Research Article

Characteristics of high- and low-risk individuals in the PRIORITY study: Urinary proteomics and mineralocorticoid receptor antagonism for prevention of diabetic nephropathy in type 2 diabetes

Running title: Baseline characteristics in the PRIORITY study

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Conflict of interests:

M.L. has equity interest in Novo Nordisk A/S. PR reports having given lectures for Astra Zeneca, Bayer and Boehringer Ingelheim, and has served as a consultant for AbbVie, Astra Zeneca, Bayer, Eli Lilly, Boehringer Ingelheim, Astellas, Janssen, and Novo Nordisk, all fees given to Steno Diabetes Center Copenhagen, and has equity interest in Novo Nordisk. FP reports having received research grants from AstraZeneca and

Novartis and lecture fees from Novartis, Eli Lilly, MSD, AstraZeneca, Sanofi and Boehringer Ingelheim and having served as a consultant for Astra Zeneca, Bayer, Amgen, Novo Nordisk and MSD. H.M. is the co-founder and co-owner of Mosaiques Diagnostics.

Novelty statement:

- This paper describes baseline data from the first prospective multicentre study using the proteomics classifier CKD273 for risk stratification in individuals with normoalbuminuria and type 2 diabetes.
- Previously, post-hoc analyses have shown that CKD273 identifies individuals at high risk of developing DKD. This study demonstrates that the associations between the CKD273 proteomic pattern and traditional risk factors for DKD are weak with small numerical differences for the traditional risk factors. CKD273 may provide additional information on risk for DKD.
- Interesting differences among sites across Europe in prevalence of CKD273 pattern cannot be explained by traditional risk factors for DKD.

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Abstract

Aims

To compare clinical baseline data in individuals with type 2 diabetes and normoalbuminuria, at high- or low-risk for diabetic kidney disease (DKD) based on the urinary proteomics classifier CKD273.

Methods

Prospective, randomized, double-blind, placebo-controlled international multicentre clinical trial and observational study in participants with type 2 diabetes and normoalbuminuria, stratified into high- or low-risk groups based on CKD273 score. Here we present clinical baseline data in the whole cohort and by risk groups. By univariate and logistic regression the associations between CKD273 and traditional risk factors for DKD are evaluated.

Results

From 15 centres 1777 participants were included, with 12.3% having a high-risk proteomic pattern. Participants in the high-risk group (n=218), were more likely men, were older, had longer diabetes duration, lower eGFR and higher urine albumin-to-creatinine ratio (UACR) than low-risk participants (n=1559, $p<0.02$). Numerical differences were small and univariate regression analyses of CKD273 vs. each baseline variable demonstrated weak associations ($R^2 < 0.04$). In a logistic regression model including clinical variables known to be associated with DKD, eGFR, gender, logUACR and use of RAS-blocking agents remained significant determinants of CKD273 high-risk group, AUC 0.72 (95% CI: 0.68-0.75, $p<0.01$).

Conclusions

In this population of individuals with type 2 diabetes and normoalbuminuria, traditional DKD risk factors differed slightly between participants at high- and low-risk for DKD, based on CKD273. These data suggest that CKD273 may provide additional prognostic information over and above the parameters routinely available in the clinic. Testing the added value will be subject to our ongoing study.

Key words: screening, nephropathy, clinical trials

Introduction

Diabetic kidney disease (DKD) is a frequent and costly complication of diabetes. Despite established therapies, this complication is associated with substantial cardiovascular morbidity and mortality and is the leading cause of end stage renal disease (ESRD) in the Western world (1). DKD is in clinical practice diagnosed by albuminuria and/or decrease in estimated glomerular filtration rate (eGFR). Although treatment with RAS blocking agents in persons with micro- and macroalbuminuria and control of cardiovascular risk factors has improved outcome (2, 3), the prognosis is still poor. Use of the aldosterone receptor antagonist spironolactone on top of RAS inhibition has previously been shown to effectively further reduce albuminuria (4-6). However, long-term as well as larger studies with hard endpoints such as ESRD are missing.

Previous studies with RAS inhibition for prevention of microalbuminuria have shown conflicting results (7-10). To our knowledge, no studies using spironolactone as prevention of microalbuminuria have been conducted. Currently, there are no recommendations for prevention of development of microalbuminuria in diabetes, except for optimal control of metabolic and cardiovascular risk factors.

Biomarkers based on pathways leading to development and progression of DKD, have the potential to identify subjects at high risk of progression to renal complications. This would allow for early intervention only in a population at increased risk, thus allowing for better allocation of treatment. In 2010, Good et al. identified CKD273 a urinary biomarker pattern including 273 peptides significantly associated with overt kidney disease (11). This proteomics based pattern detected initiation and progression of DKD earlier than the currently used indicators (12-15), well preceding change in albuminuria class. However, all previous data on CKD273 derive from analysis of stored samples and post hoc analyses of previously conducted studies.

In the ongoing “Proteomic prediction and renin angiotensin aldosterone system inhibition prevention of early diabetic nephropathy in type 2 diabetic participants with normoalbuminuria” (PRIORITY) study we address the following questions: first, to validate that the proteomic classifier CKD273 can predict development of microalbuminuria in persons with type 2 diabetes and normoalbuminuria prospectively; second to determine whether intervention with a mineralocorticoid receptor antagonist (spironolactone) on top of standard therapy can reduce the risk of developing microalbuminuria in individuals with a high-risk CKD273 score.

For the present manuscript, the primary objective is to evaluate clinical data in individuals stratified according to CKD273 risk pattern in this first prospectively collected study population applying CKD273-based risk stratification. Secondly, to evaluate associations between CKD273 and traditional risk factors for DKD and compare high- and low-risk participants across centres to explore potential heterogeneity at study baseline.

Materials and methods

Study Design

PRIORITY is an investigator-initiated, prospective, randomized, double-blind, placebo-controlled international multicentre clinical trial and observational study in persons with type 2 diabetes and normoalbuminuria funded by the European Commission's Seventh Framework programme. The detailed rationale, study design and methods for PRIORITY have been published elsewhere (16).

Briefly, persons aged 18-75 with type 2 diabetes, preserved kidney function and normoalbuminuria, were included. The participants were required to fulfil the following inclusion criteria: normoalbuminuria (urine albumin-to-creatinine ratio (UACR) <30 mg/g) in at least two out of three consecutive morning void urine samples and eGFR >45 ml min^{-1} 1.73 m^{-2} at screening. Participants were stratified into high- or low-risk groups based on their CKD273 score, of a single urine sample collected at screening. High-risk was defined as CKD273-classifier score >0.154, low-risk as ≤0.154 as previously described (15, 16). Participants in the high-risk group were stratified based on use of RAS blocking agents and randomly assigned to either spironolactone 25 mg once daily or placebo, on top of standard care. The participants in the low-risk group are followed on standard care. The study period has been extended from 3 to 4.5 years in a protocol amendment, primarily due to delayed recruitment. Based on expected higher progression rates to microalbuminuria due to extension in treatment/observation time as well as new knowledge on treatment effect (10), estimated sample size was revisited with preserved statistical power. All participants are planned for a final visit in autumn 2018.

The protocol and amendments have been approved by the respective national competent authorities using in part the Voluntary Harmonisation Procedure. A positive opinion by the responsible ethical committees was obtained for each participating clinical site. All participants provided written

informed consent at screening and again after the protocol amendment. The study is conducted in accordance with the International Conference on Harmonisation – Good clinical practice (ICH-GCP), Declaration of Helsinki. An external independent data monitoring committee (DMC) will monitor safety throughout the study. EU Clinical Trials Register (EudraCT: 2012-000452-34) and http://www.clinicaltrial.gov (NCT02040441).

Biochemical and other analyses

At baseline biochemical samples for measurement of creatinine, HbA_{1c}, potassium, sodium, and lipids were analysed at the local routine laboratory at each study centre by standardised methods. eGFR was calculated at the local study centre and centrally by the CKD-EPI equation based on locally measured creatinine with a standardised method. UACR was measured at the central laboratory at Steno Diabetes Center Copenhagen using Vitros® 5600 MicroSlide. Samples were shipped frozen on dry-ice from study centres. Confirmed microalbuminuria was defined as UACR >30 mg/g in at least two of three first morning voids with 30% increase (geometric mean) in UACR from 'run-in-phase', or >40 mg/g (geometric mean).

Urine proteomics was performed by applying capillary electrophoresis mass spectrometry (CE-MS) analysis at Mosaiques Diagnostics in Hannover, Germany. In brief, this provides data on >1000 identified proteins or peptides and a predefined renal risk profile based on 273 peptides (CKD273). The limit of detection for individual peptides is ~ 1 fmol and mass resolution is above 8000, enabling resolution of monoisotopic mass signals for $z \leq 6$. Details on the analysis have previously been described (16, 17).

Medical history

Data collections regarding concomitant medication, medical history, smoking status and diabetes duration were based on local medical records and self-reporting. Hypertension was defined as medical history of hypertension or concomitant treatment with antihypertensive agents at baseline. Dyslipidaemia was defined as dyslipidaemia in the medical history or concomitant treatment with lipid-lowering agents.

Statistical analysis

Continuous variables are reported as means with standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for skewed data and are compared between groups using an unpaired t-test, skewed data are log transformed before comparison between groups. A chi-square test is used for comparison of categorical data. Correlations between baseline variables and CKD273 score are calculated from a linear regression model and presented as coefficients of determination (R^2) and beta-coefficients. Prediction of CKD273 high-risk group is calculated from clinical variables in a logistic regression model, including known risk factors for DKD (age, gender, diabetes duration, systolic blood pressure, eGFR, logUACR, HbA_{1c}, smoking, retinopathy and use of RAS-blocking agents) and in individual models with logUACR, eGFR and use of RAS-blocking agents. A receiver operating characteristic (ROC) curve based on the logistic regression model including known risk factors for DKD is presented. A two-tailed *p* value of <0.05 is considered significant. SAS Enterprise Guide version 7.1 (7.100.1.2711) (64-bit) by SAS Institute, Inc., Cary, NC, USA is used for statistical analysis.

Results

Enrolment

From March 25th 2014 through end of inclusion on August 31st 2016, a total of 2276 persons from 15 study centres in 10 countries were screened and 1777 participants were included. Of those, 218 participants were in the high-risk group and 1559 participants were in the low-risk group. The proportion of participants in the high-risk group in the whole study population was 12.3%. The high-risk rates varied considerably between study centres, ranging from 0% to 27% (Fig. s1).

The screening failure rate was 22% and varied between sites from 6% to 32%. The main reason for screening failure was presence of microalbuminuria with UACR >30 mg/g (n=133), followed by HbA_{1c} <48 mmol/mol (6.5%) or >119 mmol/mol (13%) (n=71) and declining to participate (n=58) as shown in the study flow diagram (Fig. s2). Individuals who were not included in the study had lower eGFR (p<0.01), higher UACR (p<0.01) and higher potassium (p<0.01) compared to included individuals (supplementary table s1). Screening failure was therefore most commonly due to previously unrecognised kidney disease at baseline.

Baseline characteristics and medication

In total, 1777 participants were included for proteomic assessment. Participants with a high-risk pattern differed from those with a low-risk pattern: high-risk participants were more likely men, were older, had longer diabetes duration, lower eGFR and higher UACR ($p<0.02$), (Table 1). As mentioned, there was a wide range in the proportion of high-risk participants between sites, but there were no systematic differences in the traditional risk markers for DKD between centres (supplementary table s2). With regards to baseline medication, there were also differences between the high- and low-risk groups (Table 2). Biguanides were more commonly used in the high-risk group than in the low-risk ($p<0.03$), ACEi was used more frequently in the high-risk than in the low-risk group, whereas the use of ARB was lower in the high-risk group ($p<0.01$). The baseline concomitant medication divided by study sites is listed in supplementary table s3.

Medical history

In the entire study population, 13% had a history of background diabetic retinopathy, 3% of proliferative diabetic retinopathy and 4% of diabetic maculopathy. Laser treatment before baseline was performed in 4%. At baseline 68% had a history of hypertension, 50% of dyslipidaemia and 12% of ischemic heart disease. No difference was detectable in the history of diabetic retinopathy or diabetic maculopathy between high- and low-risk groups ($p>0.62$), however the high-risk group differed from the low-risk group with more participants having a history of hypertension, dyslipidaemia and ischemic heart disease ($p<0.02$), (Table 1). The medical history according to study sites is shown in supplementary table s4.

Correlation analysis with established risk factors

Univariate regression analyses of CKD273 vs each baseline variable demonstrated weak associations with age, diabetes duration, BMI, systolic blood pressure, eGFR, UACR, HDL cholesterol and triglycerides ($p<0.04$), (Supplementary table s5). The strongest association was seen for UACR with R^2 of 0.04 and beta of 0.014 ($p<0.01$) and for eGFR with R^2 of 0.03 and beta of -0.005 ($p<0.01$), suggesting at maximum 4% and 3% of the variation in CKD273 score could be explained by the variables eGFR and UACR, respectively. Scatterplots of CKD273 and UACR

(Fig. s3) and of CKD273 and eGFR (Fig. s4) are provided in the supplementary material. In a logistic regression model predicting CKD273 risk stratification to the high-risk group, the area under the curve (AUC) for eGFR was 0.61 (95% CI: 0.56-0.65) ($p<0.01$), for logUACR 0.62 (95% CI: 0.58-0.66) ($p<0.01$) and for treatment with RAS blocking agents (either ACEi or ARB), the AUC was 0.64 (95% CI: 0.61-0.66) ($p<0.01$). In one model including a combination of ten known risk factors for DKD (gender, diabetes duration, systolic blood pressure, eGFR, logUACR, HbA_{1c}, smoking, retinopathy and use of RAS-blocking agents) the AUC was 0.72 (95% CI: 0.68-0.75) ($p<0.01$) (Fig. 1). In this model gender, eGFR, logUACR and use of RAS-blocking agents remained significant determinants of CKD273 high-risk group ($p<0.01$).

Discussion

In this study, we describe baseline data of the PRIORITY study, prospectively applying the urinary proteomic based CKD273 kidney disease risk classifier in a large population of individuals with normoalbuminuria and type 2 diabetes. The ability of CKD273 to add prognostic information beyond the already available clinical data including eGFR and albuminuria (within the normal range) has previously been demonstrated in post hoc analyses (13-15, 18). However, the current ongoing study aims to further verify these findings and to assess feasibility of this approach in the clinical setting. The aim of the current analysis is to evaluate if high-and low-risk participants based on CKD273 in this setting, are easily differentiated with the standard clinical data, in order to assess the potential added value of the classifier.

The study included people with type 2 diabetes and normoalbuminuria, from 15 sites in 10 European countries, on average with relatively long disease duration; in accordance with this, one third were being treated with insulin. Overall, participants had reasonably well controlled HbA_{1c}, lipids and blood pressure, with more use of ARB or ACEi, and normal kidney function with low albumin excretion and eGFR within the normal range. Small numerical differences were seen in baseline variables between the high- and low-risk groups. In particular, UACR, which is currently the best predictor of progression of DKD, was 5 (3–8) mg/g in the low-risk and 7 (4–12) mg/g in the high-risk group, being statistically, but not clinically, different. Weak correlations were seen between CKD273 and single baseline variables, with associations explaining <5% of the variability, suggesting that the proteomics score cannot be fully explained by established risk factors associated

with DKD. When combining the known traditional risk factors for DKD in one model, association with high-risk CKD273 score was seen.

Previous studies, all post-hoc analyses of cohorts collected for other purposes without applying a standardised protocol for collection, storage, transportation or analysis of samples, showed that a high CKD273 score was associated with progression of renal disease in persons without diabetes (18-20). Other studies focused on CKD273 as a risk predictor specifically in a population with diabetes. Zürbig et al. demonstrated that CKD273 predicted progression from normo- to microalbuminuria 1.5 years before microalbuminuria occurred and that progressors from micro- to macroalbuminuria could be identified by the classifier 3-5 years before disease progression in adjusted models (12). At baseline there was a trend towards progressors being older, male, with higher urine albumin excretion rate, lower eGFR and higher systolic blood pressure compared to non-progressors. This is similar to what we find in the current PRIORITY study. The findings were confirmed by Roscioni et al. also in a small case-control study, demonstrating that CKD273 predicted development of albuminuria stage on top of eGFR in a three year period, also when adjusting for baseline urinary albumin excretion and eGFR (21). In DIRECT-Protect 2, in participants with type 2 diabetes, 9.8% were identified as high-risk, a lower rate than in the current study; however a higher cut-point for the CKD273 score was applied (15). The participants had similar urinary albumin excretion rate and blood pressure at baseline; however, they were younger, had shorter diabetes duration than the current population and a lower eGFR, which could partly explain the lower than expected high-risk rate in PRIORITY. Pontillo et al. investigated a large population primarily diagnosed with diabetes (type 1 and 2) with eGFR decline >5 ml min^{-1} 1.73 m^{-2} per year as the primary endpoint (14). The authors reported that for baseline ranges of eGFR >70 ml min^{-1} 1.73 m^{-2}, CKD273 had a superior predictive value to urinary albumin excretion for fast eGFR decline. These findings support the use of CKD273 in the present study population with relatively high eGFR.

The average prevalence of the high-risk pattern was 12.3%, but ranged from 0 to 27% across centres. Although standardised procedures including sampling protocols are described for all centres, slight differences in sample handling and variances in diet and lifestyle between countries may occur, potentially influencing the urine proteome. The performance of CKD273 across centres was investigated by Siwy et al. in a case-control study where cases had macroalbuminuria and/or eGFR <45 ml min^{-1} 1.73 m^{-2} (22). The performance was similar across sites (AUC value 0.89-1.00).

The considerable variation in the high-risk CKD273 pattern rate between centres in PRIORITY might be explained by different recruitment strategies between centres, but when looking at the variation in baseline characteristics, there is no clear trend in the variables explaining the varying rates of high-risk individuals.

The study demonstrated that it is feasible to have the results of the proteomics analysis within three days and therefore to use the test in a clinical setting. The CE-MS analysis is a high-end technology and the cost is higher than testing for urinary albumin. However, if it proves to predict microalbuminuria and progression to microalbuminuria can be prevented or delayed with selected preventive treatment, it may well be cost effective (23). Moreover, as the technology is developed further, the expectation is that the cost may be reduced.

We recognise some limitations in our study. Data concerning medical history and other baseline parameters was partly self-reported, however monitored in accordance with good clinical practice (GCP). Even though the same in- and exclusion criteria were applied, it may have had an impact that some centres included participants from primary care, whereas others came from secondary care settings. However, the differences seen between sites might reflect the nature of a multicentre setting and thus the study population will provide a more generalizable result. The risk stratification to high- and low-risk was based on proteomics analysis of one urine sample. We expect that the variation is limited due to the large number of individual peptides included in the pattern (11), this issue has however not been extensively studied. Microalbuminuria is an accepted clinically relevant surrogate for DKD, although not an approved hard endpoint. However, in studies for prevention, it is nearly impossible to analyse hard endpoints since follow up of participants would last for decades. The major strengths of the study are the well-described phenotype of a large population with type 2 diabetes and the prospective design with 4.5 years planned follow-up.

In conclusion, in participants with type 2 diabetes and normoalbuminuria, established risk factors for DKD differed only slightly, with numerically small differences, between high- and low-risk participants, grouped according to the CKD273 score. Moreover, a limited correlation was seen between CKD273 and baseline variables, indicating that the proteomics score may not be explained by established risk factors and may thereby contribute additional information to the measures currently available in the clinic. Whether the classifier adds prognostic information compared to the clinical data will be evaluated with the follow-up of this cohort.

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Table 1 Baseline characteristics of the total study population and by CKD273 subgroup

	Included N = 1777	Low-risk[a] N = 1559	High-risk[b] N = 218	P-value (high vs. low)
Gender, men	1106 (62)	955 (61)	151 (69)	0.02
Age, years	63 [57-68]	63 [57-68]	64 [59-68]	< 0.01
Known diabetes duration, years	12 (8)	11 (8)	14 (8)	< 0.01
Body mass index, kg/ m^2	30 (5)	30 (5)	31 (5)	0.28
Systolic blood pressure, mmHg	133 (12)	133 (12)	135 (12)	0.03
Diastolic blood pressure, mmHg	78 (9)	78 (9)	79 (9)	0.51
Heart rate, bpm	75 (11)	74 (11)	75 (12)	0.36
eGFR, ml min^{-1} 1.73 m^{-2}	87 (16)	88 (15)	81 (17)	< 0.01
UACR, mg/ g	5 [3 – 9]	5 [3 – 8]	7 [4 – 12]	< 0.01
Potassium, mmol/ L	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)	0.08
Sodium, mmol/ L	140 (2)	140 (2)	140 (3)	0.89
HbA_{1c}, mmol/ mol	57 (12)	57 (12)	59 (13)	0.03
HbA_{1c}, %	7.4 (1.1)	7.3 (1.1)	7.5 (1.2)	0.03
Total cholesterol, mmol/ L	4.4 (1.0)	4.4 (1.0)	4.4 (1.1)	0.90
HDL cholesterol, mmol/ L	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	0.49
LDL cholesterol, mmol/ L	2.4 (0.9)	2.4 (0.9)	2.4 (1.0)	0.78
Triglycerides, mmol/ L	1.6 [1.1-2.3]	1.6 [1.1-2.3]	1.7 [1.2-2.6]	0.10
Smoking status	Current: 243 (14) Never: 980 (55) Former: 547 (31) Unknown: 6 (< 1)	Current: 223 (14) Never: 861 (55) Former: 468 (30) Unknown: 6 (< 1)	Current: 20 (9) Never: 119 (55) Former: 79 (36) Unknown: 0 (0)	0.20
Diabetic retinopathy	No: 1458 (82) Simplex: 229 (13) Proliferative: 56 (3) Unknown: 33 (2)	No: 1283 (82) Simplex: 198 (13) Proliferative: 48 (3) Unknown: 29 (2)	No: 175 (80) Simplex: 31 (14) Proliferative: 8 (4) Unknown: 4 (2)	0.88
Maculopathy	No: 1656 (93) Yes: 79 (4) Unknown: 41 (2)	No: 1451 (93) Yes: 69 (4) Unknown: 38 (2)	No: 205 (94) Yes: 10 (5) Unknown: 3 (1)	0.62
Laser treatment	No: 1674 (94) Yes: 76 (4) Unknown: 26 (1)	No: 1470 (94) Yes: 66 (4) Unknown: 22 (1)	No: 204 (94) Yes: 10 (5) Unknown: 4 (2)	0.86
Hypertension	1360 (77)	1152 (74)	208 (95)	< 0.01
Dyslipidaemia	1007 (57)	869 (56)	138 (63)	< 0.01
Ischemic heart disease	221 (12)	183 (12)	38 (17)	0.02
Congestive heart disease	19 (1)	18 (1)	1 (< 1)	0.35
Other cardiac diseases[c]	153 (9)	129 (8)	24 (11)	0.18
Stroke	75 (4)	63 (4)	12 (6)	0.31
Peripheral arterial disease[d]	58 (3)	51 (3)	7 (3)	0.96

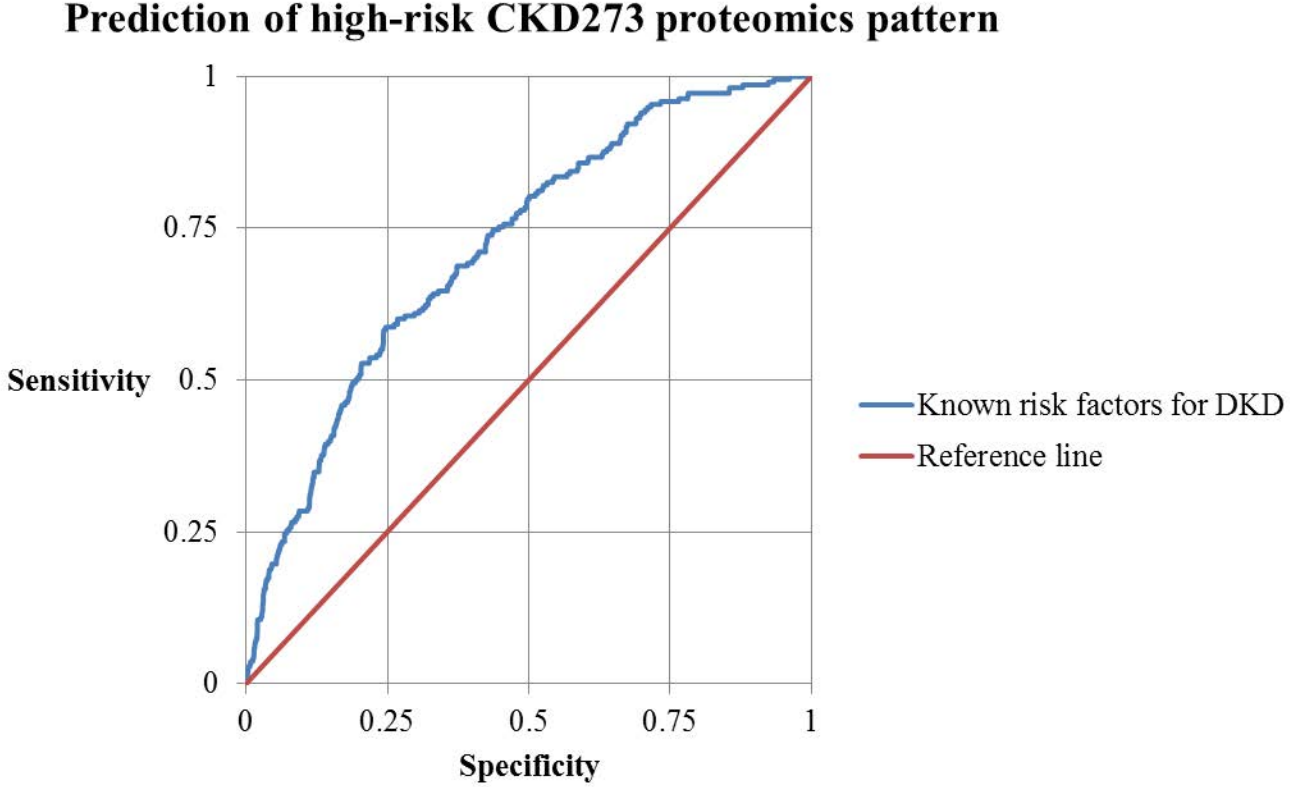
Mean (SD) or median [IQR] for continuous variables, n (%, rounded) for categorical variables. P value for chi-square test for categorical variables and unpaired t-test for continuous variables. [a]CKD273-classifier below or equal to the cut-point of 0.154. [b]CKD273-classifier above the cut-point of 0.154. eGFR denotes estimated glomerular filtration rate, UACR Urine Albumin-to-Creatinine Ratio. [c]Other cardiac diseases include arrhythmias, cardiomyopathies, aortic stenosis and valve stenosis. [d]Peripheral arterial disease include amputations, aneurisms and carotid stenosis.

Table 2 Baseline medication of the whole study population and by CKD273 subgroup

Medication	Included N = 1777	Low-risk[a] N = 1559	High-risk[b] N = 218	P-value (high- vs. low-risk)
Insulin of any kind	623 (36)	541 (35)	82 (38)	0.37
Biguanides	1407 (81)	1219 (80)	188 (86)	0.03
Sulphonylureas	424 (24)	359 (24)	65 (30)	0.09
GLP-1 analogues	278 (16)	241 (16)	37 (17)	0.71
SGLT-2 inhibitors	123 (7)	103 (7)	20 (9)	0.21
DDP4 inhibitors	263 (15)	224 (15)	39 (18)	0.13
Glitazones	73 (4)	64 (4)	9 (4)	0.67
ACE inhibitors	623 (36)	471 (31)	152 (70)	< 0.01
ARB	536 (31)	491 (32)	45 (21)	< 0.01
Alpha-blockers	76 (4)	62 (4)	14 (6)	0.13
Beta-blockers	467 (27)	387 (25)	80 (37)	< 0.01
Calcium channel blockers	392 (22)	335 (22)	57 (26)	0.19
Loop diuretics	75 (4)	62 (4)	13 (6)	0.22
Thiazides	486 (28)	394 (26)	92 (42)	< 0.01
Statins	1182 (68)	1032 (68)	150 (69)	0.80
Fibrates	103 (6)	85 (6)	18 (8)	0.08
Aspirin	567 (32)	466 (31)	101 (46)	< 0.01
Other anti-platelet agents[c]	130 (8)	106 (7)	24 (11)	0.04

N (%, rounded) GLP-1 denotes glucagon-like peptide-1, SGLT-2 sodium-glucose cotransporter-2, DDP-4 dipeptidyl peptidase-4, ACE angiotensin-converting-enzyme and ARB angiotensin-II-receptor blockers. P value for chi-square test. [a]CKD273-classifier below or equal to the cut-off of 0.154. [b]CKD273-classifier above the cut-point of 0.154. [c]Other anti-platelet agents include Warfarin, Non-vitamin K-antagonistic oral anticoagulants (NOAC) and Clopidogrel.

Figure 1 Receiver operating characteristics curve of known risk factors for diabetic nephropathy (gender, diabetes duration, systolic blood pressure, eGFR, UACR, HbA1c, smoking, retinopathy and use of RAS-blocking agents) predicting CKD273 risk strata. Gender, UACR, eGFR and use of RAS-blocking agents show significant predictive value ($p < 0.01$), AUC = 0.72 (95 % CI: 0.68 to 0.75) for the model.


Prediction of high-risk CKD273 proteomics pattern
Sensitivity
1
0.75
0.5
0.25
0
0
0.25
0.5
0.75
1
Specificity
Known risk factors for DKD
Reference line