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Progression of nephropathy in type 2 diabetic patients

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Background. Nephropathy in type 2 diabetes is the single most common cause of end-stage renal disease (ESRD), but the decline in kidney function varies considerably between individuals, and determinants of renal function loss, early in the course of renal disease, have not been clearly identified.

Methods. In a prospective observational study, we followed 227 (60 female) Caucasian type 2 diabetic patients with nephropathy for 6.5 (range 3 to 17) years from a baseline glomerular filtration rate (GFR) of 83 (SD30) mL/min/1.73m^2 with 7 (range 3 to 22) measurements of GFR (^{51}Cr-EDTA) per patient. We evaluated determinants of (1) rate of decline in GFR, (2) risk of doubling in serum creatinine or ESRD, and (3) mortality using potential risk factors at baseline and during follow-up.

Results. The mean (SD) rate of decline in GFR was 5.2 (4.1) mL/min/year. In multivariate regression analysis, higher baseline albuminuria, systolic blood pressure (SBP), hemoglobin A1c, GFR, age, and degree of diabetic retinopathy were significantly associated with increased rate of decline in GFR (R^2_{adj} 0.24). During follow-up, elevated mean albuminuria, SBP, hemoglobin A1c, and lower hemoglobin, heavy smoking, and presence of diabetic retinopathy were significantly associated with increased decline in GFR (R^2_{adj} 0.26). During follow-up, 63 patients had a doubling in serum creatinine or developed ESRD, and 79 patients died, primarily due to cardiovascular disease. In Cox regression analysis, higher baseline albuminuria, hemoglobin A1c, and SBP, together with lower GFR and hemoglobin, were significantly associated with shorter time to doubling of serum creatinine or ESRD. Higher baseline albuminuria, hemoglobin A1c, SBP, and age were significantly associated with increased mortality.

Conclusion. Our long-term prospective study of type 2 diabetic patients with nephropathy has revealed several modifiable risk factors of enhanced progression in kidney disease and increased mortality.

Diabetic nephropathy develops in approximately 40% of all type 2 diabetic patients and is characterized by persistent albuminuria, elevated blood pressure, and a progressive decline in kidney function leading toward end-stage renal disease (ESRD). In addition, these patients have a high risk of cardiovascular disease, which further increases with deteriorating renal function [1].

In the past, diabetic renal disease in type 2 patients was viewed as a rather benign condition in comparison with type 1 diabetes [2]. However, it has become evident that type 2 diabetes constitutes the single most frequent cause of ESRD in the Western world, and the world-wide prevalence is anticipated to increase considerably due to the epidemic growth in the number of patients developing type 2 diabetes [1]. Additionally, type 2 diabetes occurs more frequently at a younger age, and because these individuals live longer with diabetes, a higher percentage may be expected to develop diabetic renal disease and ESRD.

In both type 1 and type 2 diabetic patients with nephropathy, a large interindividual variation in the rate of decline in glomerular filtration rate (GFR) has been reported [3–10]. This highlights the need for identification of risk factors for loss of renal function early in the course of nephropathy. The identification of risk factors influencing the long-term prognosis is important for the creation of new powerful treatment modalities impeding the development of ESRD. In type 1 diabetic patients with nephropathy, several factors associated with an increased rate of decline in renal function have been identified in a large prospective study [3]. In type 2 diabetic patients with nephropathy, data on the early course of renal disease come from observational studies of limited sample size [5–7, 9, 10] and studies of specific ethnic groups at high risk of diabetes and its complications (Pima Indians and Asians) [8, 11, 12]. Baseline risk factors for the composite end point of doubling of serum creatinine or ESRD have been evaluated in proteinuric type 2 diabetic patients with impaired renal function participating in a study documenting the renoprotective capacity of an angiotensin II receptor blocker during a follow-up period of 3.4 years [13]. However, there are no large studies with prolonged follow-up evaluating risk factors for progression in renal disease by applying direct measurement

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of GFR repeated regularly over time. Furthermore, factors linked to mortality have not been evaluated in type 2 diabetic patients with nephropathy.

The aim of our long-term prospective observational study carried out early in the course of renal disease was to evaluate potential risk factors associated with enhanced loss of renal function, and all-cause mortality in a large consecutive cohort of albuminuric Caucasian type 2 diabetic patients.

Patients

Since 1983, all patients with diabetes and persistent macroalbuminuria (albuminuria ≥300 mg/24 hr in at least two out of three consecutive 24-hour urine collections) have had their kidney function monitored with one yearly determination of GFR at Steno Diabetes Center in Gentofte, Denmark. The data is kept in a registry which included 411 Caucasian type 2 diabetic patients with persistent macroalbuminuria. A total of 45 patients were excluded from the present study due to clinical or laboratory evidence of nondiabetic renal disease. Among the remaining 366 type 2 diabetic patients with nephropathy we included in the present study all patients who had at least three years of follow-up, at least three measurements of GFR, and who had no clinical or laboratory evidence of nondiabetic nephropathy or urinary tract disease ($N = 227$). These 227 patients were followed until death or 2003, and determinants of renal function loss and mortality were evaluated in these patients. The remaining 139 patients had less than three years of follow-up on renal function, and were excluded from the analysis of determinants of renal function loss. However, these 139 patients were included together with the above-mentioned 227 patients in an additional analysis of baseline predictors of mortality.

Type 2 diabetes was diagnosed in patients treated by diet alone or by diet combined with oral hypoglycemic agents, in patients treated with insulin when body mass index (BMI) was above normal (≥25 kg/m^2 in women, ≥27 kg/m^2 in men) at the time of diagnosis and diabetes onset was after the age of 40 years, or in patients treated with insulin when BMI was normal and a glucagon-stimulated C-peptide value was equal to or above 0.60 pmol/mL.

During follow-up, patients were seen at Steno Diabetes Center for routine visits approximately three to four times a year. Body weight, postprandial blood glucose, hemoglobin A1c, blood pressure, and 24-hour urinary albumin excretion were determined at each visit, GFR, serum creatinine, and hemoglobin were determined once a year, and cholesterol was determined at least every second year. No sodium or protein restriction was applied during the study. During follow-up, the treatment goal for hemoglobin A1c was 7.5%, as recommended by the Danish Medical Association. Arterial hypertension was diagnosed and treated according to the World Health Organization's criteria (≥160/95 mm Hg) until 1995, and thereafter according to the American Diabetes Associations criteria (≥140/90 mm Hg) [14]. Until 2001, no specific class of antihypertensive agents was recommended. The most commonly prescribed agents included diuretics, angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, and beta blockers. After 2001, agents blocking the renin-angiotensin-system were recommended as initial drugs of choice [15–17]. Low-dose aspirin and statins were not used systematically in type 2 diabetic patients with macroalbuminuria until 2002 at Steno Diabetes Center.

Methods

GFR was measured after a single intravenous injection of ^{51}Cr-EDTA (3.7 MBq) at 08.30 a.m. by following the plasma clearance of the tracer for 4 hours [18]. The results were standardized for 1.73m^2 body surface area. The surface area calculated at the initial GFR measurement was used to standardize all subsequent GFR measurements for 1.73m^2 body surface area. The mean intra-individual day-to-day coefficient of variation in GFR is 4% in our laboratory.

Diabetic retinopathy was assessed by direct ophthalmoscopy until 1993, and thereafter by fundus photography, both after pupillary dilatation. The degree of retinopathy was classified as nil, simplex, or proliferative. Diabetic maculopathy was graded as simplex retinopathy.

The method used for measurement of hemoglobin A1c from venous blood samples has changed over the years: from 1983 to 1988, high-performance liquid chromatography (HPLC) ion exchange and isoelectric focusing were used [19]. These methods have a normal range of 4.1% to 6.1% and 4.1% to 6.4%. From 1989, hemoglobin A1c was measured with HPLC (Bio-Rad Diamat; Bio-Rad, Richmond, CA, USA) with a normal range of 4.1% to 6.4%. The correlation between this method and the two previous methods were as follows: $r = 0.983$ ($N = 194$) and $r = 0.931$ ($N = 119$), respectively. Finally, from 1994, another HPLC-based method was used (Bio-Rad Variant; Bio-Rad). The normal range remained unchanged (4.1% to 6.4%), and the coefficient of correlation between the latter and present method was $r = 0.993$ ($N = 161$).

Serum creatinine was measured using a reaction rate kinetic technique eliminating pseudocreatinine [20]. Serum cholesterol and hemoglobin were measured with standard laboratory techniques that were unchanged during the study.

Albuminuria was quantitated using radioimmunoassay from 1983 to 1990 (sensitivity 0.5 mg/L, coefficient of variation 9%) [21], and from 1990 to 1997 by enzyme immunoassay (sensitivity 1.1 mg/L, coefficient of variation

8%) [22]. A close correlation between radioimmunoassay and enzyme immunoassay ($r = 0.99$) was documented before changing the methods. From 1997, the DAKO Turbidimetric method (Carpinteria, CA, USA) was used to measure urinary albumin excretion rate. This method is closely correlated with enzyme immunoassay ($r = 0.99$) and has a coefficient of variation of 5%.

Blood pressure was measured in the sitting position after approximately 5 to 10 minutes rest with a standard mercury sphygmomanometer using an appropriate cuff size.

Smoking history was assessed from patient's records. At baseline, patients were classified as smokers if they smoked more than one cigarette per day, and as heavy smokers when smoking 20 cigarettes or more per day. During follow-up patients were classified as smokers when smoking one or more cigarette per day for more than 50% of the follow-up period, and as heavy smokers if they smoked 20 cigarettes per day for more than 50% of the follow-up period.

ESRD was defined as the need for dialysis or renal transplantation and doubling of serum creatinine as an increase of 100%, and to at least 177 μmol/L, as suggested previously [23].

In all patients included in the study, we obtained information on survival status/date of death in 2003 from the Danish registry of death. Among the 227 patients with at least three years of follow-up on renal function, death certificates were obtained to establish the cause of death. In patients who died with ESRD, the cause of death was coded as ESRD independent of the cause of death on the death certificate.

Statistical analysis

Values are expressed as mean (SD) unless otherwise stated. Albuminuria was logarithmically transformed before analysis because of the positively skewed distribution, and is given as geometric mean (95% CI). Mean values during follow-up of albuminuria, systemic blood pressure, hemoglobin A1c, hemoglobin, and serum cholesterol were calculated as the mean of yearly averages.

Linear regression analysis (least squares method) was used to determine the rate of decline in GFR for each patient, using all measured GFR values during follow-up. Multiple linear regression models using backward selection was performed to evaluate the impact of variables at baseline and during follow-up on the rate of decline in GFR. The validity of the regression analysis was confirmed by demonstrating a normal distribution (with a mean of zero) of the residuals.

Cox proportional hazards regression model with backward selection of baseline variables were used to predict time from inclusion in the study until doubling in baseline creatinine or ESRD and time to death. Results are expressed as rate ratio with 95% confidence interval. The proportional hazard assumption was assessed by scatterplots of the partial residuals of the covariates and time to event. Once the full regression model for the combined end point of doubling of serum creatinine or development of ESRD was determined, each significant risk factor in the model was divided into tertiles, and the cumulative incidence of doubling of serum creatinine or development of ESRD were plotted using Kaplan-Meier curves.

Unpaired Student t test was used to compare continuous variables.

P values < 0.05 (two-sided) were considered significant.

All calculations were performed using SPSS 12.0 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics of the 227 (60 female) type 2 diabetic patients with nephropathy are shown in Table 1. At baseline, systemic blood pressure was 158 (19)/86 (10) mm Hg, albuminuria 726 (122 to 4319) mg/24-h, and hemoglobin A1c 8.8 (1.6)%. Renal function was generally well preserved, with a GFR of 83 (30) mL/min/1.73m^2, and only six patients had a GFR below 30 mL/min/1.73m^2.

Renal function was evaluated over a median (range) period of 6.5 (3 to 17) years, with 7 (3 to 22) GFR determinations per patient. During follow-up, the mean (SD) rate of decline in GFR was 5.2 (4.1) mL/min/year, systemic blood pressure 154(15)/82 (7) mm Hg, albuminuria 581 (70 to 4775) mg/24-h, hemoglobin A1c 8.9 (1.3)%, serum cholesterol 5.7 (1.2) mmol/L, hemoglobin 8.4 (1.0) mmol/L, and body mass index 30.5 (5.3). At the end of follow-up, the mean (SD) number of antihypertensive agents prescribed per patient was 2.5 (1.1).

Determinants of rate of decline in GFR

Baseline predictors of the rate of decline in GFR were evaluated in multivariate linear regression analysis, which revealed that higher baseline values of albuminuria, systolic blood pressure, hemoglobin A1c, GFR, and age, together with increasing severity of diabetic retinopathy (nil/simplex/proliferative) were significantly associated with an increased rate of decline in GFR (Table 2). Gender, known duration of diabetes or nephropathy, diastolic blood pressures, pulse pressure, BMI, smoking, heavy smoking, serum cholesterol, or hemoglobin had no statistically significant impact on the rate of decline in GFR and were excluded from the final model. Figure 1 illustrates the unadjusted (univariate) and adjusted (multivariate) impact on the rate of decline in GFR of each of the six baseline variables, which were

Table 1. Baseline characteristics of 227 type 2 diabetic patients with nephropathy followed for 6.5 years

	Total $N = 227$
Age *years*	57 (8)
Male gender *N* (%)	167 (74)
Known duration of diabetes *years*[a]	11 (0 to 32)
Known duration of nephropathy *years*[a]	1 (0 to 11)
Body mass index kg/m^2	30.0 (5.3)
Diabetic retinopathy *N* (%)	
Nil	68 (30)
Simplex	112 (49)
Proliferative	47 (21)
Smoking *N* (%)	
None smokers	146 (64)
≥1 and <20 cigarettes per day	38 (17)
≥20 cigarettes per day	43 (19)
History of cardiovascular disease *N* (%)	
Stroke	26 (12)
Myocardial infarction	22 (10)
Coronary-artery-bypass-graft-intervention or percutanous-coronary-intervention	9 (4)
Amputations	18 (8)
Surgical intervention for peripheral ischemia	4 (2)
Glucose-lowering treatment *N* (%)	
Diet alone	15 (7)
Oral hypoglycemic agents	98 (43)
Insulin	80 (35)
Oral hypoglycemic agents and insulin	34 (15)
Antihypertensive agents per patient *N*	1.6 (1.1)
Type of antihypertensive medication *N* (%)	
ACE inhibitor	111 (49)
Angiotensin II receptor blocker	11 (5)
Calcium channel blocker	52 (23)
Beta-blocker	34 (15)
Diuretic	153 (67)
Other	8 (4)
GFR $mL/min/1.73m^2$	83 (30)
Creatinine $\mu mol/L$[a]	106 (45 to 276)
Albuminuria *mg/24-h*[b]	726 (122 to 4319)
Blood pressure *mm Hg*	
Systolic	158 (19)
Diastolic	86 (10)
Hemoglobin A1c %	8.8 (1.6)
Serum cholesterol *mmol/L*	5.9 (1.5)
Hemoglobin *mmol/L*	8.7 (1.1)

Data are mean (SD).
[a]Median (range); [b]geometric mean (95% CI).

significantly associated with rate of decline in GFR, with continuous variables separated in quintiles.

The impact of variables during the follow-up period on the rate of decline in GFR was evaluated by multivariate regression analysis using mean values during follow-up. This multivariate analysis revealed that higher mean values of albuminuria, systolic blood pressure, and hemoglobin A1c, lower values of hemoglobin, heavy smoking, and presence of diabetic retinopathy during follow-up, together with baseline age and baseline GFR were significantly associated with an increased rate of decline in kidney function (Table 2). Diastolic blood pressure, pulse pressure, and cholesterol levels during follow-up, gender and class of antihypertensive treatment for more than 50% of the follow-up period (ACE inhibitor or angiotensin II receptor blocker vs. other antihypertensive agents) were not associated with rate of decline in GFR and were excluded from the final model.

Among 23 patients without diabetic retinopathy at the end of the study, the rate of decline in GFR was only 2.6 (3.2) $mL/min/1.73m^2$ compared with 5.5 (4.1) $mL/min/1.73m^2$ in the remaining patients ($P < 0.001$).

None of the potentially modifiable markers of increased rate of decline in renal function either at baseline or during follow-up showed a significant threshold, suggesting that the best fit of the data is linear or curve linear.

Time to doubling in baseline serum creatinine or development of ESRD

During follow-up, 63 (28%) of the patients doubled their baseline serum creatinine, and 15 (7%) patients developed ESRD. The Cox proportional model revealed that the following baseline variables predicted time to the composite end point of doubling of serum creatinine or development of ESRD: albuminuria, hemoglobin A1c, systolic blood pressure, GFR, and baseline hemoglobin (Table 3) Gender, age, known duration of diabetes or nephropathy, degree of diabetic retinopathy, diastolic blood pressure, pulse pressure, BMI, smoking, or serum cholesterol were initially introduced in the model but were subsequently excluded due to lack of statistical significance.

To illustrate the individual influence of baseline albuminuria, hemoglobin A1c, systolic blood pressure, GFR, and hemoglobin on doubling in serum creatinine or development of ESRD, Kaplan-Meier curves are presented on Figure 2, with baseline variables stratified into tertiles.

Cardiovascular disease and all-cause mortality

During follow-up of the 227 patients, new nonfatal cardiovascular events including acute myocardial infarction in 38 (17%) patients, stroke in 35 (15%), coronary artery bypass grafts or percutaneous coronary intervention in 17 (7%), amputations in 13 (6%), and surgical intervention for peripheral ischemia in 7 (3%) patients occurred.

During the follow-up period, 79 (35%) of the 227 patients died. Causes of death included cardiovascular disease in 55 patients, ESRD in 14, cancer in 6, and other various causes in 4 patients. Among the 79 patients who died during follow-up, the median (range) survival time from entry to the study was 6.5 (3 to 16) years. A Cox proportional hazard model revealed that the following baseline variables influenced time to death (all cause mortality): age, albuminuria, hemoglobin A1c, and systolic blood pressure (Table 4). There was no statistical significance of gender, known duration of diabetes or nephropathy, degree of diabetic retinopathy, diastolic blood pressure, pulse pressure, BMI, smoking, hemoglobin, or serum cholesterol.

Table 2. Baseline and follow-up variables associated with increased rate of decline in GFR in 227 type 2 diabetic patients with nephropathy followed for 6.5 years

Variable	Slope (95% CI)	*P* value
Dependent variable		
Rate of decline in GFR *mL/min/year*		
Baseline model		
Independent variables at baseline		
Albuminuria $\log_{10}$	3.58 (2.22 to 4.9)	<0.001
Systolic blood pressure per 10 mm Hg	0.33 (0.03 to 0.63)	0.02
Hemoglobin A_{1c} per 1%	0.67 (0.32 to 1.02)	<0.001
Age per 10 years	0.82 (0.01 to 1.58)	0.03
GFR per 10 mL/min/1.73 m^2	0.60 (0.41 to 0.81)	<0.001
Level of diabetic retinopathy nil/simplex/proliferative	1.64 (0.80 to 2.48)	<0.001
R^2_{adjusted} 0.24		
Follow-up model		
Independent variables		
Mean albuminuria during follow-up $\log_{10}$	2.00 (0.92 to 3.10)	<0.001
Mean systolic blood pressure during follow-up per 10 mm Hg	0.51 (0.14 to 0.88)	0.010
Mean hemoglobin A_{1c} during follow-up per 1%	0.39 (0.01 to 0.77)	0.040
Mean hemoglobin during follow-up per mmol/L	−0.70 (−1.34 to −0.07)	0.030
Diabetic retinopathy at end of follow-up present/absent	2.13 (0.49 to 3.77)	0.011
Smoking ≥20 cigarettes a day during follow-up *yes/no*	1.33 (0.01 to 2.64)	0.048
Baseline age per 10 years	0.77 (0.05 to 1.48)	0.035
Baseline GFR per 10 mL/min/1.73 m^2	0.55 (0.33 to 0.75)	<0.001
R^2_{adjusted} 0.26		

Variables excluded from the baseline model: gender, duration of diabetes and nephropathy, diastolic blood pressure, BMI, smoking, heavy smoking, serum cholesterol, and hemoglobin. Variables excluded from the follow-up model: diastolic blood pressure and cholesterol levels during follow-up, gender, and class of antihypertensive treatment.

An additional analysis of all 366 (257 males) type 2 diabetic patients with nephropathy and no known nondiabetic renal disease revealed that 194 (53%) patients were dead in 2003. The median (range) duration of follow-up on survival was 6 (0 to 17) years in these 366 patients. A Cox proportional hazard model revealed that baseline age, albuminuria, hemoglobin A1c, and GFR influenced time to death, whereas there was no statistical significance impact of systolic blood pressure or other baseline variables (data not shown).

DISCUSSION

In the present long-term prospective observational cohort study of 227 type 2 diabetic patients followed early after the onset of nephropathy, we identified several modifiable variables including albuminuria, hemoglobin A1c, systolic blood pressure, hemoglobin, and heavy smoking to be independently associated with enhanced progression in renal disease, as evaluated by rate of decline in GFR and by the time to doubling in baseline serum creatinine or ESRD. Furthermore, baseline albuminuria, hemoglobin A1c, and systolic blood pressure independently predicted time to death, which was primarily due to cardiovascular disease.

In order to obtain a valid determination of the rate of decline in GFR, it has previously been suggested that the applied GFR method should have good accuracy and precision, repeated measurement should be performed every 6 to 12 months, and the observation period should extend at least two years [24]. These requirements were met in our cohort consisting of all 227 Caucasian type 2 diabetic patients with nephropathy who were followed with annual GFR measurements for at least three years. Whereas at least three years of follow-up was required to establish a valid rate of decline in renal function, this requirement would bias mortality toward survivors due to exclusion of patients who died before three years of follow-up. Therefore, we performed an additional analysis of baseline predictors of mortality, including all type 2 diabetic patients with nephropathy irrespective of the duration of follow-up on renal function. In this cohort of 366 type 2 diabetic patients with nephropathy, baseline age, albuminuria, hemoglobin A1c, and GFR were found to have a significant impact on mortality. The impact of age, albuminuria, and hemoglobin A1c on mortality was similar to what was found among the 227 patients with at least three years of follow-up on renal function.

Patients with clinical, laboratory, or biopsy-proven evidence of nondiabetic nephropathy or urinary tract disease were excluded from our study. A clinical diagnosis of diabetic nephropathy can be made in a patient with diabetes on the basis of persistent albuminuria, the presence of diabetic retinopathy, and the absence of any clinical or laboratory evidence of other kidney or urinary tract disease [1]. In type 2 diabetic patients with nephropathy, the prevalence of biopsy proven nondiabetic nephropathies varies from 5% to 30% depending on the enrollment criteria of the patients [25–27]. The highest prevalence of nondiabetic kidney disease has been documented in patients lacking retinopathy. Underlying differences in renal pathology among the studied patients may

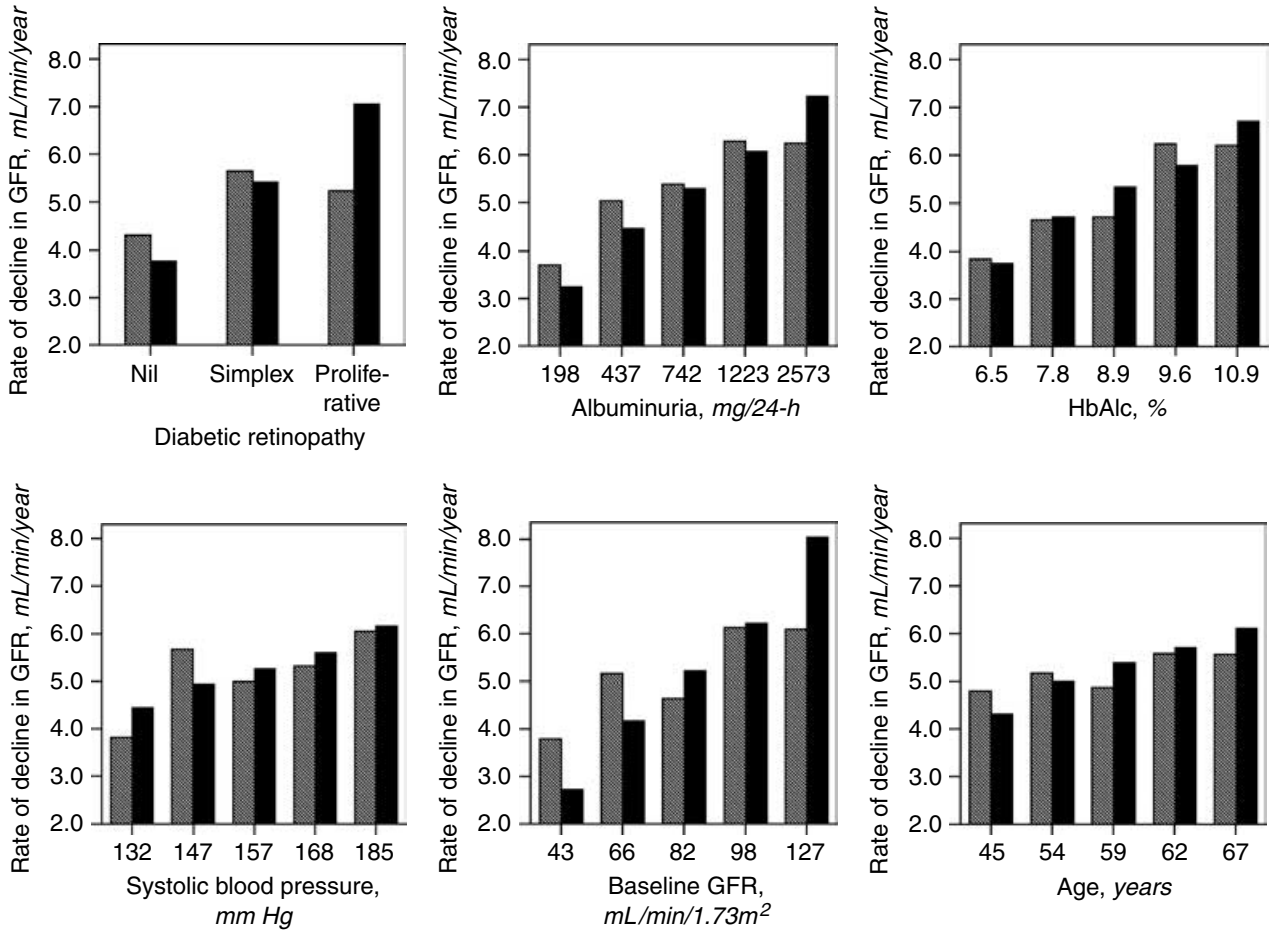

Fig. 1. Impact of baseline parameters: level of diabetic retinopathy, albuminuria, hemoglobin A1c, systolic blood pressure, glomerular filtration rate (GFR), and age on the rate of decline in GFR (continuous variables are separated into quintiles). Crossed bars show the unadjusted association with the rate of decline in GFR and solid bars show the adjusted association with rate of decline in GFR. Adjustment has been made for the other five variables significantly associated with rate of decline in GFR.

Table 3. Baseline predictors of time to doubling of baseline serum creatinine (to at least 177 μmol/L) or end-stage renal disease in 227 type 2 diabetic patients with nephropathy followed for 6.5 years (Cox proportional hazard model)

Baseline	Hazard ratio (95% CI)	*P* value
Albuminuria log10	7.35 (3.35 to 15.70)	<0.001
Systolic blood pressure per 10 mm Hg	1.23 (1.07 to 1.38)	0.001
HbA1c per 1%	1.48 (1.21 to 1.80)	<0.001
Hemoglobin per 1 mmol/L	0.75 (0.57 to 0.98)	0.030
Baseline GFR per 10 mL/min	0.86 (0.74 to 0.96)	<0.010

During follow-up, 63 (28%) of the patients doubled their baseline serum creatinine, and 15 (7%) patients developed ESRD. The following baseline variables were excluded due to lack of statistical significance: age, gender, diabetes duration, diastolic blood pressure, BMI, serum cholesterol.

contribute to the variation in renal progression rates, since biopsy studies of type 2 diabetic patients have demonstrated a slower rate of decline in GFR in nondiabetic nephropathies as compared to patients with diabetic renal disease [28].

Studies of albuminuric patients with type 1 or type 2 diabetes not receiving early antihypertensive treatment demonstrate that decline in GFR ranges from 10 to 14 mL/min/year [1, 8]. Our study demonstrates that early antihypertensive treatment can reduce albuminuria and the rate of decline in GFR (5.2 mL/min/year) in albuminuric type 2 diabetic patients with rather well-preserved kidney function, which confirms and extends findings in previous studies [4, 7, 9, 10, 29]. Even more importantly, our data are in close agreement with the rate of GFR decline reported in the angiotensin II receptor blocker group of the Irbesartan Diabetic Nephropathy Trial (IDNT) and Reduction of End Points in NIDDM with the Angiotensin II Receptor Antagonist Losartan (RENAAL) study: 5.5 mL/min/year and 4.4 mL/min/year, respectively [16, 17].

We found that the degree of diabetic retinopathy at baseline independently predicted the rate of decline in GFR during follow-up. This was primarily due to a very slow rate of decline in GFR in the relatively small

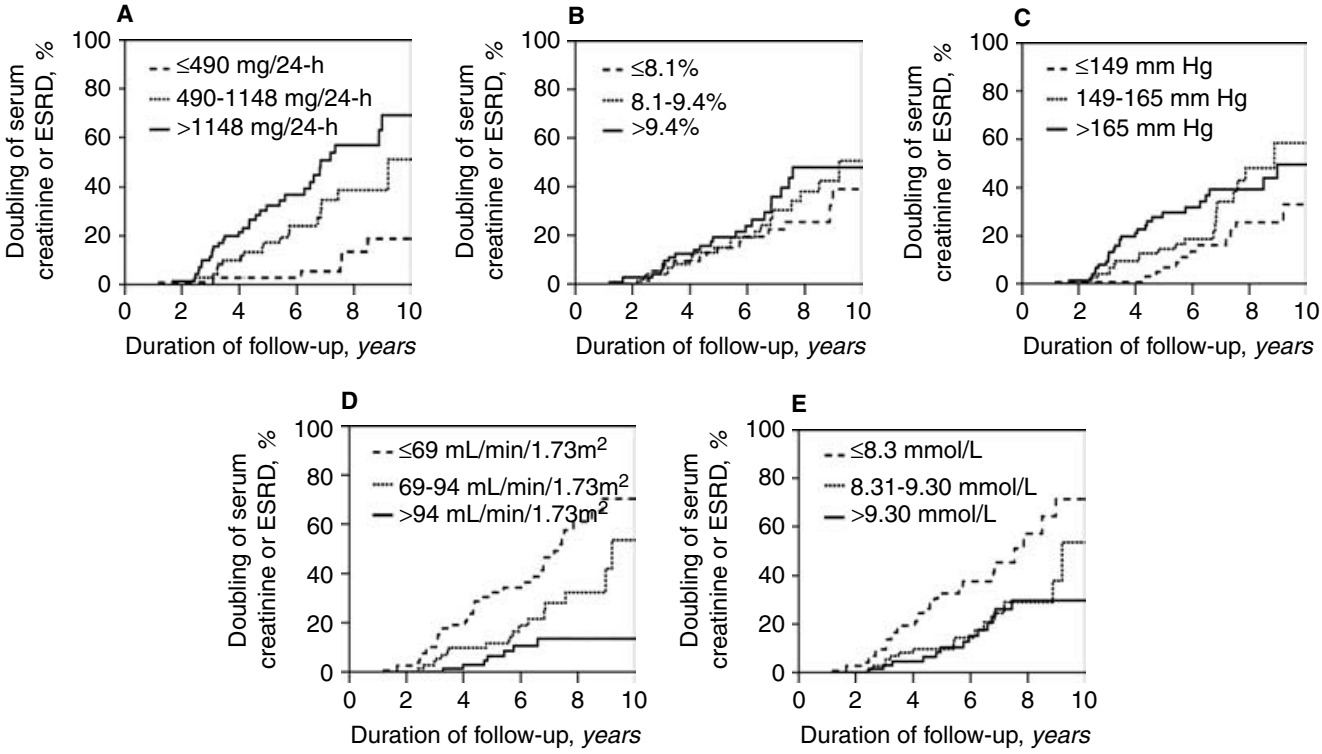

Fig. 2. Kaplan-Meier curves of the percentage of patients with doubling in serum creatinine or end-stage renal disease (ESRD) during follow-up stratified by tertiles of baseline: (*A*) albuminuria, (*B*) HbA1c, (*C*) systolic blood pressure, (*D*) GFR, and (*E*) hemoglobin.

Table 4. Baseline predictors of time to death (all-cause mortality) in 227 type 2 diabetic patients with nephropathy followed for 6.5 years (Cox proportional hazard model)

Baseline	Hazard ratio (95% CI)	*P* value
Age per 10 years	1.82 (1.32 to 2.63)	<0.001
Albuminuria $\log_{10}$	2.56 (1.34 to 4.88)	<0.01
Systolic blood pressure per 10 mm Hg	1.14 (1.00 to 1.29)	0.049
HbA1c per 1%	1.24 (1.05 to 1.47)	<0.01

During follow-up a total 79 (35%) patients died. Causes of death included cardiovascular disease in 55 patients, ESRD in 14, cancer in 6, and other various causes in 4 patients. The following baseline variables were excluded due to lack of statistical significance: age, gender, diabetes duration, diastolic blood pressure, BMI, serum cholesterol, GFR, degree of retinopathy, smoking, hemoglobin.

subgroup of 23 patients who did not develop diabetic retinopathy, whereas patients who developed diabetic retinopathy during follow-up had a rate of decline in GFR similar to patients with diabetic retinopathy present at baseline. Our study has confirmed and extended the finding from a recent study dealing with the impact of diabetic retinopathy on progression in kidney function in albuminuric type 2 diabetic patients [9]. The greater rate of decline in renal function in patients with increasing severity of diabetic retinopathy may be explained by the more severe renal lesions, since the degree of retinopathy has been correlated to renal structural abnormalities and functional impairment [10, 25, 30].

Albuminuria has previously been regarded as a marker of the extent of glomerular damage, but experimental data suggest that proteinuria per se may contribute to glomerular and tubulointerstitial lesions [31]. In our study, baseline albuminuria was an independent predictor for progression in renal disease, and the level of albuminuria during follow-up was independently associated with the rate of decline in renal function which is in agreement with previous findings in diabetic and nondiabetic renal disease [3, 4, 11, 32–34]. When risk factors for progression in renal disease were evaluated in a post-hoc analysis of 1513 patients with type 2 diabetes and impaired renal function enrolled in RENAAL study, baseline albuminuria was the strongest and most consistent independent risk factor for time to reach the composite end point of doubling in serum creatinine or developing ESRD [13]. In our study, the level of baseline albuminuria also predicted increased all-cause mortality. Several studies have previously demonstrated increased all-cause mortality rates with increasing levels of albuminuria, when comparing patients with normo-, micro-, and macroalbuminuria, and even slightly increased urinary albumin excretion rate within the upper-normal range has been shown to be associated with increased mortality rates [35, 36]. The excess mortality is due to cardiovascular disease, and it has previously been suggested that the presence of albuminuria

is a marker of generalized vascular dysfunction leading to increased atherosclerosis [37].

In type 2 diabetes, elevated blood pressure has previously been demonstrated to be an independent risk factor for development and progression of diabetic nephropathy, and several studies have demonstrated that antihypertensive treatment reduces the risk of developing nephropathy and slows the progression in renal injury once nephropathy has developed [38]. Accordingly, recent guidelines suggest an aggressive treatment of arterial blood pressure with a target blood pressure of 130/80 mm Hg [39]. In our study, systolic but not diastolic blood pressure was associated with increased progression in renal disease, which is in accordance with previously published data [4, 5, 9, 40]. This is likely due to the fact that type 2 diabetic patients primarily suffer from isolated systolic hypertension. In contrast to findings in a previous study of type 2 diabetic patients with nephropathy and impaired renal function, diastolic blood pressure was not negatively correlated to progression in renal disease [40]. Furthermore, pulse pressure at baseline and during follow-up did not have an independent effect on renal disease progression in our study of patients with rather well preserved GFR. We could not demonstrate any specific renoprotective effects by treatment with agents blocking the renin-angiotensin system. However, our study was not designed to evaluate treatment effects due to the risk of confounding by indication in observational studies [41]. Randomized double-blind, clinical trials of type 2 diabetic patients with early or advanced renal disease have clearly demonstrated specific renoprotective effects by angiotensin II receptor blockers as compared with conventional antihypertensive agents not blocking the renin-angiotensin system [15–17].

Our study suggests an association between hyperglycemia and progression in renal disease, which we previously reported in 301 type 1 diabetic patients with diabetic nephropathy followed at Steno Diabetes Center during the same time period [3]. In other studies of type 2 diabetic patients with nephropathy some reported a correlation between hyperglycemia and increased renal function loss [10, 29, 42], whereas other studies have not found such an association [4, 5, 9, 11, 13]. The discrepancy between the findings may in part be due to small sample size, and consequently, lack of statistical power. One exception is the previously mentioned post-hoc analysis of baseline predictors in the RENAAL trial, which did not find any impact of baseline hemoglobin A1c on the time to development of renal end points [13]. The discrepancy in relation to our results may be because patients included in the RENAAL study all had severe nephropathy with reduced renal function at baseline, whereas patients in our study were followed earlier in the course of renal disease. Our study suggests that hyperglycemia plays a role for the progression early in the course of nephropathy, but it may be that the impact decreases over time with deteriorating renal function when other risk factors for progression, such as albuminuria and hypertension, are more markedly increased and thus have a relatively greater impact on renal outcome.

Smoking has previously been associated with an increased risk of developing diabetic nephropathy [43–45]. In our study, we could not demonstrate a significant impact of smoking on the rate of decline in GFR when patients were stratified into smokers and nonsmokers. However, when patients were stratified into heavy smokers (smoking 20 cigarettes a day or more) and nonheavy smokers (individuals smoking less than 20 cigarettes a day and nonsmokers), heavy smokers had a significantly greater rate of decline in GRF. This finding confirms and extends the results from a study of 182 Japanese type 2 diabetic patients with nephropathy and impaired renal function (>133 μmol/L), where smoking increased the risk of progression to ESRD [11]. Smoking at baseline was not a risk factor for renal outcome in the RENAAL study [13]. However, the numbers of cigarettes smoked was not quantified, and it was not taken into account that some patients may have stopped smoking during follow-up. Furthermore, a high cardiovascular mortality was seen in the RENAAL study, and it is possible that smokers may have been more likely to die of cardiovascular disease before reaching the renal end points.

Anemia has been reported to be a frequent and unrecognized companion to diabetes in particular when nephropathy develops [46]. It has been suggested that anemia occurs more frequently and develop earlier in diabetic compared to nondiabetic renal disease due to a predominance of renal interstitium damage, systemic inflammation, and autonomic neuropathy in diabetic renal disease [47]. Increased concern of the possible deleterious effects of hypoxia-induced organ damage in type 2 diabetic patients has been raised since the finding of an increased risk of adverse renal outcome in patients with even modest degrees of anemia [13]. In our study of patients with generally well-preserved kidney function at inclusion, baseline hemoglobin even within the normal range predicted time to doubling of serum creatinine or ESRD independently of other risk factors including baseline GFR, although baseline hemoglobin did not correlate to the subsequent rate of decline in GFR. Future interventional studies of anemic patients with normal or moderately reduced renal function is needed to establish if anemia correction could retard progression in renal disease or reduce the frequent occurrence of ischemic cardiovascular disease.

In agreement with our findings, previous studies in type 2 diabetic patients have failed to demonstrate a significant correlation between serum cholesterol and rate of decline in GFR [4, 5, 11–13, 29]. In contrast, we have previously demonstrated elevated cholesterol to be an independent

risk factor for enhanced rate of decline in GFR in type 1 diabetic patients with nephropathy [3]. The lack of association between cholesterol and all-cause mortality in our study may be due to the influence of other risk factors.

We did not find any significant impact of gender on progression of renal disease or survival. Consequently, the male preponderance seen in our study is not likely to affect the generalizability of our data to populations with a higher prevalence of females. The frequency of males in our study is comparable to a cross-sectional study of unselected type 2 diabetic patients attending another Danish diabetes hospital, where 80% of patients with proteinuria were male [48]. The male preponderance is in part due to an unequal gender distribution among diabetic patients in the Danish population, with more males than females having diabetes [49]. Furthermore, male gender has been demonstrated to be an independent risk factor for development of incipient and overt diabetic nephropathy [36].

Among the 227 patients included in our observational study, 173 of the patients did not participate in any clinical trials during the follow-up period. The remaining 54 of the patients were enrolled in two different long-term clinical intervention trials of two to three years' duration at the Steno Diabetes Center during the follow-up period. One trial was comparing the renoprotective effects of ACE inhibitors versus beta-blockers, the other trial compared angiotensin II receptor blockers versus conventional antihypertensive treatment. Consequently, it cannot be excluded that a Hawthorne effect (i.e., improved prognosis due to participation in clinical trials irrespective of the intervention) may have improved the outcome in these patients. However, the rate of decline in GFR among patients participating in clinical trials was comparable to patients who were trial naïve. This may in part be explained by the relatively short duration of follow-up in the clinical trial compared to the overall follow-up period of 6.5 years in the present study.

The available data suggest that multifactorial intervention targeting blood pressure, albuminuria, hyperglycemia, smoking, hyperlipidemia, and, potentially, anemia, is needed in order to improve the prognosis in type 2 diabetic patients with nephropathy. The success of such a combined approach in delaying development and progression of micro- and macrovascular complications has been demonstrated in microalbuminuric type 2 diabetic patients [50, 51].

CONCLUSION

Our long-term prospective study of proteinuric type 2 diabetic patients has revealed several modifiable risk factors for progression of renal disease, including albuminuria, hemoglobin A1c, systolic blood pressure, hemoglobin, and heavy smoking. Furthermore, baseline albuminuria, hemoglobin A1c, and systolic blood pressure independently predicted time to death, which was primarily due to cardiovascular disease.

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