Diabetologia
https://doi.org/10.1007/s00125-019-4812-6

ARTICLE

Effect of duration and burden of microvascular complications on mortality rate in type 1 diabetes: an observational clinical cohort study

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Received: 10 August 2018 / Accepted: 17 December 2018

Abstract

Aims/hypothesis The role of burden and duration of multiple microvascular complications on mortality rate has not been explored in detail in type 1 diabetes. Taking complication burden and time-updated duration into account we aimed to quantify mortality rate in individuals with and without microvascular complications.

Methods This observational clinical cohort included 3828 individuals with type 1 diabetes attending the Steno Diabetes Center Copenhagen in 2001–2013. We used information on mortality and detailed clinical measures of microvascular complications from electronic patient records. Poisson models were used to model mortality rates according to complication burden.

Results During 26,665 person-years of follow-up, 503 deaths occurred. Compared with individuals without microvascular complications, the mortality rate ratio was 2.20 (95% CI 1.79, 2.69) for individuals with diabetic kidney disease, 1.72 (95% CI 1.39, 2.12) for individuals with neuropathy and 1.02 (95% CI 0.77, 1.37) for individuals with retinopathy, all adjusted for calendar time (year/month/day), age, duration of diabetes, sex, HbA_{1c}, LDL-cholesterol, BMI, smoking status, systolic blood pressure, use of antihypertensive and lipid-lowering medication, and cardiovascular disease status. In individuals with two complications or more, the risk of mortality did not exceed the combined risk from each individual complication. Mortality rate ratios increased immediately after diagnosis of neuropathy and diabetic kidney disease. Mortality rate ratios were independent of the duration of neuropathy and retinopathy, while the mortality rate associated with diabetic kidney disease reached a stable level after approximately 3 years.

Conclusions/interpretation Neuropathy and diabetic kidney disease are strong and independent risk markers of mortality in type 1 diabetes, whereas no evidence of higher mortality rate was found for retinopathy. We found no indication that the mortality risk with multiple complications exceeds the risk conferred by each complication separately. The duration spent with microvascular complications had only a marginal effect on mortality.

Keywords Complication · Duration · Microvascular complications · Mortality · Type 1 diabetes

Abbreviations

CVD	Cardiovascular disease
EPR	Electronic patient record
ETDRS	Early Treatment Diabetic Retinopathy Study
UACR	Urinary albumin/creatinine ratio

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00125-019-4812-6) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Published online: 16 January 2019

Research in context

What is already known about this subject?

- Individuals with type 1 diabetes have an excess risk of mortality, which is partly explained by microvascular complications
- In assessing the impact of complications on mortality rate in type 1 diabetes, previous studies mainly considered one complication at a time and overlooked the effect of clustering of complications and duration spent with complications

What is the key question?

- How does the total microvascular complication burden and duration spent with microvascular complications affect mortality rate in type 1 diabetes?

What are the new findings?

- Individuals with type 1 diabetes and diabetic kidney disease had a 2.2-fold higher mortality rate than individuals with type 1 diabetes without complications, whilst individuals with type 1 diabetes and neuropathy had a 1.7-fold higher rate of mortality. No excess in mortality rate was observed in individuals with type 1 diabetes and retinopathy vs those with type 1 diabetes without complications
- Mortality risk in individuals with more than one diabetic complication consists of the combined risk estimates and is weakly dependent on duration since diagnosis of the microvascular complication

How might this impact on clinical practice in the foreseeable future?

- Our findings indicate that multi-state models including diabetic kidney disease, retinopathy and neuropathy and duration spent with these complications can give a clinically relevant estimate of all-cause mortality in individuals with type 1 diabetes

Introduction

The rate of mortality in individuals with type 1 diabetes continues to exceed that of the background population by 3- to 4-fold [1, 2], despite markedly improved clinical management, which has led to substantial declines in mortality rates and has reduced the gap in life expectancy between individuals with and without diabetes [3–5]. The excess mortality rate is primarily associated with prolonged exposure to hyperglycaemia [6, 7]. However, higher mortality rates are still seen in individuals with well-controlled type 1 diabetes compared with the background population [8, 9]. Microvascular complications are associated with the excess mortality risk in type 1 diabetes, especially diabetic kidney disease, the effects of which are well documented [5, 10]. Most studies on the impact of microvascular complications on mortality rate in type 1 diabetes that have been published to date have been limited to a single complication, without accounting for the great heterogeneity in the development of complications that individuals with type 1 diabetes experience throughout their life [11]. Importantly, we do not know how the total microvascular complication burden links with mortality in type 1 diabetes, or how duration of microvascular complications affects mortality risk.

Multi-state models have the advantage of providing detailed perspectives on the longitudinal development of multiple microvascular complications and their association with mortality in the same time window [12, 13]. This statistical method handles the movement of individuals through different disease states, while accounting for the accrued follow-up time in each state. Using this method, this study aimed to quantify rate of all-cause mortality in type 1 diabetes with or without microvascular complication(s) in a large clinical cohort. Specifically, we examined how all-cause mortality depends on the concurrent microvascular complication burden (complication burden at each time point in the observation window). Moreover, we assessed whether identified associations between microvascular complications and mortality rates in type 1 diabetes vary according to the duration of microvascular complications.

Methods

Data sources and study population This clinical cohort consisted of individuals with type 1 diabetes from the Steno Diabetes Center Copenhagen. Data was extracted from electronic patient records (EPR) and the Danish Register of Causes of Death [14]. These data sources were linked for the period 2001–2013 through personal identification numbers in the Danish Civil Registration System [15].

Type 1 diabetes was defined based on the epidemiological phenotype requirements as implemented in the Danish National Quality database: age under 30 years at diagnosis

and glucose management with insulin treatment at diagnosis. Individuals aged 30 years or above at diagnosis with randomly obtained non-fasting low C-peptide values (according to laboratory-specific reference values) or glutamic acid decarboxylase (GAD) 65 antibody positivity, both in combination with a need for insulin to control blood glucose concentrations, were also classified as having type 1 diabetes.

Study participants were included at the first date on which they had a clinical examination of diabetic kidney disease, retinopathy, and neuropathy registered in the EPR. If the date of the first registration for diabetic kidney disease, retinopathy and neuropathy differed, the latest screening date for any of the microvascular complications was used as the inclusion date. Included individuals were followed until emigration, the censoring date of 30 September 2013 (end of study period) or death. Individuals without an examination of any of the microvascular complications were excluded from the study.

The presented data collection was approved by the Danish Data Protection Agency (J. No.: 2007-58-0015 and 2012-58-0009). According to Danish law, anonymised analyses of databases do not require informed consent.

Study variables and definitions of complications The outcome of the present study was death from any cause. Information on date of birth, immigrant status (first- or second-generation immigrant), diagnosis of diabetes and microvascular complications was available in the EPR. We also obtained information on self-reported lifestyle habits (alcohol consumption, smoking and physical activity habits), BMI, estimated glomerular filtration rate (eGFR), blood pressure, and biochemistry laboratory information on HbA_{1c}, serum creatinine, HDL- and LDL-cholesterol, total cholesterol and triacylglycerol, at clinical visits. We also had information on prescription of antihypertensive medication and lipid-lowering medication.

Data on diabetic kidney disease, retinopathy and neuropathy was also obtained from the EPR. Diabetic kidney disease was assessed every 3–4 months. The definition of diabetic kidney disease included both eGFR under 60 ml min^{-1} [1.73 m]$^{-2}$ and urinary albumin/creatinine ratio (UACR) exceeding 3.5 mg/mmol in a spot urine or albumin excretion >30 mg/24 h in two urine specimens within the previous 12 months. Neuropathy was assessed annually with a biothesiometer and graded according to published age-specific thresholds [16] to classify any peripheral neuropathy defined by bilateral abnormal sensory modalities on the big toes. Retinal images were obtained annually or every other year (depending on previous status) and graded by a trained nurse or, if necessary, a medical specialist, according to the Early Treatment Diabetic Retinopathy Study (ETDRS) scale [17]. Retinopathy was defined as at least moderate non-proliferative changes in either eye.

Statistical analysis Clinical characteristics of individuals at entry are presented as medians (25th percentile, 75th percentile) for continuous variables or as frequencies and proportions for categorical variables.

The exact date of onset of microvascular events was unknown as microvascular status was assessed at clinical visits (i.e. at interval-censored transition dates). We used two different approaches to establish the most accurate transition date for microvascular complications. First, for individuals with a prior negative assessment, we used the first clinical assessment with abnormal measure as the transition date, even if the assessment date was prior to inclusion in the study. Second, for individuals who entered the study with complications (no prior negative assessment) we assumed that all individuals had no complications at diabetes diagnosis; for these individuals, transition dates were imputed solely using the distribution of diabetes duration from the onset of diabetes to the diagnosis of each specific newly diagnosed complication. We performed all analyses in 60 imputed datasets and summarised the obtained estimates using Rubin's rules [18]. The distribution of diabetes duration at diagnosis of each complication is shown in electronic supplementary material (ESM) Fig. 1.

Individuals were followed-up independent of complication state at entry. Complications were considered irreversible and we did not distinguish the order in which complications occurred. Individuals could change state (transition) during follow-up and thereby contribute follow-up time in different states (Fig. 1). As an example, an individual could start without any complications. If retinopathy was later diagnosed at one of the clinical visits, the individual would change to the 'retinopathy state' in our models. The time spent without complications was assigned as exposure time to the 'no complication state', while time spent with retinopathy was assigned to the 'retinopathy state'. If an individual died, the event was counted as an outcome for the exposure state of the individual at that point in time.

In the present study, we modelled occurrence rates; the likelihood function for this type of observation is (proportional to) a Poisson likelihood. Follow-up time was split into 6 month intervals and each interval was assigned the value of the covariates age, time since diagnosis of type 1 diabetes, time since complication onset, the response variables status at the end of the interval (censoring or event type) and length of the interval (risk time). The modelling of mortality rates was done by Poisson models using mortality as outcome and the log of the risk time as offset, modelling the effect of the timescales (age, diabetes duration and time since diagnosis of microvascular complications) as smooth functions using natural splines (and adjusting for baseline levels of other covariates) [12]. The logarithmic transformation was based on the natural logarithm ($\log_e$). We tested for interactions by sex. We considered three different model structures: structure I included

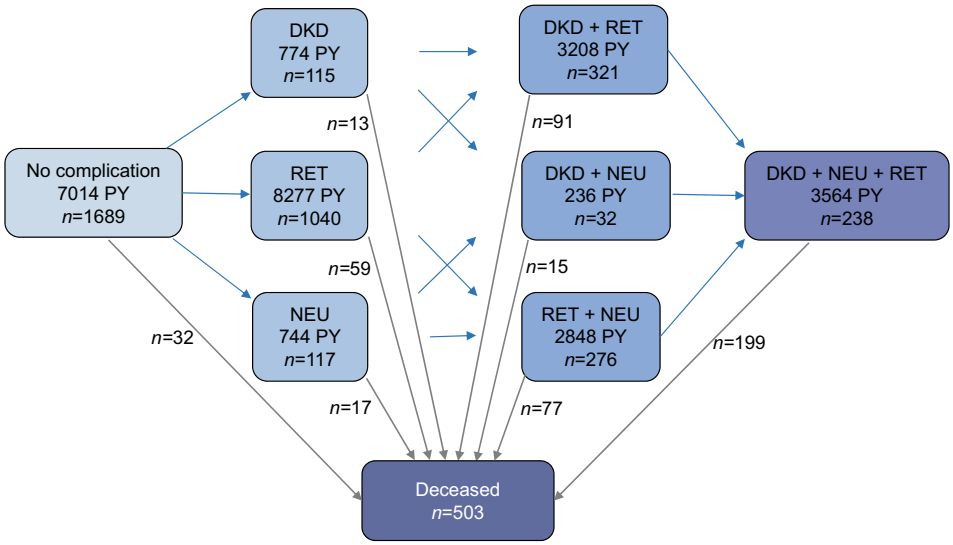

Fig. 1 Flow diagram showing number of participants in each complication state, person-years of follow-up and number of events (death) during follow-up. Each box shows a complication state with person-years of follow-up and number of participants who entered the study in that state. Individuals could enter the study in any state and could change status during the study according to concurrent burden. Therefore, during the study period, one individual could contribute follow-up time to a variety of states according to their individual history of complications. Complications were considered irreversible (the patient flow goes from left to right with evolving complication burden). Blue arrows, transitions from one complication state to another; grey arrows, death during follow-up at each state (*n* value for individuals who died in each state are given by grey arrows). DKD, diabetic kidney disease; PY, person-years; RET, retinopathy; NEU, neuropathy

only the number of microvascular complications (0–3 complications); structure II included main effects of complication type without interactions; and structure III used a separate variable for each of the eight possible complication states. Likelihood ratio tests were used to compare models and select the most parsimonious model structure for subsequent analysis.

Adjustments were made in three steps the first adjustment level included concurrent calendar time (year/month/day as a linear term), age and duration of diabetes as restricted cubic splines. It also included sex, and HbA_{1c} level at entry in the study (adjustment level 1). Furthermore, we adjusted for BMI, systolic blood pressure, LDL-cholesterol, use of antihypertensive medication, use of lipid-lowering medication and smoking status at entry in the study (adjustment level 2). Finally, the status of cardiovascular disease (CVD) at entry in the study was added in the ultimate adjustment (adjustment level 3).

Four representative patient profiles were constructed to illustrate how mortality rises according to diabetes duration, age and evolving microvascular complication burden, and duration since diagnosis of microvascular complications (Fig. 3).

We performed four separate sensitivity analyses; first, we replaced retinopathy defined as at least moderate non-proliferative changes with a definition using at least severe non-proliferative or proliferative retinal changes (grade 3 or higher on the ETDRS score). Second, we replaced the definition of diabetic kidney disease with UACR >35 mg/mmol on two occasions within a year and/or eGFR <60 mL min^{-1} $[1.73\ m]^{-2}$. Third, we replaced the definition of diabetic kidney disease with a definition solely based on UACR >3.5 mg/mmol on two occasions within a year. Fourth, we performed a sensitivity analysis that included only individuals without microvascular complications at entry ($n = 1689$).

Statistical analyses were performed in R, version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria; www.R-project.org) using the mitml package, the mitools package, and the Epi package for the definition of the multi-state model [12, 19].

Results

Participant characteristics The total population comprised 5031 individuals with type 1 diabetes. Individuals without assessment of diabetic kidney disease, neuropathy or retinopathy were excluded, leaving 3828 individuals for analysis with a total follow-up time of 26,665 person-years. ESM Table 1 shows descriptive characteristics of included and excluded individuals at first assessment in the study period. The age- and sex-adjusted morality rates in included and excluded individuals are similar (data not shown, but as an example, the mortality rate per 1000 person-years for an excluded 50-year-old woman was 14 (95% CI 12, 17), whilst for an included woman of the same age it was 12 (95% CI 11, 14); $p = 0.08$).

Table 1 shows descriptive characteristics of individuals included in the study by prevalence of any complications at

Table 1 Baseline characteristics of the Steno cohort by complication status at entry into the study

Variables	No complications	Any complication(s)
N	1689	2139
Sex (male)	871 (51.6)	1187 (55.5)
Age (years)	38.9 (27.4, 51.6)	49.8 (39.0, 59.9)
Age at diagnosis (years)	25.0 (15.0, 38.0)	21.0 (12.0, 34.0)
HbA_{1c} (mmol/mol)	63 (55, 73)	69 (61, 78)
HbA_{1c} (%)	7.9 (7.2, 8.8)	8.5 (7.7, 9.3)
Total cholesterol (mmol/l)	4.7 (4.1, 5.3)	4.9 (4.4, 5.6)
LDL (mmol/l)	2.5 (2.0, 3.1)	2.7 (2.2, 3.2)
HDL (mmol/l)	1.6 (1.3, 1.9)	1.6 (1.3, 2.0)
eGFR (ml min^{-1} [1.73 $m]^{-2}$)	102 (89, 116)	90 (73, 103)
Systolic blood pressure (mmHg)	128 (118, 138)	136 (124, 150)
Diastolic blood pressure (mmHg)	78 (71, 84)	79 (71, 85)
BMI (kg/m^2)	24.2 (22.2, 26.4)	24.6 (22.5, 27.0)
Insulin dose (U day^{-1} kg^{-1})	0.6 (0.5, 0.8)	0.6 (0.5, 0.7)
Antihypertensive medication	294 (17.4)	1189 (55.6)
ACE inhibitor	167 (9.9)	770 (36.0)
ARB	66 (3.9)	257 (12.0)
β-blocker	26 (1.5)	124 (5.8)
Calcium channel antagonists	79 (4.7)	336 (15.7)
Anti-diuretic medication	128 (7.6)	663 (31.0)
Statins	201 (11.9)	454 (21.2)
Insulin pump	80 (4.7)	80 (3.7)
CVD	88 (5.2)	446 (20.9)
Immigrant[a]	118 (7.0)	118 (5.5)
Regular alcohol consumption	1477 (88.1)	1917 (90.7)
Regular exercise	1186 (70.7)	1355 (64.2)
Ever smoker	933 (55.5)	1492 (70.5)
Complication burden[b]		
DKD	–	706 (33.0)
Neuropathy	–	663 (31.0)
Retinopathy	–	1875 (87.7)
Neuropathy duration (years)[c]	–	4.1 (1.9, 7.5)
Retinopathy duration (years)[c]	–	5.6 (2.9, 9.4)
DKD duration (years)[c]	–	4.1 (1.7, 7.7)

Data are medians (25th percentile, 75th percentile) or *n* (%)

[a] Defined as first- or second-generation immigrant

[b] The sum does not add up to 100% as some individuals may have more than one complication at entry

[c] Data from 60 imputed datasets

ARB, angiotensin II receptor blocker; DKD, diabetic kidney disease

entry into the study. Individuals with any complications at entry were older ($p < 0.001$) and diagnosed with diabetes earlier in life ($p < 0.001$) than individuals without complications. A larger fraction of those with complications were male ($p = 0.017$) and had an unhealthier lifestyle (a higher proportion smoked and was physically inactive; $p < 0.001$ for both). Furthermore, a higher proportion of those with microvascular complications at entry also had CVD ($p < 0.001$) and were being prescribed antihypertensive drugs ($p < 0.001$) and/or lipid-lowering medication (statins; $p < 0.001$).

The observed median screening interval (25th percentile, 75th percentile) was 120 days (84, 214) for diabetic kidney disease, 388 days (217, 618) for retinopathy and 433 days (337, 567) for neuropathy. An overview of follow-up time and number of deceased individuals in each complication state, along with the number of individuals in each

Table 2 Mortality rates and rate ratios for all-cause mortality according to concurrent microvascular complication burden

Complication state	Person-years of follow-up	Deceased (*n*)	Crude mortality rate per 1000 person-years (95% CI)	Mortality rate ratio (95% CI)[a]		
				Adjustment level 1	Adjustment level 2	Adjustment level 3
No complications	7014	32	4.56 (3.23, 6.45)	REF	REF	REF
DKD	774	13	16.79 (9.75, 28.92)	2.52 (2.07, 3.05)	2.17 (1.77, 2.67)	2.20 (1.79, 2.69)
Retinopathy	8277	59	7.13 (5.52, 9.20)	1.10 (0.83, 1.44)	1.06 (0.79, 1.42)	1.02 (0.77, 1.37)
Neuropathy	744	17	22.86 (14.21, 36.76)	1.74 (1.42, 2.12)	1.82 (1.47, 2.24)	1.72 (1.39, 2.12)
DKD and retinopathy	3208	91	28.37 (23.10, 34.84)	2.76 (2.01, 3.78)	2.30 (1.65, 3.22)	2.25 (1.61, 3.14)
DKD and neuropathy	236	15	63.69 (38.40, 105.65)	4.37 (3.38, 5.63)	3.94 (3.00, 5.17)	3.77 (2.88, 4.95)
Retinopathy and neuropathy	2848	77	27.04 (21.63, 33.80)	1.90 (1.38, 2.62)	1.92 (1.37, 2.70)	1.76 (1.25, 2.47)
DKD, neuropathy and retinopathy	3564	199	55.83 (48.59, 64.15)	4.79 (3.41, 6.72)	4.18 (2.91, 6.01)	3.86 (2.68, 5.56)

Person-years and events (deaths) are pooled medians from 60 datasets, while mortality rates and mortality ratios are pooled from all 60 datasets using Rubin's rules

[a] Estimated from model structure II (includes separate effects from each complication type without interactions)

Adjustment level 1: Adjusted for calendar time (linear effect), age (spline model with four parameters), duration of diabetes (spline model with three parameters), sex and HbA_{1c} at first assessment in the study period

Adjustment level 2: model 1+BMI, smoking status, systolic blood pressure, LDL-cholesterol and antihypertensive medication and lipid-lowering medication at first assessment in the study period

Adjustment level 3: model 2+CVD status at first assessment in the study period

Estimates for multiple complications are extracted from the model by multiplication of the effect from each complication separately. Logarithmic transformation is based on the natural logarithm ($\log_e$). Calculations are based on exact numbers, resulting in minor rounding errors

DKD, diabetic kidney disease

complication state at study commencement, is shown in Fig. 1. Follow-up time in complication states ranged from 236 to 8277 person-years. The number of deaths ranged from 13 to 199. In total, 706 individuals had diabetic kidney disease (with or without another complication) at inclusion and 546 individuals developed diabetic kidney disease during follow-up; 1875 individuals had retinopathy (with or without another complication) at inclusion and 723 individuals developed retinopathy during follow-up; and 663 individuals had neuropathy (with or without another complication) at inclusion and a further 523 individuals developed neuropathy during follow-up (Table 1 and Fig. 1).

Mortality rate and mortality rate ratio from multi-state models Model structure evaluation showed that model structure II (including each complication type separately as main effects without interactions) was clearly superior to model structure I (including only the number of microvascular complications) ($p < 0.001$), but it was not inferior to model structure III (separate parameters for each of the eight states) ($p = 0.247$). Furthermore, mortality rate ratios from model structure II and model structure III were highly comparable (data not shown). Hence, further analyses were based on model structure II. We found no evidence for interaction between sex and complication status on mortality ($p = 0.46$). Mortality rates by sex at 53 years (the mean age at the end of follow-up) for each complication state are presented in ESM Table 2.

Table 2 presents the crude mortality rate and mortality rate ratios from model structure II with different levels of adjustment. A total number of 503 deaths occurred during the follow-up period. Among them, 318 had diabetic kidney disease (defined as UACR >3.5 mg/mmol and/or eGFR <60 ml min^{-1} [1.73 m]$^{-2}$), 426 had retinopathy (defined as at least moderate non-proliferative changes in the retina) and 308 had neuropathy (above age-specific threshold). At adjustment level 1, adjusting for calendar time (year/month/day), age, duration of diabetes, sex and HbA_{1c}, we saw a clear association between both diabetic kidney disease and neuropathy and mortality, but no effect of retinopathy was seen. Further adjustment for well-known modifiable risk factors (adjustment level 2) did not greatly change the associations, nor did additional adjustment for CVD status (adjustment level 3). At adjustment level 3, the mortality rate ratio for individuals with diabetic kidney disease was 2.20 (95% CI 1.79, 2.69), whilst for neuropathy it was 1.72 (95% CI: 1.39, 2.12) and for retinopathy it was 1.02 (95% CI: 0.77, 1.37), relative to individuals without microvascular complications. Mortality rate ratios for individuals with more than one complication compared with individuals without any microvascular complications was extracted from the model by multiplication of the effect from each complication separately (Table 2). As an

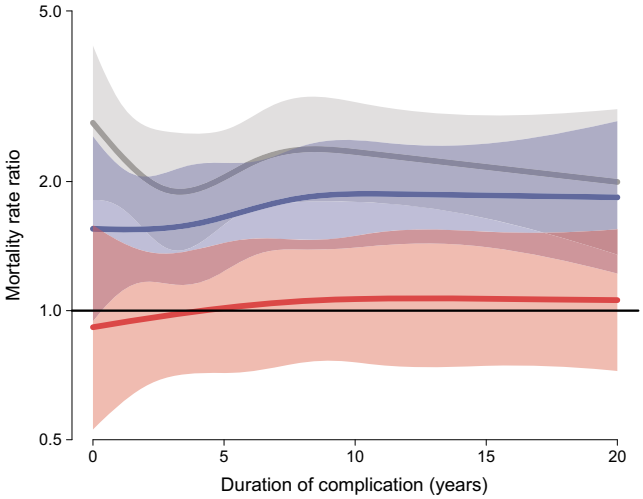

Fig. 2 Mortality rate ratios for individuals with one microvascular complication compared with individuals without microvascular complications as a function of duration of complications. Pooled estimates by Rubin's rules from 60 datasets with imputed event times. Estimates are from the fully adjusted model (structure II, adjustment level 3), adjusted for calendar time (linear effect), age (spline model with four parameters), duration of diabetes (spline model with three parameters), sex, HbA_{1c}, BMI, smoking status, LDL-cholesterol, systolic blood pressure, use of antihypertensive medication, use lipid-lowering medication and CVD status at first assessment in the study period. The *y*-axis is on a log scale ($\log_e$). Grey line, diabetic kidney disease; blue line, neuropathy; red line, retinopathy. Shaded areas indicate 95% CIs

example, individuals with all three microvascular complications had a mortality rate ratio of 3.86 (95% CI: 2.68, 5.56) at adjustment level 3 ($2.20 \times 1.72 \times 1.02 = 3.86$); this was an effect driven by diabetic kidney disease and neuropathy.

We extended the simple fixed-time effect model in order to explore the effect of duration of diabetes, current age and duration of microvascular complications on mortality rate. ESM Fig. 2 shows the absolute mortality rates for individuals without complications as a function of age and diabetes duration, modelled at rates for the year 2012 for individuals diagnosed at different ages (20, 30 and 40 years), stratified by sex. The mortality rate roughly increased exponentially with age and diabetes duration. Figure 2 shows the mortality rate ratios in individuals with diabetic kidney disease, neuropathy or retinopathy compared with individuals without any microvascular complications, as a function of duration of the respective complication. Retinopathy did not have an effect on mortality rate. In contrast, the mortality rate ratio for neuropathy increased immediately after diagnosis and then stabilised at approximately 1.7-fold higher than the rate in individuals without complications. Diabetic kidney disease also immediately raised the mortality rate by a factor of 2.8 compared with those without complications. However, during the first 3 years after diagnosis of diabetic kidney disease, mortality rate ratio decreased, stabilising at ~2.2-fold higher than individuals without complications.

To illustrate the effects of age, microvascular complication burden, duration of diabetes and duration of complications on mortality, estimated mortality rates per 1000 person-years for four hypothetical complication scenarios are presented in Fig. 3. Mortality rates were shown to increase exponentially with age and duration of diabetes in individuals without complications. However, when an individual was diagnosed with diabetic kidney disease or neuropathy, their mortality rate was elevated to a higher level compared with individuals who remained complication free (Fig. 3a, b). The higher mortality rate continued to increase as a nearly constant exponential function of age until the next complication developed, after which point the mortality rate was elevated to an even higher level (Fig. 3c, d).

Sensitivity analysis Using a stricter definition of diabetic kidney disease (UACR >35 mg/mmol on two occasions within a year and/or eGFR <60 ml min^{-1} [1.73 m]$^{-2}$), the mortality rate ratio was 2.39 (95% CI 1.92, 3.00) for individuals with type 1 diabetes and diabetic kidney disease compared with individuals with type 1 diabetes without complications. Excluding eGFR in the definition of diabetic kidney disease resulted in marginally fewer people being considered as having diabetic kidney disease; nonetheless, the conclusions from this analysis did not differ from the conclusions of our main analysis, indicating that our results are highly robust. A stricter definition of retinopathy, which included only severe non-proliferative or proliferative retinal changes, had only a marginal impact on our estimates. Restricting analyses to individuals without any microvascular complications at entry ($n = 1689$) yielded effectively the same estimated effects but with wider CIs. Compared with individuals without any complications, mortality rate ratios of 2.27 (95% CI 1.37, 3.75) for diabetic kidney disease, 1.04 (95% CI 0.63, 1.70) for retinopathy and 1.61 (95% CI 0.92, 2.82) for neuropathy were observed. The mortality rate as a function of duration of microvascular complications was highly robust (data not shown).

Discussion

This study shows that all-cause mortality in individuals with type 1 diabetes varies according to their evolving microvascular complication burden. Compared with individuals without complications, individuals with neuropathy had a 1.7-fold higher mortality rate and individuals with diabetic kidney disease had a 2.2-fold higher mortality. We found no evidence of an association between retinopathy and mortality rate in type 1 diabetes. We are able to show that the increased mortality risk in individuals with type 1 diabetes certainly depends on the age of the individual and the duration of diabetes, while it only marginally depends on the duration of each of the diabetes-

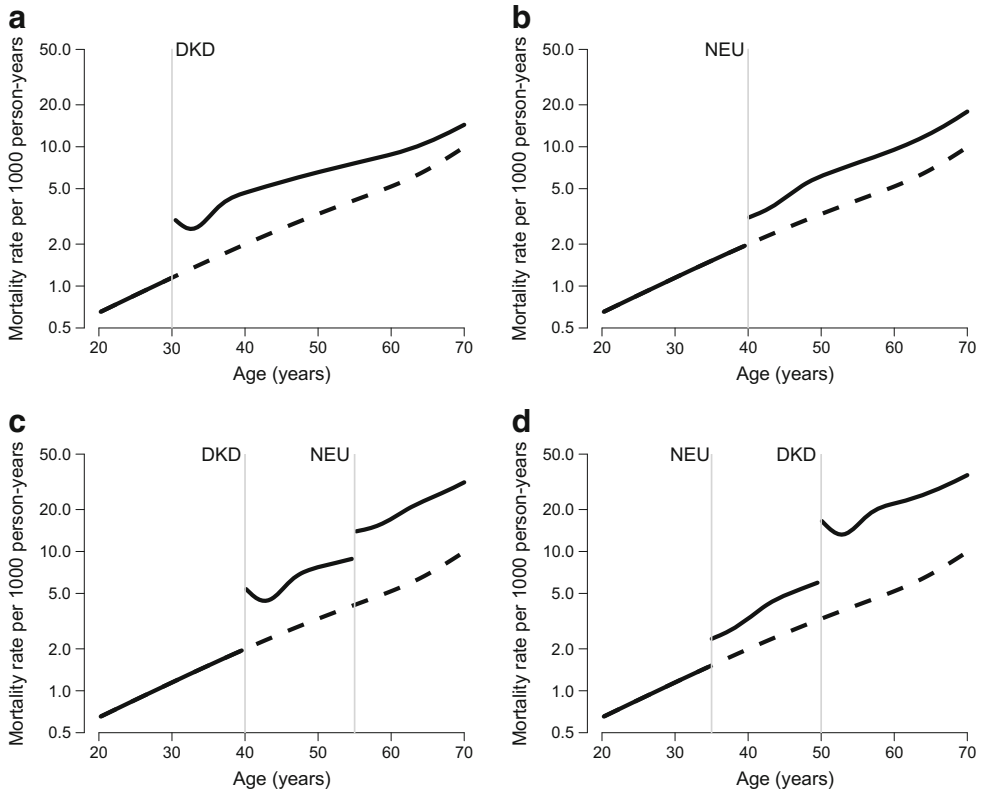

Fig. 3 Hypothetical examples of mortality rates with different complication burdens. Four modelled examples of how mortality rate (per 1000 person-years) changes with age and sequence of microvascular complications (modelled at rates for the year 2012). (**a**) Diabetic kidney disease diagnosed at age 30. (**b**) Neuropathy diagnosed at age 40. (**c**) Diabetic kidney disease diagnosed at age 40 and neuropathy diagnosed at age 55. (**d**) Neuropathy diagnosed at age 35 and diabetic kidney disease diagnosed at age 50. Solid line, fictitious patient profile; dashed line, profile of patient with the same specifications as shown with solid line but with no complications during follow-up. Mortality rates are estimated for a hypothetical individual who was male, non-smoking, diagnosed with diabetes at age 20, without CVD, not using antihypertensive medication or lipid-lowering medication at any time during follow-up, and who had an HbA_{1c} value of 57 mmol/mol (7.4%), a BMI of 25 kg/m^2, LDL-cholesterol of 2.5 mmol/l and systolic blood pressure of 130 mmHg. Data are pooled estimates using Rubin's rules from 60 datasets with imputed event times; estimates are derived from the fully adjusted model (structure II, adjustment level 3). The *y*-axis is on a log scale ($\log_e$). DKD, diabetic kidney disease; NEU, neuropathy

related microvascular complications. The mortality risk in individuals with more than one complication is simply the product of the risk estimates from each separate complication; no evidence was found of additional risk from having more than one complication.

Large clinical trials have shown that intensive control of blood glucose lowers mortality risk in individuals with type 1 diabetes [6, 7, 20]. However, even with glycaemic levels near those recommended, an increased mortality risk remains [8, 9]. Our study is unique in that we assessed both the separate risk and the combined mortality risk associated with all three microvascular complications, while taking duration since diagnosis of complications into account. Although we did not find evidence for a statistical interaction between the microvascular complications, this does not mean that a biological interaction does not exist.

The excess mortality risk in individuals with diabetic kidney disease, both with short and long duration of diabetes, has been long established [5, 7, 10, 21–23]. Mortality rates in individuals with diabetic kidney disease and type 1 diabetes in our study was slightly lower than previously reported, but it was still comparable with the previously published rates. There may be several explanations for the lower mortality rate with diabetic kidney disease observed in our study, including differences in the composition of the population and also recent improvements in available treatments and quality of care; our study was conducted in a clinical setting and all individuals were offered highly specialised and standardised care. Moreover, mortality rates have been shown to be on a general downward trend over recent years [3–5] and studies conducted in the past were likely to find a stronger association between diabetic kidney disease and risk of death in type 1 diabetes. Based on the sensitivity analyses, the choice of diagnostic criteria for diabetic kidney disease that was used in our study does not seem to explain the lower mortality rate ratios observed with this complication, as compared with findings from other studies. Our results emphasise diabetic kidney disease as a clear and strong risk factor for mortality in type 1 diabetes. The association was found to be slightly stronger immediately after diagnosis but stabilised after 3 years.

We showed a clear association between diabetic neuropathy and mortality in type 1 diabetes. Few other studies have reported on mortality rates in individuals with type 1 diabetes and peripheral neuropathy [10, 20] and those that do show discrepant results. The explanation for these differences remains elusive, but it could be related to the diversity in the criteria for the definition of neuropathy. First, clinical trials often include symptoms in the definition of neuropathy [10, 24], whereas our criterion is solely based on an abnormal biothesiometry measure, which may classify more individuals as having neuropathy. However, despite our more inclusive definition, the impact of this complication on mortality rates in type 1 diabetes is clear. Second, glycaemic control, a strong determinant of both neuropathy and mortality, was suboptimal in our cohort. We adjusted for a wide range of potential confounders but we cannot exclude the possibility of residual confounding. Our results suggest that neuropathy should be considered a clear risk factor for mortality in type 1 diabetes, and that the mortality risk increases immediately after diagnosis.

Retinopathy did not appear to be associated with mortality risk in type 1 diabetes. Based on the literature, we would have expected to find a higher mortality rate in the individuals with type 1 diabetes and retinopathy compared with individuals without this complication [25–27]. In a previous study, we found retinopathy to be predictive of the occurrence of diabetic kidney disease and neuropathy in type 1 diabetes [28]. Since previous studies in this field did not examine multiple complications, many of their participants with retinopathy will also have had neuropathy and/or diabetic kidney disease. Although this approach is valid, the effects of the different complications are likely to have been combined under the retinopathy label and, thus, may have led to inaccurate conclusions. Our multi-state analytical design allows for more thorough separation of the effects of different complications. However, it should be noted that, in our study individuals with any given complication could have subclinical levels of other complications. Other studies have found an association between proliferative retinopathy and mortality risk [29, 30]. An even stricter definition of retinopathy might have enabled us to support these findings; however, such detailed information was not available in the dataset at hand.

Adjustment by calendar time, age, duration of diabetes, sex and HbA_{1c} reduced all mortality rate ratios compared with the crude mortality rate ratios (adjustment level 1). There was little effect following adjustment for well-known modifiable cardiovascular risk factors (adjustment level 2). As CVD was expected to be a strong confounder, adjustment for CVD at baseline (adjustment level 3) was expected to weaken the association between the microvascular complications and mortality; however, that was not the case.

Of all retinopathy cases, 27.8% were detected during follow-up, whilst of all neuropathy cases, 44.1% were detected during follow-up and of all diabetic kidney disease cases, 43.6% were detected during follow-up. Since the majority of retinopathy cases were present at baseline, while the other complications were more likely to develop during follow-up, it was important to consider the possibility that CVD adjustment had a stronger impact on the mortality risk linked to retinopathy. However, this seems not be the case, as the effect of retinopathy on mortality rate was virtually unchanged in models with or without CVD adjustment.

The cohort used is clinic-based and all events were properly accounted for because of highly structured protocols for regular clinical examinations. Moreover, all deaths in our study population were recorded in the Danish registration system. This implies that the follow-up data for all complication states and outcome (death) was practically complete. The duration effect was less precisely estimated in individuals with complications at entry in the study. We imputed 60 datasets to optimise our estimates for the effects of complication duration on mortality rates. This approach ensured maximal use of the available observation time. The sensitivity analysis including only individuals without complications showed that this approach introduced very limited bias but increased the power of the study. A major advantage of the Poisson models with smooth parametric effects is that we could adequately deal with multiple-related time scales, such as age, calendar time, duration of diabetes and duration spent with complication(s). It is normal practice for individuals with type 1 diabetes in Denmark to attend a tertiary care centre, which limits the risk of referral bias in our study. Our results are generalisable and highly relevant to other countries with similar healthcare systems.

Our study focuses on all-cause mortality rather than on cause-specific mortality. We chose this approach because the excess mortality seen in type 1 diabetes is not solely explained by higher mortality from CVD [31] or acute complications (i.e. ketoacidosis or hypoglycaemia). Furthermore, routine certification of causes of death is subject to heterogeneous sources of error [32], which do not affect all-cause mortality. Microvascular complications might lead to increased risk of CVD, which in turn could mediate the higher mortality rate associated with microvascular complications. In the current study, we adjusted only for baseline status of CVD. However, the role of CVD on mortality rate in type 1 diabetes during follow-up should be further investigated in future studies.

Excluded individuals were younger ($p < 0.001$) and had shorter duration of diabetes ($p = 0.007$). Even though clinicians at the Steno Diabetes Center work according to clinical protocols, we have to consider the possibility that clinicians may tend to send this group of individuals for microvascular screening less frequently. If this were the case, the resulting selection could lead to bias in that excluded individuals would have a lower absolute mortality

rate as they are characterised by younger age and shorter diabetes duration compared with included individuals, and they would also have a lower complication burden. However, when we compared the mortality rates, only a minor non-significant difference between excluded and included individuals was observed (mortality rate ratio for excluded individuals compared with included individuals was 1.1; $p = 0.08$), indicating that the age-dependent selection process is unlikely to constitute a major source of bias.

Conclusion

We found no indication that individuals with type 1 diabetes and multiple complications have a mortality risk exceeding the risk conferred by each complication separately. Thus, mortality rates in individuals with more than one diabetic complication consists of the risk estimates from all separate complications combined. Neuropathy and diabetic kidney disease were found to be independent risk factors for mortality in individuals with type 1 diabetes, whereas we found no excess risk of mortality associated with retinopathy. Exploring the impact of the duration since diagnosis of complications adds new and valuable knowledge to the field. Although mortality rates are highly dependent on the age of the individual and diabetes duration, we showed that the duration of microvascular complications had only a marginal effect on mortality rate ratios, especially after a few years from diagnosis of these complications. Since our study shows that attention to the evolving and heterogeneous microvascular complication burden provides a more detailed perspective on the risk of mortality in type 1 diabetes, this knowledge could facilitate implementation of better integrated risk models in daily clinical practice.

Acknowledgements L. Niedziella (Research Unit for General Practice, Aarhus, Denmark) is thanked for proofreading the manuscript.

Data availability Data are available from the senior author (MEJ) on request.

Funding LB is funded by an unrestricted grant from the Innovation Fund Denmark. LB, AH and DRW are funded by an unrestricted grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation. The study sponsors were not involved in the writing of the report or the decision to submit the manuscript for publication. The study sponsors were not involved in the design of the study; the collection, analysis and interpretation of data; writing the report; or the decision to submit the report for publication.

Duality of interest LB, MC and AH declare no duality of interest relevant to their contribution to this work. DRW, BC and MEJ own shares in Novo Nordisk A/S. MEJ has received research funding from AstraZeneca and Amgen AB.

Contribution statement LB, MEJ and DRW designed the study and wrote the manuscript. MC designed the study and reviewed and edited the manuscript. LB and AH analysed the data and reviewed and edited the manuscript. BC contributed software solution for multi-state analysis and reviewed and edited the manuscript. LB is the guarantor of this work and confirms full access to all the data and had the final responsibility for data integrity, accuracy of data analysis and decision to submit for publication. All authors have approved the final version of this manuscript.

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