Diabetic nephropathy : from histological findings to clinical features

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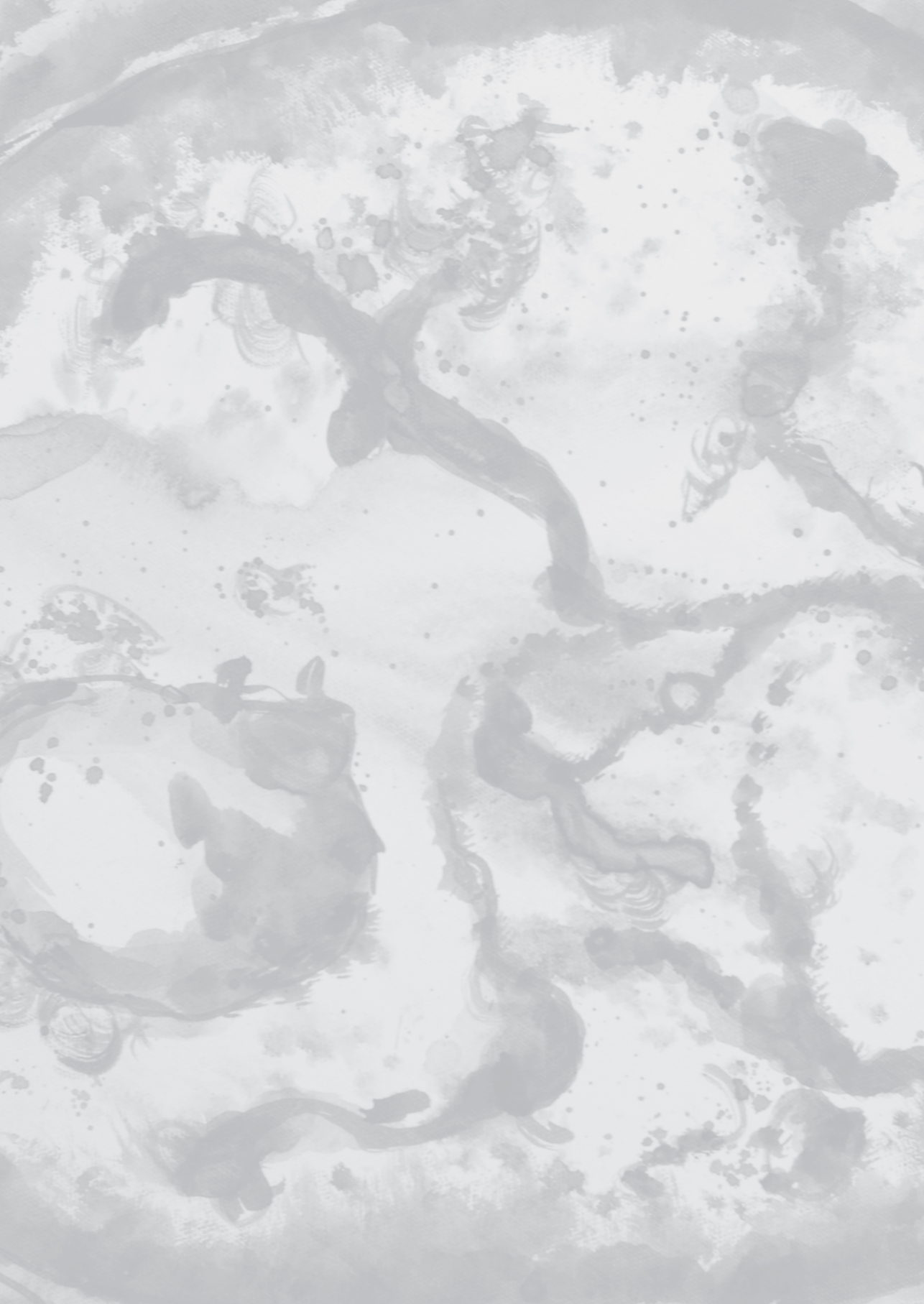

An autopsy study suggests that diabetic nephropathy is underdiagnosed

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2

ABSTRACT

The reported prevalence of diabetic nephropathy (DN) among diabetes patients varies widely. Most studies use the presence of microalbuminuria for clinical onset of DN in absence of a histopathological evaluation. In this autopsy study, we collected and analyzed data from a cohort of patients with type 1 and 2 diabetes. We determined the prevalence of histologically proven DN in patients with or without clinical manifestations of renal disease. We also examined the distribution among histopathological classes with respect to clinical parameters.

Renal tissue specimens from autopsies and clinical data were collected retrospectively from 168 patients with diabetes. The histopathological classification for DN was scored as well as interstitial and vascular parameters.

In this cohort, 106 of 168 patients had histopathological changes in the kidney characteristic of DN. Twenty of the 106 histologically proven DN cases did not present with DN associated clinical manifestations within their lifetime. Glomerular and interstitial lesions were associated with renal function, but not with proteinuria.

In this study, the prevalence of histologically proven DN was higher than previously appreciated, and we found a relatively high proportion of DN that was clinically underdiagnosed yet histologically proven, suggesting that DN lesions may develop before the onset of clinical findings. We also found that underdiagnosed DN may encompass all histopathological classes except the sclerotic class.

INTRODUCTION

Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease [1-3] that develops in approximately 10-30% of patients with diabetes mellitus. The reported prevalence of DN depends upon the type of diabetes, the duration of diabetes, and ethnicity. DN presents approximately ten years after the onset of type 1 diabetes (T1D) [4]; in contrast, the time of onset of DN among patients with type 2 diabetes (T2D) is highly variable [5]. The prevalence of clinically diagnosed DN in T1D varies between 5-20% [6-8] and in T2D between 25%-35%, based on microalbuminuria or proteinuria [5, 7]. Data on renal pathology in patients with DN is relatively limited, as a renal biopsy is performed only in cases in which the renal disease's manifestations cannot be explained sufficiently by the presence of clinically suspected DN [9, 10]. Relatively few studies on DN confirmed clinical manifestations of DN by renal biopsy [11-14].

It is generally considered that the clinical onset of DN is characterized by microalbuminuria. However, some studies suggest that a reduction in glomerular filtration rate (GFR) may precede the development of microalbuminuria [15-18]. Relatively little is known about the amount and severity of histological lesions in the kidney prior to the clinical onset of DN. Thickening of the glomerular basement membrane and mesangial changes were described in 1985 by Mauer *et al.* as the earliest histological manifestations of DN [19]. A recent study with normoalbuminuric T1D patients showed that greater glomerular basement membrane width is an independent predictor for progression to DN [20]. Nodular sclerosis is often encountered in patients with substantial proteinuria. Interestingly, there is evidence that to some extent, lesions of DN in T1D may be reversible [21, 22].

A histopathological classification for DN was launched in 2010 [23]. The original study included a substudy on interobserver agreement amongst pathologists, showing good agreement for the evaluation of the classes. Over the years, several clinical validation studies appeared [11, 13, 14], of which the most recent study [11] showed that the severity of glomerular and interstitial lesions is significantly associated with renal outcomes in patients with DN. In a smaller study, interstitial lesions—but not glomerular lesions—were determined to be a significant predictor of renal prognosis [14]. Studies which include diabetic patients who underwent a renal biopsy may be subject of selection bias regarding the moment in time at which the biopsy was taken [13, 24]. To avoid such a selection bias, we obtained tissue samples from autopsies rather than from biopsy samples.

In this autopsy study, we collected and analyzed data from a unique cohort of patients with T1D or T2D, and we determined the prevalence of histologically proven DN in patients with or without clinical manifestations of renal disease. Virtually none of the included patients underwent a renal biopsy during their lifetime. The aim of this study was to investigate the prevalence of histopathologically proven DN in patients with diabetes with or without clinical signs of DN, and its distribution over histopathological classes. Furthermore, we investigated which clinical parameters were related to the histopathological classes in patients with and without clinical manifestations of DN.

METHODS

Patients were included retrospectively from autopsies performed in 1984 through 2004 via the database of the pathology archives at Leiden University Medical Center for autopsy material. The primary inclusion criteria were the presence of either T1D or T2D in patients who were over the age of 18 years at the time of autopsy. We initially included 204 patients via our search in Delphic. We excluded 11 patients because their medical history revealed that they received a pancreas and/or kidney transplant. In nine cases, renal tissue blocks were not available, and these patients were excluded as well. Renal autopsy tissues from the remaining 184 cases were prepared for light microscopy; 16 of these samples were excluded due to poor tissue quality. Thus, 168 patients were included in the clinical histopathological analysis.

Clinical data

The clinical information was obtained via the medical records available at Leiden University Medical Center and via the patients' general practitioners. Approval was obtained from the medical ethics committee of Leiden University Medical Center to obtain relevant clinical data from the patients' practitioners for at least one year prior to the patient's death (the response rate from the practitioners was 80%). The following laboratory parameters were included: serum creatinine, eGFR (calculated using the MDRD formula), microalbuminuria (30-300 mg/L), proteinuria (>300 mg/L) via 24-hour urine or dipstick test, systolic and diastolic blood pressure, serum hemoglobin, serum cholesterol, and serum glycated hemoglobin. These data were collected retrospectively from the period starting one year before the patient's death. Data that reflected a stable representation of the serum and/or urine levels were included. Data were excluded if clearly affected by an unstable clinical condition. The decision to exclude data was made in consultation with an experienced nephrologist. We also obtained data regarding co-morbidities, duration of diabetes, medication history, hypertension, smoking, and diabetic complications. The causes of death were obtained from the autopsy reports.

Diagnosis of DN

The presence or absence of clinical DN was determined via the medical records of each patient. DN was considered present when the 24-hour urine was positive for more than 30mg/L albuminuria and/or dipstick was positive between + and ++++ in a stable period of diabetes during the year before death.

Absence of clinical diagnosis of DN

Absence of clinical DN (absence of albuminuria) was determined via the medical records of each patient. Clinical DN was considered absent when the urine dipstick tests were reported as negative or trace and/or when the 24-hour urine contained less than 30mg/L albuminuria in a stable period of diabetes during the year before death. In some cases the data was too limited to either confirm or rule out a clinical diagnosis of DN. These cases were registered as missing and were not used in the analysis.

Matched control group

A non-diabetic control group (N=40), which was matched to the underdiagnosed DN group (N=20 patients) with respect to gender, age, hypertension, and smoking habit was created to distinguish histological changes of underdiagnosed DN from age-related pathology. Each underdiagnosed DN patient had two matched control subjects for which clinical data was collected. The renal tissue specimens from the 40 control subjects were stained with hematoxylin and eosin (H&E), Periodic-acid Schiff (PAS), and silver in order to determine whether this group contained histological lesions.

Histopathology

Renal tissue was fixed in 10% buffered formalin and embedded in paraffin. Slices were cut at 1-µm and 3-µm thickness and stained with H&E, PAS, and silver stain (for the 1-µm thick sections).

Renal tissue specimens containing ≥100 glomeruli were scored by two investigators who were blinded with respect to the patients' clinical data. Glomerular lesions, interstitial lesions, and vascular lesions were scored in accordance with the established histopathological classification for DN [23]. Patients without histological lesions in our study were designated as class 0 DN. In addition, the following glomerular lesions were noted and scored as either present or absent: FSGS, cholesterol emboli, any other glomerular lesions, capsular drops, and hyalinosis of the glomerular vascular pole.

We evaluated all 45 cases with class III DN in order to determine the percentage of mesangial sclerotic nodules in each case. The percentage of mesangial sclerotic lesions was defined as the percent of glomeruli with at least one nodule in the total number of

non-sclerotic glomeruli in the tissue specimen. Additionally, we evaluated the percentage (and range) of mesangial expansion in the underdiagnosed cases. We determined the amount of mesangial expansion in either mild (class IIa) or severe (class IIb) DN in 100 glomeruli per case.

Transmission electron microscopy

Formalin-fixed, paraffin-embedded tissue blocks were reprocessed for electron microscopy. Two areas containing open glomeruli were identified on the corresponding microscopic slide, and 2-mm punches were made within these areas in the corresponding paraffin block. The paraffin was melted overnight in a 70°C oven, and the tissue was deparaffinized. After the paraffin was removed, the tissue was washed for 20 min in 0.1 M sodium cacodylate buffer (pH 7.4) containing 3% sucrose (w/v), post-fixed for 90 min in 2% osmium tetroxide diluted 1:1 with 2% potassium ferrocyanide, and dehydrated in ethanol at 70, 80, and 90% (1 hour each), followed by two one-hour incubations in 100% ethanol. The tissue was then transferred to propylene oxide for 10 min, embedded in epon, and polymerized in a 70°C oven for 30 hours. The epon blocks were trimmed, 1-µm thick survey sections were stained with toluidine blue solution, and ultrathin (100-nm thickness) sections of at least one glomerulus were cut using a Leica Ultracut UCT ultra microtome. The ultrathin sections were collected on copper grids. The sections were contrast-stained with uranyl acetate (7 min) and Reynold's lead citrate (7 min) (both from Sigma) and examined using a JEOL JEM-1011 electron microscope operating at 60 kV. Images were digitized using a MegaView III camera, and the glomerular basement membrane was measured using the Soft Imaging Solutions program (Olympus).

For all cases in our study we had to use paraffin tissue. Because reprocessing of paraffin tissue for electron microscopy causes artefactual glomerular basement membrane thinning, an additional 34% was added to the glomerular basement membrane width as recommended in a previous study of Nasr *et al*. [25], who showed that the mean reduction in glomerular basement membrane thickness in paraffin-embedded material was 34% in DN. Taking this calculation into account, the cut-off levels described by Tervaert *et al.* [23] could then be used in our study.

Statistical analysis

The SPSS statistical software package, version 20.0 (IBM, Armonk, NY) was used for all statistical analyses. Statistical differences between groups were analyzed by ANOVA and a univariate general linear model with polynomial contrast for linear trend. Differences with a p-value <0.05 were considered statistically significant. The histopathological

data were analyzed using the chi-square test. The data in the tables are presented as a percentage or as the standard error of the mean (SEM).

Ethics

All tissue samples were coded and then handled and analyzed anonymously in accordance with the Declaration of Helsinki.

RESULTS

The baseline characteristics of the 168 included patients are summarized in Table 1. The cohort contained 17 patients with T1D and 127 patients with T2D; in 24 cases, the type of diabetes was unclear. The mean age of the 168 patients was 69 years, and 55% of the cohort was male. The histopathological examination revealed lesions that were consistent with DN in 106 patients. For 21 patients, the clinical data were insufficient for determining whether they had received a clinical diagnosis of DN. In 65 of 106 patients, their clinical diagnosis of DN was in accordance with the histological lesions, i.e. a clinical diagnosis of DN had been made before death and lesions consistent with DN were found at autopsy. In 20 of the 106 patients with histological signs of DN at autopsy, no clinical diagnosis of DN was made prior to death, as neither microalbuminuria nor proteinuria had been observed during these patients' lifetime. The 20 underdiagnosed DN patients had multiple negative dipsticks and/or no albuminuria (<30mg/24h) found in the 24h urine in the year before death (Table 2). Of these 20 patients, the histopathological classification revealed that 7 patients had class I DN, 5 patients had class IIa DN, 3 patients had class IIb DN, and 5 patients had class III DN (Figure 1). Table 3 summarizes the characteristics of patients with diagnosed and underdiagnosed DN, as well as the matched control group. The decades of death varied among the underdiagnosed patients (one patient before 1990, 15 patients between 1990-2000 and four patients between 2000-2004), so the care of diabetes did not seem to influence this phenomenon. Eight patients were diagnosed clinically as having DN; however, no lesions consistent with DN were found in their renal tissues at autopsy.

Table 1. Baseline characteristics of the cohort

Baseline characteristic	Percentage or mean (SEM)
Gender (% males)	54.8
Age, years	69.3 (0.96)
T1D (%)	10.1
Duration of diabetes, years	13.69 (1.27)
eGFR, ml/min/1.73 m^2	53.14 (2.85)
Microalbuminuria or proteinuria (%)	49.4
Creatinine serum, µmol/L	136.21 (11.23)
Hb, mmol/L	7.12 (0.13)
HbA1c, (% units)	8.2 (0.82)
Cholesterol, mmol/L	4.97 (0.24)
Death by CV event (%)	47.6
Systolic pressure, mmHg	134 (2.63)
Diastolic pressure, mmHg	75.6 (1.30)

SEM, standard error of the mean; T1D, type 1 diabetes; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; CV, cardiovascular

Table 2. Clinical diagnosis of 20 underdiagnosed patients

	DN class	Method for absence of albuminuria	Stage of CKD	ACE inhibition	Hypertension	History of smoking
1	1	24h urine	3	Yes	Yes	No
2	1	Dipstick tests	2	Missing	Yes	Not described
3	1	Dipstick tests	1	No	Yes	Yes
4	1	Dipstick tests	1	No	Yes	No
5	1	24h urine	1	No	No	No
6	1	24h urine	1	No	Yes	Not described
7	1	Dipstick tests and 24h urine	2	Missing	No	Yes
8	2a	Dipstick tests	3	No	Yes	Not described
9	2a	Dipstick tests	2	No	Yes	Yes
10	2a	Dipstick tests		No	Yes	Yes
11	2a	Dipstick tests	3	No	No	Not described
12	2a	Dipstick tests	3	No	No	Yes
13	2b	Dipstick tests	4	Yes	Yes	Not described
14	2b	Dipstick tests	1	No	No	Yes
15	2b	Dipstick tests	3	Yes	Yes	No
16	3	Dipstick tests	3	Missing	Missing	Not described
17	3	Dipstick tests	3	No	No	No
18	3	Dipstick tests and 24h urine	3	No	No	Yes
19	3	Dipstick tests	3	No	No	Yes
20	3	Dipstick tests	Missing	Missing	Missing	Not described

DN class, histopathological classification of diabetic nephropathy; Stage of CKD, GFR estimated by KDIGO guidelines; Dipstick tests were reported as negative or trace, 24h urine was <30mg/L albuminuria

Table 3. Characteristics of patients with diagnosed and underdiagnosed DN

Baseline characteristic	Diagnosed DN (N=86)	Underdiagnosed DN (N=20)	*p*-value*
Gender (% males)	53.4	65	0.327
Age, years	69.8	65	0.043
T1D (%)	12	10.5	0.853
Duration, years	13.4	14	0.594
eGFR, ml/min/1.73 m^2	50.6	69.2	0.381
Microalbuminuria or proteinuria (%)	60.8	0.00	<0.0001
Creatinine serum, µmol/L	168	121	0.523
Hb, mmol/L	7.1	6.8	0.634
HbA1c, (% units)	9.2	7.3	0.351
Cholesterol, mmol/L	5.1	4.3	0.405
Death by CV event (%)	48	45	0.832
Systolic blood pressure, mmHg	136	129	0.196
Diastolic blood pressure, mmHg	76	73	0.745
Anti-hypertensive medication (%)	53.1	56.3	0.773
ACE inhibitor or ARB (%)	26.8	18.8	0.494

SEM, standard error of the mean; T1D, type 1 diabetes; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; CV, cardiovascular; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker. Measured using the chi-square test

Histopathological lesions

Lesions consistent with DN were found in 63% of the tissue specimens (106/168). Twenty-two of the samples had class I DN, which is characterized by a thickened glomerular basement membrane as determined by electron microscopy. The glomerular basement membrane width can change due to hypertension, but in this cohort there was no significant difference between patients with and without hypertension between class I and class 0 (p=0.904). Therefore, it was unlikely that increased glomerular basement membrane width was caused by hypertension. Thirty-three samples had class II DN, characterized by mesangial expansion; 21 of these samples were class IIa, and 12 samples were class IIb. Forty-five samples had class III DN, characterized by the presence of nodular sclerosis. The remaining six samples had lesions that were consistent with class IV DN, characterized by more than 50% of glomeruli with global sclerosis. We found that the percentage and range of glomeruli with mesangial sclerotic nodules was significantly lower in the underdiagnosed group compared to the diagnosed group. Overall, the mean percentage of nodules in the 45 cases with class III DN was 22.9% (range: 2.6-67.6%). In the diagnosed and underdiagnosed groups, the mean percentage of nodular sclerosis was 24.4% (range: 5.6-67.6%) and 10.9% (range: 3.0-26.2%), respectively (p=0.031).

In addition, we examined mesangial expansion in the underdiagnosed group. The five patients with class IIa DN had mesangial expansion in 40, 51, 51, 57, and 60% of glomeruli (mean: 51.8%). The three patients with class IIb DN had mesangial sclerosis in 92, 93, and 100% of glomeruli (mean: 95%).

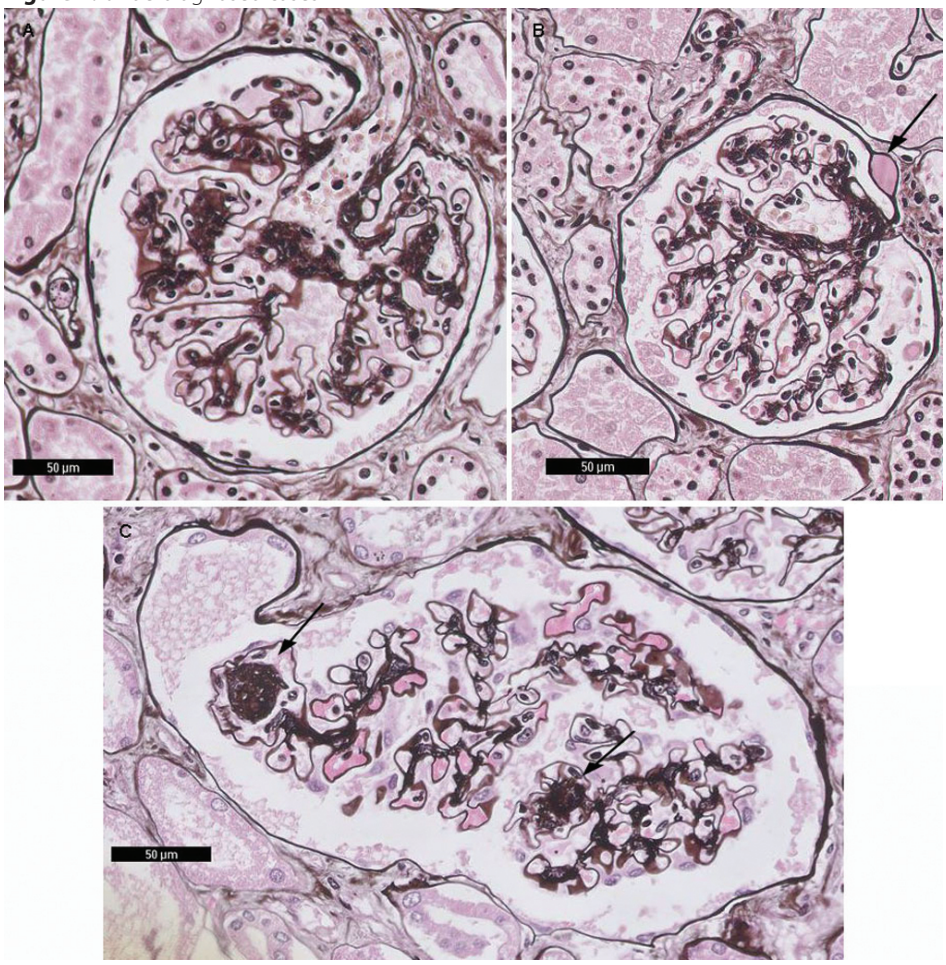
Glomerular lesions are associated with other histopathological lesions in DN, including interstitial fibrosis and tubular atrophy (IFTA) (p<0.001) and lesions like arteriosclerosis, hyalinosis, capsular drops and glomerular hyalinosis (p=0.238, p<0.001, p=0.004, p<0.001, respectively). IFTA, arteriosclerotic lesions in the arterioles, and hyalinosis were more prevalent among the patients with histologically proven DN than among the patients who had no DN lesions (i.e., class 0 DN) (p=0.007, p=0.017, and p<0.001, respectively). There were significantly more IFTA, arteriosclerotic lesions and hyalinosis with more severe DN (p<0.001 for both arteriosclerotic lesions and hyalinosis). In contrast, no significant correlation was found between the degree of arteriosclerosis and the histopathological class of DN (p=0.238). The number of capsular drops and glomerular hyalinosis increased significantly with the increasing severity of DN (p=0.004 and p<0.001, respectively). The underdiagnosed group contained significantly more capsular drops compared to the diagnosed group (p<0.001) (Figure 1 B, Supplementary Table1).

DN was absent in the control group. The amount of IFTA, arteriosclerosis, and hyalinosis of interstitial arterioles did not differ between the underdiagnosed DN patients

and controls (Supplementary Table 2). Hyalinosis of the glomerular vascular pole was sporadically present in 10 of the forty controls, but occurred significantly more often in the underdiagnosed DN group where it was present in 15 of the 20 cases ($p<0.0001$).

Focal segmental glomerulosclerosis not otherwise specified (FSGS, NOS) was present in 15 cases; 13 of these 15 patients had evidence of DN at autopsy. Six patients had cholesterol emboli; three of these six patients also had histologically proven DN. Two patients had mild glomerulonephritis suggestive of post-infectious glomerulonephritis, but no histologically proven DN, and three patients had pyelonephritis; two of these three patients had histologically proven DN.

Figure 1. Underdiagnosed cases



A: class II DN, **B**: capsular drop (arrow), **C**: class III DN (arrows indicate nodular sclerosis), Scale bars = 50 µm

Clinical data

The eGFR (estimated GFR) values and the presence of microalbuminuria, proteinuria, or other diabetic complications did not differ between the patients with T1D and the patients with T2D. A significant association between diabetes duration and DN class was found (p=0.035). Specifically, a significant difference was found between class 0 and class III patients (p=0.004) and between class IIa and class III patients (p=0.008). However, no linear trend over the classes was identified (p=0.44). Also the severity of IFTA correlated with the duration of diabetes (p=0.02). The eGFR values decreased significantly as DN class increased (p=0.006) Also, when the eGFR was staged by KDIGO CKD guidelines, there was a linear trend (p=0.001), and eGFR was linearly correlated with DN class (p<0.001). The eGFR values were inversely correlated with IFTA (p=0.001) and arteriosclerosis (p=0.002), but not with arteriolar hyalinosis. No significant correlation was found between microalbuminuria and/or proteinuria and the presence of DN (p=0.150). eGFR staged by KDIGO guidelines correlated significantly with albuminuria in a linear trend (p=0.001). Microalbuminuria and/or proteinuria was correlated linearly with IFTA (p<0.001). In addition, IFTA was inversely correlated with hemoglobin levels (p<0.001).

The medical records revealed that 29 out of 113 patients had received therapy with an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker. These 29 patients were distributed equally between the patients with and without clinical manifestations of DN (p=0.494). Data were sparse with respect to diabetic retinopathy; diabetic retinopathy was specifically noted as present in 14 patients and absent in one patient. All 14 patients, in whom diabetic retinopathy was recorded, had histologically proven DN (two had class IIa, ten had class III, and two had class IV); only one of these 14 patients was clinically underdiagnosed with DN. One patient with a clinically documented absence of both DN and diabetic retinopathy had histological evidence of class III DN at autopsy. Finally, cardiovascular-related death showed a significant linear trend with DN class (p=0.008), but not with IFTA (p=0.130).

DISCUSSION

In our diabetic cohort of 168 patients, histopathological changes attributable to DN were present in the kidney samples of 106 patients. In 20 of 106 histologically proven DN patients, clinical manifestations associated with DN had been absent during their life. This may indicate that renal lesions consistent with DN may develop before the onset of clinical abnormalities. Our data also show that an underdiagnosed DN may encompass all classes except the sclerotic class, as in the 20 patients with histopathologically proven DN who had no signs of DN during their lives, classes I, II and III were

diagnosed in respectively 7, 8, and 5 patients. Of note, next to the absence of albuminuria in several tests in 10 cases the absence of clinical DN was explicitly stated in their medical record. In this study, microalbuminuria or proteinuria was not associated with the presence of histologically proven DN.

Interestingly, the presence of microalbuminuria and/or proteinuria was correlated with IFTA, but not with the severity of DN. These findings might be explained by the capacity of healthy tubular epithelium to reabsorb proteins from the glomerular filtrate, thereby disguising glomerular protein leakage. Only after interstitial damage has occurred, the resulting loss of reabsorption capacity causes the onset of proteinuria, explaining why severe glomerular damage consistent with DN can occur prior to the onset of microalbuminuria or proteinuria [26].

In our cohort, the incidence of non-DN–related renal disease was relatively low. Based on light microscopy, 26 patients (15.5%) had lesions consistent with other renal abnormalities. The reported prevalence of non-diabetic renal disease detected on renal biopsy was previously reported up to 79% [13, 24, 27, 28]. However, this relatively high prevalence may have been influenced by selection bias with respect to the reasons for performing the renal biopsy.

Our cohort of autopsy cases enabled us to observe the manifestations of diabetes in the kidney by histology at various stages of the disease, irrespective of the clinical manifestations present in the patient prior to death. We can only compare our study results with those of clinical validation studies with respect to the clinical data at the time of tissue sampling, because per definition, our study lacks a clinical follow-up. Previous clinical validation studies yielded conflicting results with respect to the correlation between glomerular classes, interstitial lesions, and clinical parameters [11-14]. The strong association found in our study between eGFR and the classes of DN as well as between DN classes and interstitial and vascular lesions are in line with the previous studies by An *et al*. [11] and Oh *et al*. [13], who observed similar associations. In our retrospective study, diabetic retinopathy was not a clinical indicator for the presence of DN. However, data regarding the specific presence or absence of diabetic retinopathy were restricted to only 15 patients. Recently, Zhang *et al*. [29] investigated the clinical characteristics and predictive factors of subclinical DN in T2D patients and found that these patients had increased renal size, abnormal levels of tubular injury markers, high blood pressure, and abnormal circadian rhythm. In our retrospective study, data on these parameters were too limited for further evaluation.

In our study, we had the huge benefit of being able to study at least 100 glomeruli per patient, which are approximately ten-fold more glomeruli than usually available in a renal biopsy sample. In a substantial percentage of cases, we observed that explicit lesions such as nodular sclerosis were either relatively sparse or concentrated in specific areas of tissue. Thus, it is conceivable that these histopathological patterns are partly responsible for the underrepresentation of DN in renal biopsy studies. Given the large amount of tissue available, we were not only certain about the presence or absence of DN, but also in case of DN of its distribution over the four classes and the severity of interstitial lesions.

Despite the advantages discussed above with respect to our unique cohort, our study also has some limitations that merit discussion. The cohort may suffer from selection bias, because all patients included underwent an autopsy whereas not all deceased patients in the Netherlands are autopsied and those who are were mostly hospitalized before death. Our samples were taken from autopsies performed in 1984 through 2004; although we attempted to retrieve all of the clinical information available for each patient, some of the data were incomplete, and some information was missing due to the fact that medical records were sometimes destroyed after 15 years. The observed correlations between clinical and pathological parameters are based on a population of mainly Caucasians with DN. Therefore, one should be cautious with extrapolating these results to other populations. Nodular lesions similar to those seen in class III DN may occur in patients with hypertension and a history of smoking but without diabetes (typically elderly males) [30]. It seems unlikely that our results were influenced by this entity because only 2 patients in the underdiagnosed group were smokers and they did not have hypertension. Moreover, the subjects in the matched control group did not reveal histological changes characteristic of DN.

In conclusion, we found a high proportion of clinically underdiagnosed yet histologically proven DN. The potential clinical benefit of identifying this underdiagnosed group of patients remains to be determined. For example, the ability to diagnose DN in an early stage might enable clinicians to begin a specific therapeutic regimen that could slow disease progression and may ultimately prevent the onset of end-stage renal disease. Clinical studies should be designed in order to investigate whether initiating such therapeutic regimens in an early phase of DN can affect the course of renal complications associated with diabetes. Equally important is the need to develop diagnostic tools that could be used to accurately detect currently underdiagnosed patients.

DISCLOSURE

All authors declare that they have no conflict of interest.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Comparison between histological parameters of diagnosed and underdiagnosed DN patients

	Underdiagnosed DN (N=20)	Diagnosed DN (N=86)	*p*-value (χ^2 test)
DN classification (I/II/III/IV)	(7/8/5/0)	(15/25/40/6)	<0.0001
Global glomerulosclerosis (mean, %) †	9.95 (19.8)	17.03 (19.8)	0.89
IFTA (0/1/2/3)	(4/11/3/2)	(12/49/12/13)	0.87
Arteriosclerosis (0/1/2/3)	(2/11/5/2)	(6/45/20/15)	0.85
Hyalinosis (0/1/2/3)	(6/12/2/0)	(22/30/19/15)	0.06
Glomerular hyalinosis of the vascular pole (present/absent)	(15/5)	(12/74)	0.23
Capsular drops (present/absent)	(11/9)	(12/74)	<0.0001

†, *p*-value based on Student's t-test

Supplementary Table 2. Matched parameters and histological parameters of underdiagnosed DN patients and controls

		Underdiagnosed DN (N=20)	Controls (N=40)*	*p*-value (χ^2 test)
Matched parameters	Gender (male)	13/20	26/40	1.000
	Age (year)	65.45	65.28	0.504
	Hypertension absent/present	44.4/55.56	52.6/47.4	0.567
	Smoking history (yes/no/missing)	40/25/35	27.5/32.5/40	0.608
Histological parameters	Global glomerulo sclerosis (mean, %) (SD) (range) †	8.25 (11.5) (1-40%)	6.25 (6.3) (0-25%)	0.445
	IFTA (0/1/2/3) (%)	(20/55/15/10)	(40/47.5/12.5/0)	0.121
	Arteriosclerosis (0/1/2/3) (%)	(10/55/25/10)	(22.5/65/12.5/0)	0.086
	Hyalinosis (0/1/2) (%)	(30/60/10)	(52.5/30/17.5)	0.082
	Glomerular hyalinosis of the glomerular vascular pole (absent/present) (%)	(25/75)	(75/25)	<0.001

Controls were matched for gender, age, hypertension, and smoking habit; †, p-value based on Student's t-test