Improved prognosis of diabetic nephropathy in type 1 diabetes

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The natural history of diabetic nephropathy offered an average survival of only 5–7 years. During the past decades, multiple changes in therapy and lifestyle have occurred. The prognosis of diabetic nephropathy after implementing stricter control of blood pressure (including increased use of long-term renin–angiotensin system inhibition), lipids, and glycemia, along with less smoking and other lifestyle and treatment advancements, is inadequately analyzed. To clarify this, we studied 497 patients with type 1 diabetes and diabetic nephropathy at the Steno Diabetes Center and compared them with previous data, obtained using identical criteria at our hospital. The glomerular filtration rate, measured yearly by ^{51}Cr-EDTA plasma clearance, was a mean of 71 ml/min per 1.73 m^2 at baseline. The mean glomerular filtration rate decline was significantly reduced by 19% (95% confidence interval 5–34) from previously 4.0 to 3.3 ml/min per 1.73 m^2/year. During a median follow-up of 9.1 years, 29% of participants doubled their plasma creatinine or developed end-stage renal disease. Mortality risk was similar to our prior study (hazard ratio 1.05 (0.76–1.43). However, after age adjustment, as both diabetes and nephropathy onset occurred later in life, mortality was reduced by 30%. Risk factors for decline in glomerular filtration rate, death, and other renal end points were generally in agreement with prior studies. Thus, with current treatment of nephropathy in type 1 diabetes, the prognosis and loss of renal function has improved along with better control of modifiable risk factors.

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KEYWORDS: diabetic nephropathy; diabetes mellitus; end-stage renal disease; glomerular filtration rate; mortality; progression of chronic renal failure

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Diabetic nephropathy (DN) is a major complication of diabetes, characterized by elevated urinary albumin excretion rate (UAER), increase in blood pressure (BP), and a relentless decline in renal function. During the natural course of DN, mean survival after the onset of persistent proteinuria was 5–7 years.[1,2] Despite better treatment, including antihypertensive agents and dialysis, DN is still the leading cause of end-stage renal disease (ESRD) in industrialized countries. Furthermore, the increased mortality observed in diabetes occurs mainly in patients with DN[3] and is primarily due to cardiovascular disease (CVD)[4] and not only the result of ESRD.

Antihypertensive treatment[5,6] and particularly inhibition of the renin–angiotensin system (RAS) has become a cornerstone in the treatment of patients with diabetes and albuminuria. This is based on randomized studies showing RAS inhibition to delay renal end points and death.[7–10] However, the prognosis with longer-term clinical use is inadequately analyzed.

For patients with type 1 diabetes (T1DM) and DN, RAS inhibition became a fully implemented part of standard therapy after reinforcement of local guidelines in 2000. These guidelines also stressed the importance of control of BP, lipids, glycemia, and smoking.

This current study evaluates the loss of renal function and prognosis of patients with T1DM and DN from 2000 to 2010 by assessing change in ^{51}Cr-EDTA plasma clearance (glomerular filtration rate (GFR)), progression to ESRD, and mortality rate. A multistate model is used to account for CVD, the competing risks of ESRD, and death. Results are compared with patients identified and followed up with the same criteria and methods, at the same hospital,[11,12] before these guidelines.

RESULTS

Study participants

We identified 497 eligible patients with T1DM and DN. The mean (s.d.) baseline GFR was 71 (32) ml/min per 1.73 m^2, hemoglobin A_{1c} (HbA_{1c}) was 9.1 (1.4) %, and median (interquartile range) albumin excretion rate (UAER) was 483 (193–1089) mg/24 h. At baseline, 74% received RAS inhibition; however, 91% were prescribed RAS inhibition for

at least half of their follow-up and 80% were prescribed RAS inhibition for 80% of follow-up. Median follow-up time was 9.1 (5.1–10.5) years. Compared with our prior follow-up cohort used for the comparison of mortality rates ($n = 199$),[12] our current cohort developed diabetes 4.8 (95% confidence interval: 1.0–11.8, $P < 0.01$) years later and had an onset of DN 6.4 (1.1–12.7, $P < 0.01$) years later, causing 1.6 (0.8–9.3, $P = 0.02$) years longer diabetes duration before DN onset. Table 1 compares the characteristics of both cohorts.[12]

For analyses of GFR decline, three measurements of GFR over 3 years were required, leaving 315 patients (63%) with sufficient follow-up. Table 2 shows characteristics for this subgroup and our prior cohort used to compare the rate of decline in GFR ($n = 301$).[11] RAS inhibition was prescribed over half of the study duration in 96% and since 2001 in >90% at any time point, compared with 60% in the past.[11] In addition, most risk factors such as BP, UAER, cholesterol, and smoking were better controlled in the recent cohort. GFR was lower and glycemic control unchanged (Table 2).

Clinical end points

The whole cohort ($n = 497$) was followed up for a median of 9.1 (5.1–10.5) years. The combined end point of either doubling of plasma creatinine or ESRD was reached by 30%

Table 1 | Characteristics for the whole current cohort in comparison with our prior follow-up cohort used for comparison of mortality rates among patients with type 1 diabetes and diabetic nephropathy

Cohort	Current	Prior follow-up[12]	*P*-value
Time period (years)	2000–2010	1993–2003	
Patients (number)	497	199	
Baseline characteristics			
Male (%)	62	61	0.87
Age (years)	48 (12)	41 (10)	0.001
Diabetes duration (years)	30 (11)	28 (8)	0.32
Nephropathy duration (years)	8 (3–14)	8 (5–11)	0.76
Retinopathy: nil/simplex/proliferative (%)	15/35/50	0/31/69	0.001
Smoking (%)	36	50	0.001
Body mass index (kg/m^2)	21.8 (3.4)	24 (3.3)	0.001
Prior cardiovascular disease (%)	21	11[a]	
Insulin requirements (IE/kg per day)	0.7 (0.5–0.9)	NA	NA
Baseline measurements			
GFR (ml/min per 1.73 m^2)	71 (32)	74 (34)	0.17
Albuminuria (mg/24 h or mg/g creatinine)[b]	483 (193–1089)	796 (342–2079)	0.001
Blood pressure (mm Hg)	142 (17)/80 (9.1)	151 (23)/86 (13)	0.001
HbA_{1c} (%)	9.1 (1.4)	9.5 (1.5)	0.001
Hemoglobin (mmol/l)	8.0 (1.0)	8.2 (1.1)	0.13
Total cholesterol (mmol/l)	5.2 (1.1)	5.6 (1.2)	0.001
HDL cholesterol (mmol/l)	1.6 (0.5)	1.5 (0.5)	0.001
LDL cholesterol (mmol/l)	3.0 (1.0)/2.9 (2.3–3.5)	3.5 (1.1)	0.001
LDL/HDL ratio	2.1 (1.0)	NA	NA
Triglyceride (mmol/l)	1.2 (0.8–1.8)	1.2 (0.9–1.7)	0.63
Plasma creatinine (μmol/l)	106 (90–140)[c]	103 (82–134)	0.03
Other information			
Age at diabetes onset (years)	18.1 (12.8)	13.3 (8.8)	0.001
Age at nephropathy onset (years)	38.8 (13.4)	32.4 (10.4)	0.001
Prescribed with medicine[d]			
RAS inhibition (%)	91	53	
ACE inhibition (%)	65	53	
Angiotensin receptor blocker (%)	26	0	
Beta blocker (%)	19	14	
Diuretics (%)	80	65	
Calcium channel blocker (%)	38	22	
Other antihypertensive drugs (%)	8	NA	
Statins (%)	59	0	
Acetylsalicylic acid (%)	72	11	

Abbreviations: ACE, angiotensin-converting enzyme; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not available; RAS, renin–angiotensin system.

Data are presented as mean (s.d.), median (interquartile range), or frequency in percent. *P*-values from *t*-test, Kruskal–Wallis test, or χ^2-statistic.

[a]Only including history of acute myocardial infarction and stroke.

[b]All patients previously had sustained macroalbuminuria, expressed as minimum 2/3 measurements of 24-h albumin >300 mg or albumin/creatinine ratio >300 mg/g. At baseline, some patients had values below 300.

[c]Our plasma creatinine values were, for the sake of comparison, converted to Jaffe, as this was used in the prior cohort. Median in isotope dilution mass spectrometry (IDMS) values was 94 (79–126) μmol/l for our cohort.

[d]For the current cohort, this is prescription for at least half of the study duration, whereas for the prior cohort we only have baseline information.

Table 2 | Characteristics for the two cohorts of patients with type 1 diabetes and diabetic nephropathy included in the analysis of rate of decline in GFR

Cohort	Current ΔGFR cohort	Prior ΔGFR cohort[11]	*P*-value
Time period (years)	2000–2010	1983–2000[a]	
Patients (number)	315	301	
Baseline variables			
Male (%)	61	64	0.42
Age (years)	47.0 (10.9)	36.2 (11.0)	<0.001
Diabetes duration (years)	30.3 (10.4)	22.3 (8.3)	<0.001
Diabetic nephropathy duration (years)	9 (4–15)	3 (1–5)	<0.001
Retinopathy: nil/simplex/proliferative (%)	14/32/53	0/33/67	<0.001
Smoking (%)	36	54	<0.001
Body mass index (kg/m^2)	21.6 (3.1)	23.9 (3.0)	<0.001
GFR (ml/min per 1.73 m^2)	77.6 (28.7)	89.1 (28.0)	<0.001
Albuminuria (mg/24 h (or mg/g creatinine))[b]	433 (167–950)	629 (304–1334)	<0.001
Blood pressure (mm Hg)	140.4 (16.6)/78.7 (8.3)	139.7 (18.5)/85.3 (9.3)	0.62/<0.001
HbA_{1c} (%)	9.2 (1.4)	9.3 (1.5)	0.24
Hemoglobin (mmol/l)	8.2 (0.9)	NA	
Total cholesterol (mmol/l)	5.3 (1.0)	5.8 (1.3)	<0.001
Plasma creatinine (μmol/l)	100 (88–125)[c]	83 (69–106)	<0.001
Other information			
Age at diabetes onset (years)	16.7 (12.2)	13.9 (8.6)	<0.001
Age at nephropathy onset (years)	37.6 (0.7)	32.7 (0.6)	<0.001
Time span of GFR measurements (years)	7.8 (2.4)	7.5 (3.5)	0.33
GFR measurements (number)	7.1 (2.8)	NA	
Annual decline in GFR (ml/min per 1.73 m^2)	3.3 (3.1)	4.0 (4.1)	<0.01

Abbreviations: GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; NA, not available.
Data are presented as mean (s.d.), median (interquartile range), or frequency in percent. *P*-values from *t*-test, Kruskal–Wallis test, or χ^2-statistic.
[a]Recruitment from 1983 to 1997, follow-up from 1983 to 2000.
[b]All patients previously had sustained macroalbuminuria, expressed as minimum 2/3 measurements of 24-h albumin >300 mg or albumin/creatinine ratio >300 mg/g. At baseline, some patients had values below 300.
[c]Plasma creatinine was measured with Jaffe until 2004; for the sake of comparison we converted all our measurements to Jaffe; the isotope dilution mass spectrometry (IDMS) median (interquartile range) for our current GFR cohort was 88 (77–111) μmol/l at baseline.

($n=148$, (17% ($n=83$) received renal replacement therapy (RRT) and 23% ($n=116$) doubled plasma creatinine)), 27% ($n=135$) died, and 0.3% ($n=16$) emigrated. Crude mortality risk after DN onset was similar to our previous follow-up study,[12] with a hazard ratio (95% confidence interval) from Cox regression of 1.05 (0.76–1.43), $P=0.78$. As patients in the prior cohort were >6 years younger at the onset of DN, age was adjusted for, resulting in a 30% risk reduction (0.70 (0.51–0.98), $P=0.04$) for the present cohort. Replacing the timeline with diabetes duration gave similar hazard ratio (0.90 (0.66–1.12), $P=0.51$, and 0.68 (0.49–0.94), $P=0.03$) for the two models, respectively.

A total of 113 patients from the previous study were included in our current study. Excluding recurring subjects (long-time survivors) in the current cohort only resulted in hazard ratio of 1.12 (0.81–1.55 $P=0.49$, and 0.76 (0.54–1.07, $P=0.12$)).

As CVD registration and ascertainment differs in the cohorts, CVD rates are not comparable. Supplementary A online describes CVD occurrence in the whole current cohort.

Rate of decline in GFR

Median follow-up time with GFR measurements was 8.6 (5.6–9.9) years, and the median number of measurements were 7.[5–9] The mean rate of decline in GFR was 3.26 (3.1) ml/min per 1.73 m^2 per year. Compared with our prior cohort,[11] the decline was reduced by 19% (5–34) from 4.0 (4.1) ml/min per 1.73 m^2 per year ($P<0.01$).

A total of 125 patients were included in both study periods. These patients maintained a stable rate, suggesting persistent effect of intervention, as decline in GFR was 2.4 (4.4) ml/min per 1.73 m^2 per year in the former and 2.6 (4.3) ml/min per 1.73 m^2 per year in the current period (paired *t*-test: $P=0.61$).

In a sensitivity analysis of incidence cohorts, GFR decline was compared for patients with either new-onset DN during the past 10 years of the previous follow-up ($n=66$) or new-onset DN during the latter follow-up period ($n=60$); the rate of decline in GFR before 2000 was 5.6 (5.4) ml/min per 1.73 m^2 per year and was reduced by 18% to 4.4 (3.8) ml/min per 1.73 m^2 per year in the latter period ($P=0.19$).

Figure 1 shows the unadjusted effects of 1-s.d. change in continuous baseline or follow-up variables on decline in GFR. Furthermore, male gender ($P=0.02$), chronic kidney disease stage ($P<0.01$), and smoking ($P<0.01$) were associated with faster decline in GFR. Table 3 shows the multivariate models with adjusted effects of baseline and follow-up variables on decline in GFR. The variable effects were substantially unchanged by alternative selection strategies (Supplementary B online).

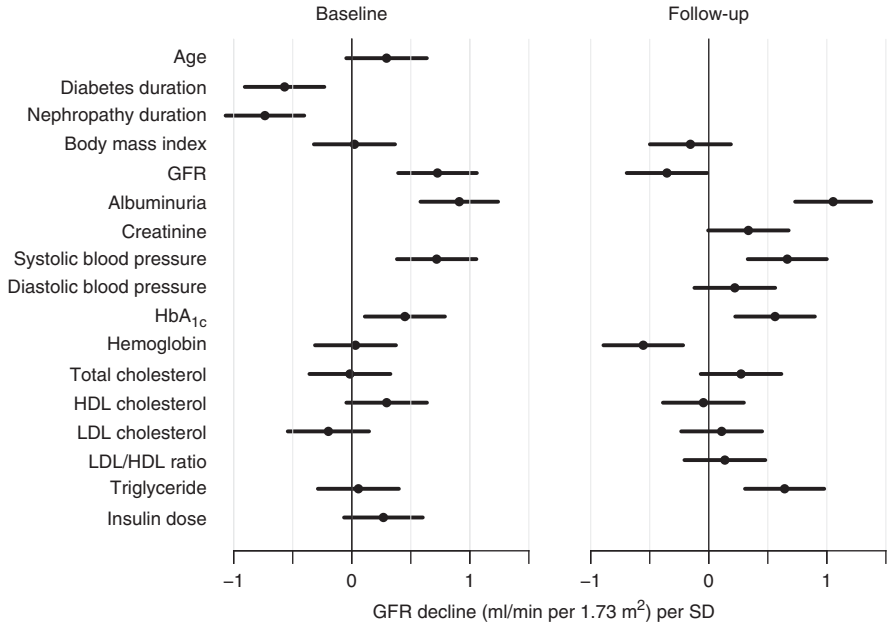

Figure 1 | Unadjusted effects of 1-s.d. change in continuous baseline variables to the left and follow-up variables to the right on the rate of decline in glomerular filtration rate (GFR) (ml/min per 1.73 m^2 per year) in our current cohort of 315 patients with type 1 diabetes and diabetic nephropathy. Variables on the right side of the vertical bar are linked with a more rapid decline in GFR, whereas those on the left side are linked with a slower GFR decline.

Table 3 | Effects of baseline and follow-up variables on annual decline in GFR for patients with type 1 diabetes and diabetic nephropathy in multivariate models, based on the current ΔGFR cohort

	Difference of/between:	Effect on decline in GFR (ml/min per 1.73 m^2 per year)	*P*-value
Baseline parameters[a]			
Smoking	Yes/no	0.79 (0.11–1.47)	<0.01
Age[b]	10 years	0.50 (0.14-0.86)	<0.01
HbA_{1c}	1%	0.42 (0.19-0.65)	<0.001
Albuminuria	100%	0.39 (0.22-0.57)	<0.001
LDL/HDL ratio	1	−0.38 (−0.72 to −0.03)	0.03
GFR	10 ml/min per 1.73 m^2	0.29 (0.17-0.41)	<0.001
Systolic blood pressure	10 mm Hg	0.28 (0.07-0.50)	0.01
Sex[b]	Men/women	0.25 (0.41 to −0.91)	0.46
Follow-up parameters[c]			
Hemoglobin	1 mmol/l	−0.65 (−0.25 to −1.05)	<0.01
HbA_{1c}	1%	0.50 (0.19-0.80)	<0.01
Albuminuria	100%	0.42 (0.27-0.57)	<0.01
Systolic blood pressure	10 mm Hg	0.32 (0.06-0.58)	0.02

Abbreviations: GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Adjusted r^2 for baseline model = 0.24.
[b]Age and sex were forced into the baseline model.
[c]Adjusted r^2 for the follow-up model = 0.18.

Multistate model

To account for competing risk of pre-ESRD death and ESRD, we developed a multistate model illustrated in Figure 2. The number of transitions between different clinical states and the corresponding progression rates in percent/year are indicated on the arrows. The annual mortality rate was 1.4% for DN only, after a CVD event it increased to 5.1%, after ESRD to 5.6%, and after both CVD and ESRD the rate further increased to 19.1% (Figure 2). The rate for post-ESRD mortality was derived by adding the number of transitions from the ESRD groups to mortality (the red arrows: 45 + 14) and dividing by total person-years in the previous states (235.5 + 250) or 12.2%. Similarly, pre-ESRD mortality, corresponding to the green arrows, was 2.3%, and the progression rate to ESRD (blue arrows) was 4.5% per year.

The model was used to assess adjusted relative risk of putative clinical predictors on the competing risk of either pre-ESRD mortality or ESRD (including doubling of plasma creatinine), corresponding to the green and blue arrows in Figure 2. Results are given in Figure 3. Prior CVD and smoking were the strongest predictors of pre-ESRD mortality, with the addition of lower GFR. The risk of ESRD was significantly affected by male gender, higher UAER, systolic BP, HbA_{1c}, and lower GFR and hemoglobin.

The 10-year cumulative probability of being dead or alive, with or without CVD and/or ESRD (including doubling of plasma creatinine) based on our findings, is shown in Figure 4. It illustrates the risk for a patient with better (Figure 4a) or worse (Figure 4b) control of risk factors (Table 4 shows risk factor values). Figure 4c and d depict similar risk probabilities for patients with CVD at baseline. Worse control of risk factors approximately tripled the estimated fraction of patients who progressed to ESRD in 10 years (40% vs. 13%

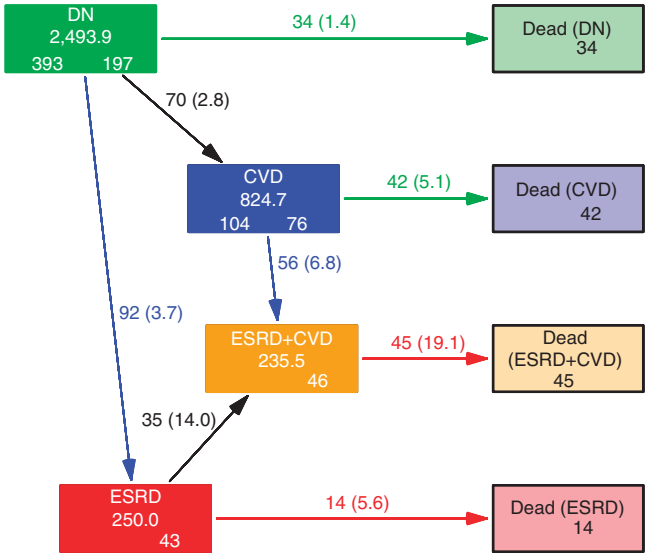

Figure 2 | For patients with type 1 diabetes and diabetic nephropathy (DN), follow-up time was divided into time spent in the following clinical states: DN, cardiovascular disease (CVD), end-stage renal disease (ESRD), and ESRD + CVD in chronologic order, as shown in this multistate model with age, diabetes duration, and DN duration as underlying time scales. Numbers in boxes represent the sum of person-years in the state; numbers on the arrows are the number of patients who alter state (transitions) and rates in percent/year. Patients with CVD at baseline started in the CVD state (blue box); otherwise, all patients started in the DN state (green box). Green, diabetic nephropathy (DN) only; blue, cardiovascular disease (CVD); red, end-stage renal disease (ESRD), including doubling of p-creatinine; yellow, ESRD and CVD. Framed boxes with lighter colors represent death in the above-mentioned states. Coloring of the boxes corresponds to the coloring of clinical states in Figure 4.

without CVD, and 40% vs. 14% with prior CVD) and more than doubled the cumulative 10-year risk of death (32% vs. 11% without CVD and 48% vs. 23% with prior CVD). For model patients with CVD, the 10-year probability of dying was higher than reaching ESRD. Within 10 years from baseline, approximately one-third of patients with ESRD but without CVD at baseline had died, compared with half of the patients with ESRD and baseline CVD.

DISCUSSION

Our long-term observational study in which patients with T1DM and DN were followed up during 2000–2010 showed a significantly improved prognosis for survival and preservation of renal function compared with prior equivalently selected and followed cohorts at our hospital. We demonstrate better control of several modifiable risk factors such as BP, blood glucose and lipids, greater use of RAS inhibition, and less smoking. Age-adjusted mortality risk after DN onset was reduced by 30% and mean rate of decline in GFR diminished by 19% to now 3.3 (0.17) ml/min per 1.73 m^2 per year compared with prior cohorts[11,12] and the effect was long-lasting. This is noteworthy as these prior studies, using the same methods, already demonstrated improved prognosis after routinely implementing antihypertensive treatment, with a GFR decline of 4 ml/min per 1.73 m^2 per year and a median survival of 21.7 years after the onset of DN[12] compared with the natural history of DN with a GFR decline of 10–20 ml/min per 1.73 m^2 per year and a median survival of 5–7 years.[1,2]

Previous randomized double-blind controlled trials of up to 5 years duration demonstrated benefit of blocking RAS in patients with T1DM and DN.[7,9,13] No randomized controlled trial has been powered to evaluate the long-term effect of RAS inhibition in DN. In contrast, long-term use was suggested to increase renal event rates in a register-based study,[14] although the study was debated.[15] In our prior cohort,[11] RAS inhibition was used in up to 60% of the patients.The present study was not designed to compare RAS inhibition with other antihypertensive treatments, as it was observational and almost all received RAS inhibition (91%), but it demonstrates improved prognosis for patients under current treatment, where most receive long-term RAS inhibition, in addition to improved risk factor control. The use of incidence cohorts had been superior but not possible owing to low numbers. Similarly, a comparison with a concurrent cohort under other regimens would have minimized the effects of secular trends. However, no such cohort was available. Only a randomized study could elucidate the true impact of different treatment aspects.

In our study, plasma creatinine doubled in 23% of patients during a median follow-up of 9.1 years. During 3 years, 12% of patients with T1DM and DN treated with captopril reached this end point in the Captopril Study by the Collaborative Study Group.[9] More recently, the Finn-Diane Study[16] and the Joslin Clinic[17] published data on patients with T1DM and DN. Incidence of ESRD was higher than in our cohort, despite our use of a combined end point with doubling of creatinine (see Table 5). However, their pre-ESRD mortality rates were lower–perhaps explained by our cohort being considerably older and having higher GFR. The joint incidence of pre-ESRD death and RRT was similar, as were post-ESRD mortality rates. They[16,17] already showed that low GFR favors ESRD, whereas with better renal function the risk of death is prevailing. This corresponds with our data. As illustrated from the major impact of age on mortality when comparing our own cohorts, differences in age are clearly of substantial impact; subsequently, age and GFR differences make comparison difficult.

We are not aware of recent studies using 'measured true' GFR in unselected patients with DN on current treatment. As mentioned, the present finding of a rate of decline of 3.3 (3.1) ml/min per 1.73 m^2 per year is a 19% reduction compared with patients from our institution followed up before 2000.[11] In the Captopril Study,[9] the decline in measured 24-h creatinine clearance was 11 (21)% per year in the captopril group and 17 (20)% per year in the control group. Although the data cannot be directly compared, our patients are losing 3.3 ml/min per 1.73 m^2 per year of an initial mean of 77 ml/min per 1.73 m^2, corresponding to 4% in the first year.

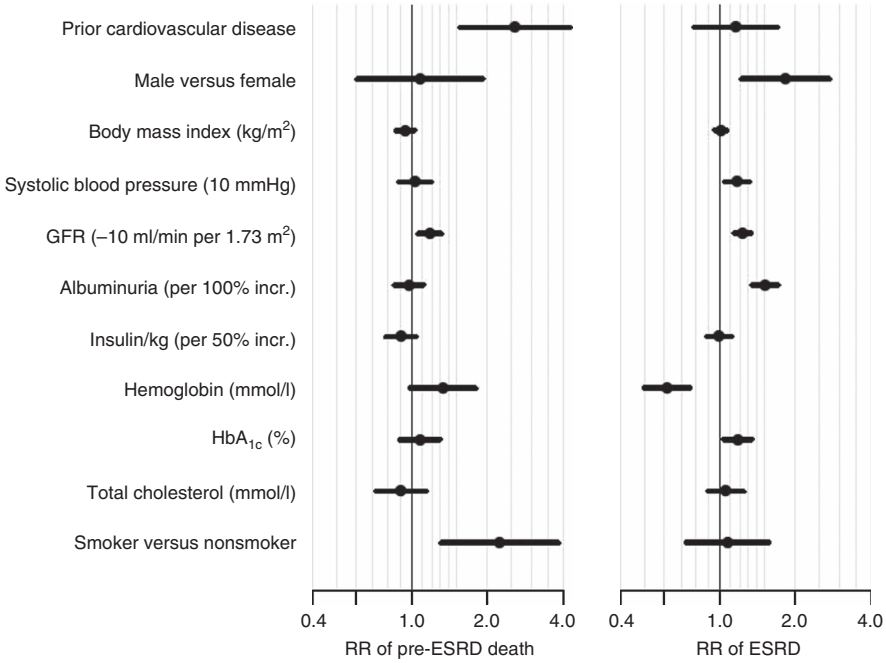

Figure 3 | Estimated relative risk scores (RR) for pre-end-stage renal disease (ESRD) mortality or ESRD, including doubling of p-creatinine, in a multivariate model showing the relative risk and 95% confidence intervals for clinical predictors for patients with type 1 diabetes and diabetic nephropathy. The risks are adjusted for the duration of diabetes and nephropathy, as well as age.

The slower decline in GFR compared with prior studies fits well with the relatively constant incidence of RRT for patients with diabetes in Denmark since 2002,[18,19] even though the diabetes prevalence doubled between 1997 and 2007.[20] However, the effect of higher diabetes prevalence on ESRD incidence will not be evident until decades after diabetes onset. Data from United States and Catalonia demonstrate that age-specific incidences of ESRD in the diabetic population have decreased since the late 1990s and 2002, respectively.[21,22] Similarly, the relative incidence of RRT due to T1DM has declined in Finland,[23] Moreover, onset of RRT was postponed from 35 to 45 years from 1990 to 2007,[23] akin to our cohort's increased age and longer diabetes duration at DN onset, as was seen at Joslin.[17] Conversely, recently published data suggest that despite constant rates ESRD onset might just be delayed in T1DM,[24] which could lead to a steep rise in ESRD incidence when the growing diabetes population ages.

Our model (Figure 4) underlines that control of modifiable risk factors, as identified in our previous cohort,[11] has a major impact on the estimated fraction of patients who progress to ESRD or die, as relatively small differences in multiple risk factors triple the risk of ESRD and double the mortality risk.

The main factors associated with developing ESRD or doubling of creatinine were basically the same as those associated with GFR decline. The effects of glycemic control,[25–28] UAER or proteinuria,[26,29–31] hemoglobin,[29] BP,[26,28,30,32] and gender[33] were seen for both end points. Meanwhile, GFR, age, smoking, and lower low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio were only related to decline in GFR. With the exception of LDL/HDL ratio, this is generally compatible with earlier studies.[4] Previous CVD[33] or macrovascular disease,[16] smoking,[34–36] and lower GFR[35,37] are known risk factors for mortality. Furthermore, chronic kidney disease[35,38,39] and smoking[34] add to CVD risk and thereby increase mortality. However, we must acknowledge that only 18–24% of the variability in the rate of GFR decline could be explained by our models.

The fact that cholesterol had no impact could be owing to improved treatment, as 65% were in long-term statin treatment, thereby reducing variability and thus potential as predictor. The influence of lower LDL/HDL ratio may be an artifact of statin treatment, possibly identifying residual risk for statin users as we expected the reciprocal contribution. Nonetheless, statin treatment was not independently associated with progression. Possibly an exposure for half the study period is too coarse a measure for an effect to be detected.

Levels of UAER and BP had influence on progression of renal end points, but no significant effect on pre-ESRD mortality risk. Similarly, previous CVD—the major risk factor for mortality—had no effect on progression of renal disease. Indeed, it appears from Figure 3 as if these end points could be driven by different pathways. Had we evaluated either end point disregarding the other, instead of using our multistate model, this may not have been evident, as the effect of predictors may be confounded by the occurrence of the other end point. This should be considered when interpreting and comparing studies using different methods.

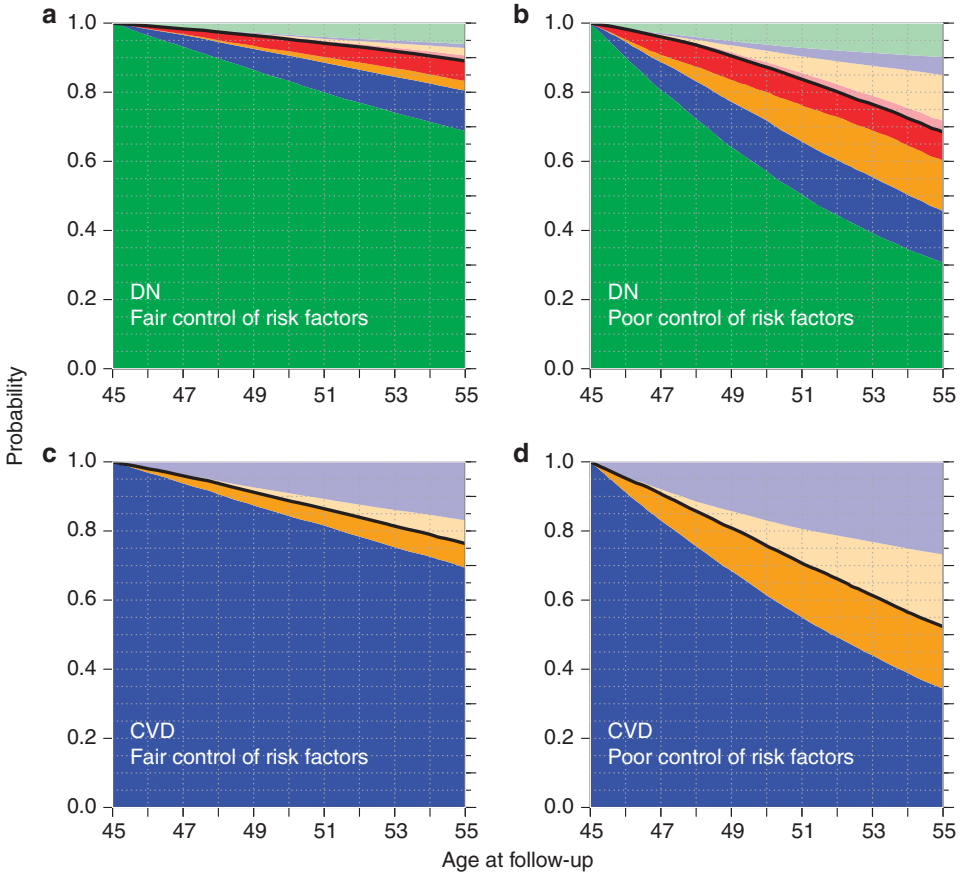

Figure 4 | Probabilities of being in different clinical states during the first 10 years after study entry for patients with type 1 diabetes and diabetic nephropathy. Panel **a** and **b** represent patients without prior cardiovascular disease (CVD), whereas **c** and **d** show patients with CVD at baseline. In **a** and **c**, patients were given better control of risk factors than otherwise comparable patients in **b** and **d**. Table 4 shows the values of cofactors. Color coding refers to the clinical states, as shown in Figure 2. Black line, overall survival curve; red, end-stage renal disease (ESRD) or doubling of p-creatinine; blue, CVD; yellow, ESRD (including doubling of p-creatinine); and CVD, green, diabetic nephropathy only. Light colors represent death in the corresponding states.

Table 4 | Values of clinical variables used for modeling 10-year cumulative risk probabilities (Figure 4) for patients with type 1 diabetes and diabetic nephropathy

	No prior CVD		Prior CVD	
Figure	4a	4b	4c	4d
Control of risk factors	Better	Worse	Better	Worse
Gender	Male	Male	Male	Male
Age (years)	45	45	45	45
Diabetes duration (years)	25	25	25	25
Nephropathy duration (years)	5	5	5	5
Smoking	No	Yes	No	Yes
Body mass index (kg/m^2)	22	22	22	22
Insulin requirements (IE/kg per day)	0.75	0.75	0.75	0.75
GFR (ml/min per 1.73 m^2)	70	70	70	70
Albuminuria (mg/24 h)	300	1000	300	1000
Systolic blood pressure (mm Hg)	130	150	130	150
HbA_{1c} (%)	7.5	9.0	7.5	9.0
Hemoglobin (mmol/l)	8	8	8	8
Total cholesterol (mmol/l)	4.5	5.5	4.5	5.5

Abbreviations: CVD, cardiovascular disease; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}.
Model patients were given the above clinical values in order to demonstrate different 10-year cumulative risk probabilities in Figure 4. Values were selected to be close to the mean in our cohort.

To obtain valid determination of the rate of decline in GFR, the applied method should have good accuracy and precision, repeated measurements should be performed, and the observation period should last at least 2 years.[40] Our study fulfills these requirements. However, owing to the prerequisite of 3 years of follow-up and three measurements of GFR, patients with recent onset of DN and those who progress to ESRD or death within 3 years are not eligible. However, as the inclusion criteria were equivalent to our former study,[11] results are comparable. The sole difference was the exclusion of patients without retinopathy in the prior study. With similar criteria applied to the present cohort, the decline in GFR was 3.28 (3.20) ml/min per 1.73 m^2 per year, comparable with the rate for those excluded ($P = 0.80$).

As some patients appeared in both study periods, our results should be interpreted cautiously. Thus, we performed an analysis excluding recurring subjects from the current cohort only, which gave similar hazard ratios for death, albeit not significant. Although this introduced a bias due

Table 5 | Comparison of studies with rate of hard end points for patients with type 1 diabetes and diabetic nephropathy

Cohort	Current cohort	Joslin[17]	FinnDiane[16]
Recruitment period (years)	2000–2010	2001–2004	1995–2006
Number of patients (number)	497	198	592
Follow-up (years)	9.1 (5.1–10.5)	902 Person-years	Median 9.9
Male gender (%)	62	54	59
Age (years)	48 (12)	42 (10)	42 (10)
Diabetes duration (years)	30 (11)	27 (9)	29 (8)
Nephropathy duration (years)	8 (3–14)	—	—
Prior cardiovascular disease (%)	21	—	20
Smoking (%)	36	21	—
GFR (ml/min per 1.73 m^2)	71 (32)[a]	60 (30)	52 (26)
Albuminuria (mg/24 h or mg/g)	483 (193–1089)	778 (465–1795)[b]	Macroalbuminuria
Systolic blood pressure (mm Hg)	142 (17)	131 (17)	145 (20)
HbA_{1c} (%)	9.1 (1.4)	8.7%	9.0 (1.5)
Total cholesterol (mmol/l)	5.2 (1.1)	5.2 (1.3)	5.4 (1.1)
RAS inhibition use (%)	91	82	75–88[c]
Incidence ESRD (rate (events))	4.5 ($n=148$)[d]	6.8 ($n=61$)	5.1 ($n=210$)
Pre-ESRD mortality (rate (events))	2.3 ($n=76$)	1.1 ($n=10$)	1.4 ($n=56$)
Post-ESRD mortality (rate (events))	12.2 ($n=59$, from 148)	12.6 ($n=17$, from 61)	NA ($n=65$, from 210)

Abbreviations: ACE, angiotensin-converting enzyme; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; RAS, renin-angiotensin system.
Values are expressed as median (interquartile range), mean (s.d.), or otherwise specified. The rates are given in % per year, equivalent to cases per 100 patient-years.
[a]Real measured GFR with ^{51}CR-EDTA plasma clearance, whereas the others are estimated GFR estimates.
[b]Not stated whether this is range or interquartile range.
[c]75% received ACE inhibition and 13% received angiotensin receptor blockers, and thus RAS inhibition use must be between 75 and 88%.
[d]Our incidence rate includes doubling of serum creatinine. Thus, they are higher than incidence rates for ESRD alone. Only 17% ($n=83$) of our cohort developed ESRD, whereas 29% ($n=148$) reached the combined end point of ESRD or doubling of creatinine.

to exclusion of long-time survivors in our cohort, excluding recurring subjects from both cohorts would greatly aggravate the mortality rate of the previous cohort. We also applied ssensitivity analysis of GFR decline in new-onset DN in the two periods; other use of incidence cohorts was, as previously stated, not possible.

We have attempted to minimize the bias due to the change in creatinine method in 2004 by transforming earlier creatinine measurements. We additionally find nearly the same progression promoters for the development of ESRD, including doubling of creatinine, as for decline in ^{51}Cr-EDTA-based GFR. As in our prior studies,[11,12] lipid parameters were collected regardless of fasting state.

In summary, for patients with T1DM and DN, the survival and preservation of renal function has improved—coinciding with enhanced control of modifiable risk factors and long-term RAS inhibition.

MATERIALS AND METHODS

Study design and cohort

At Steno Diabetes Center, all patients with DN are offered yearly measurements of GFR using ^{51}Cr-EDTA plasma clearance.[41] In a retrospective setting, we identified all patients with T1DM and DN with measurements of GFR from 01 January 2000 to 31 December 2010 and combined information from our laboratory database, patient files, and national databases from inclusion (date of first GFR determination after 01 January 2000) until 31 December 2010, death, or emigration. DN was defined as persistent macroalbuminuria (>300 mg/24 h or albumin/creatinine ratio >300 mg/g) in two out of three consecutive samples without clinical or laboratory evidence of other kidney disease. The date for fulfilling the DN criteria was set as the date of second macroalbuminuric sample.

The end points were development of ESRD or doubling of plasma creatinine with the competing risk of mortality, and annual rate of decline in GFR. Although only patients followed up with GFR for a minimum of 3 years and at least three measurements were eligible for analyses of the rate of decline in GFR, all were included in analyses of the other end points.

For comparison of mortality rates at Steno Diabetes Center, we used a follow-up study of a previous T1DM cohort followed up from 1993 to 2003 at our center.[12] Similarly, the results of GFR decline were compared with a Steno cohort followed up from 1983 to 2000 (recruitment from 1983 to 1997).[11] The present cohort was selected on the basis of identical criteria and analyzed similarly.

Data sources and clinical assessment

Demographic, clinical, and laboratory information originated from our clinic's electronic records and laboratory database. The Danish National Registry on Regular Dialysis and Transplantation provided information on kidney transplantations and dialysis treatments, the National Patient Registry supplied ICD codes from hospitals contacts, and the Danish Register of Causes of Death provided information on death.

All patients were routinely examined with HbA_{1c}, BP, and UAER 3–4 times yearly in our clinic and with yearly GFR, serum hemoglobin, creatinine, and cholesterol. Intra-individual coefficient of variation in GFR is 3.9%.[42] Urinary albumin was analyzed by immunologic methods (turbidimetry). Creatinine was changed from modified Jaffe to an enzymatic reaction on 1 September 2004. BP was measured in the sitting position after 5–10 min rest, with a mercury sphygmomanometer or an oscillometric device (A&D Medical, San Jose, CA) using an appropriate cuff size. Laboratory values attained at inclusion were used as baseline. If unavailable, the closest measurement in time, within 1 year before baseline and 4 months after, was applied. To overcome day-to-day

variation, we determined the weighted mean of BP and UAER (geometric) from 1 year before baseline until 1 month after.

Follow-up and end-point evaluation

Rate of decline in GFR was determined as the regression coefficient, using all measurements during the study. Other follow-up values were expressed at weighted mean of all measurements. ESRD was defined as dialysis or renal transplantation. Doubling of plasma creatinine was determined as an increase of 100% from baseline, if it reached the minimum value of 150 µmol/l, after transforming Jaffe measurements to IDMS values.[43] CVD included stroke, acute myocardial infarction, ischemic heart disease, and heart failure, on the basis of ICD-10 and ICD-8 codes and on operational codes for coronary artery bypass graft and percutaneous coronary intervention. Death date as reported in the Danish Register of Causes of Death.

Statistical analysis

For comparison of means, medians, and proportions, we applied the *t*-test, Kruskal–Wallis test, and χ^2-statistic, respectively. Statistical significance was an α-level < 0.05 on two-sided tests. Univariate associations for baseline and follow-up variables with rate of change in GFR were assessed using linear regression. Continuous variables were standardized to show the effect of 1-s.d. increase. Factors with *P*-value below 0.2 in univariate models were entered in a multivariate model chosen by adjusted corrected Akaike's information criterion and backward selection, as backward selection was used in our prior study.[11] Age and gender were forced into the model of baseline variables. To avoid inclusion of related variables, we selected age rather than diabetes or nephropathy duration, GFR rather than chronic kidney disease stage or creatinine, systolic BP rather than diastolic BP, and LDL/HDL ratio instead of total LDL, and HDL cholesterols. Creatinine, UAER, albumin/creatinine ratio, triglyceride, and dosage of insulin/kg per day were log2 transformed to comply with model assumptions. We fitted a Cox regression model with staggered entry for the combined data set of our current and previous cohort,[12] with DN duration or diabetes duration as underlying time-scale using cohort membership as categorical variable, with and without age as a linear effect. We reported the hazard ratio of the new cohort versus the old. For database management and the above-mentioned statistical analysis, we used SAS Enterprise Guide 4.3 (SAS Institute, Cary, NC).

We set up a multistate model classifying the follow-up (follow-up time and events) in the categories DN, CVD, ESRD (including doubling of plasma creatinine), and ESRD + CVD, along with death from each of these states. Patients with CVD before DN entered follow-up in the CVD state, and we subdivided the ESRD state by previous CVD. Occurrence of CVD and ESRD in combination was considered together regardless of which event occurred first. Patient follow-up was split in intervals of 2 months' length, and the time scales age, diabetes duration, and time since DN onset were computed at the start of each interval. Transition rates between states were modeled using a Poisson model with natural splines for time scales (age, diabetes duration, DN duration) using log-length of the intervals as offset.[44] For the analysis of pre-ESRD death and ESRD, we included the following baseline covariates, as they have previously been associated with the following end points: systolic BP, GFR, UAER, hemoglobin, total cholesterol and smoking status (yes/no), BMI, and insulin/kg. Proportionality of rates was tested using likelihood-ratio tests; the most appropriate model was found to be the one with separate baseline intensities for rates of death and ESRD, and CVD as a time-dependent covariate with proportional effects along the time scales. We modeled the rate of CVD from DN using only age, diabetes duration, gender, and HbA_{1c}, and the mortality in ESRD patients using age, time since ESRD, and previous CVD occurrence.

We used a simulation method to compute the cumulative risks of death and ESRD (state occupancy probabilities) under this model for hypothetical patients who differed in their control of risk factors (Table 4). All calculations of rates and cumulative risk were performed by the Lexis machinery implemented in the Epi package (version 1.1.52)[44,45] for R (version 3.0.1).[46] Supplementary C online reports full account of rate analyses. The study was executed in accordance with ethical standards.

DISCLOSURE

PR has participated in advisory boards for Abbvie, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, MSD, and Novo Nordisk. H-HP has participated in advisory boards for Abbvie, Astra Zeneca, and Novartis. PR, H-HP, BC, MLJ, and GA own shares in Novo Nordisk. The other authors declared no competing interests.

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