Title: Presence of retinopathy and incident kidney and cardiovascular events in type 2 diabetes with normoalbuminuria – a post-hoc analysis of the PRIORITY randomized clinical trial

Authors: Viktor Rotbain Curovic[1], MD, Nete Tofte[1], PhD, Morten Lindhardt[1,2,3], PhD, Katarina Adamova[4], MD, Stephan J.L. Bakker[5], Prof., Joachim Beige[6], Prof., Joline W.J. Beulens [7, 8, 9,10], Prof., PhD, Andreas L. Birkenfeld[11], MD, Gemma Currie[12], PhD, Christian Delles[12], MD, Prof., Ingo Dimos[13], MD, Lidmila Francová[14], MD, Marie Frimodt-Møller[1], PhD, Peter Girman[15], MD, Rüdiger Göke[16], MD, Tine W. Hansen[1], PhD, Tereza Havrdova[15], MD, Adriaan Kooy[17], MD, Gozewijnw D. Laverman[18], Prof., Harald Mischak[19], Prof., Gerjan Navis[5], Prof., Giel Nijpels[20], Prof., Marina Noutsou[21], MD, Alberto Ortiz[22], MD, Aneliya Parvanova[23], PhD, Frederik Persson[1], DMSc, John R. Petrie[24], Prof., Piero L. Ruggenenti[23], PHD Femke Rutters[7, 9], MD, Ivan Rychlík[14], Prof., Justyna Siwy[19], PhD, Goce Spasovski[25], MD, Marijn Speeckaert[26], Prof., Matias Trillini[23], MD, Petra Zürbig[19], PhD, Heiko von der Leyen[27], Prof., Peter Rossing[1,3], Prof., on the behalf of the PRIORITY Study Group

Affiliations:

[1]Steno Diabetes Center Copenhagen, Herlev, Denmark
[2]Department of Medicine, Copenhagen University Hospital – Holbæk, Holbæk, Denmark
[3]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[4]University Clinic of Endocrinology, Diabetes and Metabolic Disorders, Skopje, Northern Macedonia
[5]Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[6]Division of Nephrology and KfH Renal Unit, Hospital St Georg, Leipzig, Germany; Martin-Luther University Halle, Wittenberg, Germany
[7]Amsterdam UMC, location Vrije Universiteit Amsterdam, Department of Epidemiology and Data Science, Amsterdam, The Netherlands
[8]Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands
[9]Amsterdam Public Health, Amsterdam, The Netherlands
[10]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[11]Department of Internal Medicine IV, Division of Endocrinology, Diabetology, and Nephrology, University Hospital Tübingen, Tübingen, Germany
[12]School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, UK.
[13]Diabetespraxis, Leipzig, Germany.
[14]Department of Internal Medicine, Charles University, Third Faculty of Medicine, Prague, and Faculty Hospital Královské Vinohrady, Prague, Czech Republic.
[15]Diabetes Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

[16]Diabetologische Schwerpunktpraxis, Diabetologen Hessen, Marburg, Germany.
[17]Bethesda Diabetes Research Center, Hoogeveen, Netherlands
[18]Department of Internal Medicine/Nephrology, Ziekenhuisgroep Twente Hospital, Almelo, Netherlands.
[19]Mosaiques Diagnostics GmbH, Hannover, Germany.
[20]Department General Practice and Elderly Care, Amsterdam, Netherlands.
[21]Diabetes Center, 2nd Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Hippokratio General Hospital, Athens, Greece.
[22]Instituto de Investigacion Sanitaria de la Fundacion Jiménez Díaz UAM, Madrid, Spain.
[23]Department of Renal Medicine, Clinical Research Centre for Rare Diseases "Aldo e Cele Daccò": Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Ranica, Bergamo, Italy.
[24]School of Health and Wellbeing, University of Glasgow, Glasgow, UK
[25]Department of Nephrology, Cyril and Methodius University in Skopje, Skopje, North Macedonia.
[26]Department of Nephrology, Ghent University Hospital, Ghent, Belgium.
[27]Orgenesis Germany GmbH, Munich, Germany

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Corresponding author:

Viktor Rotbain Curovic

Steno Diabetes Center Copenhagen, Borgmester Ib Juuls Vej 83, 2730 Herlev, Denmark

+4531344451; viktor.rotbain.curovic@regionh.dk

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Abstract

Aims: Baseline diabetic retinopathy (DR) and risk of development of microalbuminuria, kidney function decline, and cardiovascular events (CVEs) in type 2 diabetes.

Methods: Post-hoc analysis of the PRIORITY study including 1758 persons with type 2 diabetes and normoalbuminuria followed for a median of 2.5 (IQR: 2.0-3.0) years. DR diagnosis included non-proliferative and proliferative abnormalities, macular oedema, or prior laser treatment. Cox models were fitted to investigate baseline DR presence with development of persistent microalbuminuria (urinary albumin-creatinine ratio >30mg/g); chronic kidney disease (CKD) G3 (eGFR <60 mL/min/1.73m^2); and CVE. Models were adjusted for relevant risk factors.

Results: At baseline, 304 (17.3%) had DR. Compared to persons without DR, they were older (mean ±SD: 62.7±7.7 vs 61.4±8.3 years, p=0.019), had longer diabetes duration (17.9±8.4 vs. 10.6±7.0 years, p<0.001), and higher HbA_{1c} (62±13 vs. 56±12 mmol/mol, p<0.001). The adjusted hazard ratios of DR at baseline for development of microalbuminuria (n=197), CKD (n=166), and CVE (n=64) were: 1.50 (95%CI: 1.07, 2.11), 0.87 (95%CI: 0.56, 1.34), and 2.61 (95%CI: 1.44, 4.72), compared to without DR.

Conclusions: Presence of DR in normoalbuminuric type 2 diabetes was associated with an increased risk of developing microalbuminuria and CVE, but not with kidney function decline.

Keywords

Type 2 diabetes; diabetic retinopathy; chronic kidney disease; albuminuria; cardiovascular disease; risk stratification

Background

One of the mains concern for a person diagnosed with type 2 diabetes is the considerable risk of developing debilitating and potentially fatal diabetic complications over time (1). Improvements in pharmacological agents, multifactorial treatment, and diabetes care have radically progressed, reducing the incidence of diabetes related complications (2). However, despite this success a residual risk remains. While increased blood glucose is the main driving factor for the development of diabetes complications in type 1 and type 2 diabetes alike, the interplay between the various complications is still not fully understood. Diabetic retinopathy (DR), a microvascular complication leading to proliferation in retinal vessels, leaky vessels with oedema formation, and potentially blindness if left untreated, is one of the most feared complications for an individual with diabetes and a leading cause of blindness in adults in developed countries (3). Another microvascular diabetes complication, chronic kidney disease (CKD), is the single largest cause of kidney failure and dialysis in developed countries (4). Furthermore, cardiovascular events (CVE) are frequent macrovascular complications and the leading cause of death in type 2 diabetes (5).

DR and CKD in diabetes specifically affects microvascular tissue and are commonly present in long-term diabetes, to the extent that they have been considered as different manifestations of the same vascular complication (6, 7). In type 1 diabetes, DR almost invariably precedes CKD, a pattern that can also be found for type 2 diabetes, albeit to a lower degree (8). While an association between DR and CKD has been shown in prospective studies, only a few of them have investigated the association between DR and the development of CKD in otherwise uncomplicated diabetes, none in normoalbuminuric type 2 diabetes, and none simultaneously investigating the association between DR and CVE (9).

Recent studies have suggested that DR and CKD in diabetes might lack a common molecular interplay and physiology (10, 11). Therefore, we have investigated their relationship, utilizing data from the PRIORITY study; a prospective multi-center observational study with an embedded randomized clinical trial, including 1775 individuals with type 2 diabetes and normoalbuminuria. The study demonstrated that the urinary proteomic classifier – CKD273 – predicts onset of microalbuminuria and CKD in type 2 diabetes (12). In the present study we aimed to evaluate whether, and to which extent, the presence of DR at baseline is associated with the onset of microalbuminuria, kidney function decline, and development of CVE in individuals with type 2 diabetes and normoalbuminuria.

Methods

Study design and participants

The details of the PRIORITY study design and population have been previously described (12, 13). In short, individuals aged 18-75 with type 2 diabetes, preserved kidney function, and normoalbuminuria were recruited from 15 highly specialized diabetes medical centers in 10 European countries. The clinical study protocol and informed consent documents were reviewed and approved by the respective local independent ethics committees and competent authorities, respectively. Every patient gave written informed consent prior to the conduct of any study-related procedures. This study was registered (EudraCT 20120-004523-4; ClinicalTrials.gov NCT02040441) and is completed. The main inclusion criteria were urinary albumin-creatinine ratio (UACR) <30 mg/g of at least two out of three consecutive first-morning void urine samples and an estimated glomerular filtration rate (eGFR) >45 mL/min/1.73m^2. Historical UACR status was not referred. The main exclusion criteria were treatment with dual renin-angiotensin-aldosterone system blockade or mineralocorticoid receptor antagonist, or heart failure requiring treatment with a mineralocorticoid receptor antagonist. The complete list of inclusion and exclusion criteria can be found in Supplementary Table 1. In the original study, all participants were stratified by low- (≤0.154) or high-risk (>0.154) urinary proteomic classifier (CKD273) status. The classifier is based on 273 peptides differentially present in urine in people with vs without CKD (14). High-risk participants were included in a nested randomized clinical trial and subsequently randomized to receive daily spironolactone 25 mg or placebo. Low-risk participants were seen once yearly, and high-risk participants once quarterly for the study duration of median 2.5 (IQR: 2.0-3.0) years. At all visits, local blood and urine sampling was performed and vital parameters were measured, and adverse events were recorded by the interviewing investigator using subject-reported information and electronic medical records. The primary outcome was the development of persistent

microalbuminuria (moderately increased albuminuria: UACR>30 mg/g in two out of three consecutive measurements.

The present study aimed to assess baseline DR status with: 1) the primary outcome – development of confirmed microalbuminuria (UACR>30 mg/g) in at least two of three first morning voids with a 30% increase (geometric mean) in UACR from 'run-in-phase' samples, or >40 mg/g (geometric mean), 2) development of CKD grade 3 (G3), defined as eGFR <60 mL/min/1.73m^2 based on two consecutive serum creatinine measurements; 3) development of a composite endpoint of CVE defined as non-fatal myocardial infarction, stroke, coronary artery bypass graft, percutaneous coronary intervention, hospitalization for heart failure, or all-cause mortality; and 4) yearly eGFR decline. The CKD G3 endpoint was only applicable for participants with a baseline eGFR >60 mL/min/1.73m^2; Therefore, a total of 111 participants were excluded from this endpoint calculation due to baseline eGFR<60 mL/min/1.73m^2.

Procedures

Demographics including age, sex, diabetes duration, smoking, medical history, and concomitant medical treatment were collected at the baseline visit by the interviewing investigator. Likewise, the interviewing investigator evaluated DR status at baseline based on participant information and the participant's electronic medical record. Participants were included from highly specialized diabetes medical centers in which participants were subjected to regular retinal photo assessments graded by trained specialized staff according to local protocols. UACR was measured centrally (at Steno Diabetes Center Copenhagen, Gentofte, Denmark) at all visits using a Vitros 5600 MicroSlide (Orto Clinical Diagnostics, Raritan, NJ, USA). eGFR was determined using serum creatinine values measured centrally using the 2009 Chronic Kidney Disease Epidemiology Collaboration equation (15). HbA1c and potassium were analyzed at the participating center's local routine laboratory

using standardized methods. Urinary proteomics were performed by capillary electrophoresis mass spectrometry by Mosaiques Diagnostics (Hannover, Germany) using previously described methods (12), assessing a predefined renal risk profile based on 273 peptide fragments (CKD273).

Statistical analysis

Baseline values are presented as mean ± standard deviation (SD), median (inter-quartile range) if non-normally distributed, and n (%) if categorical. Comparisons between groups were performed using one-way analysis of variance, Kruskal-Wallis rank sum test, and χ^2-test, respectively. Cox proportional hazard models were used to investigate associations between baseline presence of DR, alone or stratified by CKD273 risk profile, and time to development of persistent microalbuminuria, CKD G3, or the composite cardiovascular endpoint. Results are presented as hazard ratios (HR) with 95% confidence intervals (95% CI) with participants without DR at baseline being the reference. Decline in eGFR was derived using all available eGFR measurements for all participants (yearly measurements for low-risk participants and quarterly for high-risk) and calculating an estimated yearly decline using individual linear regression models. To calculate the estimated yearly decline, participants were required to have a minimum of three eGFR measurements across a minimum of 6 months to acquire an acceptable estimate. All adjusted models included the following covariates: sex, baseline age, diabetes duration, HbA1c, systolic blood pressure, eGFR, UACR, and CKD273 risk status. Adjustments in the analysis of the CVE endpoint also included baseline LDL cholesterol, body mass index, and history of ischemic heart disease and stroke. Baseline DR, alone or stratified by CKD273 risk profile was investigated categorically using linear regression models, in association with the calculated yearly eGFR decline. Models stratified by DR and CKD273 risk profiles were subjected to a limited adjustment including only HbA_{1c} and diabetes duration due to low event rates and significant risk of over-adjustment. Cumulative incidence plots were drawn, and differences between groups are in these figures calculated using log-rank test.

Sensitivity analyses were performed, first by further adjustment of all models with renin-angiotensin-aldosterone system inhibitor, statin, aspirin, glucagon like peptide 1 receptor agonist, and sodium-glucose co-transporter 2 inhibitor treatment; and second by substratification of baseline DR into non-proliferative and proliferative DR. Furthermore, a sensitivity analysis was performed investigating DR status at baseline in association with difference in eGFR slope in which only values from month 3 (first visit after randomization) and forward were included, only in the high-risk group. This to address the possible acute influence spironolactone administration might have had on eGFR. All statistical analyses and data visualizations were performed using R v. 4.1.0 (R Core Team, 2021) and RStudio v. 1.4.1 (RStudio Team, 2021).

Results

Original enrollment of the PRIORITY study occurred between March 25, 2014, and Sept 30, 2018. Of the 1775 participants included in the PRIORITY study, 1758 had baseline information regarding DR status while the status was unknown for 17 (Supplementary Figure 1). Of the 1758 participants, 304 (17.3%) had DR at baseline, with 231 (76.0%) classified as non-proliferative DR (NPDR), 56 (18.4%), as proliferative DR (PDR), and 17 (5.6%) as background or non-classified DR (only maculopathy or history of laser treatment). Furthermore, 79 (26.8%) participants had maculopathy at baseline, and 76 (25.0%) had a history of laser therapy. 1454 participants did not have DR at baseline. Participants with DR at baseline had, on average, longer diabetes duration (17.7±8.4 vs 10.5±7.0 years), higher HbA1c (62±13 vs. 56±12 mmol/mol (7.8±1.2 vs. 7.3±1.1 %)), lower eGFR (85±16 vs. 87±15 ml/min/1. 73m^2), lower diastolic blood pressure (76±10 vs. 79±8 mmHg), and lower LDL cholesterol (2.22±0.83 vs. 2.45±0.94 mmol/l) than participants without DR. In addition, history of ischemic heart disease was higher in participants with DR than in those without DR (18% vs. 11%, $p<0.001$). However, the proportion with a history of stroke was identical (4% vs. 4%, $p=1.000$). Participants with DR at baseline were also more likely to be treated with insulin, glucagon like peptide 1 receptor agonists, or sodium-glucose co-transporter 2 inhibitors compared to those without DR (Table 1). These overall associations were intact when stratifying baseline characteristics by both presence of DR and urinary proteomic risk classifier status (Supplementary Table 1). Baseline characteristics were also largely identical for the subset of participants with eGFR >60 mL/min/1.73m^2 at baseline, in which development of CKD G3 was assessed (Supplementary Table 3).

Median follow-up was 2.5 (IQR: 2.0-3.0) years for all outcomes. During this time, 198 (11.3%) participants progressed to microalbuminuria, 166 (9.4% of the 1675 participants with eGFR >60 mL/min/1.73m^2 at baseline) to CKD G3, and 64 (3.6%) had a CVE. An overview of the events

comprising the CVE endpoint is presented in Supplementary Table 4. Results from the primary Cox proportional hazards models can be seen in Table 2. Presence of DR at baseline was significantly associated with progression to persistent microalbuminuria in both unadjusted (HR: 1.81 (95% CI: 1.32, 2.48), p<0.001) and adjusted (1.50 (1.07, 2.11), p=0.018) models compared to participants without DR. No association was found with progression to CKD G3, neither in unadjusted (0.95 (0.63, 1.42), p=0.801) nor in adjusted (0.87 (0.56, 1.34), p=0.55) models. The risk of developing CVE was more than 2.6-fold increased for participants with DR compared to those without, irrespective of adjustment (unadjusted: 2.74 (1.64, 4.57), p<0.001; adjusted: 2.61 (1.44, 4.72), p=0.002). The cumulative incidence for all dichotomous endpoints, stratified by the presence of DR at baseline, can be seen in Figure 1, showing a higher event rate for microalbuminuria and CVE in participants with DR. When sub-stratifying the baseline DR, similar associations were found for NPDR, both before and after adjustment (adjusted HR (95% CI): microalbuminuria: 1.53 (1.06, 2.22), p=0.024; CKD G3: 0.69 (0.41, 1.17), p=0.173; and CVE: 2.49 (1.33, 4.67), p=0.004) when compared to no DR. Although having similar HRs, PDR was not associated with endpoints when compared to no DR, in any of the adjusted models, presumably due to the low number of participants with PDR at baseline (n=56) (Supplementary Table 5). Furthermore, we subdivided participants into strata by the combined presence of DR and CKD273 risk status at baseline. Results were largely confirmatory with the primary analyses; compared with no DR and low-risk status, no DR and high-risk status at baseline was associated with development of all endpoints but not with a steeper eGFR slope. Presence of DR and low-risk status at baseline were associated with increased risk of microalbuminuria and CVE, but not CKD G3 and eGFR slope. Finally, the presence of DR and high-risk status compared to no DR and low-risk status at baseline was associated with all

outcomes with magnitude higher HRs for the development of microalbuminuria and CVE (Table 3, Figure 2).

Further investigation of the relationship between baseline DR status and yearly eGFR change (mean for entire population: -1.01 (-1.27, -0.76) mL/min/1.73 m^2/year) showed no difference in yearly eGFR slope of individuals with DR at baseline compared to those without (adjusted β estimate: -0.01 (-0.61, 0.58), p=0.968), Table 2). To account for a possible acute influence spironolactone administration might have had on eGFR, a sensitivity analysis was performed including only measurements from month 3 and forward, in the high-risk group. Results were unchanged compared to the main analysis. When sub-stratifying baseline DR, no differences in yearly eGFR slopes were observed neither in NPDR nor in PDR (Supplementary Table 5). Likewise, when the analysis considered the population stratified by both baseline presence of DR and CKD273 risk status, no significant associations were observed in any combination of baseline presence of DR or risk status compared to no DR and low-risk status (Table 3).

Additional adjustment for renin-angiotensin-aldosterone system inhibitors, statins, aspirin treatment, sodium-glucose co-transporter 2 inhibitor treatment, glucagon like peptide 1 receptor agonist treatment, or randomization group (for the nested trial) did not affect any of the above-described results significantly (Supplementary Table 6).

Sub-analysis combined microalbuminuria and CKD G3 development

Of 198 (microalbuminuria) and 166 (CKD G3) events, 27 participants experienced both endpoints. The association between presence of DR at baseline and the development of the combined microalbuminuria and CKD G3 endpoint did not result in significant associations neither in crude nor in adjusted models. We also performed two further analyses, including only participants who had developed either CKD G3 or microalbuminuria. We assessed the relationship between baseline DR

and the development of both microalbuminuria and CKD G3. In both subsets, no significant associations were found between DR and the development of the combined endpoint neither crude nor adjusted models (Supplementary Table 7).

Discussion

We have demonstrated that the presence of DR in people with type 2 diabetes and normoalbuminuria was associated with increased risk of development of microalbuminuria and CVE across a median follow-up of 2.5 years. Interestingly, DR was not associated with an increased risk of developing impaired kidney function in this relatively short observation period. CKD in individuals with diabetes, and type 1 diabetes in particular, has long been considered closely associated with the presence of DR (6). This relationship is so deeply established, that clinical diabetic kidney disease can solely be diagnosed in persons with diabetes and macro- (severe) albuminuria, presence of DR, and absence of clinical or laboratory evidence of other kidney diseases. Previous studies, however, have primarily focused on macroalbuminuric CKD in diabetes, thereby highlighting a severe form of kidney impairment that was not included in this study and with which DR has been strongly associated. In addition, our results showed no association between the presence of DR and a steeper yearly eGFR slope, irrespective of the progression of the albuminuria grade during follow-up.

In our study, participants with DR at baseline had longer diabetes duration, but similar age and were more likely to have a history of ischemic heart disease. These factors did, however, not affect the risk effect size or significance, indicating certain robustness of the findings. We have previously demonstrated that participants identified at high risk based on the CKD273 classifier had a higher risk for development of microalbuminuria or CKD G3. In this study, we demonstrate that the combination of DR and a high risk CKD273 profile indicates an additive effect on the risk for development of microalbuminuria and CVE, compared to the presence of DR or a high-risk CKD273 profile alone. Given the much fewer individuals in each group, confidence intervals were large, and no adjustments were performed; thus, no firm conclusion should be drawn from these analyses. However, as we have previously shown, urinary proteomics, and CKD273 specifically,

were not associated with baseline DR grade or progression in DR in both type 1 and type 2 diabetes (10). These results may reflect two pathogeneses leading to the development of microalbuminuria.

Our results beg the question of how specific microalbuminuria is to CKD in type 2 diabetes or if it instead is a phenotype of general vascular damage. This has been suggested before, as it has been shown that more than 30% of people with type 2 diabetes and eGFR <60 mL/min/1.73m^2 had non-albuminuric CKD (16, 17). On a molecular level, recent studies have supported the distinction between DR and CKD (10, 11). A study by Hong et al., which investigated the relationship between baseline DR and development of kidney impairment or cardiovascular disease over 14 years, found a significantly higher risk of cardiovascular disease compared to CKD (18), suggesting that DR may be primarly associated with cardiovascular disease, rather than kidney disease. Even in type 1 diabetes, few metabolite similarities were discovered using exploratory omics-based methods in two studies that used the same cohort and metabolomics platform (19, 20). Ribitol and ribonic acid, both derivatives of ribose, which in turn is active in the pentose phosphate pathway and highly influenced by hyperglycemia (21), were positively correlated to presence of DR (19), a finding also identified in type 2 diabetes (22). Similarly, ribonic acid was significantly associated with future kidney function decline after adjustment for clinical covariates and multiple testing (20), but no metabolites independent of hyperglycemia influence were identified. Furthermore, another recent study investigating proteomic biomarkers for risk of kidney disease found very little association with prevalence, incidence, or progression of DR in a population of 958 individuals included in the Fremantle Diabetes Study (23).

While the current study does not provide a comprehensive answer to the relationship between DR and diabetic kidney disease and vice versa, it does support the idea that a distinction between these two microvascular complications may be indicated. Alternatively, albuminuric CKD and non-albuminuric CKD in type 2 diabetes may be separate diseases and should be targeted differently,

especially at an early stage. The sooner this can be fully elucidated, and the respective pathophysiologies charted, the sooner more targeted solutions might be proposed and applied in the treatment and prevention of DR and kidney disease. It is also important to note that our findings do not invalidate previous research linking DR and CKD in diabetes, but rather that the complete picture may be more complex than previously assumed while emphasizing that our study is limited to normoalbuminuric type 2 diabetes

Our study also has its limitations. Mainly, no standardized retinal photography was performed at baseline to grade DR and thus the presence of DR at baseline was assessed by the interviewing physician at the participating center. This is somewhat ameliorated by the fact that all participating centers and investigators specialized in diabetes treatment and care, leading to a supposed higher validity of the DR diagnosis. Notwithstanding, a formal, homogenous, retinal examination across the entire cohort would be preferred. The study is a large European multi-center cohort of individuals with type 2 diabetes and normoalbuminuria which increases the generalizability of the results across a western population, albeit the homogenous racial composition of the participants as well as the strict inclusion and exclusion criteria inherently limits the applicability of the study in other populations. Finally, despite the relatively large number of CKD events in the study, the short follow-up of 2.5 years could potentially have influenced the negative association with DR at baseline, as development of advanced CKD often requires longer follow-up.

Conclusions

We have demonstrated that the presence of DR in a population of individuals with type 2 diabetes and normoalbuminuria is mainly a risk factor for the development of microalbuminuria and cardiovascular disease. At the same time, it is not associated with kidney function decline, implying that DR is an indicator of microvasculopathy in general and appears to be a prognostic factor. Our

results may lead to a more precise risk assessment of individuals with type 2 diabetes. It furthers the notion that DR and impaired kidney function may not be markedly interlinked.

List of abbreviations

DR – diabetic retinopathy

CKD – chronic kidney disease

CVE – cardiovascular events

UACR – urinary albumin-creatinine rate

eGFR – estimated glomerular filtration rate

CKD273 – urinary proteomic renal risk classifier based on 273 peptide fragments

G3 – grade 3

NPDR – non-proliferative diabetic retinopathy

PDR – proliferative diabetic retinopathy

Declarations

Ethics approval and consent to participate

The clinical study protocol and informed consent documents were reviewed and approved by the respective local independent ethics committees and competent authorities at each participating study site. Every patient gave written informed consent prior to the conduct of any study-related procedures. This study was registered (EudraCT 20120-004523-4; ClinicalTrials.gov NCT02040441) and is completed. The study complied Good Clinical Practice and with the Declaration of Helsinki.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to Danish GDPR legislation. Scrambled, anonymized datasets could be available from the corresponding author on reasonable request.

Competing interests

NT is a full-time employee of Novo Nordisk A/S. TWH has equity in Novo Nordisk A/S. ML has received research support from Boehringer Ingelheim, Bayer and Merck Sharp & Dohme, and lecture fees from AstraZeneca, Bayer and Boehringer Ingelheim. All fees are given to Holbaek hospital. FP has served as a consultant, on advisory boards or as educator for AstraZeneca, Novo Nordisk, Boehringer Ingelheim, Sanofi, Mundipharma, MSD, Novartis, Amgen and has received research grants to institution from Novo Nordisk, Boehringer Ingelheim, Amgen and AstraZeneca.
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Mundipharma-UAM of diabetic kidney disease and the Catedra Astrazeneca-UAM of chronic kidney disease and electrolytes. HM is the cofounder and co-owner of Mosaiques Diagnostics GmbH. JS and PZ are employees of Mosaiques Diagnostics GmbH. MF-M has received speaker fees or support for medical meetings and research grants from AstraZeneca and Novo Nordisk and speaking fees from Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Novartis, Baxter, and Sanofi-Aventis. IR has received honoraria for lectures of AstraZenec, Bayer, Boehringer Ingelheim, and Mundipharma. PR has received research support and personal fees from AstraZeneca, Bayer and Novo Nordisk, and personal fees from Astellas Pharma Inc., Bayer, Boehringer Ingelheim, Eli Lilly and Company, Gilead, Merck, Merck Sharp & Dohme, Mundipharma, Sanofi, and Vifor Pharma. All fees are given to Steno Diabetes Center Copenhagen. All other authors declare no competing interests that could be perceived as affecting the impartiality of the reported results.

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Author contributions

VRC, TWH, and PR designed the study. All authors contributed to data collection. VRC performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to the

interpretation of the results. All authors contributed to critical revision and review of the manuscript. All authors approved the final version of the manuscript.

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Variable	No DR	DR	p
N	1454	304	
Age, years	61.4 (8.4)	62.7 (7.7)	0.010
Male, n (%)	899 (61.8)	198 (65.1)	0.310
Non-White	46 (3.2)	14 (4.6)	0.278
Diabetes duration, years	10.5 (7.0)	17.7 (8.4)	<0.001
Low-risk CKD273 pattern, n (%)	1279 (88.0)	263 (86.5)	0.545
Retinopathy grade, n (%)			
Non-proliferative	-	231 (76.5)	
Proliferative	-	56 (18.5)	
Maculopathy, n (%)	-	79 (26.8)	
History of laser therapy, n (%)	-	76 (25.8)	
HbA1c, mmol/mol	56 (12)	62 (13)	<0.001
HbA1c, %	7.3 (1.1)	7.8 (1.2)	
eGFR, mL/min/1.73m^2	87 (15)	85 (16)	0.010
UACR, mg/g	5 [3, 8]	6 [4, 10]	<0.001
CKD273, arbitrary unit	-0.34 (0.42)	-0.26 (0.38)	0.002
Systolic BP, mmHg	133 (12)	134 (12)	0.250
Diastolic BP, mmHg	79 (8)	76 (10)	<0.001
BMI, kg/m2	30.3 (5.0)	30.6 (5.2)	0.487
LDL, mmol/l	2.45 (0.94)	2.22 (0.83)	<0.001
Smoker, n (%)	656 (45.2)	126 (41.7)	0.291
RAAS-inhibitor treatment, n (%)	909 (62.5)	232 (76.3)	<0.001
Metformin treatment, n (%)	1092 (78.6)	240 (79.7)	0.709
Insulin treatment, n (%)	365 (26.3)	167 (55.5)	<0.001
GLP1-RA treatment, n (%)	200 (14.4)	71 (23.6)	<0.001
SGLT2-inhibitor treatment, n (%)	91 (6.5)	30 (10.0)	0.050
History of IHD, n (%)	157 (11)	58 (18)	<0.001
History of stroke, n (%)	62 (4)	13 (4)	1.000

Table 1: Baseline characteristics stratified by presence of diabetic retinopathy (DR) at baseline. Data are presented as mean (standard deviation), median [inter-quartile range], or n (%). P-values were calculated using Student's t-test, Kruskal-Wallis rank sum test, and the $\chi 2$-test for continuous, non-normal continuous, and categorical variables, respectively. HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; UACR: urinary albumin-creatinine rate; CKD273: urinary proteomics classifier; BP: blood pressure, BMI: body mass index, RAAS: renin-angiotensin-aldosterone system; GLP1-RA: glucagon like peptide 1 receptor agonist; SGLT2: sodium-glucose co-transporter 2; IHD: ischemic heart disease.

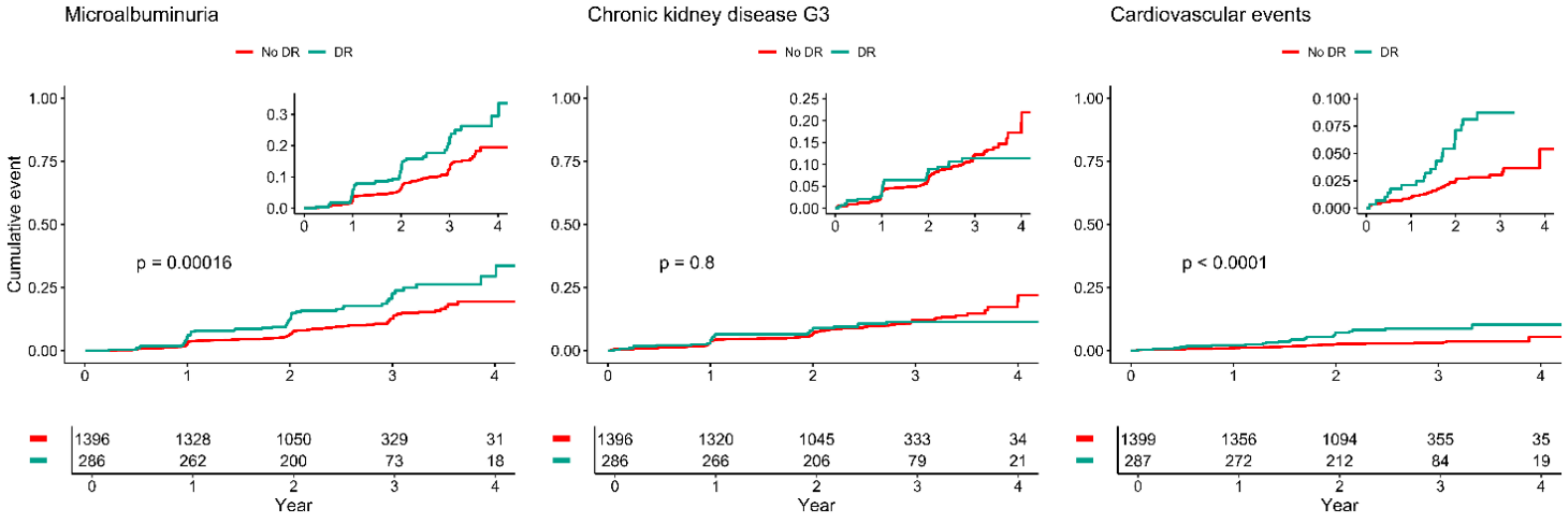
Outcome	Presence of DR Unadjusted HR (95% CI)	p	Presence of DR Adjusted HR (95% CI)	p
Microalbuminuria (n=198)	1.81 (1.32, 2.48)	<0.001	1.50 (1.07, 2.11)	0.018
CKD G3 (eGFR<60 mL/min/1.73m^2) (n=166)	0.95 (0.63, 1.42)	0.801	0.87 (0.56, 1.34)	0.553
CVE (n=64)	2.74 (1.64, 4.57)	<0.001	2.61 (1.44, 4.72)	0.002
	Presence of DR Unadjusted mL/min/1.73m^2/year		Presence of DR Adjusted mL/min/1.73m^2/year	
eGFR slope difference	-0.13 (-0.45, 0.70)	0.669	-0.01 (-0.61, 0.58)	0.968

Table 2. Presence of diabetic retinopathy (DR) with the development of microalbuminuria, chronic kidney disease (CKD) G3, or cardiovascular events (CVE), and difference in yearly estimated glomerular filtration (eGFR) slope, compared to no diabetic retinopathy. Associations to dichotomous outcomes were estimated using Cox proportional hazards models and association to yearly eGFR slope with linear regression. Adjustments included sex, baseline age, diabetes duration, HbA1c, systolic blood pressure, eGFR, urinary albumin-creatinine rate, and urinary proteomic risk classifier status. Adjustments for the CVE endpoint also included baseline LDL cholesterol, body mass index, and ischemic heart disease and stroke history.

	Microalbuminuria n=198 HR (95% CI)	p	CKD G3 n=166 HR (95% CI)	p	CVE n=64 HR (95% CI)	p		Difference in eGFR slope, ml/min/1.73m^2 Mean yearly change: -1.01 (-1.27, -0.76)	p
Unadjusted									
No DR/Low Risk (n=1279)	Reference								
No DR/High Risk (n=175)	3.68 (2.57, 5.26)	<0.001	3.41 (2.34, 4.95)	<0.001	1.34 (0.57, 3.2)	0.503		-0.63 (-1.36, 0.10)	0.092
DR/Low Risk (n=263)	1.67 (1.14, 2.46)	0.009	0.87 (0.52, 1.43)	0.578	2.58 (1.46, 4.56)	0.001		0.10 (-0.52, 0.71)	0.761
DR/High Risk (n=41)	7.46 (4.57, 12.18)	<0.001	3.43 (1.79, 6.59)	<0.001	4.53 (1.77, 11.59)	0.002		-0.22 (-1.64, 1.20)	0.760
Adjusted (limited)									
No DR/Low Risk (n=1279)	Reference								
No DR/High Risk (n=175)	3.46 (2.41, 4.98)	<0.001	3.45 (2.36, 5.05)	<0.001	1.28 (0.53, 3.06)	0.581		-0.63 (-1.37, 0.10)	0.092
DR/Low Risk (n=263)	1.46 (0.97, 2.21)	0.072	0.90 (0.53, 1.53)	0.710	2.30 (1.22, 4.31)	0.010		0.09 (-0.57, 0.76)	0.780
DR/High Risk (n=41)	6.40 (3.81, 10.73)	<0.001	3.55 (1.81, 6.94)	<0.001	4.04 (1.52, 10.70)	0.005		-0.23 (-1.67, 1.21)	0.757

Table 3: Unadjusted analyses of baseline presence of diabetic retinopathy and CKD273 risk status in relation to development of dichotomous outcomes (microalbuminuria, chronic kidney disease (CKD) G3, and cardiovascular events (CVE)) and yearly estimated glomerular filtration (eGFR) slope. Association to dichotomous outcomes were estimated using Cox proportional hazards models, and association to yearly eGFR slope with linear regression. Limited adjustment was performed including baseline values of glycated hemoglobin and diabetes duration.

Figure 1: Cumulative event plot illustrating the event rate of microalbuminuria, chronic kidney disease G3, and cardiovascular events stratified by presence of diabetic retinopathy (DR) at baseline. Each plot is presented with a complete y-axis and a minor embedded plot with a non-complete y-axis.



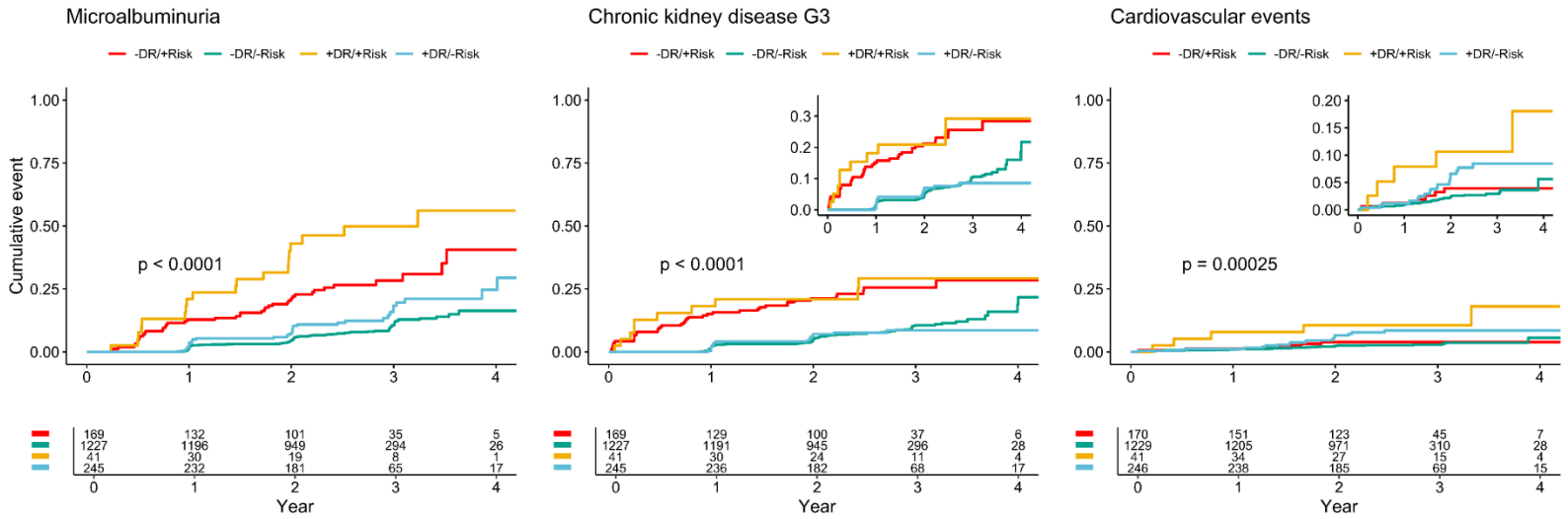


Figure 2: Cumulative event plot illustrating the development of microalbuminuria, chronic kidney disease G3, and cardiovascular events stratified by presence of diabetic retinopathy (DR) and CKD273 risk status at baseline. Presence/non-presence of DR and high/low risk are presented as +/-, respectively. The plots showing the cumulative events for chronic kidney disease and cardiovascular events are presented with complete y-axes as well as with minor embedded plots with non-complete y-axes. P-value is derived from log-rank tests.