Causes of albuminuria in patients with type 2 diabetes without diabetic retinopathy

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Background. The causes of albuminuria in patients with type 2 diabetes are heterogeneous and are scantily investigated, particularly if the patient has a lack of diabetic retinopathy. Therefore, we evaluated the structural background of albuminuria in a large consecutive group of Caucasian patients with type 2 diabetes without retinopathy.

Methods. Three hundred forty-seven consecutive patients with type 2 diabetes with persistent albuminuria (>300 mg/24 h) were recorded. Fundus photo (80%) and ophthalmoscopy were performed. Ninety-three (27%) had no retinopathy, and a kidney biopsy was performed in 52 (56%) of these patients. An insufficient tissue sample was obtained in one patient. The biopsies were evaluated by three masked nephropathologists.

Results. The biopsies revealed diabetic glomerulopathy in 69% of the patients (28 males and 7 females), while the remaining 31% (95% CI, 18 to 44) had either nondiabetic glomerulopathies such as glomerulonephritis ($N = 7$, 6 males and 1 female, 13%) or normal glomerular structure ($N = 9$, 7 males and 2 females, 18%). No significant differences in sex, age (56 ± 8 vs. 53 ± 10 years, mean SD), body mass index (30 ± 4 vs. 31 ± 8 kg/m^2), known duration of diabetes (6 ± 6 vs. 4 ± 3 years), GFR (95 ± 29 vs. 89 ± 31 mL/min/1.73 m^2), albuminuria (1304 ± 169 to 4731 vs. 1050 ± 181 to 5176 mg/24 hours), blood pressure (150/87 ± 16/9 vs. 145/89 ± 16/9 mm Hg), prevalence of hypertension (89 vs. 100%), hemoglobin A$_{1c}$ (8.2 ± 1.6% vs. 9.0 ± 2.5%), and serum total cholesterol (7.1 ± 2.4 vs. 6.3 ± 1.6 mmol/L) were found between patients with and without diabetic glomerulopathy.

Conclusions. Albuminuric patients with type 2 diabetes without diabetic retinopathy have a prevalence of biopsies with normal glomerular structure or nondiabetic kidney diseases of approximately 30%. A separation between diabetic and nondiabetic glomerular lesions was not possible based on demographic, clinical, or laboratory data. Consequently, such patients may require further evaluation, including a kidney biopsy.

In patients with type 2 diabetes, a clinical diagnosis of diabetic nephropathy can be made if the following criteria are fulfilled: persistent albuminuria (>300 mg/24 hours in at least 2 out of 3 consecutive, sterile, nonketotic, 24-hour urine samples), presence of diabetic retinopathy (simplex or proliferative), and the absence of any clinical or laboratory evidence of other kidney or renal tract disease [1]. Biopsy studies have shown that albuminuric patients with type 2 diabetes without retinopathy frequently suffer from nondiabetic kidney disease [1–5], but the prevalence is not known. Furthermore, the potential role of demographic, clinical, and laboratory data in separating the causes of albuminuria has not been evaluated. Consequently, it is not possible to establish the causes of albuminuria in patients with type 2 diabetes without retinopathy if a kidney biopsy is not performed.

A correct diagnosis and a careful examination of the glomerular and interstitial structure in patients with diabetes give important information on the course of glomerular filtration rate (GFR), risk of end-stage renal disease (ESRD) development, and optimal treatment modalities for the different renal diseases in diabetic patients [6–12].

The aim of our study was to evaluate the cause of renal disease in a cohort of consecutive albuminuric patients with type 2 diabetes without retinopathy. Second, we evaluated the potential role of demographic, clinical, and laboratory data in separating diabetic from nondiabetic nephropathies, and finally, we evaluated the interrelationship between kidney structure and some of the previously mentioned data.

An interim report on a subset ($N = 20$) of the present ($N = 52$) patients has previously been presented [1].

METHODS

Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent albuminuria (>300 mg/24 hours in at least 2 out of 3 consecutive,
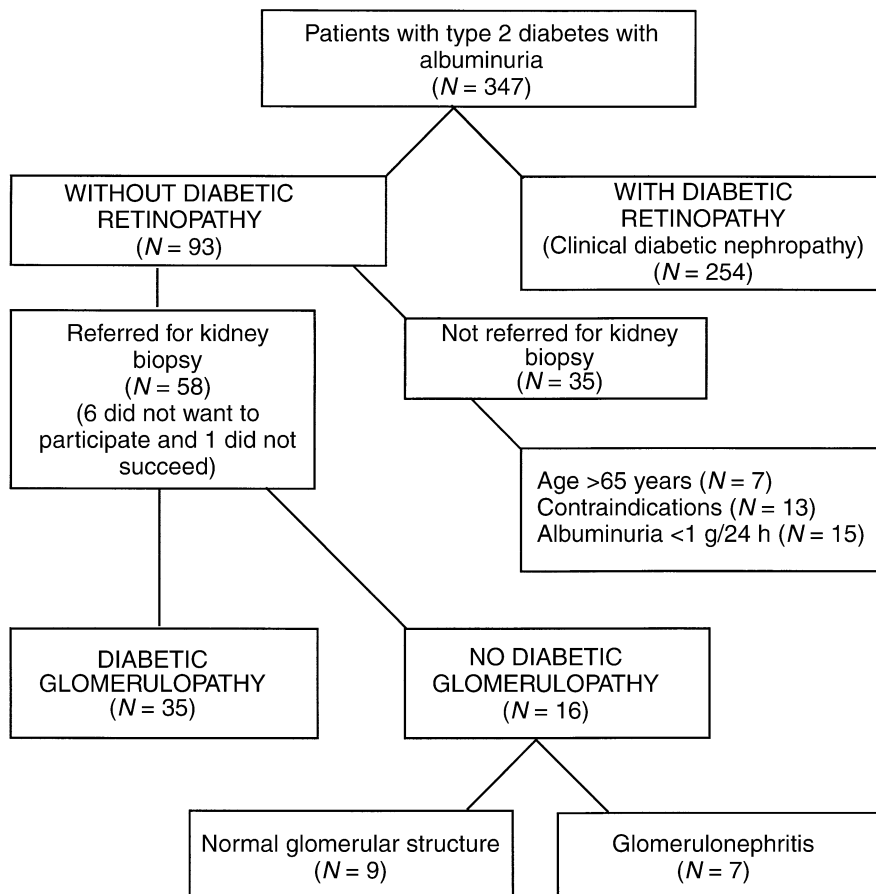
Fig. 1. Selection procedure and findings in patients with type 2 diabetes who were referred to kidney biopsy.

sterile, nonketotic, 24-hour urine samples), presence of diabetic retinopathy (simplex or proliferative), and the absence of any clinical or laboratory evidence of other kidney or renal tract disease [1].

Retinopathy was assessed following pupillary dilation with direct ophthalmoscopy until 1989, when fundus photography became common. Retinopathy was graded as nil, simplex, or proliferative; however, 80% percent of the patients in this study were evaluated solely by fundus photography.

Patients were considered to have type 2 diabetes if they were treated with diet alone or in combination with oral hypoglycemic agents, or if they were treated with insulin and had an onset of diabetes after the age of 40 years and a body weight in excess of the ideal body weight at the time of diagnoses [13]. All insulin-treated patients who were lean at the time of diagnosis had a glucagon test performed, and type 2 diabetes was diagnosed if a stimulated C-peptide value was equal to or above 0.60 pmol/mL [13].

Patients

All Caucasian ($N = 347$) patients with type 2 diabetes with persistent albuminuria (>300 mg/24 hours in at least two out of three consecutive, sterile, nonketotic, 24-hour urine samples) who had been or was attending the outpatient clinic at Steno Diabetes Center between 1978 and 1998 were recorded.

Two hundred fifty-four patients had diabetic retinopathy (simplex or proliferative), and 93 patients had no diabetic retinopathy. Fifty-eight of the patients without retinopathy were referred for a kidney biopsy, but six of these patients later decided not to participate. After 1992, our nephrologist decided not to perform kidney biopsy in patients with type 2 diabetes with albuminuria of less than 1 g/24 hours. Consequently, 15 patients without retinopathy and with albuminuria of <1 g/24 hours were not evaluated. The remaining 20 patients were not referred for a kidney biopsy because of the following reasons: (*1*) four patients died shortly after onset of albuminuria, (*2*) seven patients were more than 65 years old, and (*3*) contraindications were present in nine patients (4 had a solitary kidney, 1 had a contracted kidney, and 4 patients were treated with anticoagulant because of cardiovascular disease) as shown in Figure 1. All patients gave informed consent, and the study was performed in accordance with the Helsinki Declaration.

Laboratory techniques

Albuminuria was measured by radioimmunoassay in all urine samples until 1992 [14]. After 1992, an enzyme-linked immunosorbent assay (ELISA) method was used [15]. The correlation between the two methods was $r = 0.99$ [15]. The serum creatinine concentration was assayed by a kinetic Jaffe method [16].

Glomerular filtration rate was measured after a single intravenous injection of 3.7 MBq ^{51}Cr-labeled ethylene-diaminetetraacetic acid (EDTA) by determination of the radioactivity in venous blood samples taken from the other arm 180, 200, 220, and 240 minutes after the injection [17, 18]. The small underestimation (10%) of ^{51}Cr-EDTA clearance versus clearance of inulin was corrected for by multiplying EDTA clearance by 1.10 [17]. The results were standardized for 1.73 m^2 body surface area. This method is precise (coefficient of variation 4%) and does not require frequent timed urine collections as do the classic renal clearance procedures [17, 18].

All plasma clearance studies were carried out between 9:00 a.m. and 1:30 p.m. Patients had their usual breakfast and morning medication before the investigation, which was carried out with the patient in the supine position. They drank 200 mL tap water per hour during the clearance study.

Arterial blood pressure was measured with a clinical sphygmomanometer after 10 minutes of rest. The measurements were taken with an appropriately sized cuff. Diastolic blood pressure was measured at the disappearance of the Korotkoff sounds (phase V). Arterial hypertension was diagnosed according to the World Health Organization criteria, systolic blood pressure ≥160 mm Hg, and/or diastolic blood pressure ≥95 mm Hg, or if antihypertensive treatment was being prescribed. Mean arterial blood pressure (MABP) was calculated as diastolic blood pressure plus one third of the pulse amplitude.

Body mass index was calculated as body weight/height^2 (kg/m^2).

Serum total cholesterol was measured using conventional laboratory techniques.

Hemoglobin A_{1c} (HbA$_{1c}$) was determined by using a DIAMAT Analyzer (Bio-Rad, Hercules, CA, USA) until 1995 and thereafter by Variant Bio-Rad. The normal range for HbA$_{1c}$ was 4.1 to 6.1% for both methods, with a positive bias of 0.1 to 0.2% between the methods.

Neuropathy was measured by peripheral vibration perception threshold using a biothesiometer (Biomedical Instrument Co., Newbury, OH, USA).

Microscopic examination of the urinary sediment was performed in a midstream specimen in all patients with positive dipsticks for hemoglobin. Hematuria was defined as three or more erythrocytes per high-power field urinalysis of two or more sterile urine samples.

Biopsies

A renal biopsy was performed in 52 patients without diabetic retinopathy but with persistent albuminuria. Twenty of these patients were enrolled in our initial cross-sectional study [1]; insufficient material was obtained in one patient.

The biopsies were evaluated by three masked nephropathologists. To exclude/minimize bias with respect to the presence of diabetic kidney lesions and to increase possible variability in the morphometric analyses, we mixed biopsies from patients with diabetes with 14 biopsies from subjects (6 women and 8 male) without diabetes, with normal renal function, and normal structure by light microscopy (LM), having a mean ± SD age of 43 ± 12.4 and a range of 20 to 61 years.

Furthermore, this gave us the opportunity to see whether the upper normal range of Vv (mes/glom), measured by our method, in our biopsies from patients without diabetes with normal kidney structure differs from other reports.

Seven biopsies were from patients suffering from monosymptomatic albuminuria, and the remaining seven biopsies were from kidney donors. All morphometric measurements were made by one of the authors (S.O.).

Light microscopy

The tissue was fixed in 4% formaldehyde buffered to pH 7.0 ("Lillie's fluid"), embedded in Paraplast® (Fisher Company, Fair Lawn, NJ, USA). Following the usual procedures of our laboratory, 4, 3, and 2 μm serial sections were cut and stained with hematoxylin eosin, periodic-acid Schiff, picric acid + Sirius red, Masson's trichrome, and silver methenamine + hematoxylin eosin. Only 2 μm sections were used for semiquantitative and quantitative measurements.

Diffuse diabetic glomerulopathy, hyaline arteriolosclerosis, tubular atrophy, and interstitial fibrosis were semiquantitatively estimated. The final reading (0, +1, +2, +3) of the three nephropathologists sitting together was made by agreement at a video screen attached to the microscope. The number of totally sclerotic glomeruli was counted and expressed as a percentage of the total number.

Morphometry

The mesangial volume fraction [mesangium in a fraction of the total glomerular volume (Vv (mes/glom))] was measured at LM by point counting using a computer-assisted stereological system (GRID; Interactivision, Denmark). Magnification on the video screen was ×1650. The grid had 5 × 6 points. The number of points hitting the mesangium (matrix as well as cells) was expressed as the fraction of the points hitting the reference space, which was the glomerulus defined as the circumscribed
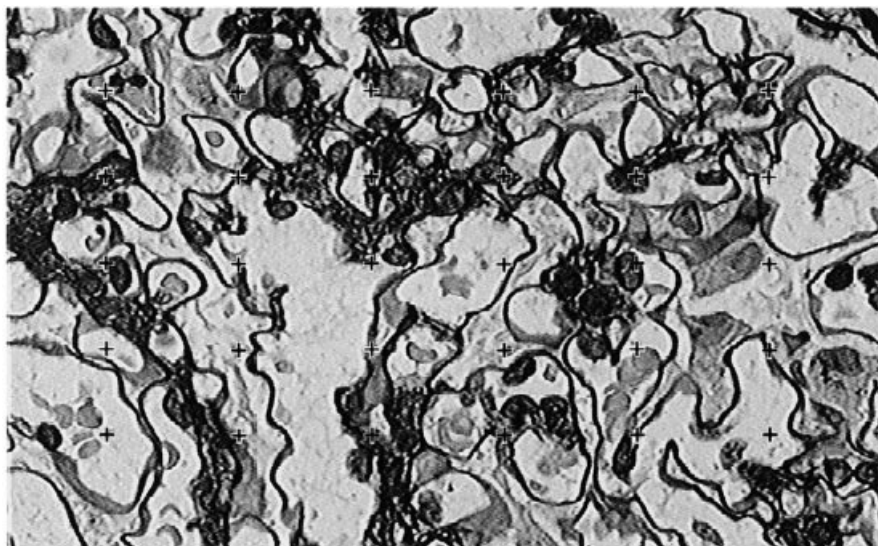
Fig. 2. Silver methenamine-stained section of glomerulus with superimposed grid for estimation of mesangial volume of the total glomerular volume [Vv (mes/glom)].
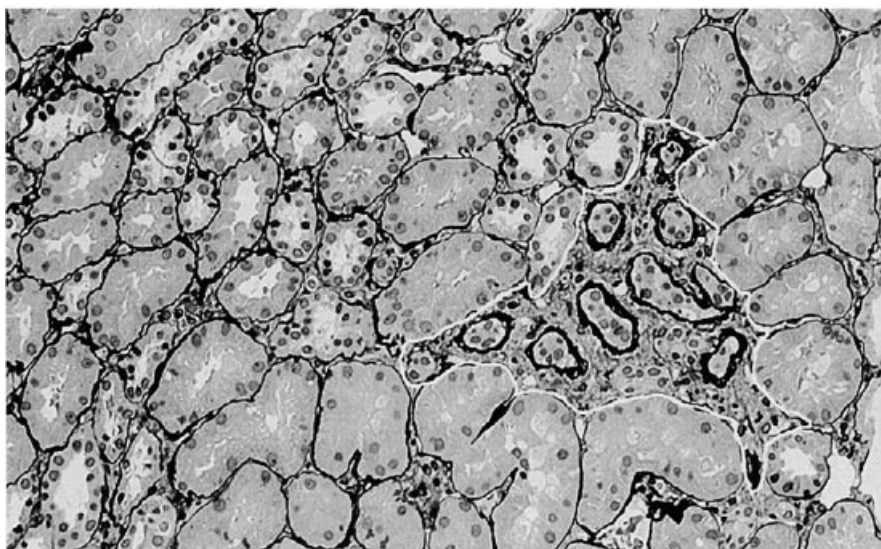

Fig. 3. Fibrotic scar circumscribed (white line) for measurement of fractional area (silver methenamine staining).

minimal polygon. The measurements were made on one silver methenamine-stained 2 μ section from each biopsy. One field from each of 6 to 10 glomeruli was measured (corresponding to 180 to 300 reference points), excluding totally or partially sclerosed glomeruli (Fig. 2). Five biopsies contained less than six open glomeruli, but had a minimum of 4. In these biopsies, two different fields in each glomerulus were measured. Using this LM technique on plastic sections from 31 biopsies from patients with diabetes and correlating the data with EM morphometry on the same biopsies, a statistically significant correlation was found ($r = 0.81$, $P < 0.0001$; R. Østerby, personal communication).

Interstitial fibrosis and tubular atrophy always occurred in sharply defined focal areas (Fig. 3). Each fi-

brotic scar was circumscribed, and their areas were automatically computed by the system. The sum of these areas was expressed as a percentage of the total cortical area in the biopsy. In order not to miss any diffuse interstitial fibrosis outside of these focal areas, we also measured the relative interstitial volume by point counting. The interstitial tissue including capillaries was expressed as a fraction of the cortical labyrinth (cortex minus glomeruli and vessels larger than capillaries). Tubular basement membranes were not included in the interstitial tissue.

Immunofluorescence microscopy

Kidney specimens were frozen using dry ice, embedded in Tissue-Teck (Miles, Naperville, IL, USA) gelatin,

and 2 μm sections were cut at approximately −24°C on a Leitz Histocryotome (Wetzlar, Germany). The direct immunofluorescent staining technique was applied using FITC-conjugated rabbit or goat antisera specifically reactive to human IgG, IgM, and IgA, as well as complement C1q, C3, and C4 [19].

Electron microscopy

Tissue had been processed for electron microscopy (EM) in 24 out of the 51 kidney biopsies. The tissue was fixed in 3% glutaraldehyde at pH 7.4, followed by dehydration in increasing concentrations of ethanol. After fixation in 1% osmium tetroxide and embedding in Epon, ultrathin sections were cut on an LKB I ultramicrotone and examined on a Philips 100 electron microscope. In all cases obtained before 1989, only the written reports were available (N = 18), and blocks and ultrathin sections had been discarded.

Statistical analysis

Values are expressed as mean ± SD or median (range). Values for albuminuria were logarithmically transformed before performing the statistical analysis because of their positively skewed distribution. All comparisons of normally distributed parameters were done with a Student's *t*-test (unpaired design). Fisher's exact test and chi-square test were used to evaluate proportions or dichotomous variables. Linear regression analysis was used to analyze for correlations. All calculations were made using SPSS for Windows (SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was considered significant (two tailed).

RESULTS

The kidney biopsy revealed diffuse (N = 34) or nodular (N = 1) diabetic glomerulopathy in 69% (95% CI, 56 to 82) of the patients. In the remaining 16 patients (31%; 95% CI, 18 to 44), a normal glomerular structure was found in 9 (18%) and 7 (13%) biopsies that had different types of glomerulonephritis (discussed later in this article).

The clinical features in relationship to the three previously mentioned groups of patients are shown in Table 1. There were no significant differences in sex, age, body mass index, antidiabetic treatment, antihypertensive treatment, HbA$_{1c}$, serum total cholesterol, known duration of diabetes, albuminuria, GFR, serum creatinine, blood pressure, prevalence of hypertension, prevalence of cardiovascular disease (CVD), and level of peripheral neuropathy between patients with diabetic glomerulopathy or glomerulonephritis, nor between patients with diabetic glomerulopathy or normal glomerular structure. Thirty-four percent of the patients with diabetic glomerulopathy already had albuminuria at the time of diagnosis of diabetes compared with 50% of the patients without diabetic glomerulopathy (*P* = NS).

There were significantly more males than females with diabetic (*P* < 0.01) or nondiabetic glomerulopathies (*P* < 0.05).

Fifteen patients with albuminuria <1 g/24 hours were not referred for a kidney biopsy. The male:female ratio (%) in the 15 patients were 87:13 compared with 80:20 in the 51 patients who were evaluated (*P* = NS). Furthermore, no significant difference was found with respect to the previously mentioned demographic, clinical, and laboratory data between the two groups.

The mean ± SD (range) number of glomeruli in patients without diabetes was 17 ± 6.9 (9 to 31) and 14 ± 8.8 (4 to 51) in the biopsies from patients with diabetes.

All of the 51 biopsies from patients with diabetes showed linear fluorescence for IgG in peripheral capillary walls [20].

Table 2 shows the histologic findings, including mophometric results, deposits of immunoglobulins, and complement fractions and their localization in patients with diabetic glomerulopathy. The number ± SD (range) of glomeruli in these biopsies was 16 ± 8.9 (4 to 51).

Two patients (patients 4 and 21) with diabetic glomerulopathy and well-preserved kidney function (GFR, 63 and 129 mL/min/1.73 m^2) had persistent hematuria. Apart from diabetic glomerulopathy, no other possible cause was revealed by the urological/nephrological examinations.

Table 3 shows the histologic findings in patients with normal glomerular structure. The number of glomeruli in these biopsies was 12 ± 7.9 (4 to 30).

Table 4 shows the histologic findings in the seven patients with structural changes indicating glomerulonephritis. The number of glomeruli in these patients was 13 ± 6.5 (4 to 22). Three had typical IgA nephropathy consisting of mild mesangial proliferation with marked diffuse and global mesangial IgA deposits (patients 49, 50, and 51). No tissue was available for EM from patients 50 and 51; the EM in patient 49 did not show immunoglobulin deposits. In addition, patients 49 and 51 had severe diabetic glomerulopathy with scattered nodules, whereas patient 50 had mild diffuse diabetic glomerulopathy. Two other patients probably had IgA nephropathy, although not with typical abnormalities (45 and 48). These two patients had diffuse, global, and mild to moderate mesangial IgA deposits, but no immunglobulin deposits were detected on EM. In the biopsy from patient 46, some corpuscles had adhesions between the glomerular tuft and Bowman's capsule in otherwise normal corpuscles, which was interpreted as an indication of healed glomerulonephritis without activity. There were moderate IgM deposits in the mesangial regions. Patient 47 had moderate mesangial proliferation without diabetic sclerosis and C3 deposits in the glomeruli, but no immu-

Table 1. Baseline data in 51 albuminuric patients with type 2 diabetes without diabetic retinopathy, with biopsy-proven diabetic or nondiabetic glomerulopathy or with normal glomerular structure

| | Renal structure | | | |
	Diabetic glomerulopathy	Glomerulonephritis	Normal glomerular structure	P value
Sex *male/female*	28/7	6/1	7/2	NS
Age *years*	56 (8.0)	51 (15)	54 (6)	NS
Body mass index *kg/m²*	30 (4.4)	30 (5.2)	32 (9.8)	NS
Antidiabetic treatment (diet/oral hypoglycemic/insulin) %	23/57/20	33/50/17	22/67/11	NS
Hemoglobin A1c %	8.2 (1.6)	9.1 (2.9)	8.9 (2.5)	NS
Serum total cholesterol *mmol/L*	7.1 (2.4)	6.4 (1.6)	6.3 (1.7)	NS
Known duration of diabetes *years*	4.0 (0–24)	6.0 (1–10)	4.0 (0–7)	NS
Interval between known onset of proteinuria and first GFR *years*	1.0 (0–9)	3.0 (0–6)	0 (0–5)	NS
Albuminuria *mg/24 h*	1362 (313–5499)	1431 (770–4143)	1300 (341–5460)	NS
GFR *mL/min/1.73 m²*	95 (29)	83 (37)	94 (28)	NS
Serum creatinine *μmol/L*	86 (51–148)	89 (52–186)	95 (53–104)	NS
Systolic blood pressure *mm Hg*	150 (16.2)	148 (12)	144 (19)	NS
Diastolic blood pressure *mm Hg*	87 (8.5)	91 (10)	88 (9)	NS
Prevalence of hypertension %	89	100	100	NS
Prevalence of CVD %	32	17	29	NS
Peripheral vibration perception threshold *mV*	18 (8–50)	18 (8–50)	21 (10–50)	NS

Data are mean (SD) or median (range). *P* values calculated between patients with diabetic glomerulopathy or normal glomerular structure, and between patients with diabetic glomerulopathy or glomerulonephritis all were nonsignificant (NS).

noglobulins by immunofluorescent microscopy and no deposits on EM were found. We considered this case to be possible mesangial proliferative glomerulonephritis.

Patients with diabetic glomerulopathy had significantly higher Vv (mes/glom) as compared with patients without diabetic glomerulopathy 0.34 ± 0.07 versus 0.27 ± 0.08, respectively ($P < 0.01$). No significant differences were found in fraction of focal interstitial fibrosis and tubular atrophy of the cortical area (0.11 ± 0.14 vs. 0.10 ± 0.16), diffuse interstitial volume (19.0 ± 6.7 vs. 18.2 ± 6.0), and percentage of sclerotic glomeruli (17 ± 17.5 vs. 22 ± 21.5) between patients with and without glomerulopathy, respectively. The individual values of Vv (mes/glom) in patients with diabetic glomerulopathy, normal structure, or glomerulonephritis are shown in Figure 4. In our 14 patients without diabetes and without glomerular lesions, the upper normal range of mesangial volume fraction was 0.26 (Fig. 4).

Twelve patients in all three groups had normal Vv (mes/glom). If diabetic glomerulopathy is defined as Vv (mes/glom) exceeding the range of our patients without diabetes, four patients with diabetic glomerulopathy based on the subjective criteria belong to the normal group, and conversely, two patients in that group must be regarded as cases of diabetic glomerulopathy.

A comparison between our semiquantitative and morphometric values demonstrated a highly significant correlation ($r = 0.79$, $P < 0.0001$). The individual values of Vv (mes/glom) correlated with semiquantitative grade of severity of the glomerulopathy are shown in Figure 5.

In our patients with diabetes with mesangial volume fraction above or below 0.26 (upper normal limit), no significant differences were found in any of the demographic, clinical, and laboratory variables.

The interrelationships between a known duration of diabetes, albuminuria, GFR, MABP, and renal structural parameters are shown in Table 5. We found a significant inverse correlation between the mesangial volume fraction [Vv (mes/glom)] and GFR in patients with or without diabetic glomerulopathy, and between percentage of sclerotic glomeruli and GFR in patients with or without glomerulopathy. The fraction of focal interstitial fibrosis and tubular atrophy of the cortical area (FF) only showed a significant inverse correlate with GFR in the patients without diabetic glomerulopathy. A significant correlation in both groups was found between mesangial volume fraction and albuminuria. In patients with nondiabetic glomerulopathies, a strong, significant correlation was found between the percentage of sclerotic glomeruli and known duration of diabetes.

No significant association was found between Vv (mes/glom) and FF ($r = 0.12$) in patients with diabetic glomerulopathy, while a strong, significant association between Vv (mes/glom) and FF ($r = 0.75$, $P < 0.01$) was found in patients without diabetic glomerulopathy.

DISCUSSION

This consecutive cohort study of albuminuric patients with type 2 diabetes without diabetic retinopathy showed a high prevalence of normal glomerular structure and nondiabetic glomerulopathies of 31% (95% CI, 18 to 44). Normal glomerular structure was found in 18%, and different types of glomerulonephritis (predominantly

Table 2. Renal pathology in 35 albuminuric patients with type 2 diabetes without diabetic retinopathy and diabetic glomerulosclerosis

		Light and electron microscopy						Immune deposits[a]	
Patient no.	Diabetic glomerulosclerosis	Grading of severity of DGS	Vv *mes/glom*	FF	S/G %	Arteriolar hyalinosis	Nondiabetic glomerular lesions	Immuno-globulins	Complement fractions
01	Diffuse	1	0.24	0.02	10	3	Absent	0	0
02	Diffuse	1	0.31	0.01	0	1	Absent	0	0
03	Diffuse	1	0.38	0.25	25	2	Absent	0	0
04	Diffuse	3	0.39	0.14	15	3	Absent	IgA, IgM	0
05	Diffuse	2	0.42	0.04	29	2	Absent	0	C3
06	Diffuse	1	0.27	0.03	11	1	Absent	0	0
07	Nodular	3	0.56	0.16	13	1	Absent	No tissue	
08	Diffuse	1	0.25	0.58	20	1	Absent	IgM	C1c
09	Diffuse	1	0.25	0.11	6	1	Absent	IgA, IgM	0
10	Diffuse	2	0.41	0.02	9	3	Absent	0	0
11	Diffuse	1	0.28	0.10	0	2	Absent	0	0
12	Diffuse	2	0.37	0.04	8	2	Absent	IgM	0
13	Diffuse	2	0.38	0.04	25	2	Absent	IgA, IgM	0
14	Diffuse	3	0.38	0.03	40	3	Absent	No tissue	
15	Diffuse	1	0.31	0.55	80	3	Absent	No tissue	
16	Diffuse	1	0.30	0.01	0	1	Absent	0	0
17	Diffuse	1	0.27	0.09	0	2	Absent	IgA	0
18	Diffuse	1	0.32	0.09	0	3	Absent	ND	ND
19	Diffuse	1	0.30	0.05	0	3	Absent	IgA	0
20	Diffuse	1	0.30	0.06	44	3	Absent	0	0
21	Diffuse	3	0.36	0.09	19	3	Absent	IgA	C3, C4
22	Diffuse	1	0.30	0.04	4	3	Absent	No tissue	
23	Diffuse	1	0.34	0.11	13	2	Absent	ND	ND
24	Diffuse	1	0.37	0.01	5	3	Absent	0	C3
25	Diffuse	1	0.29	0.06	6	1	Absent	0	0
26	Diffuse	2	0.36	0.26	42	3	Absent	IgA, IgM	0
27	Diffuse	1	0.27	0	0	1	Absent	0	0
28	Diffuse	1	0.38	0.14	23	2	Absent	IgA	0
29	Diffuse	1	0.30	0.07	50	2	Absent	No tissue	
30	Diffuse	2	0.43	0.08	30	–	Absent	0	0
31	Diffuse	2	0.36	0.09	17	1	Absent	IgM	0
32	Diffuse	1	0.24	0.08	20	1	Absent	ND	ND
33	Diffuse	1	0.30	0.01	9	2	Absent	0	0
34	Diffuse	2	0.53	0.38	18	3	Absent	IgA	0
35	Diffuse	1	0.33	0.08	13	1	Absent	IgA, IgM	0

Abbreviations are: DGS, diabetic glomerulosclerosis; Vv (mes/glom), mesangial volume of the total glomerular volume; FF, fractional area of focal interstitial fibrosis and tubular atrophy of cortical area; S/G, percentage of sclerosed glomeruli of the total number of glomeruli in each biopsy; Slight = 1, medium = 2, severe = 3; ND, no data.

[a] All deposits of immunoglobulins and complement fractions were mild or questionable, frequently only present in a few glomeruli and without characteristic pattern

Table 3. Renal pathology in 9 albuminuric patients with type 2 diabetes without diabetic retinopathy, with normal glomerular structure

|		Light and electron microscopy					Immune deposits[a]	
Patient no.	Diabetic glomerulosclerosis	Vv *mes/glom*	FF	S/G %	Arteriolar hyalinosis	Nondiabetic glomerular lesions	Immuno-globulins	Complement fractions
36	Absent	TI	0.08	33	1	Absent	0	0
37	Absent	0.23	0.08	17	1	Absent	0	0
38	Absent	0.27	0.04	0	1	Absent	0	0
39	Absent	0.23	0.01	0	0	Absent	0	0
40	Absent	0.26	0	0	2	Absent	0	0
41	Absent	0.30	0.08	8	1	Absent	0	0
42	Absent	0.19	0.06	33	2	Absent	IgA	0
43	Absent	TI	0.07	50	3	Absent	IgM	C1, C3, C4
44	Absent	0.17	0.20	33	2	Absent	ND	ND

Abbreviations are: Vv (mes/glom), mesangial volume of the total glomerular volume; FF, fractional area of focal interstitial fibrosis and tubular atrophy of cortical area; S/G, percentage of sclerosed glomeruli of the total number of glomeruli in each biopsy; Slight = 1, medium = 2, severe = 3; ND, no data; TI, technical not possible.

[a] All deposits of immunoglobulins and complement fractions were mild or questionable, frequently only present in a few glomeruli and without characteristic pattern

Table 4. Renal pathology in 7 albuminuric patients with type 2 diabetes without diabetic retinopathy and with glomerulonephritis

| | | | Light and electron microscopy | | | | | Immunofluorescence microscopy | | | |
| | | | | | | | | Immune deposits | | Localization | |
Patient no.	Diabetic glomerulo-sclerosis	Grading of severity of DGS	Vv *mes/glom*	FF	S/G %	Arteriolar hyalinosis	Nondiabetic glomerular lesions	Immuno-globulins	Complement fractions	Mesangium	Capillary
45	Absent		0.22	0.01	14	2	Mild mesangial prolif. GN (IgA?)	IgA, IgM	C3	+	+
46	Absent		0.24	0.05	20	3	GN sequelae	IgM	0	+	+
47	Absent		TI	0.01	8	3	Mild mesangial prolif. GN	0	C3	+	+
48	Diffuse	1	0.31	0.02	TI	0	Probable IgA nephropathy	IgA, IgM	0	+	+
49	Nodular	2	0.37	0.24	56	3	IgA nephropathy	IgA, IgM	0	+	+
50	Diffuse	1	0.23	0	0	1	IgA nephropathy	IgA, IgM	C3, C4	+	+
51	Nodular	3	0.47	0.66	64	3	IgA nephropathy	IgA, IgM	C3	+	+

Abbreviations are: DGS, diabetic glomerulosclerosis; Vv (mes/glom), mesangial volume of the total glomerular volume; FF, fractional area of focal interstitial fibrosis and tubular atrophy of cortical area; S/G, percentage of sclerosed glomeruli of the total number of glomeruli in each biopsy; Slight = 1, medium = 2, severe = 3; ND, no data; TI, technical not possible.
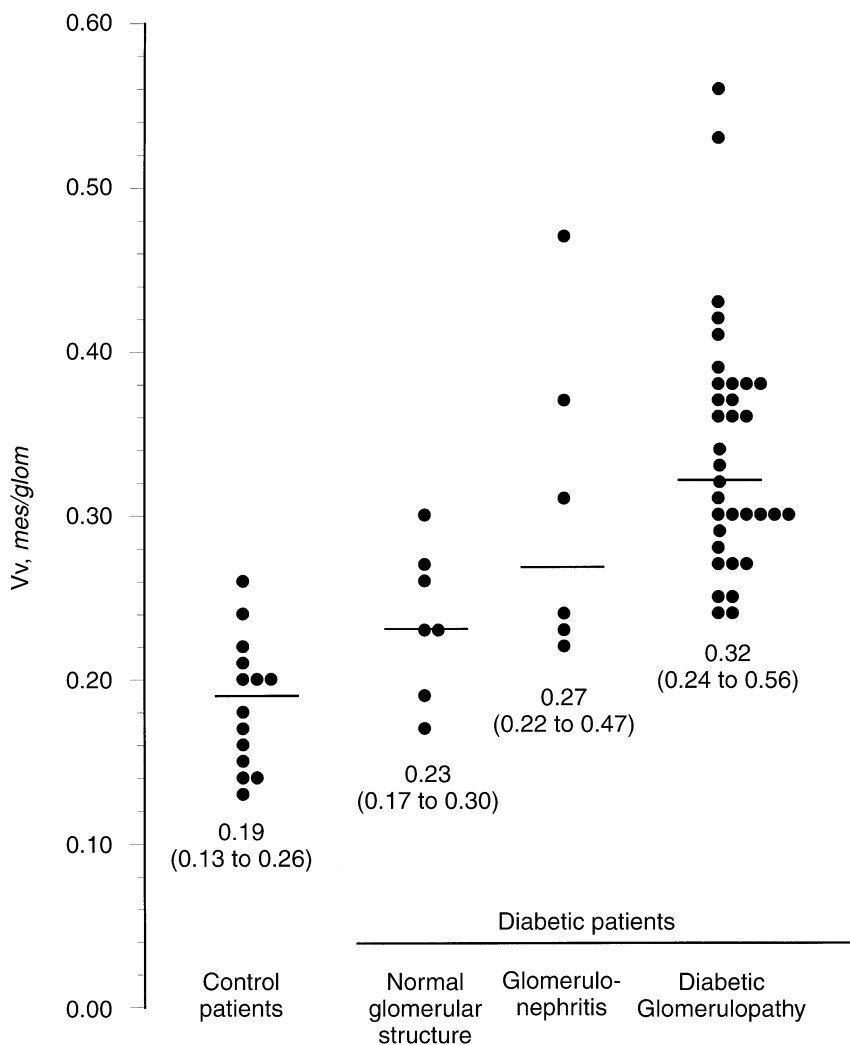
Fig. 4. Mesangial volume expressed as a fraction of total glomerular volume in patients with type 2 diabetes with diabetic glomerulopathy, glomerulonephritis, and normal glomerular structure and in control patients. Figures indicate the median (range) in the respective groups.
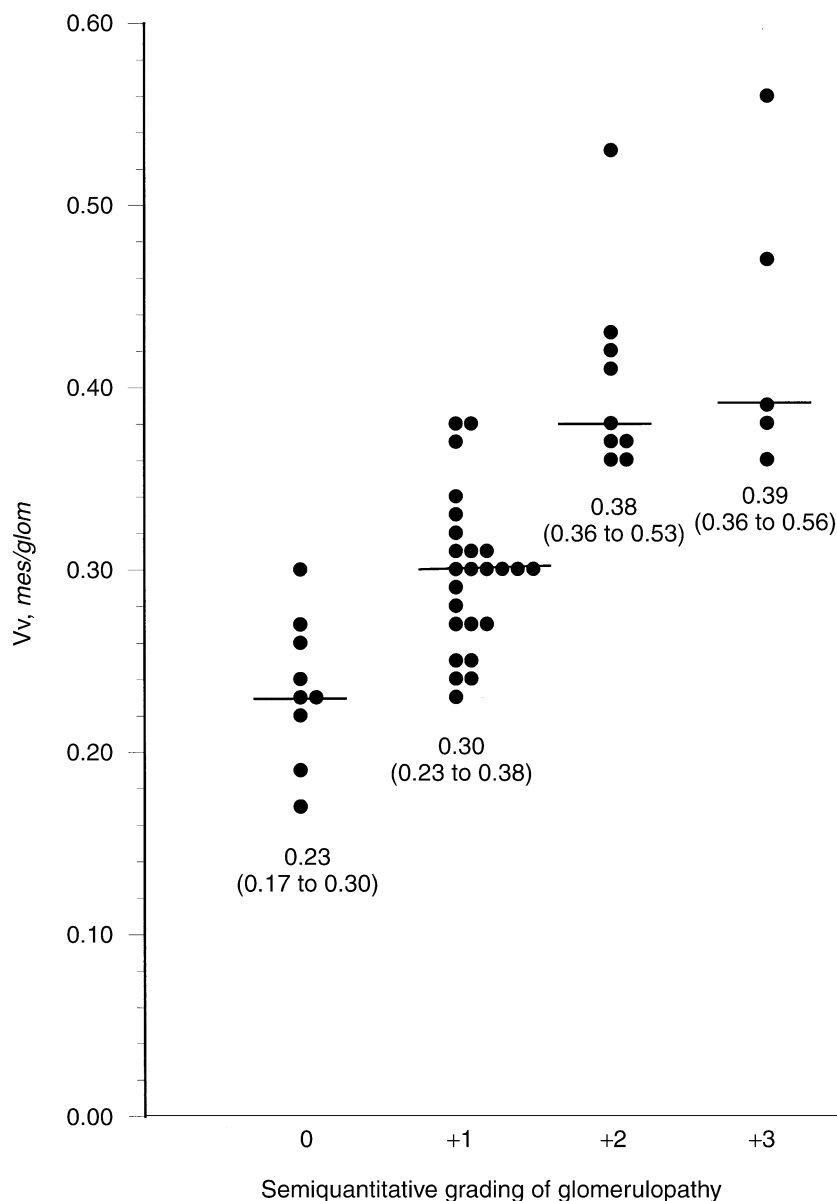
Fig. 5. Mesangial volume expressed as a fraction of total glomerular volume in albuminuric patients with type 2 diabetes. Patients were divided in accordance to a semiquantitative estimation of the severity of the glomerulopathy (nil, 0; slight, +1; medium, +2; and severe, +3).

IgA nephritis) were found in 13%. Regardless of whether the glomerular changes in the aforementioned group are responses to the diabetic state or represent other diseases, they are, nonetheless, quite atypical of what has come to be described as classic diabetic glomerulopathy, and we have preferred to keep our diabetic patients apart from this group. Patients with or without diabetic glomerulopathy were not significantly different with regards to demographic, clinical, and laboratory data at baseline, and it must be emphasized that the sole indication for kidney biopsy in our study was the presence of albuminuria and the lack of diabetic retinopathy. In other words, we had no clinical suspicion of a nondiabetic nephropathy. Consequently, it was not possible to predict the causes of albuminuria or to obtain any prognostic information on the kidney disease by solely analyzing the clinical data.

Type 2 diabetes is a male-predominated disease. The male:female ratio (%) in all patients with type 2 diabetes attending our outpatient clinic was 56:44. The percentage in type 2 patients with clinical diabetic nephropathy (presence of diabetic retinopathy and albuminuria >300 mg/24 h) was 68:32, whereas the male:female ratio of 80:20 was demonstrated in the present albuminuric patients without retinopathy. In our previous cross-sectional study [1], the male:female ratio in patients without retinopathy was 89:11. The male predominance in the albuminuric patients in our studies was more marked compared with previous investigations [21–23]; however, these studies included patients with retinopathy. No

Table 5. Interrelationships between clinical and laboratory findings and renal lesions in 51 albuminuric patients with type 2 diabetes without diabetic retinopathy

	Known duration of diabetes *years*		Albuminuria (log$_{10}$) *mg/24 h*		Glomerular filtration rate *ml/min/1.73 m²*		Mean arterial blood pressure *mm Hg*	
	r	P	r	P	r	P	r	P
Patients with diabetic glomerulopathy (N = 35)								
Vv *mes/glom*	+0.20	NS	+0.32	0.06	−0.43	<0.01	+0.15	NS
FF	−0.24	NS	+0.21	NS	−0.16	NS	+0.06	NS
S/G %	+0.19	NS	+0.33	0.06	−0.39	<0.05	−0.04	NS
Patients with nondiabetic glomerulopathy (N = 16)								
Vv *mes/glom*	+0.44	NS	+0.68	<0.05	−0.69	<0.01	+0.12	NS
FF	+0.34	NS	+0.42	NS	−0.54	<0.05	+0.41	NS
S/G %	+0.67	<0.01	+0.20	NS	−0.65	<0.01	+0.49	0.06

Abbreviations are: Vv (mes/glom), mesangial volume of the total glomerular volume; FF, fractional interstitial area of focal fibrosis and tubular atrophy of cortical area; S/G, percentage of sclerosed glomeruli of the total number of glomeruli in each biopsy.

other data are available to establish the male:female ratio in patients with type 2 diabetes with albuminuria and without retinopathy. Selection bias in the present study may be possible, but can only partly explain the male predominance, since the male:female ratio, the demographic, clinical, and laboratory data in the 51 patients included in the present study did not differ significantly from the 15 patients with <1 g albuminuria/24 hours not included. Furthermore, the number of patients with albuminuria at the time of diagnosis of diabetes was nearly the same in the two groups. All of the patients had well-preserved kidney function, and the interval between known onset of albuminuria and finally baseline evaluation in the two groups was not significantly different.

Recently, four studies applying an unbiased indication for kidney biopsy have been reported in microalbuminuric and macroalbuminuric patients with type 2 diabetes [2–5]. All of these studies included patients with and without diabetic retinopathy. Fioretto et al, by using LM, described a heterogeneity in renal structure in 34 microalbuminuric patients with type 2 diabetes: 29% had normal or near normal renal structure, and 50% of these patients had no retinopathy [3]. The same group of investigators extended these data in a cohort study of 53 patients with type 2 diabetes with microalbuminuria [4]. Forty-one percent of these patients had normal or near normal biopsies, and in 59% of these patients, no retinopathy was seen [4]. In biopsies revealing mainly interstitial changes (33%), only 31% had retinopathy, whereas all patients with obvious diabetic glomerulopathy (26%) had retinopathy [4]. In contrast to these studies [3, 4], we found a lower prevalence of normal glomerular structure, but this must be expected since we only included patients with macroalbuminuria (>300 mg/24 hours).

Cordonnier et al investigated 26 type 2 patients with albuminuria ranging from 70 to 4210 mg/24 hours, and reported nonspecific vascular and glomerular change in 15% of the patients [5]. Among 36 hypertensive, albuminuric patients with type 2 diabetes with serum creatinine between 133 and 265 μmol/L, Schwartz et al found 6% of the patients to have nondiabetic glomerulopathy [2]. The low prevalence of 6% [2] may be attributed to the inclusion criteria. All patients with diabetic retinopathy had diabetic glomerulopathy, and the two patients with nondiabetic glomerulopathy lacked diabetic retinopathy. A close relationship between the degree of diabetic retinopathy and diabetic glomerulopathy has been demonstrated in albuminuric type 2 diabetic patients [11]. In other biopsy series, the prevalence of nondiabetic glomerulopathies varies from 9 to 66% [24]; however, in all of these studies except one [1], biopsies were taken on clinical indications and were not performed on the basis of research protocols.

The prevalence of patients without diabetic glomerulopathy in the present study is, as expected, higher than in most of the previously mentioned studies in which patients with clinical diabetic nephropathy were included. Our finding of normal glomerular in 18% may, however, be a conservative estimate since the criteria for performing kidney biopsy were changed in the study period from albuminuria persistently above 300 mg to albuminuria persistently above 1 g.

The histologic classification of the renal lesions was performed according to the World Health Organization standard [25]. All biopsies were reviewed by three experienced nephropathologists in a blinded fashion. Furthermore, we mixed the 51 biopsies with 14 biopsies from nondiabetic patients with normal renal structure in order to minimize and/or exclude bias.

By routine evaluation of renal biopsies, diabetic glomerulopathy was diagnosed and semiquantitatively graded

by subjective evaluation, and this was also the method by which we made the diagnosis at the original reading of the biopsies; however, it must be regarded as inferior to quantitative morphometry. Since only a part of our biopsies included tissue for EM, we used a morphometric point count technique on LM specimens, a method that works well on high-quality 2 μ paraffin sections stained by silver methenamine. Furthermore, the conventional method of measuring the fractional volume of cortical fibrosis in biopsies with rather few and small focal fibrosis areas is very insensitive since the fibrosis will largely "disappear" in the many measuring fields outside the areas with fibrosis. We therefore also selected another objective method to evaluate fibrosis.

A comparison between the semiquantitative grading and morphometric values of mesangial expansion demonstrated a highly significant correlation; however, problems with the subjective evaluation are obvious. It may be of less practical relevance that it appears to be impossible semiquantitatively to distinguish between diffuse diabetic glomerulopathy grades +2 and +3. More important is the overlap between the morphometric values corresponding to grade 0 and +1, indicating that the cutoff point between subjectively determined normal biopsies and mild diabetic glomerulopathy is somewhat arbitrary. In fact, four patients subjectively estimated to have diabetic glomerulopathy had Vv (mes/glom) slightly below 0.26, which was within the normal range of our controls (mean value of controls, 0.18 ± 0.04, fitting well with normal values reported by ultrastructural morphometry) [26], and two patients with normal glomerular structure had a Vv (mes/glom) value above the upper normal range.

The cause of albuminuria in patients without diabetic glomerulopathy is unclear. It might conceivably be due to early, and thus very slight, diabetic changes that are undetectable by LM, but this seems less probable since early ultrastructural changes usually (at least in patients with type 1 diabetes) are present in normoalbuminuric or microalbuminuric patients [27–29], and not in patients with overt nephropathy such as our study group.

No significant difference in focal or diffuse fibrosis, the percentage of sclerotic glomeruli, or arteriolar hyaline change was demonstrated between biopsies revealing normal morphology and biopsies with diffuse glomerulopathy. Immunofluorescence microscopy was negative in most biopsies with normal glomerular structure. Only two had weak or questionable immunoglobulin deposits and only in some of the glomeruli. Atypical patterns of renal injury in patients with type 2 diabetes have been reported: no or mild diabetic glomerulopathy, but disproportionate severe renal tubulointerstitial changes, tubular atrophy and interstitial fibrosis, advanced arteriolar sclerosis, global glomerular sclerosis, and atherosclerosis of larger arteries [3, 4, 30]. The severe and dispropor-

tionate tubulointerstitial changes, which were frequent in these studies but rare in our biopsies, have the appearance of small scars and are probably sequelae to arteriolosclerosis and arteriosclerosis. These abnormalities occur in biopsies with as well as without diabetic glomerulopathy, and it is possible that such lesions may be partly responsible for albuminuria induced in the surviving and probably hyperfunctioning glomeruli in kidneys with widespread scarring. However, our patients with as well as those without glomerulopathy included cases with marked focal fibrosis. In the group without mesangial expansion, patients 8, 42, and 44 may belong to the category with atypical pattern. The comparatively weak correlation ($r = 0.28$; $P = 0.03$) between hyaline arteriolar change and focal fibrosis in our biopsies does not speak decisively against this hypothesis because of the high risk of sampling error involved in the estimation of these focal lesions.

Undetected focal segmental glomerulosclerosis (FSG) could be another explanation of albuminuria. However, our biopsies with normal glomeruli were from a general point of view representative, and their number of glomeruli had a range of 4 to 30. The possibility that FSG is occasionally responsible for albuminuria in a diabetic patient with apparently normal glomeruli cannot be totally excluded, since a biopsy is less sensitive to the diagnosis of FSG, but we regard the probability as small.

The low incidence of Kimmelstiel–Wilson nodules found in our biopsies was expected, since all our patients with diabetic nephropathy had well-preserved kidney function and early-stage diabetic nephropathy.

Albuminuria in patients without diabetes can be associated with normal glomeruli, a not very well-defined condition often called "minor change nephropathy," which includes the more well-defined entity "minor change nephrotic syndrome." Both conditions are characterized morphologically by glomeruli with normal or only minor lesions by LM and with foot process fusion as the only ultrastructural change. Seven of our 13 patients without glomerulopathy [LM normal glomerular structure and/or Vv (mes/glom) <26] were investigated with EM, and normal morphology was found in three cases. Obvious variable degrees of foot process fusion were noted in the remaining four. Fusion of the foot processes is, however, not specific and can be seen in other renal diseases associated with albuminuria [31].

Finally, albuminuria in diabetics without glomerulopathy could be caused by alterations of the molecular structure of the glomerular basement membrane and increased glomerular permeability to macromolecules (size/charge defects). Alternatively, the defect in the capillary wall barrier, as suggested by Fioretto et al, could be due to endothelial dysfunction [32]. Actually, such hypothetical diabetic capillary wall alterations could constitute the primary cause of glomerulopathy, with mesan-

gial matrix and basement membrane augmentation as an epiphenomenon. Conversely, albuminuria may participate in the initiation and progression of the tubulointerstitial lesions, as suggested by Remuzzi and Bertani [33].

The most characteristic and clinically important glomerular lesion in diabetes mellitus is mesangial expansion, and a precise quantitation of renal tissue injury combined with clinical and laboratory data can be used as a reliable predictor of the risk of ESRD [11, 34]. Mesangial expansion is closely associated with renal function in diabetic nephropathy [12], and our study confirmed and extended this information. We found a strong inverse correlation between Vv (mes/glom) and GFR in both groups; furthermore, an inverse correlation between the percentage of sclerotic glomeruli and GFR was revealed in both groups. These relationships probably resulted from the expanding mesangium compromising the structure of glomerular capillaries and reducing the filtration surface. An inverse correlation between FF and GFR and a strong correlation between FF and Vv (mes/glom) were found in the patients without diabetic glomerulopathy, indicating that the interstitial lesions are important determinants of the progression in the kidney disease, as suggested by Mauer [12].

Albuminuria is assumed to be an independent risk factor for the progression of renal diseases, and a reduction in albuminuria is important to preserve kidney function [35–38]. We found an inverse significant correlation between Vv (mes/glom) and albuminuria, indicating that albuminuria can be used as marker of the severity of the kidney disease in both groups.

Exact knowledge of the underlying cause of albuminuria may play an important role in offering the correct treatment, as demonstrated in several studies in patients with type 2 diabetes suffering from nondiabetic glomerulopathies [8–10]. If diabetic glomerulopathy is the underlying cause, improvement of glycemic control to normal or near normal levels should be attempted, since recent studies have demonstrated that glycemic control acts as progression promoter [39] and that reversal of diabetic glomerulopathy is feasible in humans [40]. Furthermore, a correct histologic diagnosis of an albuminuric condition of unknown cause may have an important prognostic impact in relation to nonfatal and fatal CVD, since diabetic nephropathy is associated with an enhanced risk [41], while this is not the case for most nondiabetic glomerulopathies per se except progression to ESRD.

Previous long-term studies have demonstrated that the course of kidney function and albuminuria differs in albuminuric (manuscript in preparation) and microalbuminuric type 2 diabetic patients with and without diabetic nephropathy [6, 34].

In conclusion, our study confirms and extends our previous findings for a high prevalence of nondiabetic

kidney diseases in albuminuric patients with type 2 diabetes without retinopathy. A separation between diabetic and nondiabetic glomerular lesions was not possible based on demographic, clinical, or laboratory data. Consequently, such patients may require further evaluation, including a kidney biopsy.

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