Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): a prospective observational study and embedded randomised placebo-controlled trial

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Summary

Background Microalbuminuria is an early sign of kidney disease in people with diabetes and indicates increased risk of cardiovascular disease. We tested whether a urinary proteomic risk classifier (CKD273) score was associated with development of microalbuminuria and whether progression to microalbuminuria could be prevented with the mineralocorticoid receptor antagonist spironolactone.

Methods In this multicentre, prospective, observational study with embedded randomised controlled trial (PRIORITY), we recruited people with type 2 diabetes, normal urinary albumin excretion, and preserved renal function from 15 specialist centres in ten European countries. All participants (observational cohort) were tested with the CKD273 classifier and classified as high risk (CKD273 classifier score >0·154) or low risk (≤0·154). Participants who were classified as high risk were entered into a randomised controlled trial and randomly assigned (1:1), by use of an interactive web-response system, to receive spironolactone 25 mg once daily or matched placebo (trial cohort). The primary endpoint was development of confirmed microalbuminuria in all individuals with available data (observational cohort). Secondary endpoints included reduction in incidence of microalbuminuria with spironolactone (trial cohort, intention-to-treat population) and association between CKD273 risk score and measures of impaired renal function based on estimated glomerular filtration rate (eGFR; observational cohort). Adverse events (particularly gynaecomastia and hyperkalaemia) and serious adverse events were recorded for the intention-to-treat population (trial cohort). This study is registered with the EU Clinical Trials Register (EudraCT 20120-004523-4) and ClinicalTrials.gov (NCT02040441) and is completed.

Findings Between March 25, 2014, and Sept 30, 2018, we enrolled and followed-up 1775 participants (observational cohort), 1559 (88%) of 1775 participants had a low-risk urinary proteomic pattern and 216 (12%) had a high-risk pattern, of whom 209 were included in the trial cohort and assigned to spironolactone (n=102) or placebo (n=107). The overall median follow-up time was 2·51 years (IQR 2·0–3·0). Progression to microalbuminuria was seen in 61 (28%) of 216 high-risk participants and 139 (9%) of 1559 low-risk participants (hazard ratio [HR] 2·48, 95% CI 1·80–3·42; p<0·0001, after adjustment for baseline variables of age, sex, HbA_{1c}, systolic blood pressure, retinopathy, urine albumin-to-creatinine ratio [UACR], and eGFR). Development of impaired renal function (eGFR <60 mL/min per 1·73 m²) was seen in 48 (26%) of 184 high-risk participants and 119 (8%) of 1423 low-risk participants (HR 3·50; 95% CI 2·50–4·90, after adjustment for baseline variables). A 30% decrease in eGFR from baseline (post-hoc endpoint) was seen in 42 (19%) of 216 high-risk participants and 62 (4%) of 1559 low-risk participants (HR 5·15, 95% CI 3·41–7·76; p<0·0001, after adjustment for basline eGFR and UACR). In the intention-to-treat trial cohort, development of microalbuminuria was seen in 35 (33%) of 107 in the placebo group and 26 (25%) of 102 in the spironolactone group (HR 0·81, 95% CI 0·49–1·34; p=0·41). In the safety analysis (intention-to-treat trial cohort), events of plasma potassium concentrations of more than 5·5 mmol/L were seen in 13 (13%) of 102 participants in the spironolactone group and four (4%) of 107 participants in the placebo group, and gynaecomastia was seen in three (3%) participants in the spironolactone group and none in the placebo group. One patient died in the placebo group due to a cardiac event (considered possibly related to study drug) and one patient died in the spironolactone group due to cancer, deemed unrelated to study drug.

Interpretation In people with type 2 diabetes and normoalbuminuria, a high-risk score from the urinary proteomic classifier CKD273 was associated with an increased risk of progression to microalbuminuria over a median of 2·5 years, independent of clinical characteristics. However, spironolactone did not prevent progression to microalbuminuria in high-risk patients.

Lancet Diabetes Endocrinol 2020

Published Online
March 2, 2020
https://doi.org/10.1016/S2213-8587(20)30026-7

See Online/Comment
https://doi.org/10.1016/S2213-8587(20)30067-X

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Funding European Union Seventh Framework Programme.

Introduction

Diabetic kidney disease is a frequent and costly complication of diabetes and a leading cause of renal failure. Additionally, diabetic kidney disease is associated with a substantially increased burden of cardiovascular disease. Globally, one in 11 adults has diabetes, and numbers are increasing.[1] Despite an observed reduction in relative risk for end-stage kidney disease in diabetes during the past three decades, the absolute number of people referred for treatment for end-stage kidney disease has more than doubled.[2] This increase probably results from the increasing prevalence of diabetes, combined with a reduction in competing cardiovascular mortality and increased eligibility for treatment of end-stage kidney disease. This situation emphasises the need for better prediction, prevention, and treatment of diabetic kidney disease.

In clinical practice, diabetic kidney disease is diagnosed by albuminuria, a decrease in estimated glomerular filtration rate (eGFR), or both. Microalbuminuria (confirmed urine albumin-to-creatinine ratio [UACR] of >30 mg/g in at least two of three consecutive urine samples) is a marker of increased risk for cardiovascular disease and end-stage kidney disease.[3] Treatment of microalbuminuria and macroalbuminuria (confirmed UACR of >300 mg/g) with renin–angiotensin–aldosterone system (RAAS) blockers and control of cardiovascular risk factors has improved outcomes,[4] but the prognosis remains poor and many still progress despite widespread prescription of these drugs as advocated by clinical guidelines. Study findings have suggested pleiotropic and kidney protective effects of SGLT2 inhibitors and potentially also GLP-1 receptor agonists, which are now recommended by guidelines in patients with type 2 diabetes with established diabetic kidney disease.[5]

Studies into prevention of microalbuminuria with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers have shown conflicting results.[6,7] Therefore, biomarkers to identify people who would benefit most from preventive therapy would be helpful. Depending on the pathophysiology underlying the biomarker, such markers could also help to guide interventions in a precision medicine approach. Good and colleagues[8] previously described a high-dimension urinary biomarker pattern composed of 273 peptides associated with overt kidney disease, CKD273. The original studies of CKD273 included people with various forms of chronic kidney disease. Risk scores have been developed with the same methods that have been optimised for diagnosis of different kidney diseases, but CKD273 has been shown to be robust across multiple causes of chronic kidney disease, including diabetic kidney disease. In retrospective studies, this proteomic classifier identified people at risk of diabetic kidney disease and progression in albuminuria class

Research in context

Evidence before this study

We searched PubMed for publications in English from between Jan 1, 1990, and June 30, 2019, using the search terms "type 2 diabetes", "normoalbuminuria", "urinary proteomics", "urinary peptidomics", "spironolactone", "mineralocorticoid receptor antagonist", "aldosterone antagonist", "albuminuria", "kidney disease", and "nephropathy". Retrospective studies of cross-sectional and longitudinal cohorts of people with type 2 diabetes and non-diabetic kidney disease have been investigated with urinary proteomics as a marker for the presence or development of kidney disease. A high-risk score based on CKD273, a urinary peptide pattern for chronic kidney disease consisting of 273 peptides, has been shown to be associated with progression of albuminuria and loss of renal function in these retrospective cohorts, but no prospective studies have been done and no studies have attempted to link this risk marker to a potential intervention. Mineralocorticoid receptor antagonists have been shown to lower urinary albumin excretion in short-term studies of patients with moderately to severely increased albuminuria, but long-term data are not available, and studies aiming to prevent progression of normoalbuminuria to microalbuminuria with these drugs have not been done.

Added value of this study

To our knowledge, this is the first prospective multicentre study assessing the multidimensional CKD273 urinary proteomic classifier for risk stratification in individuals with normoalbuminuria and type 2 diabetes. We showed that a high-risk score based on CKD273 is effective as an early marker of risk for progression to persistent microalbuminuria in a prospective study setting, and is also associated with development of impaired renal function. However, the mineralocorticoid receptor antagonist spironolactone was not shown to delay or prevent development of confirmed microalbuminuria in patients identified to be at high risk of progression on the basis of the CKD273 proteomic biomarker.

Implications of all the available evidence

A high-risk pattern from the urinary proteomic-based risk marker CKD273 is associated with early progression of diabetic kidney disease, and could add predictive value to the clinical characteristics being used in clinical practice, including urinary albumin excretion and glomerular filtration rate. However, early progression cannot be mitigated by treatment with the mineralocorticoid receptor antagonist spironolactone.

earlier than the indices currently used in clinical practice (ie, eGFR and albuminuria).[9–11] However, all data on CKD273 to date are derived from retrospective analyses of previous studies and analyses of stored samples.

RAAS blockade has been recommended in diabetic kidney disease, and more complete inhibition of the RAAS with mineralocorticoid receptor antagonists, such as spironolactone given in addition to RAAS inhibitors, might further improve renal protection.[12,13] A further reduction in albuminuria of about 20–30% was seen in short-term studies of spironolactone, which is anticipated to predict beneficial renal effects.[12,13] Long-term data from phase 3 trials focused on clinical outcomes such as end-stage kidney disease are not available; additionally, studies have not yet been done to assess the use of spironolactone to prevent the early stages of diabetic kidney disease. The components of CKD273 include collagen fragments, which are assumed to relate to early fibrosis in the kidney. Therefore, spironolactone, which is considered to be antifibrotic via blockade of aldosterone, could be a potentially useful intervention in the context of CKD273-predicted early diabetic kidney disease.

The aims of the PRIORITY (proteomic prediction and renin angiotensin aldosterone system inhibition prevention of early diabetic nephropathy in type 2 diabetic patients with normoalbuminuria) study were to show that CKD273 is associated with development of persistent microalbuminuria in people with type 2 diabetes and normoalbuminuria in a prospective study, and to determine whether intervention with a mineralocorticoid receptor antagonist (spironolactone) reduces the increased risk of developing microalbuminuria in people with a high-risk CKD273 pattern compared with placebo. Albuminuria is used as a biomarker in the clinic, in trials, and here as the endpoint for early progression of diabetic kidney disease, making it inheritably difficult for a new marker, such as CKD273, to perform better than albuminuria. Thus, we also looked at changes in eGFR as secondary outcomes, in addition to potential adverse events.

Methods

Study design and participants

A detailed rationale, study design, and methods for this study have been published elsewhere.[14] PRIORITY is an investigator-initiated, prospective, double-blind, randomised, placebo-controlled, international, multicentre clinical and observational study in people with type 2 diabetes and normoalbuminuria.

Briefly, we recruited people aged 18–75 years with type 2 diabetes, preserved kidney function, and normoalbuminuria from 15 specialist centres in ten European countries (Belgium, Czech Republic, Denmark, Germany, Greece, Italy, the Netherlands, North Macedonia, Spain, and the UK; a lists of sites and patients recruited per site are in the appendix [pp 2–3, 16]). Main inclusion criteria were normoalbuminuria (urine albumin-to-creatinine ratio [UACR] <30 mg/g) in at least two of three consecutive morning void urine samples and an eGFR of more than 45 mL/min per 1·73 m² of body surface area at screening. Data on albuminuria before the study were not collected. Key exclusion criteria were use of dual RAAS blockade or mineralocorticoid receptor antagonist, or heart failure requiring treatment with a mineralocorticoid receptor antagonist. Full inclusion and exclusion criteria are reported elsewhere.[14] Participants were stratified into high-risk or low-risk groups on the basis of their CKD273 score, which was based on a single random spot urine sample collected at screening. High risk was defined as a CKD273 classifier score of more than 0·154, and low risk as a score of 0·154 or lower, as previously described.[10,14]

Each partner in the PRIORITY project, including study sites, central CKD237 laboratory, and clinical research organisation (appendix pp 2–3) was represented in the study steering group, and the Hannover Clinical Trial Centre was responsible for data management and study monitoring. The protocol (approved on June 27, 2013) and amendments (made on March 24, and Oct 12, 2015) were approved by the respective national competent authorities (partly with reference to the Voluntary Harmonisation Procedure) and by the local institutional ethics committees (appendix pp 21–22). The protocol is available online. The study was done in accordance with the International Conference on Harmonisation Good Clinical Practice guideline and the Declaration of Helsinki. All participants provided written informed consent.

Randomisation and masking

Only participants in the high-risk group based on CKD273 score were included in the randomised trial component of the study. These high-risk participants were randomly assigned (1:1), stratified by centre and RAAS treatment (yes or no), via an interactive web-response system (appendix p 6) to either spironolactone 25 mg once daily or matching placebo, following a computer-generated randomisation scheme. Treatment allocation was double-blind, with participants and investigators masked to allocation. The medications for each treatment group were identical in appearance and were supplied in identical bottles, labelled appropriately to maintain masking within the study. The independent data monitoring committee (DMC) and the statistician supporting the committee were the only people with access to unmasked data.

Procedures

Urine proteomics testing was done by applying capillary electrophoresis–mass spectrometry analysis at the central laboratory at Mosaiques Diagnostics (Hannover, Germany) and results were available within 3 days after samples were received in the laboratory. This analysis provides data on more than 1000 identified proteins or peptides and a predefined renal risk profile based on 273 peptides (CKD273; appendix p 5).[14,15] Based on a retrospective analysis of CKD273 as a marker of

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See **Online** for appendix

For the **up to date protocol** see https://clinicaltrials.gov/ProvidedDocs/41/NCT02040441/Prot_001.pdf

progression from normoalbuminuria to microalbuminuria, a priori we re-defined a threshold for CKD273 of more than 0·154 corresponding to the 20% centile indicating high risk for progression from normoalbuminuria to microalbuminuria. All data are normalised to 28 collagen fragments in urine not affected by disease and variability is low.[16]

After the screening visit in which urine samples were collected for proteomic analysis, participants entered a run-in phase during which three consecutive morning urine samples were collected to establish baseline urinary albumin excretion. When results of the urine analyses were available, the participant would visit the study centre for the baseline visit.

Participants with a high-risk proteomic pattern were provided with study medication after random assignment to treatment (spironolactone or placebo) and continued their ongoing medication (subject to change if recommended by their primary care physician), including RAAS inhibitors, in accordance with local standards of care. They were seen for a safety visit at the study centre after 2 weeks, with local measurement of creatinine and potassium concentrations. Every 13th week, participants were seen in the clinic and provided with the study drug. At each visit, UACR was tested in three consecutive urine samples, and locally measured biochemistry was analysed.

The follow-up time of study participants was initially planned to be 3 years for all, but after an ammendment to the protocol during the study (appendix p 6), the end of the study was planned for September, 2018, such that follow-up times range from 1·5 to 4·5 years.

Participants with a low-risk CKD273 pattern were followed-up without study intervention and continued ongoing treatment in accordance with standard of care. They were seen once yearly after the baseline visit and tested for UACR in three consecutive urine samples and locally measured biochemistry.

Samples were analysed locally with standardised methods, as described previously.[17] eGFR was calculated centrally by use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. UACR was measured at the central laboratory (Steno Diabetes Center Copenhagen, Denmark [SDCC]) with a Vitros 5600 MicroSlide (Orto Clinical Diagnostics, Raritan, NJ, USA). Monitoring and evaluation of serious adverse events and adverse events of special interest (hyperkalaemia and gynaecomastia) were done at Medizinsche Hochschule Hannover Germany and an external independent DMC monitored safety (not efficacy) throughout the study on the basis of data from Hannover Clinical Trial Center (Hannover, Germany) analysed by the DMC statistician.

Efficacy and safety parameters were monitored at all visits. Safety assessments included physical examination, vital signs, laboratory tests, and adverse event monitoring. Adverse event information was collected by study personnel at each visit and recorded in the electronic case report form, with severity assessed with the Common Terminology Criteria for Adverse Events and coded with standard terms (Medical Dictionary for Regulatory Activities) to classify adverse event terms. The focus of the safety analysis was on serious adverse events, decrease in eGFR of more than 30% and 40% from baseline, hyperkalaemia, and gynaecomastia.

Outcomes

The primary objective was to confirm that urinary proteomics can be associated with development of confirmed microalbuminuria in people with type 2 diabetes and normoalbuminuria. The primary endpoint was development of confirmed microalbuminuria. Confirmed microalbuminuria was defined as a UACR of more than 30 mg/g in at least two of three first morning voids with a 30% or higher increase (geometric mean) in UACR from the run-in-phase samples, or more than 40 mg/g (geometric mean) over the study period.

A secondary objective of the primary endpoint was to investigate whether treatment with spironolactone 25 mg once daily reduces risk of transition to microalbuminuria in patients identified to be at high risk on the basis of CKD273 proteomic pattern.

Secondary endpoints were assessed in the observational and trial cohorts. Secondary endpoints were analysis of changes in the geometric mean of UACR (slope) throughout the study period in all patients by assessing the slope of albuminuria from inclusion to end of trial; development of microalbuminuria (UACR >30 mg/g) in at least one morning void urine sample instead of confirmed microalbuminuria; development of macroalbuminuria (UACR >300 mg/g) in two of three first morning void urine samples; for patients with eGFR >60 mL/min per 1·73 m² at baseline, development of chronic kidney disease stage 3—ie, eGFR <60 mL/min per 1·73 m²; development of chronic kidney disease stage 4 (eGFR <30 mL/min per 1·73 m²); change in eGFR (slope) from baseline and from 3 months after baseline to the end of the study; change in eGFR (≥40% reduction) from baseline; composite fatal and non-fatal cardiovascular outcomes (myocardial infarction, stroke, coronary intervention, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, admission to hospital for heart failure, and cardiovascular disease) and all-cause mortality during the study; and incidence of retinopathy and frequency of laser treatment for retinopathy from self-reported adverse events.

Post-hoc defined secondary endpoints were a change in eGFR of 30% or more from baseline during follow-up or a doubling in serum creatinine from baseline. Blood pressure and HbA_{1c} were evaluated during the trial as potential confounders of the primary outcome.

The safety analysis in the trial cohort focused on adverse events leading to study drug discontinuation or withdrawal from the study, or both; any serious adverse event; adverse events related to cardiovascular disease or renal disease, or related to progression of diabetic retinopathy (and

corresponding laser treatment); and laboratory abnormalities suspected by the investigators to be related to the study drug (particularly potassium). Safety outcomes of special interest in the trial cohort were hyperkalaemia (plasma or serum level of potassium >0·4 mmol/L above local upper reference), gynaecomastia, and hypotension. During hyperkalaemia, study medication was paused and could be restarted when potassium was normal.

Statistical analysis

For our study, a smaller sample size was needed for the primary objective in the observational cohort (association between CKD273 score [high risk *vs* low risk] and development of persistent microalbuminuria; n=333; appendix pp 6–7) than for the secondary objective in the intervention trial (the effect of spironolactone *vs* placebo in CKD273 high-risk participants).[10] High-risk participants were expected to comprise 15% of screened and included participants on the basis of previous retrospective analysis of similar participants.[10] On the basis of a previous study in which the short-term effects of albuminuria were reported,[13] and using the sample size formula for two proportions test (α=0·05, β=0·80), we estimated that 129 participants would be required in each trial group (spironolactone and placebo) to provide sufficient power to detect a 40% reduction in transition to microalbuminuria.

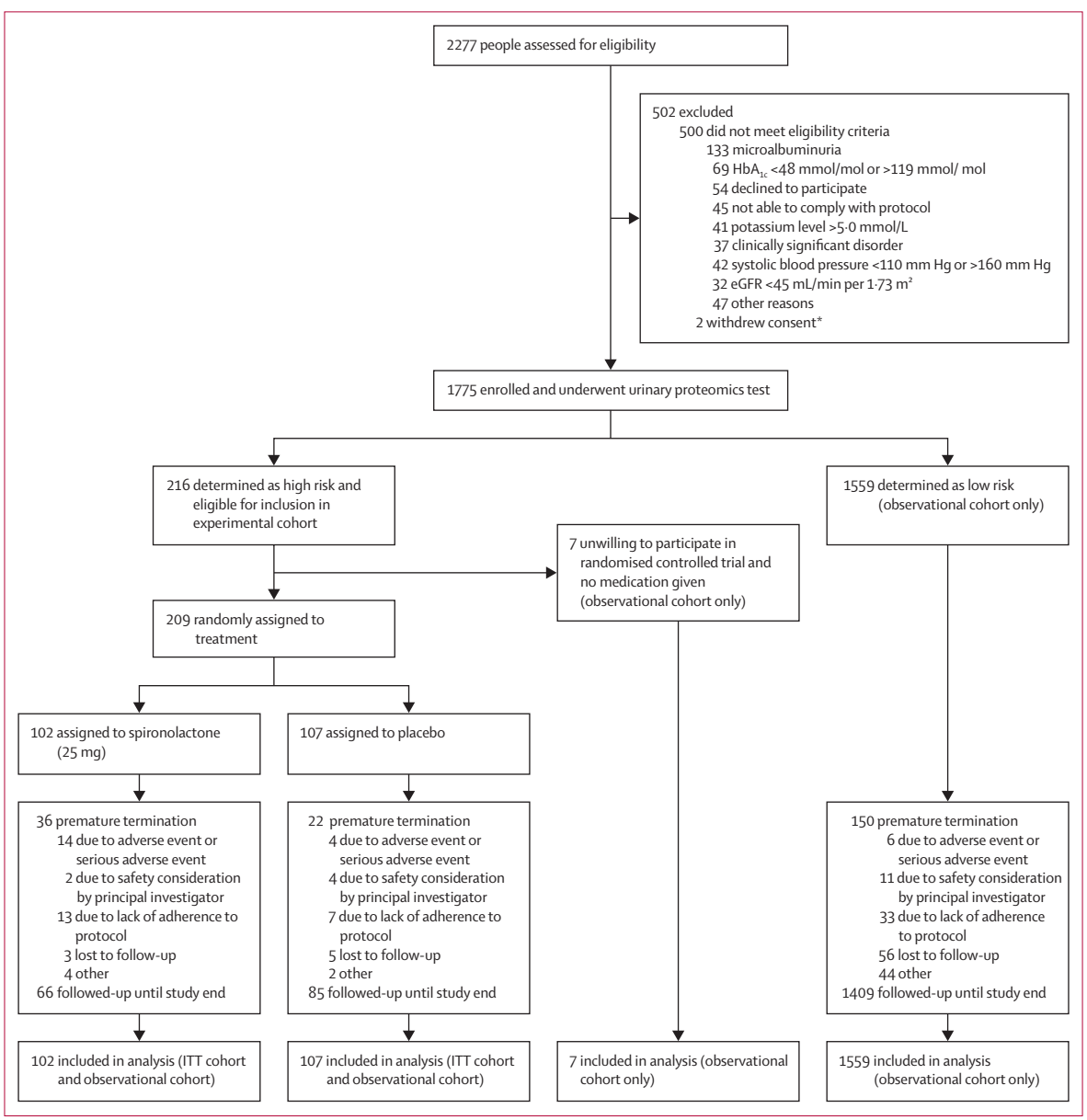

Figure 1: **Study profile**
ITT=intention-to-treat. *Consent forms were missing.

	Low-risk participants (n=1559)	High-risk participants*†	
		Spironolactone (n=102)	Placebo (n=107)
Sex			
Male	955 (61%)	69 (68%)	78 (73%)
Female	604 (39%)	33 (32%)	29 (27%)
Age, years	61 (9)	63 (6)	63 (7)
Known diabetes duration, years	11 (8)	14 (8)	14 (9)
BMI, kg/m²	30 (5)	30 (5)	31 (6)
Systolic blood pressure, mm Hg	133 (12)	135 (12)	134 (12)
Diastolic blood pressure, mm Hg	78 (9)	79 (9)	79 (9)
eGFR, mL/min per 1·73 m²	88 (15)	81 (15)	82 (19)
UACR, mg/g	5 (3 to 8)	7 (4 to 12)	7 (4 to 12)
Potassium, mmol/L	4·2 (0·4)	4·3 (0·5)	4·2 (0·4)
Sodium, mmol/L	140 (3)	139 (3)	140 (3)
HbA_{1c}, mmol/mol	57 (12)	58 (13)	59 (13)
HbA_{1c}, %	7·3 (1·1)	7·5 (1·2)	7·5 (1·2)
Total cholesterol, mmol/L	4·4 (1·0)	4·4 (1·1)	4·3 (1·1)
HDL cholesterol, mmol/L	1·2 (0·3)	1·2 (0·3)	1·2 (0·4)
LDL cholesterol, mmol/L	2·4 (0·9)	2·4 (1·1)	2·3 (1·0)
Triglycerides, mmol/L	1·6 (1·1 to 2·3)	1·8 (1·2 to 2·6)	1·7 (1·2 to 2·6)
CKD273, arbitrary units	−0·4 (0·3)	0·4 (0·2)	0·3 (0·2)
Smoking status			
Current	223 (14%)	12 (12%)	8 (7%)
Never	861 (55%)	56 (55%)	56 (52%)
Former	468 (30%)	34 (33%)	43 (40%)
Unknown	7 (<1%)	0	0
ACE inhibitor or ARB use	952 (61%)	90 (88%)	93 (87%)
Follow-up time, years	2·5 (2·0 to 3·0)	2·5 (2·1 to 3·0)	2·5 (2·0 to 3·1)

Data are mean (SD), median (IQR), or n (%). eGFR=estimated glomerular filtration rate. UACR=urine albumin-to-creatinine ratio. ACE=angiotensin-converting enzyme. ARB=angiotensin II receptor blocker *Compared with baseline description,[17] two participants were excluded in the high-risk group after inspection was not able to identify informed consent forms. †Seven high-risk participants were not randomly assigned to intervention and are not included here; baseline data for all 216 high-risk participants are in the appendix (p 15).

Table 1: **Baseline characteristics of study population by low-risk and high-risk CKD273 subgroup and assigned treatment**

We estimated that the study required 2000 participants to be included in the observational cohort to accomplish this effect size and to account for 15% withdrawal from the study. The sample size was decreased from the originally calculated total of 3280 participants, which was expected to provide 656 high-risk participants, due to a protocol amendment after a revised sample size calculation based on a review of the treatment effect of mineralocorticoid receptor antagonists.[13]

We report continuous variables as mean (SD) for normally distributed data or median (IQR) for skewed data, and compare between groups using an unpaired *t* test (with skewed data log-transformed before comparison between groups). We used a χ^2 test for comparison of categorical data. The observational cohort includes all participants with valid proteomic score (ie, passing prespecified quality control criteria) and data at baseline visit. For the primary endpoint, we did a comparison between the high-risk and low-risk groups in the observational cohort of their progression to persistent microalbuminuria using an unadjusted Cox-regression model with χ^2 test. We repeated this analysis adjusting for the baseline variables of age, sex, HbA_{1c}, systolic blood pressure, retinopathy, eGFR, and UACR at baseline. In additional analyses, we also adjusted for glucose-lowering, antihypertensive, and diuretic medication at baseline or started during follow-up, and for HbA_{1c} during follow-up. To assess the added value of the risk score, we calculated the increase in area under the curve (AUC) of the receiver operator characteristic curve (ROC) when the CKD273 score was added to the model with the baseline variables. Because high-risk individuals who were given spironolactone potentially could have a reduced rate of progression to microalbuminuria, we did a post-hoc sensitivity analysis to compare progression to microalbuminuria in high-risk participants treated with placebo with low-risk participants in the observational cohort.

For the secondary objective of the primary endpoint (effect of spironolactone in high-risk participants), we did a comparison between spironolactone and placebo treatment in the intention-to-treat trial cohort with a Cox-regression model including data on progression to confirmed microalbuminuria (the primary endpoint). We repeated the analysis adjusting for glucose-lowering, antihypertensive, and diuretic medication at baseline or started during follow-up, and for HbA_{1c} during follow-up. The intention-to-treat trial cohort consisted of all participants with a valid proteomic score with a high-risk pattern who were randomly assigned to receive study medication. We applied a linear mixed model to assess changes in UACR and eGFR over time, and for UACR with adjustment for UACR at baseline followed by truncation to weeks in the study period.

Adverse events and serious adverse events were recorded for the intention-to-treat trial population and are reported in tabulated form without significance testing; adverse event data were not collected for the observational cohort (ie, the low-risk participants who did not receive a study intervention).

We regarded a two-tailed p value of less than 0·05 to be significant. We used SAS Enterprise Guide version 7.1 for statistical analyses.

This study is registered with the EU Clinical Trials Register (EudraCT 20120-004523-4) and ClinicalTrials.gov (NCT02040441) and is completed.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and the two first authors and the study steering committee had final responsibility for the decision to submit for publication.

Results

Between March 25, 2014, and Aug 31, 2016, 2277 people were screened for eligibility and 1775 participants were included, of whom 1104 (62%) were men, and the mean age was 62 years (SD 8). The main reason for not passing screening was the presence of microalbuminuria (n=133; figure 1). Of the included participants, 216 (12%) were identified as being in the high-risk group on the basis of CKD273 proteomic pattern, with 1559 (88%) in the low-risk group. Of the high-risk participants who were eligible for inclusion in the randomised controlled trial, seven were unwilling to participate but were still included in the observational cohort. The rest of the high-risk participants were randomly assigned to spironolactone (n=102) or placebo (n=107; intention-to-treat trial cohort). Baseline characteristics for the analysable population are shown in table 1, with data for the full enrolled population shown in the appendix (p 15). By comparison with low-risk individuals, the high-risk group were more likely to be male, were older, and had a longer diabetes duration, lower eGFR, and higher UACR (p<0·02 for all; appendix pp 14–15).

The trial ended with the last study visit on Sept 30, 2018. The median follow-up time was 2·51 years (IQR 2·0–3·0). In the low-risk group, 150 (9·6%) participants did not complete the follow-up period (figure 1). Of the 209 participants in the intention-to-treat trial cohort, the median follow-up time was 2·5 years (IQR 2·0–3·1) and 36 (35%) participants in the spironolactone group and 22 (21%) in the placebo group withdrew from the study early (figure 1). During follow-up, more participants initiated treatment with glucose-lowering and blood pressure-lowering medication in the high-risk group than in the low-risk group, but no difference was seen between the treatment groups in the intention-to-treat trial cohort (appendix pp 19–20).

The primary endpoint of confirmed microalbuminuria was more frequent in high-risk individuals (61 [28%] of 216 patients) than in low-risk individuals (139 [9%] of 1559; log-rank p<0·0001; figure 2A). In a Cox-regression model, the hazard ratio (HR; high *vs* low risk) was 3·92 (95% CI 2·90–5·30; p<0·0001; table 2). After adjustment for baseline age, sex, HbA_{1c}, systolic blood pressure, retinopathy, UACR, and eGFR, the HR was 2·48 (1·80–3·42; p<0·0001; figure 2A). Adding CKD273 score to the model with these baseline variables increased the AUC of the ROC curve from 0·76 to 0·78 (p=0·0040; appendix p 8). Also, addition of CKC273 score strengthened the association (β integrated discrimination improvement [IDI] was 0·10 and relative IDI was 0·10; p=0·0009; appendix p 8). Additional adjustment for glucose-lowering or antihypertensive and diuretic medication at baseline or started during follow-up, or for HbA_{1c} during follow up, did not change the HR (data not shown; appendix pp 6–7).

In participants with an eGFR greater than 60 mL/min per 1·73 m² at baseline in the observational cohort (n=1607), development of chronic kidney disease stage 3 was more frequent in high-risk individuals (48 [26%] of 184) than in low-risk individuals (119 [8%] of 1423; HR 3·50; 95% CI 2·50–4·90, after adjustment for baseline variables; table 2). Few participants developed chronic kidney disease stage 4 (eGFR <30 mL/min per 1·73 m²) during the study period (seven [3%] in high-risk *vs* three (0·19%) in low-risk participants; HR 16·70, 4·31–64·67; p<0·0001). A decrease in eGFR of 30% from baseline was seen in 42 (19%) high-risk participants compared with 62 (4%) low-risk participants (HR adjusted for baseline eGFR and UACR: 5·15, 3·41–7·76; p<0·0001; post hoc). A 40% decrease in eGFR from baseline was seen in 15 (7%) high-risk participants compared with 22 (1%) low-risk participants (HR adjusted for eGFR and UACR: 4·84, 2·43–9·68; p<0·0001). A doubling in serum creatinine from baseline was seen in nine (4%) high-risk versus nine (1%) low-risk participants (HR adjusted for baseline eGFR and UACR: 7·49, 2·97–18·90; p<0·0001; post hoc). No participants developed end-stage kidney disease.

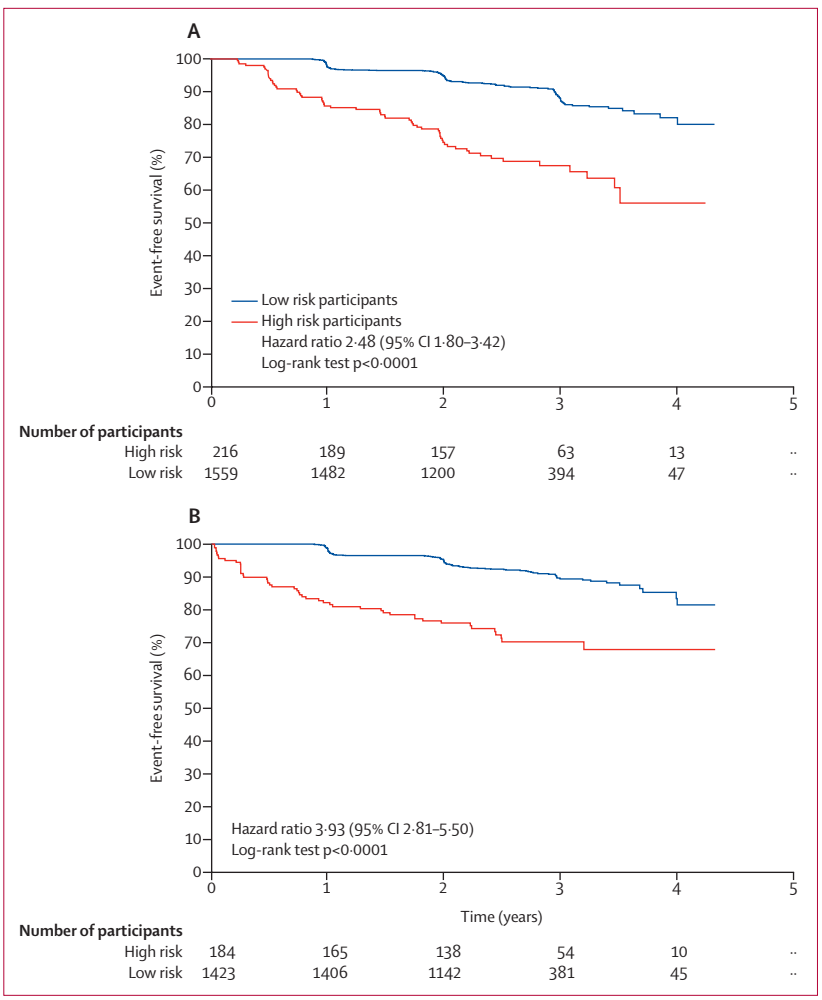

Figure 2: **Progression to renal endpoints according to CKD273 risk score status in the observational cohort**
(A) Microalbuminuria in the observational cohort. (B) Decrease in renal function and progression to chronic kidney disease stage 3 (eGFR <60 mL/min per 1·73 m²) in participants with baseline eGFR >60 mL/min per 1·73 m².

	Low-risk participants (n=1559)	High-risk participants (n=216)	Endpoint measure (95% CI)	p value
Primary endpoint				
Microalbuminuria (confirmed)	139 (8·9%)	61 (28·2%)	HR 3·92 (2·90–5·30)	<0·0001
Secondary endpoints				
Microalbuminuria (single value)	288 (18·5%)	99 (45·8%)	HR 3·68 (2·93–4·62)	<0·0001
Macroalbuminuria (confirmed)	22 (1·4%)	2 (0·01%)	HR 0·66 (0·15–2·81)	0·57
Chronic kidney disease stage 3 (eGFR <60 mL/min per 1·73 m²)*	119 (7·6%)	48 (22·2%)	HR 3·50 (2·50–4·90)	<0·0001
Fatal and non-fatal cardiovascular outcome†	53 (3·4%)	12 (5·6%)	HR 1·77 (0·92–3·22)	0·089
Ischaemic heart disease	24 (1·5%)	7 (3·2%)	HR 2·22 (0·96–5·2)	0·063
Stroke	15 (0·96%)	4 (1·9%)	HR 1·99 (0·66–6·0)	0·22
Congestive heart failure	8 (0·51%)	2 (0·93%)	HR 1·96 (0·42–9·21)	0·72
All-cause mortality	11 (0·62%)	2 (0·93%)	HR 1·41 (0·31–6·37)	0·65
Development of retinopathy or laser treatment (self-reported)	144 (9·2%)	21 (9·7%)	HR 1·02 (0·65–1·62)	0·93
Retinopathy	101 (6·5%)	14 (6·5%)	HR 0·96 (0·55–1·68)	0·89
Laser treatment for retinopathy	54 (3·5%)	9 (4·2%)	HR 1·21 (0·56–2·44)	0·60
Change in UACR, % per year	2·6 (0·85)	7·1 (1·14)	4·50 (2·70–6·20)	<0·0001
Change in eGFR, mL/min per 1·73 m² per year	0·47 (0·19)	1·37 (0·34)	0·90 (0·14–1·67)	0·206

Data are n (%) or mean (SE), unless otherwise indicated, and endpoint measures are either HRs or differences. p values are calculated from χ^2 test. eGFR=estimated glomerular filtration rate. HR=hazard ratio. UACR=urine albumin-to-creatinine ratio. *For patients with eGFR >60 mL/min per 1·73 m² at baseline. †Comparison of composite fatal and non-fatal cardiovascular outcome (myocardial infarction, stroke, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, hospital admission for heart failure or cardiovascular disease) and all-cause mortality during the study.

Table 2: **Primary and secondary endpoints in the observational cohort**

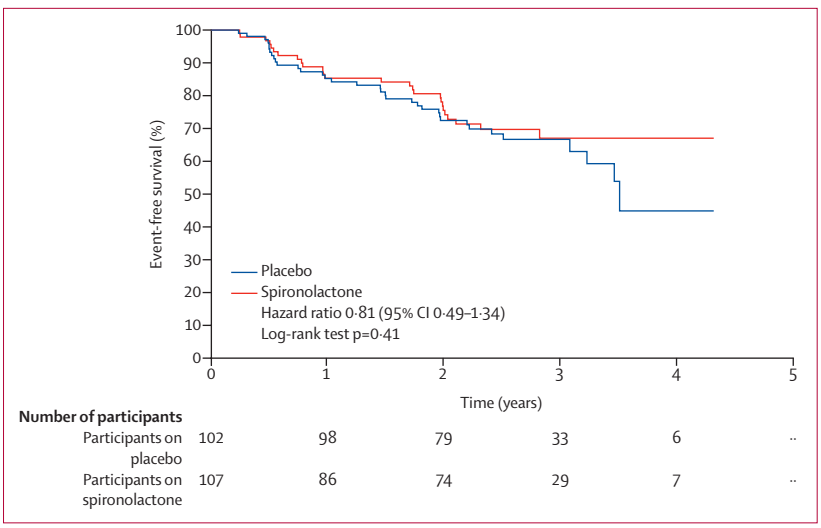

Figure 3: **Effect of spironolactone on progression to microalbuminuria in the intention-to-treat trial population (high-risk participants)**

We identified a faster progression of albuminuria in high-risk participants, after adjustment for baseline UACR, at 7·1% per year (SE 1·14), than in low-risk participants, at 2·6% per year (0·85). Similarly, decrease in eGFR was faster in high-risk participants (appendix p 10). No difference was seen in HbA_{1c} or blood pressure during the study in high risk versus low-risk participants (appendix p 9).

Fatal and non-fatal cardiovascular events were seen in 53 (3·4%) low-risk participants and 12 (5·6%) high-risk participants (HR 1·77, 95% CI 0·92–3·22; p=0·089). For all-cause mortality, 11 (0·62%) low-risk participants and two (0·93%) high-risk participants died during the study period (1·41, 0·31–6·37; p=0·65; table 2).

For the secondary study objective of assessing the effect of spironolactone in high-risk participants (as determined by CKD273), the results of the randomised controlled trial of spironolactone compared with placebo showed no significant difference between the groups in the development of confirmed microalbuminuria, with 35 (33%) of 107 participants in the placebo group developing microalbuminuria compared with 26 (25%) of 102 in the spironolactone group (HR 0·81, 95% CI 0·49–1·34; p=0·41; figure 3, table 3; appendix p 13). We had anticipated a 40% reduction in albuminuria progression, which cannot be excluded because it is within the 95% CI. Additional adjustment for glucose-lowering or antihypertensive and diuretic medications started during the study, or for HbA_{1c} during the study, did not affect the HR for the intervention (data not shown). Because spironolactone treatment could mitigate progression to microalbuminuria in high-risk participants, we did a post-hoc sensitivity analysis comparing high-risk participants assigned to placebo with low-risk participants (HR 4·23, 95 % CI 2·92–6·12; p<0·0001; appendix p 13).

Development of chronic kidney disease stage 3 (in participants with baseline eGFR >60 mL/min per 1·73 m²) was seen in 15 (17%) participants in the placebo group and 33 (36%) in the spironolactone group (HR 2·62, 95% CI 1·42–4·82; p=0·0021; appendix p 12). Development of chronic kidney disease stage 4 was seen in four (4%)

	Spironolactone group (n=102)	Placebo group (n=107)	Endpoint measures (95% CI)	p value
Primary endpoint				
Microalbuminuria confirmed	26 (26%)	35 (33%)	HR 0·81 (0·49 to 1·34)	0·41
Secondary endpoints				
Microalbuminuria (single value)	42 (41%)	57 (53%)	HR 0·76 (0·51 to 1·14)	0·18
Macroalbuminuria (confirmed)	0	2 (2%)	HR 0·00 (0·00 to 5·59)	0·52
Chronic kidney disease stage 3 (eGFR <60 mL/min per 1·73 m²)*	33 (32%)	15 (14%)	HR 2·88 (1·56 to 5·30)	0·0007
Fatal and non-fatal cardiovascular outcome†	4 (4%)	8 (7%)	HR 0·57 (0·17 to 1·88)	0·35
Ischaemic heart disease	4 (4%)	3 (3%)	HR 1·45 (0·33 to 6·70)	0·60
Stroke	0 (0%)	4 (3·7%)	HR 0·00 (0·00 to 1·59)	0·14
Congestive heart failure	1 (1%)	1 (1%)	HR 1·14 (0·071 to 18·2)	0·93
All-cause mortality	1 (1%)	1 (1%)	HR 1·13 (0·071 to 18·1)	0·93
Development of retinopathy or laser treatment (self-reported)	14 (14%)	4 (4%)	HR 2·82 (1·08 to 7·40)	0·034
Retinopathy	9 (9%)	4 (4%)	HR 2·71 (0·84 to 8·82)	0·097
Laser treatment for retinopathy	9 (9%)	2 (2%)	HR 4·22 (0·88 to 20·3)	0.073
Change in UACR, % per year	6·8 (2·5)	6·4 (2·3)	0·38 (–6·2 to 7·0)	0·91
Change in eGFR, mL/min per 1·73 m² per year	–1·52 (0·54)	–1·33 (0·49)	0·18 (–1·25 to 1·60)	0·80
Change in eGFR from week 13, mL/min per 1·73 m² per year	–1·33 (0·68)	–1·26 (0·64)	0·07 (–1·8 to 2·0)	0·94

Data are n (%) or mean (SE), unless otherwise indicated, and endpoint measures are either HRs or differences. p values are calculated from χ^2 test. HR=hazard ratio. UACR=urine albumin-to-creatinine ratio. eGFR=estimated glomerular filtration rate. *For patients with eGFR >60 mL/min per 1·73 m² at baseline. †Comparison of composite fatal and non-fatal cardiovascular outcome (myocardial infarction, stroke, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, hospital admission for heart failure or cardiovascular disease) and all-cause mortality during the study.

Table 3: **Primary and secondary endpoints in the trial intention-to-treat cohort**

participants in the placebo group and three (3%) in the spironolactone group (HR 0·83, 95% CI 0·19–3·71; p=0·806). No difference in the change in eGFR over time was seen between the two treatment groups, particularly after a small decrease from week 0 to week 13, suggesting a haemodynamic effect (table 3; appendix p 10). A decrease in eGFR of 30% occurred in 24 (24%) participants in the spironolactone group compared with 18 (17%) in the placebo group (HR 1·61, 0·87–2·96; p=0·13). A 40% decrease in eGFR from baseline was seen in eight (7%) participants in the placebo group and seven (7%) in the spironolactone group (1·00, 0·36–2·75; p=0·996). Participants who were given spironolactone had similar HbA_{1c} and blood pressure to those who were given placebo (appendix p 11).

Fatal and non-fatal cardiovascular events were seen in four (4%) participants in the spironolactone group and in eight (7%) in the placebo group (HR 0·57, 0·17–1·88; p=0·35; table 3). For all-cause mortality, one (1%) participant in the spironolactone group and one (1%) in the placebo group died (HR 1·13, 0·07–18.10; p=0·93; table 3). The death in the placebo group was due to a cardiac event (considered possibly related to study drug) and the death in the spironolactone group was due to cancer, and deemed unrelated to study drug.

Adverse events for the intention-to-treat trial groups are shown in table 4 and the appendix (pp 17–18). Of the safety events of special interest, development of gynaecomastia resulted in discontinuation of study medication in three (3%) participants in the spironolactone group and none in the placebo group, and hypotension led to discontinuation of study medication in a further three (3%) participants in the spironolactone group and one (1%) participant in the placebo group. Increased serum potassium (>5·5 mmol/L) occurred in 13 (13%)

	Spironolactone group (n=102)	Placebo group (n=107)
Any adverse events (total number)	312	321
Any adverse events (patients with at least one)	82 (82%)	86 (80%)
Adverse events leading to discontinuation of study drug	25 (25%)	10 (9%)
Any serious adverse event (patients with at least one)	34 (33%)	22 (21%)
Any serious adverse event	17 (17%)	21 (20%)
Serious adverse event considered related to study drug	2 (2%)	1 (1%)
Death	1 (1%)	1 (1%)
Events of special interest		
Hyperkalaemia based on adverse event reporting	9 (9%)	1 (1%)
Event of plasma potassium >5·5 mmol/L	13 (13%)	4 (4%)
Gynaecomastia	3 (3%)	0
Hypotension	3 (3%)	1 (1%)
Development of chronic kidney disease stage 3 (eGFR <60 mL/min per 1·73 m²)	33 (32%)	15 (14%)
Development of chronic kidney disease 4 (eGFR <30 mL/min per 1·73 m²)	3 (3%)	4 (4%)
30% decrease in eGFR from baseline	24 (24%)	18 (17%)
40% decrease in eGFR from baseline	7 (7%)	8 (7%)

Data are number of events or number (%) of participants with a specified event. eGFR=estimated glomerular filtration rate.

Table 4: **Adverse events in the trial intention-to-treat cohort**

participants in the spironolactone group and four (4%) in the placebo group.

Discussion

To our knowledge, PRIORITY is the first prospective study to use a proteomics-based signature for risk stratification followed by a risk-based intervention in diabetic kidney disease. We found that in normoalbuminuric individuals with type 2 diabetes and preserved renal function, higher CKD273 classifier scores were associated with increased risk of progression to confirmed microalbuminuria independent of clinical markers. The high-risk CKD273 pattern was also associated with a decrease in renal function, as determined by progression to chronic kidney disease stage 3 and 4 or decrease in eGFR. This finding confirms our primary hypothesis that individual risk can be assessed early in the course of type 2 diabetes on the basis of urinary proteomics. However, compared with placebo, treatment with the mineralocorticoid receptor antagonist spironolactone did not delay development of microalbuminuria or impaired renal function.

Currently, in clinical practice, confirmed microalbuminuria is used as a marker for onset of diabetic kidney disease and increased risk of cardiovascular disease, although the underlying pathology might vary. In the presence of established microalbuminuria, RAAS blockade reduces progression to macroalbuminuria,[18] and a multifactorial intervention targeting cardiovascular risk factors can reduce renal and cardiovascular morbidity and mortality.[19] Although microalbuminuria is the earliest clinical index of renal damage, histological changes might already be advanced by the time it is detectable;[20] thus earlier identification of at-risk individuals is essential to guide targeted preventive therapy.[21] Increases in urinary albumin to microalbuminuria levels or higher are not only strongly associated with progression to more serious clinical endpoints, such as clinically significant loss of renal function and eventually end-stage kidney disease, but also with an increased risk of cardiovascular complications.[22] Furthermore, use of progression to microalbuminuria as a study endpoint is currently the only option for research into early intervention aiming to prevent or delay onset of diabetic kidney disease. In addition to its clinical application in practice, the CKD273 pattern could also be used for participant enrichment of future clinical studies, helping investigators to select a high-risk population for progression of albuminuria.

Previous retrospective analyses of cross-sectional or longitudinal studies, which did not apply standardised protocols for collection, storage, transportation, or analysis of samples, showed that a high CKD273 score was associated with progression of renal disease in people with and without type 1 or type 2 diabetes.[11,23–25] In our study, not all participants with a high CKD273 risk score progressed to microalbuminuria during the study period (median follow-up 2·5 years). With longer follow-up, more participants could potentially progress to microalbuminuria, and the higher withdrawal rate in the high-risk group than in the low-risk group (28% *vs* 10%) could have led to a small underestimation of progression in the high-risk group. Findings from previous studies have suggested that an increase in CDK273 risk score precedes development of increased albuminuria within 3–5 years.[11] The increase in the AUC of the ROC curve was significant when CKD273 was added to the clinical variables. However, the clinical significance of this finding could be debated, because the change in AUC was small. The small size of the increase in AUC might result from the fact that the AUC was already high (0·76) with the clinical variables alone and that AUC is a conservative measure of added value; this suggestion is supported by the significant improvement in the discrimination index. The small increase in AUC might also suggest that the CKD273 risk score was not optimally predictive in the context of the current study design and duration. In another study that included 2672 people who were primarily diagnosed with diabetes (type 1 and 2) in which rapid decrease in eGFR during the study period was the primary endpoint,[9] CKD273 had a stronger association with change in UACR in those with baseline eGFR of more than 70 mL/min per 1·73 m², supporting the use of CKD273 in the present study population who had preserved renal function. In our study, only 12% of participants were classified as high risk according to the CKD273 score, which is less than the expected 15% based on a previous study cohort study of 700 individuals with type 2 diabetes and normoalbuminuria.[10]

The results of the proteomic analyses were available within 3 days of samples being received at the laboratory, which shows the feasibility of the test in a clinical setting. At present, urinary proteomic analysis is a high-end technology with costs that are substantially higher than those for urine albumin testing. The analysis currently costs €850 per sample and a laboratory can do the analysis on shipped samples (as in the trial) and has a scalable platform, but currently the method is only available at three locations (Hannover, Germany; Glasgow, UK; and Toulouse, France) and cannot be set up in local laboratories. Results of health economic analyses[26] previously suggested that use of CKD273 could be cost-effective in patients with type 2 diabetes at the current price. This potential cost-effectiveness requires that the CKD273 score is associated with development of microalbuminuria, and that progression can be prevented or delayed with preventive treatment.[26] Our study confirms the first of these criteria. In particular, this method could be cost saving in people at high risk of complications related to cardiovascular disease. If risk of cardiovascular disease is low, the proteomic method is not cost-effective, but most people with type 2 diabetes are at increased risk of cardiovascular disease. Irrespective of the potential cost-effectiveness, restrictions on reimbursement in health systems might still limit the use of urinary proteomic analysis in practice, particularly in settings where screening with low-cost

methods such as albuminuria and eGFR has not been implemented. Alternative uses could be in clinical trials for selection of high-risk individuals or for assessment of response to interventions.

Mineralocorticoid receptor antagonists have been shown to reduce albuminuria when added to ongoing RAAS blockade in patients with diabetes and micro-albuminuria or macroalbuminuria in short-term trials of up to 1 year.[27,28] These drugs are expected to reduce fibrosis, and, because CKD273 is largely composed of peptides related to changes in the extracellular matrix,[29] we expected spironolactone to be effective in high-risk individuals defined by CKD273 score. Our negative finding with respect to the effect of spironolactone on development of microalbuminuria could relate to study power, study duration, or absence of effect in this population. One limitation of our study was that the number of high-risk participants identified and randomly assigned to spironolactone or placebo was lower than anticipated in our sample size calculation. The event rate in the placebo group was also lower than expected, reducing study power. Decrease in eGFR over time was an additional secondary endpoint, which was similar in the spironolactone and placebo groups, particularly if calculated from week 13 after the initial decrease in eGFR with spironolactone. By contrast, more participants in the spironolactone group than in the placebo group developed chronic kidney disease stage 3, potentially due to acute haemodynamic effects, and no significant difference was seen between the treatment groups in the proportion of participants who had a 30% decrease in eGFR from baseline. Treatment was generally well tolerated, as shown by the few and relatively mild events of hyperkalaemia, and gynaecomastia or hypotension were rare events. Otherwise adverse events were considered to be unrelated to study treatment. Alternative interventions that have shown potential renal benefits in people with diabetes should be tested in individuals identified by urinary proteomics to be at high risk of diabetic kidney disease, such as non-steroidal mineralocorticoid receptor antagonists,[30] GLP-1 receptor agonists, and SGLT2 inhibitors.[5]

Our trial has some limitations. The risk stratification into high-risk and low-risk groups was based on proteomic analysis of a single urine sample and thus day-to-day variability can not be ruled out. We expect that the day-to-day variation in CKD273 risk score is not a limitation.[8] This day-to-day variability has not been extensively studied, but repeatability has been tested with 100% correct classification of cases with chronic kidney disease and controls on several occasions.[15] Additionally, the relative intra-assay coefficient of the CKD273 risk score was 7%.[15] Microalbuminuria is an accepted clinically relevant surrogate for diabetic kidney disease, but is not approved by regulatory agencies, although a recent conference with European Medicines Agency and the US Food and Drug Administration discussed observational and clinical trial data showing a strong association between changes in albuminuria and long-term renal outcomes including end-stage kidney disease.[31] Since the PRIORITY study was designed, relative changes in eGFR of 30% or 40% have been suggested as outcomes for use in clinical trials, and categorisation as high risk on the basis of CKD273 risk score was also strongly associated with these outcomes in PRIORITY, although we used one-time measurements without repeat testing for confirmation. Of these suggested outcomes, we had prespecified a 40% change in eGFR as an endpoint in the statistical analysis plan, whereas a 30% change in eGFR was a post-hoc defined endpoint. The estimated HR for progression to microalbuminuria for participants in the high-risk group in the observational study might be falsely low because half of the high-risk group were treated with spironolactone; however, spironolactone treatment had no effect on albuminuria compared with placebo, and exclusion of participants assigned to receive spironolactone did not change the HR. If spironolactone had also been tested in the low-risk group, the study power could have been increased, but such a change would also have exposed many participants without progression to the medication. Because the low-risk group was not randomly assigned to spironolactone or placebo, the randomised controlled trial part of our study cannot asses if spironolactone could be beneficial in this population or if screening and treatment is superior to non-screening, which would be particularly relevant if spironolactone had shown benefit. The major strengths of our study include a large, well phenotyped cohort and prospective study design with a median follow-up of 2·5 years. In the study protocol, additional, unmasked follow-up was anticipated, but independent of the study findings, the additional clinical follow-up has not been possible because of insufficient funding. However, we are aiming to do register-based follow-up where possible.

In conclusion, a high-risk score from the urinary proteomic classifier CKD273 was associated with an increased risk of progression to microalbuminuria and impaired renal function during a median of 2·5 years of follow-up in patients with type 2 diabetes and normo-albuminuria, independent of clinical characteristics. However, spironolactone did not prevent progression of albuminuria in these high-risk participants.

Contributors

PR, HM, HvdL, CD, and ML contributed to the design of the study. All authors contributed to data collection. PR, ML, and NT wrote the first draft of the report. All authors were involved in data analysis and interpretation, and in drafting and critically revising the report. All authors had access to study results and the first authors and corresponding author assume responsibility for the integrity and accuracy of the data reported. All authors reviewed and approved the final submitted version of the report.

Declaration of interests

JB reports honoraria and lecture fees from Amgen, Boehringer Ingelheim, and Nipro. ML has equity interest in Novo Nordisk A/S. ALB reports giving lectures for and serving on advisory boards for AstraZeneca, Boehringer Ingelheim, Novo Nordisk, and Sanofi. GC reports giving lectures and receiving meeting support from NAPP Pharmaceuticals.

AK reports research grants from AstraZeneca, MSD, and Novo Nordisk, all given to Bethesda Diabetes Research Center. AO reports research grants from Sanofi and Amgen; lecture fees from Sanofi, Amgen, Fresenius Medical Care, Amicus, and Kyowa-Kyrin; and serving as a consultant for Freeline, Sanofi, and Amicus. FP reports research grants from AstraZeneca, Novo Nordisk, and Novartis; lecture fees from Novartis, Eli Lilly, MSD, AstraZeneca, Sanofi, and Boehringer Ingelheim; and serving as a consultant for AstraZeneca, Bayer, Amgen, Novo Nordisk, and MSD. JRP reports receiving travel support and payment via the University of Glasgow for advisory and consultancy work on behalf of Novo Nordisk; non-financial support in the form of study medication (for the REMOVAL study) from Merck (Germany) and Itamar Medical; personal fees for consultancy from Eli Lilly, ACI Clinical, Pfizer, and AstraZeneca; and research grants from Janssen. MS reports giving lectures for Bayer, Boehringer Ingelheim, Siemens Healthineers, and Takeda; serving as a consultant for Vifor Fresenius; and previously being a member of an advisory board of Otsuka. HM is the cofounder and co-owner of Mosaiques Diagnostics. MN reports lecture fees from Eli Lilly, AstraZeneca, Sanofi, Novo Nordisk, and Boehringer Ingelheim. JS and PZ are employees of Mosaiques Diagnostics. PR reports giving lectures for AstraZeneca, Bayer, Novo Nordisk, Merck, and Boehringer Ingelheim; serving as a consultant for AbbVie, AstraZeneca, Bayer, Eli Lilly, Boehringer Ingelheim, Astellas, Gilead, Mundipharma, and Novo Nordisk, with all fees given to Steno Diabetes Center Copenhagen; and having equity interest in Novo Nordisk. All other authors declare no competing interests.

Data sharing

Individual, de-identified participant data are not freely available because of the risk of patient re-identification, but interested parties can request access to de-identified participant data or anonymised clinical study reports through submission of a request for access to the corresponding author, provided that the necessary data protection agency and ethical committee approvals are provided in compliance with relevant legislation.

Acknowledgments

This study was funded by the European Union Seventh Framework Programme (FP7/20072-013) under grant agreement number 279277, and was overseen by a steering committee not including members from the study funder. We thank the patients who participated in the study for their commitment and enthusiasm. We also thank the study investigators (appendix pp 2–4), laboratory technicians, and study nurses for their valuable contributions to this work, and the members of the data monitoring committee (appendix p 4), who oversaw the safety of the study.

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