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1 **Research Article**

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

5 **Running title:** Baseline characteristics in the PRIORITY study

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25

26 **Conflict of interests:**

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

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

4

5 **Novelty statement:**

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

16

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18 the study. Furthermore we would like to acknowledge sub-investigators, laboratory technicians and
19 study nurses and for their valuable contribution to this study. Names are provided in the
20 acknowledgements (supplementary).

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1 **Abstract**

2 *Aims*

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

6
7 *Methods*

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

13
14 *Results*

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

23
24 *Conclusions*

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

30
31 **Key words:** screening, nephropathy, clinical trials

1 **Introduction**

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

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

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

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

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

6

7 **Materials and methods**

8 *Study Design*

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

13 Briefly, persons aged 18-75 with type 2 diabetes, preserved kidney function and normoalbuminuria,
14 were included. The participants were required to fulfil the following inclusion criteria:
15 normoalbuminuria (urine albumin-to-creatinine ratio (UACR) <30 mg/g) in at least two out of three
16 consecutive morning void urine samples and eGFR >45 ml min⁻¹ 1.73 m⁻² at screening. Participants
17 were stratified into high- or low-risk groups based on their CKD273 score, of a single urine sample
18 collected at screening. High-risk was defined as CKD273-classifier score >0.154, low-risk as
19 ≤0.154 as previously described (15, 16). Participants in the high-risk group were stratified based on
20 use of RAS blocking agents and randomly assigned to either spironolactone 25 mg once daily or
21 placebo, on top of standard care. The participants in the low-risk group are followed on standard
22 care. The study period has been extended from 3 to 4.5 years in a protocol amendment, primarily
23 due to delayed recruitment. Based on expected higher progression rates to microalbuminuria due to
24 extension in treatment/observation time as well as new knowledge on treatment effect (10),
25 estimated sample size was revisited with preserved statistical power. All participants are planned for
26 a final visit in autumn 2018.

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

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

6

7 *Biochemical and other analyses*

8 At baseline biochemical samples for measurement of creatinine, HbA_{1c}, potassium, sodium, and
9 lipids were analysed at the local routine laboratory at each study centre by standardised methods.
10 eGFR was calculated at the local study centre and centrally by the CKD-EPI equation based on
11 locally measured creatinine with a standardised method. UACR was measured at the central
12 laboratory at Steno Diabetes Center Copenhagen using Vitros® 5600 MicroSlide. Samples were
13 shipped frozen on dry-ice from study centres. Confirmed microalbuminuria was defined as UACR
14 >30 mg/g in at least two of three first morning voids with 30% increase (geometric mean) in UACR
15 from ‘run-in-phase’, or >40 mg/g (geometric mean).

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

22

23 *Medical history*

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

1 *Statistical analysis*

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

15

16 **Results**

17 *Enrolment*

18 From March 25th 2014 through end of inclusion on August 31st 2016, a total of 2276 persons from
19 15 study centres in 10 countries were screened and 1777 participants were included. Of those, 218
20 participants were in the high-risk group and 1559 participants were in the low-risk group. The
21 proportion of participants in the high-risk group in the whole study population was 12.3%. The
22 high-risk rates varied considerably between study centres, ranging from 0% to 27% (Fig. s1).

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

1 *Baseline characteristics and medication*

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

12

13 *Medical history*

14 In the entire study population, 13% had a history of background diabetic retinopathy, 3% of
15 proliferative diabetic retinopathy and 4% of diabetic maculopathy. Laser treatment before baseline
16 was performed in 4%. At baseline 68% had a history of hypertension, 50% of dyslipidaemia and
17 12% of ischemic heart disease. No difference was detectable in the history of diabetic retinopathy or
18 diabetic maculopathy between high- and low-risk groups ($p > 0.62$), however the high-risk group
19 differed from the low-risk group with more participants having a history of hypertension,
20 dyslipidaemia and ischemic heart disease ($p < 0.02$), (Table 1). The medical history according to
21 study sites is shown in supplementary table s4.

22

23 *Correlation analysis with established risk factors*

24 Univariate regression analyses of CKD273 vs each baseline variable demonstrated weak
25 associations with age, diabetes duration, BMI, systolic blood pressure, eGFR, UACR, HDL
26 cholesterol and triglycerides ($p < 0.04$), (Supplementary table s5). The strongest association was seen
27 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 -
28 0.005 ($p < 0.01$), suggesting at maximum 4% and 3% of the variation in CKD273 score could be
29 explained by the variables eGFR and UACR, respectively. Scatterplots of CKD273 and UACR

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

10

11 **Discussion**

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

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

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

3 Previous studies, all post-hoc analyses of cohorts collected for other purposes without applying a
4 standardised protocol for collection, storage, transportation or analysis of samples, showed that a
5 high CKD273 score was associated with progression of renal disease in persons without diabetes
6 (18-20). Other studies focused on CKD273 as a risk predictor specifically in a population with
7 diabetes. Zürbig et al. demonstrated that CKD273 predicted progression from normo- to
8 microalbuminuria 1.5 years before microalbuminuria occurred and that progressors from micro- to
9 macroalbuminuria could be identified by the classifier 3-5 years before disease progression in
10 adjusted models (12). At baseline there was a trend towards progressors being older, male, with
11 higher urine albumin excretion rate, lower eGFR and higher systolic blood pressure compared to
12 non-progressors. This is similar to what we find in the current PRIORITY study. The findings were
13 confirmed by Roscioni et al. also in a small case-control study, demonstrating that CKD273
14 predicted development of albuminuria stage on top of eGFR in a three year period, also when
15 adjusting for baseline urinary albumin excretion and eGFR (21). In DIRECT-Protect 2, in
16 participants with type 2 diabetes, 9.8% were identified as high-risk, a lower rate than in the current
17 study; however a higher cut-point for the CKD273 score was applied (15). The participants had
18 similar urinary albumin excretion rate and blood pressure at baseline; however, they were younger,
19 had shorter diabetes duration than the current population and a lower eGFR, which could partly
20 explain the lower than expected high-risk rate in PRIORITY. Pontillo et al. investigated a large
21 population primarily diagnosed with diabetes (type 1 and 2) with eGFR decline $>5 \text{ ml min}^{-1} 1.73 \text{ m}^{-2}$
22 2 per year as the primary endpoint (14). The authors reported that for baseline ranges of eGFR >70
23 $\text{ml min}^{-1} 1.73 \text{ m}^{-2}$, CKD273 had a superior predictive value to urinary albumin excretion for fast
24 eGFR decline. These findings support the use of CKD273 in the present study population with
25 relatively high eGFR.

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

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

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

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

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

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1 **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

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3 Mean (SD) or median [IQR] for continuous variables, n (%), rounded) for categorical variables. P value for
4 chi-square test for categorical variables and unpaired t-test for continuous variables. ^aCKD273-classifier
5 below or equal to the cut-point of 0.154. ^bCKD273-classifier above the cut-point of 0.154. eGFR denotes
6 estimated glomerular filtration rate, UACR Urine Albumin-to-Creatinine Ratio. ^cOther cardiac diseases
7 include arrhythmias, cardiomyopathies, aortic stenosis and valve stenosis. ^dPeripheral arterial disease include
8 amputations, aneurisms and carotid stenosis.

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1 **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

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3 N (% , rounded) GLP-1 denotes glucagon-like peptide-1, SGLT-2 sodium-glucose cotransporter-2, DDP-4
 4 dipeptidyl peptidase-4, ACE angiotensin-converting-enzyme and ARB angiotensin-II-receptor blockers. P
 5 value for chi-square test. ^aCKD273-classifier below or equal to the cut-off of 0.154. ^bCKD273-classifier
 6 above the cut-point of 0.154. ^cOther anti-platelet agents include Warfarin, Non-vitamin K-antagonistic oral
 7 anticoagulants (NOAC) and Clopidogrel.

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1 **Figure 1 Receiver operating characteristics curve** of known risk factors for diabetic nephropathy
2 (gender, diabetes duration, systolic blood pressure, eGFR, UACR, HbA1c, smoking, retinopathy
3 and use of RAS-blocking agents) predicting CKD273 risk strata. Gender, UACR, eGFR and use of
4 RAS-blocking agents show significant predictive value ($p < 0.01$), AUC = 0.72 (95 % CI: 0.68 to
5 0.75) for the model.

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Prediction of high-risk CKD273 proteomics pattern

