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# The Role of Vascular Lesions in Diabetes Across a Spectrum of Clinical Kidney Disease



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**Introduction:** The clinical-histologic correlation in diabetic nephropathy is not completely known.

**Methods:** We analyzed nephrectomy specimens from 90 patients with diabetes and diverse degrees of proteinuria and glomerular filtration rate (GFR).

**Results:** Thirty-six (40%) subjects had normoalbuminuria, 33 (37%) microalbuminuria, and 21 (23%) non-nephrotic proteinuria. Mean estimated GFR (eGFR) was 65±23 (40% <60 ml/min per 1.73 $m^2$). About 170 glomeruli per patient were analyzed, and all samples included vascular tissue. Six subjects (7%) were classified in diabetic nephropathy class I, 61 (68%) in class II-a, 13 (14%) in class II-b, 9 (10%) class III, and 1 (1%) in class IV. Eighty percent to 90% of those with normoalbuminuria or microalbuminuria were classified in class II-a or II-b and <10% in class III; 52% of those with proteinuria were in class II-a, 15% in class II-b, and 19% in class III. Nodular sclerosis (57%) and mesangial expansion (15%) were more frequent in cases with proteinuria than in normoalbuminuria (28% and 8%; $P = 0.028$ and 0.017). About 20% to 30% of all cases, regardless the level of albuminuria or proteinuria or the histologic class had tubular atrophy, interstitial fibrosis, or inflammation in >10% to 20% of the sample. Moderate hyalinosis and arteriolar sclerosis were observed in 80% to 100% of cases with normoalbuminuria, microalbuminuria, proteinuria, as well as in class I, II, or III.

**Conclusions:** Weak correspondence between analytical parameters and kidney histology was found. Thus, disease may progress undetected from the early clinical stages of the disease. Finally, vascular damage was a very common finding, which highlights the role of ischemic intrarenal disease in diabetes.

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Diabetic kidney disease is a major cause of end-stage renal disease,[1,2] affecting 30% to 50% of patients on dialysis in the Western population.[1,2] These figures have not changed in the last 20 years despite improvement in treatments, including use of renin-angiotensin system blockade.[3] Thus, understanding diabetic

kidney disease is crucial to prevent the burden of end-stage renal disease.

The clinical course of diabetic kidney disease is complex and heterogeneous. It has been structured in consecutive periods from normal or high GFR (i.e., hyperfiltration), followed by accelerated loss of renal function.[4–6] Albuminuria is low (i.e., normoalbuminuria) or moderately elevated (i.e., microalbuminuria) in the early stages, whereas overt proteinuria marks the turning point of rapid GFR loss and chronic kidney disease (CKD) progression.[4–6] Microalbuminuria classically indicates "incipient" and proteinuria "overt" diabetic kidney disease.[4–6] However, this picture has been recently challenged by studies showing that patients without proteinuria may develop CKD, what has been called a *nonproteinuric phenotype* of renal disease.[7] Both renal histology and the pathogenesis of this new phenotype remain uncharacterized.

Renal histology in type 2 diabetes is also heterogeneous. Most available data come from patients with type 1 diabetes, which may not translate properly into type 2 diabetes.[6] Also, the majority of diabetic patients do not undergo a renal biopsy unless an unusual clinical presentation or course are detected. This represents a major limitation in our understanding of the pathogenesis of the disease. Some reports showed a clinical-pathologic dissociation showing that severe renal lesions, that is, nodular sclerosis in glomeruli, can be observed in patients without proteinuria.[8–10] However, the reports are frequently low numbered. Thus, several aspects of diabetic kidney disease remain unknown, such as the morphologic changes in early stages of normoalbuminuria or microalbuminuria and the histologic background of renal disease in the absence of proteinuria. Of relevance, the role of intrarenal ischemia in diabetes—a disease characterized by vascular disease—has been seldom analyzed. So, a comprehensive understanding of renal damage is still far from reaching in diabetes.

To address these points, we evaluated the renal histology of unaffected renal tissue in pieces of nephrectomy from patients with type 2 diabetes with normoalbuminuria, microalbuminuria, or proteinuria and diverse degrees of renal function.

## METHODS

This is a multicenter study including 15 centers from 6 countries and is part of the European Nephrectomy Biobank (ENBiBA) project (see participants). ENBiBA was designed to analyze the pathogenesis of renal disease in diabetes, obesity, and metabolic syndrome, conditions in which renal biopsies are rarely performed, mostly because of the low prevalence of proteinuria. To overcome this limitation, unaffected renal tissue of nephrectomy specimens were collected, as well as serum, urinary samples, and clinical data of patients with these clinical conditions. ENBiBA is an initiative of DIABESITY, a working group of the ERA-EDTA. For this analysis, we included only subjects with type 2 diabetes. The protocol was approved by the Ethics Committee of all participants centers.

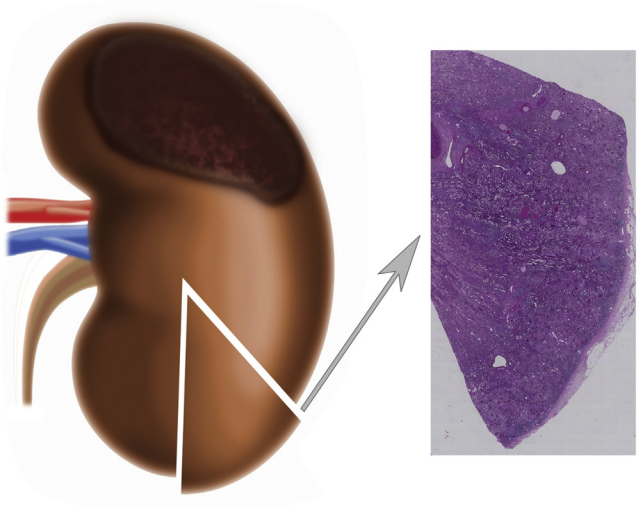

**Figure 1.** Schematic representation of sample collection from a nephrectomy specimen.

### Patients

This is a clinical-pathologic study. Patients in the waiting list for an elective nephrectomy were screened and eventually included. Inclusion criteria were as follows: type 2 diabetes, that is, fasting glucose >125 mg/dl, glycated hemoglobin (HbA1c) >6.5%, or the use of antidiabetic medications; age >18 years; and capacity to understand the informed consent. Exclusion criteria were as follows: renal disease that can jeopardize histologic analysis, for example, urinary tract obstruction, glomerulonephritis, reflux nephropathy, chronic pyelonephritis, polycystic disease, interstitial nephritis, nephrolithiasis, history of severe renal artery stenosis, acute kidney injury, or other cause of acute or chronic kidney disease; extension of the tumor to the whole kidney limiting the availability of unaffected tissue; previous radiotherapy; or episode of renal toxicity due to chemotherapy.

After surgery, a sample of renal parenchyma, including both cortical and medulla, was taken and embedded in paraffin. Laboratory analyses were performed before nephrectomy. Cancer is expected to be the major cause for nephrectomy, and so samples were taken of an area distant to the tumor (Figure 1).

## Clinical Data

We collected the following variables: weight, height, smoking habits, time with diabetes, diagnosis of diabetic nephropathy, retinopathy and neuropathy; dyslipidemia, hyperuricemia, gout, hypertension, treatments for diabetes, hypertension, dyslipidemia, hyperuricemia, cardiovascular events, laboratory analysis, albumin/creatinine ratio in urine spots, albuminuria, total proteinuria in 24-hour urine and eGFR using the CKD-EPI creatinine-based formula.

## Sampling and Histologic Variables

In each center, the pathologist took a sample of renal tissue (~ 3 × 2 × 0.5 cm) at least more than 5 cm from the tumor. The absence of neoplastic lesions or infiltrates was confirmed later in the coordinating center. Three-micrometer-thick histologic sections were processed for light microscopy according to standard techniques and stained with periodic acid–Schiff and Sirius red. Histologic evaluation was performed in 2 steps by a single pathologist (RR). First, a detailed examination of glomerular, tubular, interstitial, and vascular pathologic specimens was performed, following the BANFF classification of 1999[11-13] with modifications, as done before.[11] We analyzed (a) *number of glomeruli*, (b) *glomerular sclerosis* (nodular, segmental, diffuse, global); (c) *increased mesangial matrix*, defined as an uniform increase in mesangial matrix when the width of mesangial inter-space exceeds the length of 2 mesangial cell nuclei, classified in (i) absence, (ii) <25%, (iii) 26% to 50%, and (iii) >50% of affected nonsclerotic glomeruli, (iv) increased mesangial proliferation as the presence of 3 or 4 nuclei or more in 1 mesangial area, (v) nodular mesangial expansion, that is, round formations of matrix in general 1 or 2 per section; (c) *tubular atrophy and interstitial fibrosis*: in subgroups of <5%, 5% to 10%, 10% to 20%, >20%, etc.; (d) *inflammation*: mononuclear cell interstitial inflammation in total parenchyma (scarred and unscarred), in subgroups of <5%, 5% to 10%, 10% to 20%, >20%, etc.; and finally (e) *arterial sclerosis (fibrointimal thickness)*; and (f) *arteriolar hyalinosis* were classified as mild = 1+, moderate = 2+, or severe = 3+. The thresholds for arteriosclerosis and hyalinosis were mild (0%–25%), moderate (25%–50%), and severe (>50%). Vascular damage was analyzed in the most severely affected vessels.

To this point, the pathologist was blind to the clinical characteristics of patients. Then, patients were classified according with the Diabetic Nephropathy Classification of the Renal Pathology Society.[14] Immunofluorescence and electron microscopy were not performed.

## Definitions

*Diabetic retinopathy:* chronic progressive disease of the retinal microvasculature associated with prolonged hyperglycaemia.[15]

*Diabetic neuropathy:* a chronic, symmetrical, length-dependent sensorimotor polyneuropathy.[16] Data on diabetic retinopathy and neuropathy were based on previous diagnoses. No special test was performed to detect occult disease.

*Hypertension:* systolic or diastolic blood pressure >140/90 mmHg or the use of medications.[17]

*Dyslipidemia:* triglycerides >200 mg/dl, low-density lipoprotein cholesterol >160 mg/dl, total cholesterol >240 mg/dl, high-density lipoprotein cholesterol <35 mg/dl, or the use of medications.[18]

*Urinary protein excretion:* collected as per clinical practice in each center using either isolated urine spots or 24-hour collections. The diagnosis of normoalbuminuria, microalbuminuria or overt proteinuria was based on the following criteria: (i) urinary albumin excretion <30, 30 to 299, and >300 μg/mg creatinine, respectively for the cases with spot urine; (ii) <30, 30 to 299, and >300 mg in 24-hour urine collections; or (iii) total protein >500 mg in 24-hour urine collections for proteinuria.[6]

## Statistical Analysis

Patients were grouped based on urinary albumin or protein excretion, that is, normoalbuminuria, microalbuminuria, or overt proteinuria, or diabetic nephropathy for analysis. Continuous variables were compared using parametric or nonparametric tests when appropriate. Dichotomous variables were compared with $\chi^2$ test. For analysis, SPSS Statistics for Windows, version 17.0 (SPSS, Chicago, IL) was used.

## Sensitivity Analyses

We analyzed the effect of age (percentage >60 years), gender, medications (renin-angiotensin-aldosterone system inhibition yes/no), and GFR (percentage >60 ml/min per 1.73 $m^2$) on major findings of the study.

# RESULTS

Ninety-two patients with diabetes were included. In all, the cause of nephrectomy was urologic cancer (kidney, ureter, or renal pelvis). Two were excluded because of insufficient material for histologic analysis, that is, unaffected tissue unavailable. Thus, we evaluated a total of 90 cases. The absence of neoplastic infiltration in the sample was confirmed by the pathologist (RR).

**Table 1.** Clinical characteristics of the patients: overall and according to urinary albumin excretion—normoalbuminuria, microalbuminuria, or overt proteinuria

| | Total ($N$ = 90) | Normoalbuminuria ($n$ = 36; 40%) | Microalbuminuria ($n$ = 33; 37%) | Proteinuria ($n$ = 21; 23%) |
|---|---|---|---|---|
| Age, yr, mean (SD) | 68 ± 10 | 67 ± 10 | 69 ± 9 | 70 ± 9 |
| Gender, male | 65 (72) | 25 (67) | 22 (70) | 18 (86) |
| Weight, kg, mean (SD) | 85 ± 20 | 86 ± 21 | 83 ± 15 | 84 ± 17 |
| BMI, mean (SD) | 30 ± 6 | 31 ± 7 | 30 ± 5 | 29 ± 5 |
| Smoking habits[a] | | | | |
| Never | 36 (41) | 15 (42) | 15 (48) | 6 (30) |
| Current | 14 (16) | 5 (14) | 8 (26) | 1 (5) |
| Previous | 37 (43) | 16 (44) | 8 (26) | 13 (65)[b] |
| Time on diabetes, yr, median (IQR) | 10 (5–17) | 9 (5–13) | 10 (5–15) | 15 (5–20) |
| Fasting glucose, mg/dl, mean (SD) | 146 ± 56 | 143 ± 54 | 143 ± 60 | 150 ± 54 |
| $HbA_{1c}$, %, mean (SD) | 7 ± 1.7 | 6.5 ± 1.9 | 7.1 ± 1.2 | 7.6 ± 1.7 |
| Treatment for diabetes | | | | |
| Insulin | 22 (26) | 7 (19) | 7 (21) | 8 (38) |
| Oral agents | 71 (79) | 32 (89) | 30 (91) | 9 (43)[c] |
| Diet alone | 4 (4) | 3(8) | 1 (3) | 0 |
| Hypertension, yes | 74 (82) | 27 (75) | 28 (85) | 19 (90) |
| Blood pressure levels, mm Hg | | | | |
| Systolic | 139 (18) | 139 (19) | 139 (19) | 138 (14) |
| Diastolic | 76 (12) | 77 (10) | 75 (10) | 76 (16) |
| ACE inhibitors | 34 (38) | 12 (33) | 10 (30) | 12 (57)[d] |
| AR blockers | 24 (27) | 9 (25) | 11 (33) | 4 (19) |
| Calcium channels blockers | 29 (32) | 9 (25) | 12 (36) | 8 (38) |
| Beta-blockers | 18 (20) | 4 (11) | 7 (21) | 7 (33)[e] |
| Diuretics | 31 (34) | 10 (28) | 10 (30) | 11 (52) |
| Dyslipidemia, yes | 51 (57) | 25 (69) | 17 (51) | 9 (43)[f] |
| Total cholesterol, mg/dl, mean (SD) | 154 ± 40 | 159 ± 40 | 155 ± 44 | 145 ± 36 |
| HDL cholesterol, mg/dl, mean (SD) | 41 ± 13 | 43 ± 11 | 43 ± 15 | 36 ± 15 |
| LDL cholesterol, mg/dl, mean (SD) | 86 ± 34 | 87 ± 34 | 90 ± 37 | 78 ± 31 |
| Triglycerides, mg/dl, mean (SD) | 150 ± 60 | 147 ± 56 | 138 ± 53 | 171 ± 71 |
| Statins | 44 (49) | 22 (61) | 15 (45) | 7 (33) |
| Fibrates | 6 (7) | 2 (6) | 1 (3) | 3 (14) |
| Diabetic nephropathy | 9 (9) | 1 (3) | 3 (9) | 4 (19)[g] |
| Diabetic neuropathy | 3 (3) | 1 (3) | 0 | 2 (9) |
| Diabetic retinopathy | 7 (8) | 1 (3) | 2 (6) | 4 (20) |
| Hyperuricemia, yes | 16 (18) | 5 (14) | 7 (21) | 4 (19) |
| Uric acid levels, mg/dl, mean (SD) | 6 ± 1.6 | 5.6 ± 1.5 | 7.1 ± 1.5 | 6 ± 1.9 |
| Allopurinol | 10 (12) | 2 (6) | 5 (19) | 3 (15) |
| Gout | 4 (4) | 0 | 2 (6) | 2 (9) |
| Cardiovascular events, yes | 9 (10) | 5 (14) | 1 (3) | 3 (14) |
| eGFR: CKD-EPI, ml/min per 1.73 $m^2$, mean (SD) | 65 ± 23 | 71 ± 23 | 66 ± 19 | 51 ± 24[h] |
| <60 ml/min | 36 (40) | 11 (31) | 12 (36) | 13 (62)[i] |
| Albumin/creatinine, µg/mg, median (IQR) | | 6 (0.7–13) | 110 (50–225) | 698 (399–1058) |
| Proteinuria, mg, median (IQR) | | — | — | 1194 (595–1701) |

ACE, angiotensin-converting enzyme; AR, angiotensin receptor; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration (equation); eGFR, estimated glomerular filtration rate; $HbA_{1c}$, glycated hemoglobin; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; SD, standard deviation.
[a]3 cases with missing information.
[b]$P = 0.045$ versus microalbuminuria.
[c]$P < 0.0001$ versus normoalbuminuria and microalbuminuria.
[d]$P = 0.07$ versus normoalbuminuria.
[e]$P = 0.046$ versus normoalbuminuria.
[f]$P = 0.045$ versus normoalbuminuria.
[g]$P = 0.040$ versus normoalbuminuria-microalbuminuria.
[h]$P = 0.028$ versus microalbuminuria, $P = 0.002$ versus normoalbuminuria.
[i]$P = 0.03$ versus normoalbuminuria and microalbuminuria.
Unless otherwise noted, values are $n$ (%).

## Patient Characteristics

Age averaged 68 ± 10 years (range 45–88, 25% <60 years), 72% were male, mean time with diabetes was 10 years (interquartile range [IQR]: 5–16), and mean HbA1c was 7% ±1.7% (Table 1). Most cases had hypertension (82%), 65% were on renin-angiotensin-aldosterone system inhibition, and about half were obese (BMI > 30) and had dyslipidemia. Mean eGFR was 65±23 ml/min per 1.73 $m^2$, and 40% had values <60 ml/min per 1.73 $m^2$.

## Patients With Normoalbuminuria, Microalbuminuria, or Overt Proteinuria

Thirty-six (40%) patients had normoalbuminuria, 33 (37%) microalbuminuria, and 21 (23%) overt proteinuria. Proteinuria was mild, that is, <1.5 g in 24 hours, and no patient had nephrotic-range proteinuria (Table 1). Clinical characteristics were comparable between subjects with normoalbuminuria or microalbuminuria. Subjects with proteinuria had lower eGFR, more history of smoking, and higher use of angiotensin-converting enzyme inhibitors and beta-blockers than those with normoalbuminuria or microalbuminuria.

## Renal Histology in Patients With Normoalbuminuria, Microalbuminuria, or Proteinuria

### *Glomerular Lesions*

The median number of glomeruli per subject was 172 (116–222) and comparable between groups (Table 2). Global sclerosis was about 10% in each group. Nodular sclerosis was found in 37% of the cases and more frequent in patients with proteinuria (57%) than in those with normoalbuminuria (28%, $P = 0.028$). In patients with nodular lesions, glomeruli with nodular sclerosis were low and comparable between groups: median ~2%. All cases showed glomeruli with mesangial expansion, making it the most frequent glomerular lesion. Mesangial expansion was more frequent in cases with proteinuria (15%, IQR: 8–20) than in those with normoalbuminuria (8%, IQR: 5–14, $P = 0.017$) and comparable with those with microalbuminuria (11%, IQR: 7–17).

### *Interstitial Lesions*

Tubular Atrophy. Seventy percent to 80% of the cases irrespective of the levels of albuminuria or proteinuria had tubular atrophy in <10% of the sample (Table 2). Higher levels were observed in 11% of the cases with normoalbuminuria and in 24% in those with microalbuminuria or proteinuria ($P$ nonsignificant [ns]).

Interstitial Fibrosis. About 70% of the cases in each group had interstitial fibrosis in <10% of the sample (Table 2). Higher levels were observed in 20% of the cases with normoalbuminuria, 27% in microalbuminuria, and 29% in proteinuria ($P$ ns).

Interstitial Inflammation. Seventy percent to 80% of the cases in each group had minimal inflammatory infiltrates involving <10% of the sample (Table 2). Inflammation in >10% of the sample was comparable in subjects with normoalbuminuria or microalbuminuria, about 15%, and higher in subjects with proteinuria, 39% ($P = 0.07$ and 0.06, respectively), although of borderline significance.

### *Vascular Lesions*

Arteriolar Hyalinosis. Moderate hyalinosis was highly frequent, affecting about 80% to 90% of the cases in each group (Table 2 and Figure 2). Severe hyalinosis was more prevalent in subjects with proteinuria than in those with normoalbuminuria or microalbuminuria ($P = 0.012$ and 0.06, respectively).

Fibrointimal Thickening. Moderate arteriolar sclerosis was highly prevalent, affecting 80% of the cases (Table 2 and Figure 2). It was more frequent in subjects with microalbuminuria (94%) or proteinuria (86%) than in those with normoalbuminuria (64%) ($P = 0.013$ and 0.032, respectively).

### *Renal Function*

Patients with proteinuria had lower renal function: 51±24 ml/min per 1.73 $m^2$ than those with normoalbuminuria (71±23; $P = 0.002$) or with microalbuminuria (66±19, $P = 0.028$) (Table 1). GFR was comparable between patients with normoalbuminuria or microalbuminuria.

### *Classification of Diabetic Nephropathy*

Six subjects (7%) were classified as class I, 61 (68%) as class II-a, 13 (14%) as class II-b, 9 (10%) as class III, and 1 (1%) as class IV (Table 2). Half of the cases with class I nephropathy had normoalbuminuria, and the other half microalbuminuria or proteinuria. About 80% to 90% of the cases with normoalbuminuria or microalbuminuria were classified as class II-a or II-b and <10% as class III. Finally, 52% of the cases with proteinuria had class II-a, 15% class II-b, and 19% class III. The case with class IV had proteinuria.

## Renal Histology in the Classes of Diabetic Nephropathy

### *Glomerular Lesions*

The median number of glomeruli was >160 in all classes (Table 3). Class IV includes only 1 subject and was not considered for analysis. Global sclerosis was low and comparable between classes, 8% to 10%. In subjects with class III, the median number and percentage of glomeruli with nodular lesions were 25 (12–27) and 10% (5%–19%), respectively (Table 3). Nodular lesions were also observed in subjects with class II-a and II-b, but the very low number of glomeruli affected precluded their classification cases in class III (Table 3). The number and percentage of glomeruli with mesangial expansion was higher in subjects with class II-b compared with class II-a, but comparable between class II-b and III.

### *Interstitial Lesions*

Tubular Atrophy. Seventy percent to 80% of the subjects in classes I to III had tubular atrophy in <10%

**Table 2.** Renal histology in all subjects and in those with normoalbuminuria, microalbuminuria, or overt proteinuria

| | Total ($N$ = 90) | Normoalbuminuria ($n$ = 36; 40%) | Microalbuminuria ($n$ = 33; 37%) | Proteinuria ($n$ = 21; 23%) |
|---|---|---|---|---|
| Glomerular lesions | | | | |
| Glomeruli, *n*, median (IQR) | 172 (116–222) | 169 (121–220) | 174 (111–217) | 173 (127–252) |
| Glomeruli with global sclerosis (GS), median (IQR) | | | | |
| No. with GS | 13 (7–27) | 11 (8–24) | 12 (5–29) | 24 (7–39) |
| Percentage of total glomeruli with GS | 10 (4–17) | 8 (4–12) | 11 (4–15) | 11 (3–24) |
| Glomeruli with nodular sclerosis (NS) | | | | |
| Cases with NS, *n* (%) | 33 (37) | 10 (28) | 11 (33) | 12 (57)[b] |
| Cases without NS, *n* (%) | 57 (63) | 26 (72) | 22 (67) | 9 (43) |
| No. of glomeruli with NS[a], median (IQR) | 5 (2–19) | 5 (2–10) | 4 (2–25) | 3 (1–26) |
| Percentage of glomeruli with NS, median (IQR) | 2.20 (1–9.3) | 2.35 (0.6–1) | 2.32 (1.2–13) | 1.83 (5–14.3) |
| Glomeruli with mesangial expansion (ME), median (IQR) | | | | |
| No. with ME | 15 (10–29) | 13 (9–21) | 19 (10–29) | 24 (11–38)[c] |
| Percentage of total glomeruli with ME | 9 (6–17) | 8 (5–14) | 11 (7–17) | 15 (8–20)[d] |
| Classification of diabetic nephropathy, *n* (%) | | | | |
| Class I | 6 (6) | 3 (8) | 1 (3) | 2 (9) |
| Class II-a | 61 (68) | 23 (65) | 27 (82) | 11 (52) |
| Class II-b | 13 (14) | 7 (19) | 3 (9) | 3 (15) |
| Class III | 9 (10) | 3 (8) | 2 (6) | 4 (19) |
| Class IV | 1 (1) | 0 | 0 | 1 (5) |
| Interstitium and tubuli | | | | |
| Tubular atrophy | | | | |
| <5% | 60 (67) | 27 (75) | 23 (73) | 10 (45) |
| 5%–10% | 13 (14) | 5 (14) | 2 (6) | 6 (29) |
| 10%–20% | 14 (16) | 3 (8) | 7 (21) | 4 (19) |
| >20% | 3 (3) | 1 (3) | 1 (3) | 1 (5) |
| Interstitial fibrosis | | | | |
| <5% | 50 (54) | 23 (64) | 18 (54) | 9 (43) |
| 5%–10% | 18 (20) | 6 (17) | 6 (18) | 6 (29) |
| 10%–20% | 19 (21) | 6 (17) | 8 (24) | 5 (24) |
| >20% | 3 (3) | 1 (3) | 1 (3) | 1 (5) |
| Inflammation | | | | |
| <5% | 63 (70) | 28 (78) | 24 (73) | 11 (52) |
| 5%–10% | 8 (9) | 2 (6) | 4 (9) | 2 (29) |
| 10%–20% | 11 (12) | 2 (6) | 3 (12) | 6 (30)[e] |
| >20% | 8 (9) | 4 (11) | 2 (6) | 2 (9) |
| Vascular lesions | | | | |
| Arteriolar hyalinosis | | | | |
| Mild | 5 (6) | 3 (8) | 2 (6) | 0 |
| Moderate | 81 (90) | 33 (92) | 30 (91) | 18 (86) |
| Severe | 4 (4) | 0 | 1 (3) | 3 (14)[f] |
| Fibrointimal thickening | | | | |
| Mild | 13 (14) | 10 (28) | 2 (6) | 1 (5) |
| Moderate | 72 (80) | 23 (64)[g] | 34 (94) | 18 (86) |
| Severe | 5 (6) | 3 (8) | 0 | 2 (9) |

IQR, interquartile range.
[a]In subjects with nodular sclerosis.
[b]$P = 0.028$ versus normoalbuminuria, $P = 0.075$ versus microalbuminuria.
[c]$P = 0.014$ versus normoalbuminuria, ns versus microalbuminuria.
[d]$P = 0.017$ versus normoalbuminuria, ns versus microalbuminuria.
[e]$P = 0.056$ versus normoalbuminuria, $P = 0.069$ versus microalbuminuria (considered 10–20% and > 20% together).
[f]$P = 0.012$ versus normoalbuminuria, $P = 0.064$ versus microalbuminuria.
[g]$P = 0.032$ versus normoalbuminuria, $P = 0.013$ versus microalbuminuria

of the sample (Table 3). Higher levels of atrophy, that is, 10% to 20% or more, were observed in 15% to 30% of all groups ($P$ ns).

Interstitial Fibrosis. About 70% of the cases in each class had interstitial fibrosis in <10% of the sample. Higher levels were observed in 20% to 30% of the cases in all groups ($P$ ns).

Interstitial Inflammation. About 80% of the cases in each class had inflammatory infiltrates in <10% of the sample. The prevalence of inflammation in >10% of the sample was 20% to 30% in all groups ($P$ ns).

### *Vascular Lesions*

Arteriolar Hyalinosis. Moderate hyalinosis was highly frequent and comparably distributed among classes,

**Table 3.** Renal histology in subjects with diabetic nephropathy classes I, II-a, II-b, and III

| | Class I ($n$ = 6) | Class IIa ($n$ = 61) | Class IIb ($n$ = 13) | Class III ($n$ = 9) |
|---|---|---|---|---|
| Glomerular lesions | | | | |
| Glomeruli, *n*, median (IQR) | 221 (205–252) | 167 (115–212) | 171 (134–239) | 201 (125–270) |
| Glomeruli with global sclerosis (GS), median (IQR) | | | | |
| No. with GS | 26 (16–56) | 11 (6–26) | 13 (6–24) | 14 (6–57) |
| Percentage of total glomeruli with GS | 12 (8–25) | 10 (4–16) | 8 (4–17) | 9 (4–20) |
| Glomeruli with nodular sclerosis (NS) | | | | |
| Cases with NS, *n* (%) | 0 | 14 (23) | 9 (31) | 9 (57) |
| Cases without NS, *n* (%) | 6 (63) | 47 (77) | 4 (69) | 0 |
| No. of glomeruli with NS[a], median (IQR) | 0 | 2 (1–5) | 3 (2–10) | 25 (12–27) |
| Percentage of glomeruli with NS, median (IQR) | 0 | 1.3 (0.5–2.5) | 1.8 (1.2–5.3) | 10 (5–19) |
| Glomeruli with mesangial expansion (ME), median (IQR) | | | | |
| No. with ME | 11 (6–14)[a] | 14 (9–24) | 26 (11–42)[b] | 48 (20–56)[c] |
| Percentage of total glomeruli with ME | 5 (3–6) | 9 (6–15) | 14 (7–21)[d] | 20 (17–23)[e] |
| Interstitium and tubuli | | | | |
| Tubular atrophy | | | | |
| <5% | 4 (66) | 41 (67) | 10 (76) | 5 (56) |
| 5%–10% | 0 | 9 (15) | 1 (8) | 3 (33) |
| 10%–20% | 1 (17) | 8 (16) | 1 (8) | 1 (11) |
| >20% | 1 (17) | 1 (2) | 1 (8) | 0 |
| Interstitial fibrosis | | | | |
| <5% | 3 (49) | 36 (59) | 7 (54) | 4 (44) |
| 5%–10% | 1 (17) | 11 (18) | 3 (23) | 3 (33) |
| 10%–20% | 1 (17) | 13 (21) | 2 (15) | 2 (22) |
| >20% | 1 (17) | 1 (2) | 1 (8) | 0 |
| Inflammation | | | | |
| <5% | 4 (67) | 43 (71) | 10 (77) | 6 (67) |
| 5%–10% | 0 | 7 (1) | 0 | 1 (11) |
| 10%–20% | 0 | 6 (10) | 2 (15) | 2 (22) |
| >20% | 2 (33) | 5 (8) | 1 (8) | 0 |
| Vascular lesions | | | | |
| Arteriolar hyalinosis | | | | |
| Mild | 0 | 4 (7) | 1(8) | 0 |
| Moderate | 6 (100) | 57 (93) | 11 (84) | 7 (78) |
| Severe | 0 | 0 | 1 (8) | 2 (22)[f] |
| Fibrointimal thickening | | | | |
| Mild | 2 (33) | 10 (16) | 0 | 1 (11) |
| Moderate | 4 (67) | 47 (77) | 12 (92) | 8 (89) |
| Severe | 0 | 4 (7) | 1 (8) | 0 |
| Renal function | | | | |
| GFR: CKD-EPI, ml/min per 1.73 $m^2$, mean ± SD | 59 ± 29 | 64 ± 22 | 73±22 | 58 ± 26 |
| <60 ml/min, *n* (%) | 2 (33) | 25 (41) | 3 (23) | 5 (56) |

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration (equation); GFR, glomerular filtration rate; IQR, interquartile range; ns, nonsignificant.
A total of 89 cases were analyzed; the only case with class IV was not included.
[a]In subjects with nodular sclerosis.
[b]$P = 0.016$ versus class II-a, ns versus class III.
[c]$P = 0.001$ versus class II-a.
[d]$P = 0.036$ versus class II-a, ns versus class III.
[e]$P < 0.0001$ versus class II-a.
[f]$P = 0.002$ versus class II-b.

affecting about 80% to 100% of the cases in each class (Table 3 and Figure 2). Severe hyalinosis, which was observed in few cases, was more prevalent in class III than in class II-b: 2 (22%) vs 1 (8%), respectively ($P = 0.002$).

Fibrointimal Thickening. Moderate arteriolar sclerosis was observed, ranging from 67% for class I to 89% for class III (Table 3 and Figure 2). No significant differences were observed among classes.

### Renal Function

Estimated GFR was not different between classes of diabetic nephropathy.

## Sensitivity Analysis

Histologic changes were comparable between gender or patients with and without renin-angiotensin-aldosterone system inhibitors (data not shown).

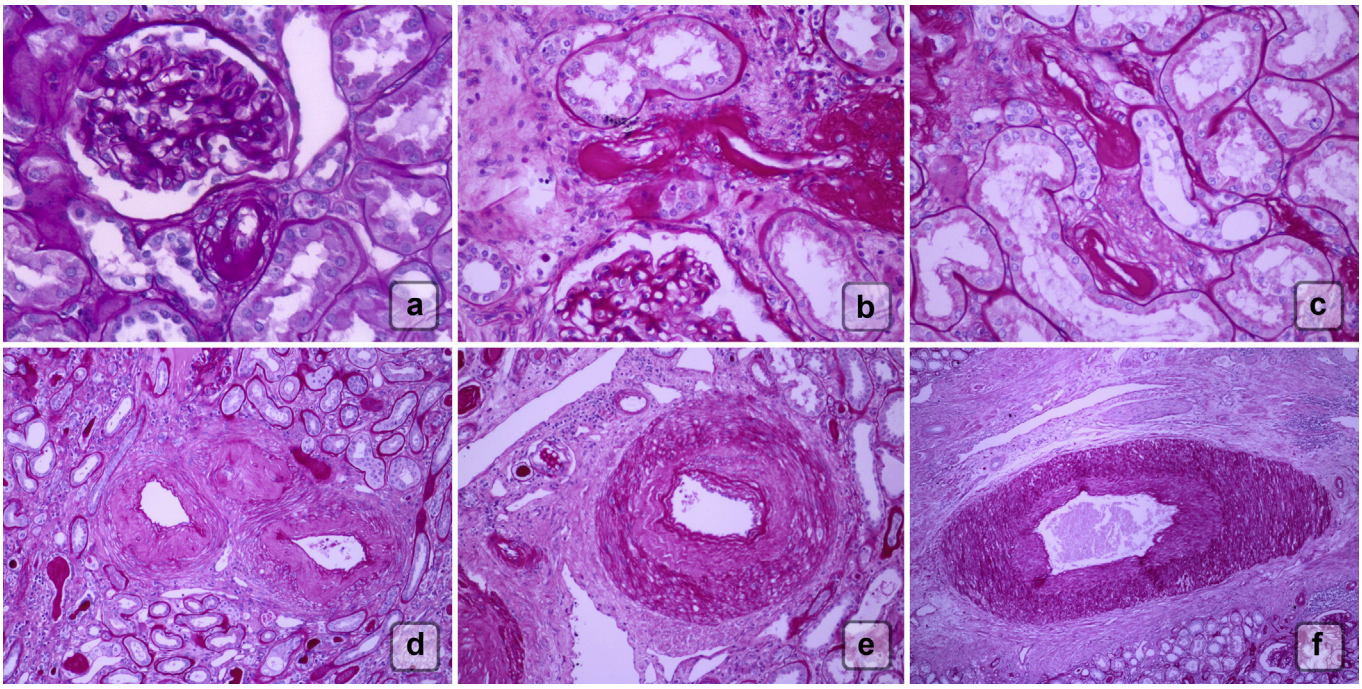


**Figure 2.** Examples of vascular lesions: (a, b, c) arteriolar hyalinosis and (d, e, f) fibrointimal thickening in patients with (d) normoalbuminuria, (a, b, e) microalbuminuria, or (c, f) non-nephrotic proteinuria.

Patients with eGFR <60 ml/min per 1.73 $m^2$ showed a larger number and percentage of glomeruli with total sclerosis (18 [IQR: 7–30] and 11% [IQR: 7%–19%] vs. 10 [5–24] and 8% [3%–13%]; $P = 0.10$ and 0.04, respectively) and more interstitial inflammation (33% vs. 13%, $P = 0.02$) than those with higher eGFR. Patients older than 60 years had a larger number and percentage of glomeruli with total sclerosis (14 [IQR: 8–30] and 8% [IQR: 3%–13%] vs. 10 [4–24] and 4% [2%–13%]; $P = 0.09$ and 0.03 respectively) and more moderate fibrointimal thickening (89% vs. 67%, $P = 0.03$) than those younger.

## DISCUSSION

We observed a lack of correspondence between renal histology and the classes of diabetic nephropathy and normoalbuminuria, microalbuminuria, or proteinuria in patients with diabetes. Advanced glomerular, tubular, and interstitial lesions were observed in patients with normoalbuminuria, whereas mild changes in the same structures were found in subjects with proteinuria. Mild glomerular changes were concomitant to severe tubular and interstitial lesions. Finally, as a novel finding, vascular damage—arteriolar hyalinosis and fibrointimal thickening—was extremely frequent and homogeneously distributed among the classes of diabetic nephropathy or stages of normoalbuminuria, microalbuminuria, or proteinuria.

Renal biopsy is not the standard of care in diabetic patients with renal disease. Histologic damage is then presupposed based on renal function, clinical evolution, and the level of albuminuria or proteinuria. This has led to an incomplete understanding of diabetic nephropathy. ENBiBA was designed to address this problem by analyzing the unaffected tissue of nephrectomy specimens and ensuring sufficient material for analysis, that is, a large number of glomeruli and small arteries and arterioles present in all samples.

Proteinuria is a risk factor for rapid disease progression in diabetic and nondiabetic renal disease.[19,20] Accordingly, patients with proteinuria showed more severe nodular glomerular sclerosis, mesangial expansion, interstitial inflammation, and lower renal function than those without. However, nearly 60% of the patients with proteinuria had mild mesangial expansion (class II-a), minimal tubular atrophy, or inflammation. By contrast, mild histologic changes and an indolent evolution of renal function are expected in patients with normoalbuminuria. Nevertheless, 10% to 20% of the patients with normoalbuminuria had nodular glomerular sclerosis (class III) and relevant (≥10%–20%) tubular lesions, that is, atrophy, fibrosis, and inflammation. Finally, microalbuminuria has been classically considered a worse situation compared with normoalbuminuria. Be as it may, most of the renal histologic parameters were comparable between patients with microalbuminuria or normoalbuminuria.

These results indicate a clinical-pathologic dissociation in diabetic nephropathy. This finding is intriguing and not easy to interpret. The fact that half of the cases with proteinuria had minor histologic changes may be

explained by the cross-sectional nature of our study that included patients at diverse points in the evolution of the disease. So, in some cases the time on proteinuria could have been not long enough to induce damage. Also, none of the cases had severe proteinuria, which may explain the lack of a more severe histologic picture. A recent study using scanning electron microscopy showed severe glomerular damage in patients with diabetes and nephrotic proteinuria: acellular glomeruli, degenerated foot processes, and ultrastructural glomerular basement membrane defects such as tunnels and cavities, and others.[21] Thus, it could be possible that nephrotic proteinuria indicates a more specific and severe phenotype of glomerular and podocyte-related damage. In any case, the different histologic damage according to the level of albuminuria or proteinuria in type 2 diabetes is worth investigating. Finally, the frequent use of antiproteinuric agents such as renin-angiotensin system blockers (65%) may have decreased the amount of urinary protein, limiting our ability to observe the association between the magnitude of albuminuria or proteinuria and the underlying histology. However, even if this were the case, the present data would then point to histologic injury that does not respond to renin-angiotensin system blockade in the same manner as analytical variables such as albuminuria/proteinuria.

On the other hand, the presence of severe lesions classically associated with proteinuria, namely, nodular sclerosis, tubular atrophy, fibrosis, and inflammation, in patients with normoalbuminuria or microalbuminuria is an unexpected finding. This speaks for a clear dissociation between the clinical presentation and the morphologic changes in the kidneys of patients with diabetes. Moreover, the fact that severe lesions can develop and evolve during the stages of normoalbuminuria or proteinuria could explain the loss of renal function in diabetic patients without proteinuria.[7] This finding is in line with studies showing advanced renal disease in patients with normoalbuminuria or microalbuminuria and normal, supranormal, or reduced GFR.[8,1,22-24] Klessens *et al*.[9] analyzed 168 necropsies of patients with diabetes, 20 of them without proteinuria. Of them, 7 (35%) were classified as class I, 5 (25%) as IIa, 3 (15%) as IIb, and 5 (25%) as class III. Finally, our study highlights the need for novel evaluations of early renal damage, that is, fibrosis, in patients with diabetic nephropathy. Recent studies showed the feasibility of urinary peptidomics, liquid biopsies, and magnetic resonance imaging as noninvasive evaluations of renal damage. Future studies are necessary to further test the validity of these approaches in the early detection of CKD in this population.[25,26]

Possibly, the most relevant finding of the study was the spread and homogeneous vascular damage observed in most cases, regardless the degree of albuminuria, the presence of proteinuria, and the class of diabetic nephropathy (Figure 2). In fact, 80% to 90% of the cases with normoalbuminuria, microalbuminuria, or proteinuria as well as those with classes II-a, II-b, or III nephropathy had moderate to severe arteriolar hyalinosis and fibrointimal thickening. In a way, this is not an unexpected finding. Diabetes is a major risk factor for cardiovascular disease,[27] and cardiovascular events are a major cause of mortality in this population. So, it is plausible that the widespread micro- and macrovascular injury that characterizes diabetes may also affect intrarenal vessels**.** Previous studies explored the role of microvascular damage in diabetes (reviewed in Eliane *et al*.[28]). Assessment of renal microcirculation is not simple. Even in standard renal biopsies, the presence of arterioles and small arteries is not granted. In our study, because of the amount of tissue evaluated, we were able to analyze renal arteries and arterioles in all subjects. Renal microvascular rarefaction, that is, a reduction in the number of vessels, has been described in patients with diabetes from the early stages of the disease.[29,30] Several factors have been involved in microvascular damage in diabetic patients such as hypertension, hyperglycemia, and endothelial damage. Albuminuria has been independently associated with cardiovascular events in diabetes, even in patients with very low levels of albuminuria.[31] In this line, the Steno hypothesis proposed that albuminuria is a marker of vascular damage, allowing the leakage of several molecules through the vascular wall as the starting point for vascular damage.[32] So, vascular disease may affect the kidneys, from the early stages of the disease, promoting chronic ischemic damage leading to tubular atrophy and interstitial fibrosis.[33] Our study is in line with a large group of cases of biopsy-proven diabetic nephropathy from Japan.[34] Furuichi *et al*.[34] found that arteriolar hyalinosis as well as arteriosclerosis with intimal thickening was particularly frequent through the CKD and diabetic nephropathy stages. Importantly, the authors concluded that lesions of nephrosclerotic lesions can be difficult to distinguish from typical diabetic nephropathy and proposed that the pathologic changes of nephrosclerosis may be included among the pathologic changes of diabetic nephropathy.[34] Also, it must be considered that ageing must have played a role in vascular damage in our cases. In fact, in the sensitivity analysis, patients older than 60 years had a larger number and percentage of glomeruli with total sclerosis and more moderate fibrointimal thickening (89% vs. 67%, $P = 0.03$) than those younger. Although this is

an expected finding, 67% of moderate atherosclerosis in younger patients is not negligible. This is in line with the finding that accelerated kidney ageing has been proposed to play a role in renal disease in patients with diabetes.[35] Finally, the high prevalence of vascular lesions seems to indicate a common background of ischemic renal disease in patients with diabetes on top of which the addition of other insults may aggravate renal damage, for example, podocyte injury with severe proteinuria, inflammation, and glomerular hyperfiltration. This novel view of renal disease in diabetes is worth investigating.

Another finding was a relevant dissociation between glomerular and interstitial lesions. A comparable degree of tubular atrophy, interstitial fibrosis, and inflammation (about 20%) was observed in subjects with mild (class II-a) or severe (class II-b) mesangial expansion or nodular sclerosis (class III). The pathogenesis of diabetic nephropathy involves many factors, including hyperglycemia, dyslipidemia, inflammation, lipotoxicity, vascular damage, and glomerular hyperfiltration, among others.[36] It may be plausible that these factors have different influences on the areas of the kidney—glomeruli, tubules, interstitium, and vessels. Also, widespread vascular damage may be causing tubular damage, because ischemia leads to interstitial fibrosis, a pathway that could be independent of glomerular damage.[28–30,34] Continuous excess of glucose reabsorption by proximal tubules may also contribute to tubulointerstitial injury, either through direct cytotoxicity or through stressing cells to increase energy consumption.[36,37] This may be directly improved by SGLT2 inhibitors.[38] It would be interesting to assess the impact of SGLT2 inhibition on renal histology findings once their use becomes widespread.[38] However, we acknowledge that these hypotheses must be proved in *ad hoc*–designed studies.

Our work has limitations. This is a cross-sectional study that precludes the analysis of the impact of the time exposed to a risk factor. None of our patients had nephrotic-range proteinuria, which limits the result to patients with severe proteinuria. Along the same lines, we have to acknowledge the variability of random "spot" urine collections. This may have changed the classification of some cases with normalbuminuria or microalbuminuria. Also, almost no patient was taking the new medications now available for the treatment of diabetes, that is, SGLT2 or GLP1 antagonists, which may not reflect the effect of more modern treatments on renal histology. The diagnosis of retinopathy was based on clinical records, which may have underestimated the real prevalence of this complication. Finally, electronic microscopy was not performed, and so we were not able to evaluate specific changes in podocytes or in the basement membranes of tubules and glomeruli.

In conclusion, we observed a clear lack of agreement between renal histology and clinical parameters in type 2 diabetes. Severe glomerular tubular and interstitial lesions were observed in patients with normoalbuminuria or microalbuminuria, whereas patients in class II-a or II-b had severe interstitial damage. This indicates that disease progression can develop within these stages, at least under the current standard of therapy. Finally, vascular damage was a very common finding, which highlights the role of ischemic intrarenal disease in diabetic nephropathy, starting from the early stages of the disease.

## DISCLOSURE

EP is a researcher of the Ramón y Cajal Program (ISCIII). AO is a consultant for Sanofi Genzyme and has received speaker fees or travel support from Astrazeneca, Amicus, Amgen, Fresenius Medical Care, Menarini, Kyowa Kirin, Alexion, Otsuka, and Vifor Fresenius Medical Care Renal Pharma and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease. BFF reports speaker fees or travel support from Abbvie, Astrazeneca, Boehringer Ingelheim, Esteve, Menarini, Mundipharma, Novartis, and Novonordisk, outside the submitted work. All the other authors declared no competing interests.

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## AUTHOR CONTRIBUTIONS

EP had the idea of the study and designed the protocol. RR did the histologic analysis of all samples. All authors collaborated in the design of the protocol. All authors contributed with samples and data of the subjects included in the study. All authors read the final manuscript of the study.

## APPENDIX

### List of Participants of ENBiBA: The European Nephrectomy Biobank Project

| Center Name | City | Country | Principal Investigator |
|---|---|---|---|
| University Clinical Centre | Maribor | Slovenia | Radovan Hojs, Sebastjan Bevc |
| Hospital Universitario Fundación Alcorcón | Madrid | Spain | Gema Fernández, Clara María Cases Corona |
| Hospital de Bellvitge | Barcelona | Spain | Maria Quero, Laia Pujol, Sergi Beato Montserrat Gomà, Josep Cruzado |
| Hospital Sant Joan Despí Moisès Broggi | Barcelona | Spain | Meritxell Ibernon |
| Rigshospitalet | Copenhagen | Denmark | Mads Hornum, Bo Feldt-Rasmussen |
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## REFERENCES


1. https://www.renalreg.org/reports/2017-twentiethannual-report.
2. Schaubel DE, Morrison HI, Desmeules M, Parsons DA, Fenton SS. End-stage renal disease in Canada: prevalence projections to 2005. *CMAJ*. 1999;160:1557–1563.
3. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA*. 2011;305:2532–2539.
4. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med*. 2002;346:1145–1151.
5. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. *Diabetes*. 1983;32(suppl 2):64–78.
6. Parving HH, Mauer M, Ritz E. Diabetic nephropathy. In: Brenner BM, ed. *Brenner & Rector's The Kidney*. 11th ed. Saunders Elsevier; 2008:1265–1298.
7. Porrini E, Ruggenenti P, Morgensen CE, et al, ERA-EDTA diabesity working group. Non-proteinuric pathways in loss of renal function in patients with type 2 diabetes. *Lancet Diabetes Endocrinol*. 2015;3:382–391.
8. Kanauchi M, Ishihara K, Nishioka H, Nishiura K, Dohi K. Glomerular lesions in patients with non-insulin-dependent diabetes mellitus and microalbuminuria. *Intern Med*. 1993;32:753–757.
9. Klessens C, Woutman T, Veraar K, et al. An autopsy study suggests that diabetic nephropathy is underdiagnosed. *Kidney Int*. 2016;90:149–156.
10. Fioretto P, Mauer M, Brocco E, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. *Diabetologia*. 1996;39:1569–1576.
11. Alexander M, Patel T, Farag Y. Kidney pathological changes in metabolic syndrome: a cross-sectional study. *Am J Kidney Dis*. 2009;53:751–759.
12. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int*. 1999;55:713–723.
13. Solez K, Colvin R, Racusen L. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8:753–760.
14. Tervaert T, Mooyaart AL, Amann K, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*. 2010;21:556–563.
15. The Royal College of Ophthalmologists. Diabetic retinopathy guidelines. Published 2012 http://www.icoph.org/dynamic/attachments/taskforce_documents/2012-sci 267_diabetic_retinopathy_guidelines_december_2012.pdf.
16. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*. 2010;33:2285–2293.
17. Chobanian A, Bakris G, Black H, et al, and the National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*. 2003;42:1206–1252.
18. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106:3143–3421.
19. Cravedi P, Remuzzi G. Pathophysiology of proteinuria and its value as an outcome measure in chronic kidney disease. *Br J Clin Pharmacol*. 2013;76:516–523.
20. Cravedi P, Ruggenenti P, Remuzzi G. Proteinuria should be used as a surrogate in CKD. *Nat Rev Nephrol*. 2012;8:301–306.
21. Conti S, Perico N, Novelli R, Carrara C, Benigni A, Remuzzi G. Early and late scanning electron microscopy findings in diabetic kidney disease. *Sci Rep*. 2018;8:4909.
22. Shimizu M, Furuichi K, Toyama T, et al. Long-term outcomes of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy. *Diabetes Care*. 2013;36:3655–3662.
23. Shimizu M, Furuichi K, Yokoyama H, et al. Kidney lesions in diabetic patients with normoalbuminuric renal insufficiency. *Clin Exp Nephrol*. 2014;18:305–312.
24. Ekinci E, Jerums G, Skene A, et al. Renal structure in normoalbuminuric and albuminuric patients with type 2 diabetes and impaired renal function. *Diabetes Care*. 2013;36:3620–3626.

25. Magalhães P, Pejchinovski M, Markoska K, et al. Association of kidney fibrosis with urinary peptides: a path towards non-invasive liquid biopsies? *Sci Rep*. 2017;7:16915.
26. Selby N, Blankestijn P, Boor P, et al. Magnetic resonance imaging biomarkers for chronic kidney disease: a position paper from the European Cooperation in Science and Technology Action PARENCHIMA. *Nephrol Dial Transplant*. 2018;33(suppl 2):ii4–ii14.
27. Leon BM, Maddox TM. Diabetes and cardiovascular disease: epidemiology, biological mechanisms, treatment recommendations and future research. *World J Diabetes*. 2015;6:1246–1258.
28. Eliane F, Wenstedt E, Vogt L. Microvascular damage and hemodynamic alterations in diabetic nephropathy, part V: microvascular involvement. In: Roelofs JJ, Vogt L, eds. *Diabetic Nephropathy: Pathophysiology and Clinical Aspects*. Springer International Publishing AG, part of Springer Nature; 2019.
29. Futrakul N, Vongthavarawat V, Sirisalipotch S, Chairatanarat T, Futrakul P, Suwanwalaikorn S. Tubular dysfunction and hemodynamic alteration in normoalbuminuric type 2 diabetes. *Clin Hemorheol Microcirc*. 2005;32:59–65.
30. Futrakul N, Futrakul P. Renal microvascular disease predicts renal function in diabetes. *Ren Fail*. 2012;34:126–129.
31. Ruggenenti P, Porrini E, Motterlini N, et al. Measurable urinary albumin predicts cardiovascular risk among normoalbuminuric patients with type 2 diabetes. *J Am Soc Nephrol*. 2012;23:1717–1724.
32. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia*. 1989;32:219–226.
33. Fine LG, Bandyopadhay D, Norman JT. Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. *Kidney Int Suppl*. 2000;75:S22–S26.
34. Furuichi K, Shimizu M, Hara A, Toyama T, Wada T. Diabetic nephropathy: a comparison of the clinical and pathological features between the CKD risk classification and the classification of diabetic nephropathy in Japan. *Intern Med*. 2018;57:3345–3350.
35. Guo J, Zheng HJ, Zhang W, et al. Accelerated kidney aging in diabetes mellitus. *Oxid Med Cell Longev*. 2020;2020:1234059.
36. Roelofs JJ, Vogt L, eds. *Diabetic Nephropathy: Pathophysiology and Clinical Aspects*. Springer International Publishing AG, part of Springer Nature; 2019.
37. Ortiz A, Ziyadeh F, Neilson E. Expression of apoptosis-regulatory genes in renal proximal tubular epithelial cells exposed to high ambient glucose and in diabetic kidneys. *J Investig Med*. 1997;45:50–56.
38. Sarafidis P, Ferro C, Morales E, et al. SGLT-2 inhibitors and GLP-1 receptor agonists for nephroprotection and cardioprotection in patients with diabetes mellitus and chronic kidney disease. A consensus statement by the EURECA-m and the DIABESITY working groups of the ERA-EDTA. *Nephrol Dial Transplant*. 2019;34:208–230.