97 ± 2.13	6.63 ± 2.22
HbA1c (%)	7.05 ± 1.63	7.17 ± 1.49	7.20 ± 1.05	7.02 ± 1.59	6.84 ± 1.61	7.04 ± 1.54
Hemoglobin (g/L)	135 ± 18	136 ± 18	125 ± 15†‡	108 ± 19*‡#	107 ± 20*‡#	119 ± 23
Progressed to ESRD	0 (0)	2 (2.3)	6 (18.8)	51 (30.4)	31 (55.4)	90 (22.7)

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; e-GFR, estimated glomerular filtration rate; FBS, fasting blood sugar; ESRD, end-stage renal disease.

Data are presented as the mean ± standard, the median with range or counts and percentages.

*P < 0.01 versus class I. †P < 0.05 versus class I. ‡P < 0.01 versus class IIa. §P < 0.05 versus class IIa. #P < 0.01 versus class IIb. ¶P < 0.01 versus class III.

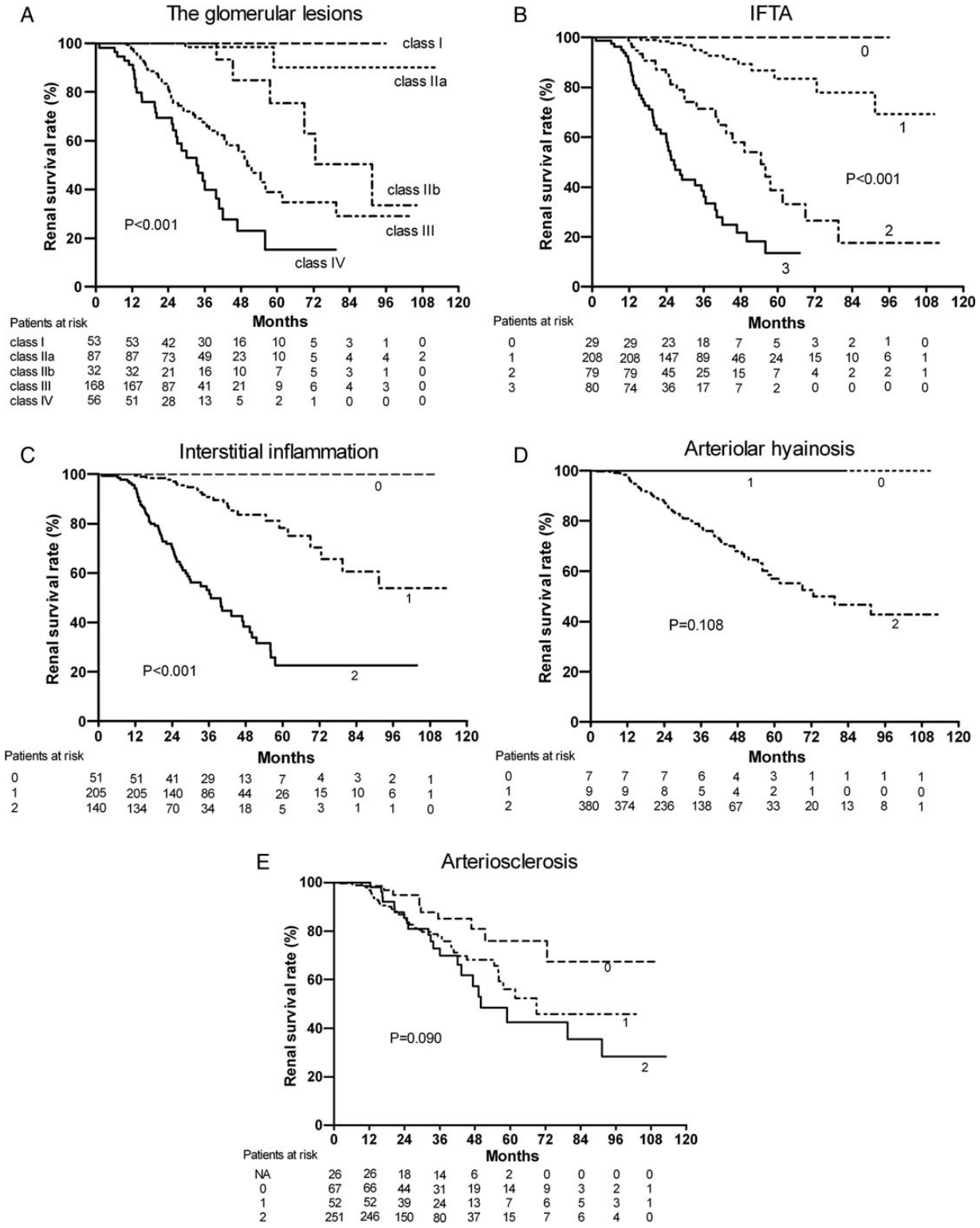

FIGURE 2: Kaplan–Meier curves of renal survival rate in patients with DN. (**A**) Renal survival rates in various glomerular pathologic classes. (**B**) Renal survival rates in IFTA. (**C**) Renal survival rates in interstitial inflammation. (**D**) Renal survival rates in arteriolar hyalinosis. (**E**) Renal survival rates in arteriosclerosis.

interstitial inflammation (score of 2). Nevertheless, 68.7% of patients with severe mesangial expansion (class IIb), and 50% of patients with nodular sclerosis (class III) had mild IFTA (scores of 0 and 1), whereas 12.7% of patients with mild mesangial expansion (class IIa) had severe IFTA (scored as 2 and 3), demonstrating a more heterogeneous histological manifestation of renal injury in patients with T2DM.

Further analysis showed that patients with mild glomerulopathy (class IIa) had a decreased e-GFR and severe anemia in those with severe IFTA (scores of 2 and 3) than those with

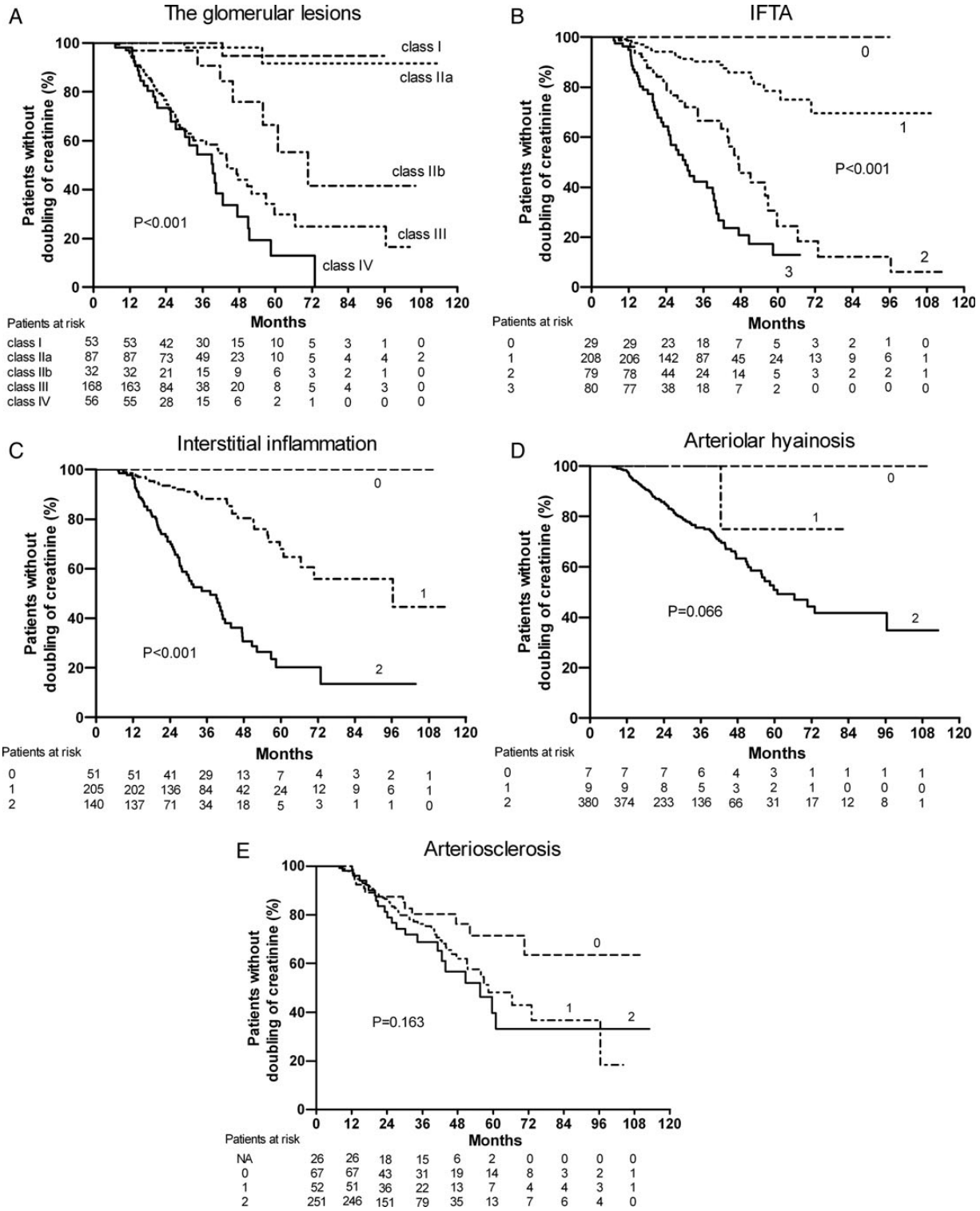

FIGURE 3: Kaplan–Meier curves of patients free from doubling of creatinine. (**A**) Percentages of patients without doubling of creatinine in various glomerular pathologic classes. (**B**) Percentages of patients without doubling of creatinine in IFTA. (**C**) Percentages of patients without doubling of creatinine in interstitial inflammation. (**D**) Percentages of patients without doubling of creatinine in arteriolar hyalinosis. (**E**) Percentages of patients without doubling of creatinine in arteriosclerosis.

mild IFTA (scores of 0 and 1) at the time of renal biopsy (Table 4). In addition, for patients with severe glomerulopathy (class IIb + III) and mild IFTA (scores of 0 and 1), the proportion of hypertension, baseline diastolic blood pressure and MAP decreased, baseline proteinuria decreased; however, the levels of fasting blood glucose and HbA1c were higher than in patients with both severe glomerulopathy (class IIb + III) and severe IFTA (scores of 2 and 3).

Significant correlations were observed between glomerular and interstitial lesions including either IFTA ($r = 0.66$) or interstitial inflammation ($r = 0.61$), suggesting a common pathogenesis for both glomerular and tubulointerstitial injury.

Table 2. Associations between pathologic parameters and renal outcomes

	Rate of renal survival				Survival from doubling of creatinine			
	Univariate		Multivariate[a]		Univariate		Multivariate[a]	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Glomerular lesions	2.99 (2.32–3.87)	<0.001	1.49 (1.10–2.02)	0.011	2.55 (2.05–3.16)	<0.001	1.38 (1.05–1.80)	0.021
IFTA	3.93 (3.01–5.12)	<0.001	1.51 (1.05–2.17)	0.028	2.98 (2.37–3.74)	<0.001	1.45 (1.03–2.03)	0.031
Interstitial inflammation	6.71 (4.27–10.53)	<0.001	1.31 (0.76–2.28)	0.332	5.21 (3.51–7.73)	<0.001	1.49 (0.90–2.45)	0.120
Arteriolar hyalinosis	7.95 (0.64–99.48)	0.108			5.07 (0.90–28.5)	0.066		
Arteriosclerosis	1.28 (0.96–1.70)	0.090			1.20 (0.93–1.56)	0.163		

IFTA, interstitial fibrosis and tubular atrophy; HR, hazard ratio; CI, confidence interval.
[a]Adjusting for baseline log-proteinuria, MAP and eGFR.

Table 3. Pathologic features of patients with different glomerular lesions

	Class I ($n = 53$)	Class IIa ($n = 87$)	Class IIb ($n = 32$)	Class III ($n = 168$)	Class IV ($n = 56$)	P for trend
IFTA						
0	32.1	10.3	6.3	0.6	0.0	<0.001
1	67.9	77.0	62.4	49.4	3.6	
2	0.0	10.3	25.0	29.8	21.4	
3	0.0	2.4	6.3	20.2	75.0	
Interstitial inflammation						
0	43.4	24.1	12.5	1.8	0	<0.001
1	54.7	66.7	68.8	51.8	16.1	
2	1.9	9.2	18.7	46.4	83.9	
Arteriolar hyalinosis						
0	9.4	2.3	0.0	0.0	0.0	<0.001
1	9.4	2.3	3.1	0.6	0.0	
2	81.2	95.4	96.9	99.4	100.0	
Arteriosclerosis						
NA	7.5	9.2	9.4	4.7	5.4	0.103
0	35.8	18.4	15.6	13.7	7.1	
1	5.7	10.3	9.4	17.3	14.3	
2	51.0	62.1	65.6	64.3	73.2	

IFTA, interstitial fibrosis and tubular atrophy.
Data are presented as percentages.

Table 4. Clinical features of patients with different glomerular and interstitial lesions

	Class IIa		P-value	Class IIb + III		P-value
	IFTA < 25% ($n = 76$)	IFTA ≥ 25% ($n = 11$)		IFTA < 25% ($n = 106$)	IFTA ≥ 25% ($n = 94$)	
Age (years)	47.9 ± 9.0	52.2 ± 7.4	0.133	50.0 ± 8.9	52.3 ± 9.1	0.078
Duration of diabetes (m)	57.0 ± 54.0	43.1 ± 40.4	0.416	119.3 ± 73.7	116.9 ± 66.0	0.806
BMI (kg/m^2)	27.2 ± 3.8	26.2 ± 1.3	0.120	24.5 ± 3.2	24.7 ± 3.6	0.715
SBP (mmHg)	134 ± 15	135 ± 8	0.945	141 ± 19	146 ± 19	0.084
DBP (mmHg)	84 ± 9	85 ± 5	0.816	82 ± 9	86 ± 11	0.007
MAP (mmHg)	101 ± 10	101 ± 5	0.765	102 ± 10	106 ± 12	0.010
History of hypertension	76.3	100.0	0.070	77.4	90.4	0.013
24-h proteinuria (g/day)	0.93 ± 1.10	1.64 ± 1.24	0.052	2.67 ± 2.74	3.54 ± 2.86	0.030
Serum creatinine (mg/dL)	0.95 ± 0.38	1.55 ± 0.78	0.029	1.07 ± 0.39	1.65 ± 0.58	<0.001
e-GFR(mL/min/1.73 m^2)	94.8 ± 28.6	59.3 ± 23.7	<0.001	79.8 ± 25.8	49.4 ± 21.5	<0.001
Serum albumin (g/L)	44.1 ± 5.2	42.4 ± 4.2	0.299	35.8 ± 6.3	34.3 ± 5.8	0.070
Cholesterol (mmol/L)	4.54 ± 1.57	4.13 ± 1.20	0.424	5.31 ± 1.60	5.46 ± 1.55	0.508
Triglyceride (mmol/L)	2.14 ± 1.05	2.22 ± 1.34	0.831	1.82 ± 0.90	1.90 ± 1.08	0.555
FBS (mmol/L)	6.54 ± 1.62	6.30 ± 1.53	0.648	7.14 ± 2.82	6.39 ± 1.99	0.029
HbA1c (%)	7.19 ± 1.57	7.04 ± 0.87	0.796	7.37 ± 1.49	6.71 ± 1.49	0.026
Hemoglobin (g/L)	138 ± 17	119 ± 16	0.001	117 ± 20	103 ± 16	<0.001

IFTA, interstitial fibrosis and tubular atrophy; HR, hazard ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; e-GFR, estimated glomerular filtration rate; FBS, fasting blood sugar; ESRD, end-stage renal disease.
Data are presented as the mean ± standard or percentages.

Correlation between glomerular and vascular lesions

The majority of patients showed obvious vascular lesions, regardless of the severity of glomerular lesions (Table 3). In class I, 81.2% of patients already had an arteriolar hyalinosis score of 2; and in classes IIa, IIb, III and IV, 95.4, 96.9, 99.4 and 100% of patients had an arteriolar hyalinosis score of 2, respectively. In class I, 51.0% of patients had an arteriosclerosis score of 2; in class IV, 73.2% of patients had an arteriosclerosis score of 2. The trend for arteriolar hyalinosis to become more pronounced occurred when there were more severe glomerular lesions ($P < 0.001$ for trend). A significant correlation was observed between arteriolar hyalinosis and the severity of glomerular lesions ($r = 0.25$). However, there was no significant correlation between arteriosclerosis and the severity of glomerular lesions.

Correlation between interstitial and vascular lesions

IFTA correlated significantly with either arteriolar hyalinosis ($r = 0.16$) or arteriosclerosis ($r = 0.14$), although the correlation coefficients were lower than those observed between glomerular and interstitial lesions. Arteriolar hyalinosis, but not arteriosclerosis, correlated with interstitial inflammation ($r = 0.15$). No significant correlation was observed between arteriolar hyalinosis and arteriosclerosis.

DISCUSSION

In this study, we evaluated the relationship between histologic changes and renal outcome in a large sample and a long-term follow-up cohort of patients with T2DM. The results revealed that the severity of glomerular lesions and IFTA were significantly associated with renal outcomes (progression to ESRD and doubling of creatinine), independent of clinical features. Interstitial inflammation influenced renal prognosis using a univariate Cox analysis but failed to be an independent risk factor. The vascular indexes were incapable of discriminating the severity of vascular lesions and did not correlate with renal outcomes. The glomerular and interstitial lesions correlated significantly among each other. However, in several patients, the severity of IFTA was dissociated from the glomerular lesions.

Glomerular lesions are the most characteristic pathologic changes of DN. Several studies have shown a significant correlation between the severity of mesangial proliferation and decreased GFR [20–23]. Whether the nodular lesions influence renal prognosis remains controversial [24–26]. In this study, we confirmed that the severity of glomerular lesions was an independent determinant of progression to ESRD and doubling of creatinine when adjusting for clinical features. Additionally, patients in class III (nodular glomerulosclerosis) had poorer renal survival than those in class IIb (severe mesangial expansion), as previously observed [25]. The results of Oh *et al.* [5] and Okada *et al.* [19] seem to be inconsistent with our study, most likely because the results are based on a limited sample size or an inclusion of patients with a small number of glomeruli (<5). In our study, we enrolled 396 patients with T2DM, including 53 patients in class I and 87 patients in class IIa, and each biopsy specimen contained more than 10 glomeruli, allowing us to make a more comprehensive evaluation of glomerular lesions with various levels of severity.

In this study, we confirmed that several glomerular lesions were significantly correlated with renal outcomes in univariate analysis. Patients with segmental sclerosis, atubular glomerulus, nodular lesions, microaneurysms, hyaline caps and fibrinoid exudation presented worse renal outcomes. When adjusted for the pathologic classification, those glomerular changes were not associated with renal outcomes whereas the glomerular classes remained an independent risk factor.

Previously, several studies evaluated the relationship between interstitial lesions and the decline of renal function in patients with T2DM [19, 21, 23, 27, 28]; however, the results were conflicting. Ruggenenti *et al.* [27] found that interstitial fibrosis significantly predicted kidney survival only in a univariate analysis. Christensen *et al.* [21] showed no correlation between focal IFTA of cortical area and the rate of decline in GFR in patients with typical diabetic glomerulopathy. Recently, Okada *et al.* [19] reported that interstitial lesions but not glomerular lesions were a significant predictor for renal prognosis in patients with T2DM and overt proteinuria. However, these results are limited by the small number of selected patients. This study demonstrated that IFTA as well as glomerular lesions constituted an independent risk factor for renal outcomes, even when adjusting for clinical features. Furthermore, when patients in classes I and IIa were excluded in the analysis, IFTA become the only independent predictor of renal outcomes, suggesting a central role in the development of advanced DN.

A chronic inflammation state is another pathologic characteristic of DN. Inflammatory mechanisms, which are important in the pathophysiology of DN, explain how metabolic and hemodynamic abnormalities in patients with diabetes mellitus translate to functional and structural kidney injury [29]. In this study, we confirmed that interstitial inflammation was observed in various periods of glomerular lesions. However, although associated with renal prognosis using a univariate analysis, interstitial inflammation was not an independent factor for renal outcomes using a multivariate Cox regression analysis.

A multicenter study of 393 renal biopsies in patients with T2DM reported that 15–16% of patients showed prevailing vascular lesions and ensuing ischemic glomerular changes. In patients with characteristic diabetic glomerulosclerosis, 84.0% had arteriolar hyalinosis and 60.3% had arteriolosclerosis [15]. This study demonstrated more obvious vascular changes in patients with T2DM and renal injury. The prevalence of severe arteriolar hyalinosis and arteriolosclerosis (score 2) were 81.2 and 51.0% in class I and 100 and 73.2% in class IV, respectively. More than one area of arteriolar hyalinosis was commonly observed in 96% of these patients, indicating that this index in the classification was incapable of discriminating lesions with various severities. In contrast to the incidence of arteriolar hyalinosis described in patients with type 1 diabetes mellitus (T1DM) according to Drummond *et al.* [30], patients in our study are older and their baseline blood pressure and proteinuria are higher. A variety of factors superimposed on hyperglycemia were likely to be involved in the pathogenesis of vascular changes in patients with T2DM [31].

A previous study showed a significant correlation between global glomerulosclerosis and the severity of arteriolar hyalinosis [32]. In a semiquantitative analysis based on the degree of the arteriolar wall permeated by PAS-positive material and the narrowing of vascular lumen, arteriolar hyalinosis was found to significantly predict kidney survival using a univariate analysis [27]. Our study has validated the correlation between the amount of arteriolar hyalinosis and glomerular changes. However, the vascular indexes in the pathologic classification were not predictive of renal outcomes, suggesting a necessity to redefine them.

Interestingly, renal lesions in patients with T2DM manifest in a heterogeneous fashion compared with those in patients with T1DM [14, 33]. It is reported that only 30% of samples from patients with microalbuminuria and 50% of samples from patients with proteinuria demonstrate typical diabetic glomerulopathy in insulin-dependent diabetes, that is, glomerular, tubulointerstitial and arteriolar changes occur in parallel [13, 18]. Generally speaking, we observed that interstitial lesions tended to become exacerbated to the extent of glomerular lesions. The glomerular pathologic classes, IFTA and interstitial inflammation showed significant correlations among each other, suggesting a common pathogenesis. However, we found that 68.7% of patients with severe mesangial expansion and 50% of patients with nodular sclerosis had mild IFTA, whereas 12.7% of patients with mild mesangial expansion showed severe IFTA, demonstrating a heterogeneous nature of DN in patients with T2DM.

We evaluated the clinical features of patients in which IFTA was not parallel with diabetic glomerulopathy. Patients with severe IFTA had a decreased e-GFR and severe anemia at the time of renal biopsy. Besides, those with severe glomerulopathy (class IIb + III) and mild IFTA had decreased blood pressure and reduced proteinuria. Proteinuria, generally reflective of glomerular damage, may accelerate tubulointerstitial damage through multiple pathways, including induction of tubular chemokine expression and complement activation that lead to inflammatory cell infiltration and sustained fibrogenesis [34, 35]. Our study indicated that the dissociation between IFTA and diabetic glomerulopathy may be closely correlated with blood pressure and proteinuria levels. Additionally, for patients in class IIb + III, fasting blood glucose and HbA1c levels were lower in patients with severe IFTA, most likely because of the decreased e-GFR and impaired glycometabolism [36].

This study has several limitations. First, it was a retrospective study and renal survival was assessed using an estimated GFR. Second, we did not evaluate the therapeutic interventions during follow-up, which may have miscellaneous impact on renal prognosis. Third, histologic changes were assessed based on a simple classification, but diabetic glomerulopathy was far more complicated than that.

In conclusion, this study showed that in patients with T2DM, the severity of glomerular lesions and IFTA were significantly associated with renal outcomes, independent of clinical features. The vascular indexes were incapable of discriminating the severity of vascular lesions and could not be used to predict renal outcomes. The glomerular and interstitial lesions correlated among each other. These results indicated that the current pathologic classification has a predictive value for renal outcome in patients with DN.

SUPPLEMENTARY DATA

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

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicting interests that are relevant to this article.

(See related article by Gesualdo and Di Paolo. Renal lesions in patients with type 2 diabetes: a puzzle waiting to be solved. *Nephrol Dial Transplant* 2015; 30: 155–157.)

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ORIGINAL ARTICLE

Omega-3 fatty acids inhibit the up-regulation of endothelial chemokines in maintenance hemodialysis patients

Adriana M. Hung[1,2], Cindy Booker[2], Charles D. Ellis[2], Edward D. Siew[1,2], Amy J. Graves[3], Ayumi Shintani[3], Naji N. Abumrad[4], Jonathan Himmelfarb[5] and Talat Alp Ikizler[1,2]

[1]CSR&D, Veterans Administration Tennessee Valley Healthcare System, Nashville, TN, USA, [2]Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN, USA, [3]Division of Biostatistics, Vanderbilt University, Nashville, TN, USA, [4]Department of Surgery, Vanderbilt University, Nashville, TN, USA and [5]Kidney Research Institute, University of Washington, Seattle, WA, USA

Correspondence and offprint requests to: Talat Alp Ikizler; E-mail: alp.ikizler@vanderbilt.edu

ABSTRACT

Background. Chronic systemic inflammation is common in patients with chronic kidney disease on dialysis (CKD5D) and has been considered a key mediator of the increased cardiovascular risk in this patient population. In this study, we tested the hypothesis that supplementation of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) will attenuate the systemic inflammatory process in CKD5D patients.

Methods. The design was a randomized, double-blinded, placebo controlled pilot trial (NCT00655525). Thirty-eight patients were randomly assigned in a 1 : 1 fashion to receive 2.9 g