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The urinary proteome as correlate and predictor of renal function in a population study

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ABSTRACT

Background. We investigate whether the urinary proteome refines the diagnosis of renal dysfunction, which affects over 10% of the adult population.

Methods. We measured serum creatinine, estimated glomerular filtration rate (eGFR) and 24-h albuminuria in 797 people randomly recruited from a population. We applied capillary electrophoresis coupled with mass spectrometry to measure multi-dimensional urinary proteomic classifiers developed for renal dysfunction (CKD273) or left ventricular dysfunction (HF1 and HF2). Renal function was followed up in 621 participants and the incidence of cardiovascular events in the whole study population.

Results. In multivariable-adjusted cross-sectional analyses, higher biomarker levels analysed separately or combined by principal component analysis into a single factor (SF), correlated ($P \leq 0.010$) with worse renal function. Over 4.8 years, higher HF1 and SF predicted ($P \leq 0.014$) lowering of eGFR; higher HF2 predicted ($P \leq 0.049$) increase in serum creatinine and decrease eGFR. HF1, HF2 and SF predicted progression from CKD Stages 2 or ≤2 to Stage ≥3, with risk estimates for a 1-SD increment in the urinary biomarkers ranging from 38 to 71% ($P \leq 0.039$). HF1, HF2 and SF yielded a net reclassification improvement of 31–51% ($P \leq 0.029$). Over 6.1 years, 47 cardiovascular events occurred. HF2 and SF, independent of baseline eGFR, 24-h albuminuria and other covariables were significant predictors of cardiovascular complications with risk estimates for 1-SD increases ranging from 32 to 41% ($P \leq 0.047$).

Conclusions. The urinary proteome refines the diagnosis of existing or progressing renal dysfunction and predicts cardiovascular complications.

Keywords: chronic kidney disease, eGFR, population science, renal function, urinary proteomics

INTRODUCTION

Chronic kidney disease (CKD) is becoming a major health problem affecting the quality of life of millions of people and draining health care resources. In the USA, CKD defined as an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 increased from 10.0% in 1988–94 to 13.1% in 1999–2004 [1]. Furthermore, the Global Burden of Disease Study 2010 collaboration estimated that worldwide 0.403 million of nearly 50 million deaths occurring annually, were attributable to CKD in 1990 and 0.736 in 2010, representing an increase by 82.3% [2]. Across all ages, over the same time span, the years lived with CKD increased by 57.1% from 2.56 to 4.02 million [3], while the disability-adjusted life years, a metric that captures both premature mortality and the prevalence of ill-health increased by 51.7% from 13.9 to 21.2 million [4]. Diagnosis of CKD before eGFR starts declining and prediction of CKD is therefore of paramount importance in the prevention of

irreversible renal dysfunction that often progresses to end-stage renal failure and causes cardiovascular complications and premature death [3, 4].

Recent publications proved the feasibility to develop multi-dimensional classifiers based on the urinary proteome that are associated with CKD [5, 6], and left ventricular dysfunction [7], but they were mainly derived in CKD patients matched with controls [8–10] or in patient cohorts with diabetes [5] or CKD [6]. In the current study, we investigated the performance of these biomarkers [5–7] in a general population. We assessed their association cross-sectionally and longitudinally in relation to renal function, and prospectively in relation to the incidence of cardiovascular complications.

MATERIALS AND MEHODS

Study population

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) [11, 12]. Recruitment started in 1985 and continued until 2004. The initial participation rate was 78.0%. The participants were repeatedly followed up [11, 12]. From May 2005 to May 2010, we mailed an invitation letter to 1208 former participants for a follow-up examination. However, 153 were unavailable, because they had died ($n = 26$), had been institutionalized or were too ill ($n = 27$), or because they had moved out of the area ($n = 100$). Of the remaining 1055 former participants, 828 renewed informed consent. The participation rate was therefore 78.5%. We excluded 31 participants from the cross-sectional analyses, because either no urine ($n = 22$) or no blood sample ($n = 9$) was available. Thus, the number of participants statistically analysed totalled 797, of whom 621 (77.9%) participated in the follow-up of renal function.

Assessment of renal function and biochemical variables

We measured the concentration of creatinine in serum, using Jaffe's method [13], with modifications described elsewhere [14, 15], on automated analysers in a single-certified laboratory. We assessed renal function from serum creatinine, eGFR computed by the Modification of Diet in Renal Disease (MDRD) [16] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [17] equations. CKD stages, defined according to the National Kidney Foundation KDOQI guideline, were eGFR ≥90, 60–89, 45–59, 30–44, 15–29 and <15 mL/min/1.73 m^2 for Stage 1, 2, 3A, 3B, 4 and 5, respectively (http://www.kidney.org/professionals/kdoqi/guidelines_ckd/p4_class_g1.htm).

At baseline, we also measured blood glucose, serum total and high-density (HDL) cholesterol, serum γ-glutamyltransferase as an index of alcohol intake, and micro-albumin in 24-h urine collections. Diabetes mellitus was a self-reported diagnosis, a fasting glucose level of at least 126 mg/dL, or use of antidiabetic agents [18]. Micro-albuminuria was a 24-h urinary excretion ranging from 30 to 300 mg and macro-albuminuria a 24-h excretion exceeding 300 mg.

Proteomic classifiers

The Supplementary data (pages 2–4) gives detailed information on the preparation and processing of urine samples. Peptide fragments identified in previous studies [7, 8] were combined into a single summary variable, using the support-vector machine-based MosaCluster software, version 1.6.5. In the present study, we used CKD273 as a multi-dimensional classifier based on 273 urinary peptide biomarkers that were significantly associated with CKD [8]. We also assessed two multi-dimensional classifiers associated with decreased left ventricular function and based on 85 (HF1) [7] and 671 (HF2) urinary peptide fragments. The peptide fragments included in CKD273 [8] have been published. The Supplementary data provide information on the peptide fragments making up HF1 (Supplementary data, Table S1) and HF2 (Supplementary data, Table S2), the peptides with known amino-acid sequence included in HF1 (Supplementary data, Table S3) and HF2 (Supplementary data, Table S4), and on the characteristics of 20 peptides shared by CKD273, HF1 and HF2 (Supplementary data, Table S5).

Other measurements

At the examination centre, nurses administered a questionnaire to collect detailed information on each participant's medical history, smoking and drinking habits and intake of medications. The conventional blood pressure was the average of five consecutive auscultatory readings obtained with the subject in the seated position. The mean arterial pressure was diastolic blood pressure plus one-third of the difference between systolic and diastolic blood pressure. Hypertension was a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic or use of antihypertensive drugs. The body mass index was weight in kilograms divided by the square of height in meters.

Ascertainment of events

Via the National Population Registry in Brussels, Belgium, we ascertained vital status of all of the participants until 31 December 2012. We obtained the International Classification of Disease codes for the immediate and underlying causes of death from the Flemish Registry of Death Certificates. We also collected information on the incidence of non-fatal events via follow-up visits with repeat administration of the same standardized questionnaire.

Fatal and non-fatal cardiac events included myocardial infarction, acute coronary syndrome, new-onset angina (stable or unstable), chronic ischaemic heart disease, coronary revascularization, heart failure, new-onset atrial fibrillation and life-threatening arrhythmias. Fatal and non-fatal cardiovascular events comprised cardiac endpoints, stroke, transient ischaemic attack, aortic aneurysm, pulmonary heart disease, arterial embolism, peripheral arterial disease and revascularization of peripheral arteries. All events were adjudicated against the medical records of general practitioners or hospitals.

Statistical analysis

For database management and statistical analysis, we used the SAS system, version 9.3 (SAS Institute, Inc., Cary, NC, USA). Means were compared using the large-sample z-test or

ANOVA and proportions by Fisher's exact test. We computed single correlation coefficients to assess unadjusted associations between variables. We searched for covariables of the renal function indices using a stepwise regression procedure with the P-values for variables to enter and stay in the models set at 0.15. We combined the three urinary proteomic variables into a single factor (SF), using the PROC FACTOR procedure implemented in the SAS software package with the method set to *principal* and rotation to *varimax*. Renal function and changes in renal function were analyses as continuous or categorical variables, using multivariable-adjusted linear regression, logistic regression and Cox modelling, as appropriate. We used Cox proportional hazard regression to model the incidence of death and cardiovascular complications as function of the baseline values of the proteomic biomarkers, renal function and other covariables. Finally, we assessed the added capacity of the urinary proteomic biomarkers to predict worsening of renal function, using the integrated discrimination improvement (IDI) and the net reclassification improvement (NRI) [19, 20]. IDI is the difference between the discrimination slopes of basic models and basic models extended with the urinary biomarker of interest. The discrimination slope is the difference in predicted probabilities (%) between cases and controls. We calculated the continuous NRI as described by Pencina *et al.* [21]. First, we predicted in each subject the 5-year risk for a worsening in renal dysfunction from a Cox proportional hazards model with and without the biomarker included. NRI is then calculated as $2 \times [P(up|case) - P(up|noncase)]$. P(up|case) is the percentage of subjects with a worsening in renal dysfunction, whose predicted probability is increased by adding the biomarker to the model. Likewise, P(up|noncase) is the percentage of subjects without a worsening in renal function, whose predicted probability is increased by adding the biomarker.

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RESULTS

Characteristics of participants

Age averaged 51.0 years (range 18–89 years) and the proportion of women was 50.7%. Of 797 participants, 338 (42.4%) had hypertension, of whom 207 (61.2%) were on antihypertensive drug treatment, and 9 (1.1%) had diabetes. Among 207 patients on treatment with antihypertensive drugs, 82 (39.6%) used diuretics, 168 (81.2%) used inhibitors of the renin system (β-blockers, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers) and 40 (19.3%) were on treatment with vasodilators (calcium channel blockers or α-blockers). Among treated patients 78 (37.7%) were on combination therapy with >1 drug class.

Table 1 lists the characteristics of participants by quartiles of the distribution of CKD273. Age, body mass index, central obesity, systolic and mean arterial blood pressure, the prevalence of hypertension and total cholesterol increased ($P \leq 0.006$) with higher category of CKD273. Among all participants, 29 (3.6%) had micro-albuminuria and 3 (0.4%) had macro-albuminuria. Renal function as assessed by serum creatinine, eGFR or 24-h urinary albumin excretion decreased ($P < 0.0001$) with higher CKD273 category. The prevalence of micro-albuminuria increased across the quartiles of the CKD273 distribution ($P < 0.0001$) with frequencies of 0.5% ($n = 1$), 1.5% ($n = 3$), 3.0% ($n = 6$) and 9.6 ($n = 19$), respectively. Macro-albuminuria occurred only in the top CKD273 quartile. The proportion of women, smokers, consumers of alcohol, diabetic patients and average heart rate did not differ ($P \geq 0.20$) across CKD273 categories. Supplementary data, Figure S1 displays the distributions of the urinary proteomic markers. Figure 1 gives the distributions of the renal function indices at baseline and follow-up.

Combination of proteomic biomarkers into a SF

The correlations of CKD273 with HF1 and HF2 were 0.47 and 0.45 ($P < 0.0001$), while that between HF1 and HF2 was 0.76 ($P < 0.0001$). To avoid collinearity in models including more than one biomarker, we derived an SF (eigenvalue, 2.13) as a linear combination of CKD273, HF1 and HF2. SF had high loadings on CKD273 ($r = 0.73$), HF1 ($r = 0.90$) and HF2 ($r = 0.89$).

Cross-sectional association of renal function with urinary proteomic markers

Continuous measures of renal function. The covariables identified by stepwise regression analysis are given in Supplementary data, Table S6, page 5. With adjustments for these covariables applied (Table 2), serum creatinine increased with CKD273 and SF ($P \leq 0.010$), whereas eGFR decreased with all four proteomic biomarkers ($P \leq 0.010$). The 24-h urinary albumin excretion increased ($P \leq 0.006$) with all urinary proteomic biomarkers. The effect sizes for 1-SD increment were 8.9% [95% confidence interval (CI): 6.5, 11.3] for CKD273, 3.4% (95% CI: 0.97, 5.8) for HF1, 5.9% (95% CI: 3.4, 8.6) for HF2 and 7.7% (95% CI: 5.1, 10.4) for SF.

Categorical measures of renal function. Categories according to eGFR derived from the MDRD formula [16], included 22.1% ($n = 176$) of participants for Stage 1, 70.0% for Stage 2 ($n = 558$), 6.9% for Stage 3A ($n = 55$), 0.9% for Stage 3B ($n = 7$) and 0.1% ($n = 1$) for Stage 4. Categories according to eGFR derived from the CKD-EPI formula [17], included 32.1% ($n = 256$) of participants for Stage 1, 59.5% ($n = 474$) for Stage 2, 6.8% ($n = 54$) for Stage 3A, 1.4% ($n = 11$) for Stage 3B and 0.2% ($n = 2$) for Stage 4.

Table 3 shows that irrespective of the eGFR formula applied, all odds ratios reached significance ($P \leq 0.05$) for all four urinary biomarkers for the risk of having a worse renal function, comparing Stages 2, ≥2, or ≥3 with Stage 1 or comparing Stage ≥3 with Stage 2. Furthermore, the risk of having a 24-h albuminuria of 30 mg or more increased ($P \leq 0.019$) with higher scores of the four biomarkers. The odds ratios associated with a 1–SD increment of the biomarker were 3.63 (95% CI: 2.31, 5.72) for CKD273, 1.63 (95% CI: 1.08, 2.46) for HF1, 2.20 (95% CI: 1.44, 3.36) for HF2 and 2.83 (95% CI: 1.81, 4.42) for SF.

Table 1. Baseline characteristics of 797 participants by fourths of the CKD273 distribution

Characteristic	<−0.82	−0.82 to −0.61	−0.61 to −0.35	≥−0.35	P-value
Number of subjects (%)					
Women	100 (50.0)	99 (49.5)	103 (52.0)	102 (51.3)	0.96
Smokers	38 (19.0)	39 (19.5)	45 (22.7)	39 (19.6)	0.78
Drinking alcohol	140 (70.0)	138 (69.0)	140 (70.7)	135 (67.8)	0.93
Hypertension	57 (28.5)	90 (45.0)‡	82 (41.4)	109 (54.8)†	<0.0001
Antihypertensive treatment	35 (17.5)	46 (23.0)	46 (23.2)	80 (40.2)‡	<0.0001
Diabetes mellitus	1 (0.5)	3 (1.5)	2 (1.0)	3 (1.5)	0.80
Mean (SD) of characteristic					
Age (years)	45.2 (14.8)	48.6 (15.7)*	51.0 (14.6)	59.4 (14.2)§	<0.0001
Body mass index (kg/m^2)	26.3 (4.4)	25.7 (4.1)	26.4 (4.1)	27.5 (4.6)*	0.002
Waist-to-hip ratio	0.86 (0.09)	0.86 (0.08)	0.88 (0.08)†	0.89 (0.08)	<0.0001
Office blood pressure (mmHg)					
Systolic pressure	125.3 (15.1)	128.8 (17.3)*	129.7 (18.8)	133.8 (18.3)*	<0.0001
Diastolic pressure	78.5 (10.3)	80.5 (10.4)	79.6 (8.5)	80.0 (9.0)	0.22
Mean arterial pressure	94.1 (11.1)	96.6 (11.1)*	96.3 (10.2)	97.9 (10.2)	0.0009
Heart rate (b.p.m.)	64.2 (9.4)	63.4 (9.5)	63.4 (9.8)	62.9 (10.5)	0.20
Biochemical data					
Serum creatinine (μmol/L)	80.5 (13.9)	83.6 (14.4)*	83.8 (12.9)	88.1 (20.6)*	<0.0001
eGFR (MDRD, mL/min/1.73 m^2)	86.8 (19.0)	81.4 (14.7)†	79.5 (15.5)	73.5 (14.1)§	<0.0001
eGFR (CKD-EPI, mL/min/1.73 m^2)	89.9 (17.9)	84.6 (15.9)†	82.2 (16.4)	74.6 (15.9)§	<0.0001
24-h albuminuria (mg)	5.0 (3.7, 6.5)	5.2 (3.7, 6.7)	6.1 (4.5, 7.5)†	8.2 (5.1, 8.8)‡	<0.0001
Total cholesterol (mmol/L)	5.12 (1.01)	5.19 (1.00)	5.37 (0.99)	5.34 (0.88)	0.006
HDL cholesterol (mmol/L)	1.41 (0.34)	1.44 (0.36)	1.43 (0.36)	1.41 (0.34)	0.008
Total-to-HDL cholesterol ratio	3.80 (1.05)	3.77 (1.02)	3.93 (1.07)	3.97 (0.99)	0.046
Blood glucose (mmol/L)	4.90 (0.73)	4.89 (0.50)	4.92 (0.90)	5.04 (0.93)	0.073
γ-Glutamyltransferase (units/L)	22 (15, 30)	21 (14, 32)	23 (16, 31)	26 (16, 37)*	0.019

eGFR, estimated glomerular filtration rate calculated according to the MDRD or CKD-EPI formulas, as described in references [16] and [17], respectively. Office blood pressure was the average of five consecutive readings. Hypertension was an office blood pressure of ≥140 mmHg systolic, or ≥90 mmHg diastolic, or use of antihypertensive drugs. For 24-h albuminuria and γ-glutamyltransferase, values are the geometric mean (inter-quartile range). Conversion factors: creatinine from μmol/L to mg/dL, multiply by 0.0113; cholesterol from mg/dL to mmol/L, multiply by 0.0259.

P-values denote the significance of the differences in prevalence rates or means across quartiles of the CKD273 distribution. Significance of the difference with the adjacent lower fourth.

*P ≤ 0.05.

†P ≤ 0.01.

‡P ≤ 0.001.

§P ≤ 0.0001.

Longitudinal association of renal function with urinary proteomic predictors

Of 797 participants, 621 (77.9%) had their renal function indices reassessed after a median interval of 4.8 years (5–95th percentile interval: 3.7, 5.4).

Continuous measures of renal function. Over follow-up, serum creatinine increased by 5.74 μmol/L (95% CI: 4.59, 6.88; P < 0.0001), whereas eGFR according to the MDRD and CKD-EPI formulae decreased by 6.89 mL/min/1.73 m^2 (95% CI: 6.05, 7.74; P < 0.0001) and 7.68 mL/min/1.73 m^2 (95% CI: 6.89, 8.47; P < 0.0001), respectively (Figure 1).

In multivariable-adjusted analyses of the changes in renal function, we accounted for baseline renal function, follow-up duration and other covariables (Table 4). In general, higher scores of the urinary proteomic biomarkers with the exception of CKD273, predicted worsening of renal function. HF1 and SF predicted (P ≤ 0.014) lowering of eGFR (MDRD and CKD-EPI). Higher HF2 predicted (P ≤ 0.049) an increase in serum creatinine and a decrease eGFR (MDRD and CKD-EPI).

Categorical measures of renal function. Over follow-up (Supplementary data, Table S7), eGFR according to the MDRD formula remained at the same Stage in 458 (73.8%) participants, moved up in 139 (22.4%) and moved down in 24 (3.9%). According to the CKD-EPI formula, these numbers were 431 (69.4%), 167 (26.9%) and 23 (3.7%), respectively. No participant proceeded to CKD Stage 5 or renal replacement therapy.

In multivariable-adjusted Cox regression (Table 5), HF1, HF2 and SF, but not CKD273 (P ≥ 0.11) predicted progression from CKD Stages 2 or ≤2 to Stage ≥3, irrespective of the formula used to estimate eGFR, with risk estimates for a 1–SD increment in the urinary biomarkers ranging from 38 to 71% (P ≤ 0.039). Figure 2 provides the multivariable-adjusted risk functions for the progression of CKD from Stage ≤2 to ≥3.

Improvement of prognostic accuracy

For a decline in eGFR from Stage ≤ 2 to ≥ 3 based on the MDRD formula, IDI reached significance (P ≤ 0.032) for HF2 and SF (Table 6). By applying the CKD-EPI formula, IDI was significant (P ≤ 0.043) for HF1, HF2 and SF. Irrespective of the formula applied, the NRI was significant (P ≤ 0.029) for HF1, HF2 and SF (Table 6).

Incidence of events

Among 797 participants who had their urinary proteome measured at baseline, the median follow-up was 6.11 years (5–95th percentile interval: 3.68, 7.36). Mortality included 8 cardiovascular and 13 non-cardiovascular deaths, and 6 deaths from

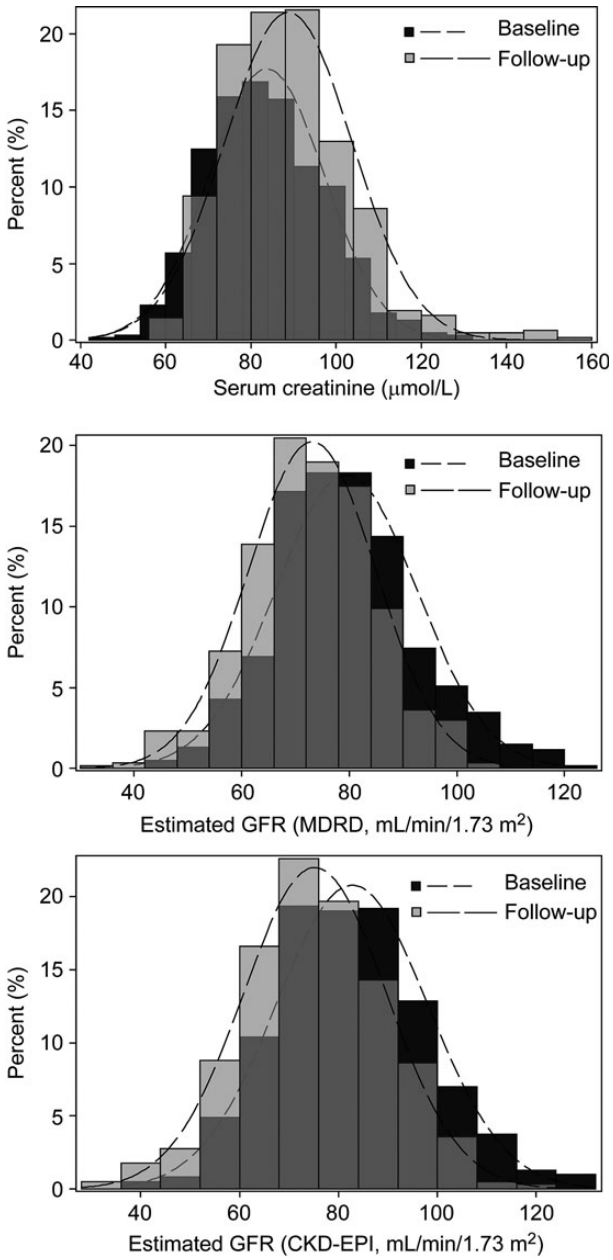

FIGURE 1: Distributions of the renal function indices at baseline and follow-up in 621 participants examined at baseline and follow-up.

undocumented causes. None of the biomarkers predicted total mortality ($P \geq 0.65$).

During the follow-up, 47 cardiovascular events occurred including 29 cardiac events (6 cases of heart failure). As shown in Supplementary data, Table S8, CKD273 did not predict these outcomes with hazard ratios for a 1–SD increase ranging from 1.14 to 1.23 ($P \geq 0.26$). Of the three other urinary proteomic biomarkers, HF1 was a weak predictor of all cardiovascular and cardiac events with hazard ratios for a 1–SD increase ranging from 1.28 to 1.41 ($0.049 \leq P \leq 0.074$). HF2 and SF significantly ($P \leq 0.047$) predicted the composite cardiovascular endpoint, irrespective of whether the model included baseline eGFR or 24-h albuminuria, or both. In fully adjusted models, including all covariables and both eGFR and 24-h albuminuria, the hazard ratios associated with a 1–SD increase were 1.40 (95% CI: 1.06, 1.89; $P = 0.018$) for HF2 and 1.32 (95% CI: 1.00, 1.74; $P = 0.047$) for SF.

DISCUSSION

To our knowledge, our current study is the first to assess the association of CKD with urinary proteomic biomarkers in a general population. The key findings can be summarized as follows: (i) in cross-sectional analyses, continuous measures of renal function and CKD stages correlated with CKD273, HF1, HF2 and SF; (ii) HF2 and SF predicted the incidence of cardiovascular complications; (iii) HF1, HF2 and SF predicted the changes of the renal function indices over time and progression of the CKD stage; (iv) optimized discrimination limits improved IDI and NRI for predicting the progression of renal dysfunction from the urinary proteomic biomarkers. NRI and IDI provide complimentary information. Indeed, adding a biomarker to a model might increase the predicted probability in cases, which means an increase in NRI, but perhaps only to a limited extend, as reflected by IDI.

Our current study moves beyond the available literature data, which were obtained in CKD patients matched with controls [8–10] or in patient cohorts with diabetes [5] or CKD ([6]). Good *et al.* derived CKD273 in a training database consisting of 609 patients with biopsy-proven CKD and 379 healthy controls [8]. Analysis of the urinary proteome yielded 634 peptides with significantly different signal between cases and controls. This set was subsequently reduced to 273 peptides with known sequence frequency. Good *et al.* reproduced in a blinded manner this biomarker pattern in an independent test database including 110 CKD patients and 34 controls. Upon unblinding, all controls and 94 patients with CKD were correctly classified, resulting in a sensitivity of 85.5% (95% CI: 77.5, 91.4) and a specificity of 100% (95% CI: 89.6, 100.0) [8].

The CKD273 proteomic marker was further studied in three prospective studies [5, 6, 9]. In a study of 44 patients with type 2 diabetes progressing to micro- or macro-albuminuria and 44 matched controls, the multivariable-adjusted odds ratio associated with CKD273 was 1.35 (95% CI: 1.02, 1.79) [9]. CKD273 significantly ($P = 0.002$) improved IDI over and beyond baseline urinary albumin excretion and eGFR [9]. In a study of 53 CKD patients, CKD273 increased with worse CKD stage and was linearly correlated with eGFR ($r = -0.64$; $P < 0.001$). Over 3.6 years of follow-up, four patients were lost to follow-up. The CKD273 score was >0.55 in all 15 patients who reached an endpoint, either dialysis ($n = 9$) or died ($n = 6$) [6]. None of the patients with a baseline CKD273 score <0.55 experienced an endpoint [6].

Several issues should be highlighted when comparing our current results with the published literature on CKD273 [5, 6, 8, 9]. First, findings in patients with advanced CKD or diabetes cannot be readily generalized to unselected people as enrolled in our population study. This might explain why in our present study the renal function indices were correlated with CKD273 in cross-sectional analyses, whereas in longitudinal analyses

Table 2. Multivariable-adjusted associations of renal function with urinary proteomic biomarkers

Explanatory variables (SD)	Serum creatinine	eGFR (MDRD)	eGFR (CKD-EPI)
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
CKD273 (0.39)	1.57 (0.57, 2.58)†	−3.83 (−4.96, −2.69)§	−4.17 (−5.30, −3.04)§
HF1 (0.92)	0.86 (−0.16, 1.88)	−1.56 (−2.75, −0.38)†	−2.09 (−3.27, −0.90)‡
HF2 (0.62)	0.90 (−0.18, 1.99)	−2.47 (−3.70, −1.24)§	−3.24 (−4.46, −2.02)§
SF (1.0)	1.43 (0.34, 2.52)*	−3.26 (−4.49, −2.03)§	−3.96 (−5.18, −2.74)§

eGFR, estimated glomerular filtration rate calculated according to the MDRD or CKD-EPI formulae, as described in references [16] and [17], respectively. Estimates given with 95% CI, express the difference in renal function associated with a 1–SD increase in the proteomic biomarkers. All associations were adjusted for mean arterial pressure, waist-to-hip ratio, smoking, log γ-glutamyltransferase (index of alcohol intake), total-to-HDL cholesterol ratio, blood glucose, log 24-h albuminuria, and use of diuretics, vasodilators (calcium channel blockers and α-blockers) and inhibitors of the renin–angiotensin system (β-blockers, angiotensin-converting-enzyme inhibitors and angiotensin receptor blockers). The association with serum creatinine was additionally adjusted for sex, age and body mass index. Significance of the associations.
$^{*}P \leq 0.05$.
$^{\dagger}P \leq 0.01$.
$^{\ddagger}P \leq 0.001$.
$^{\S}P \leq 0.0001$.

Table 3. Multivariable-adjusted associations of eGFR with urinary proteomic biomarkers

Biomarkers	Stage 2 versus Stage 1	Stage ≥3 versus Stage 1	Stage ≥2 versus Stage 1	Stage ≥3 versus Stage 2
	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
MDRD				
CKD273	1.70 (1.37, 2.11)§	2.98 (1.80, 4.94)§	1.76 (1.42, 2.18)§	1.46 (1.09, 1.95)*
HF1	1.23 (1.00, 1.53)*	1.80 (1.18, 2.75)†	1.26 (1.02, 1.56)*	1.34 (1.01, 1.79)*
HF2	1.38 (1.09, 1.73)†	2.26 (1.43, 3.59)‡	1.42 (1.13, 1.79)†	1.36 (1.02, 1.80)*
SF	1.64 (1.28, 2.09)§	2.88 (1.70, 4.87)§	1.70 (1.33, 2.17)§	1.46 (1.10, 1.93)†
CKD-EPI				
CKD273	1.73 (1.41, 2.12)§	4.07 (2.42, 6.82)§	1.82 (1.49, 2.22)§	1.54 (1.16, 2.05)†
HF1	1.28 (1.06, 1.56)*	2.29 (1.47, 3.54)‡	1.32 (1.09, 1.60)†	1.35 (1.02, 1.77)*
HF2	1.50 (1.20, 1.86)‡	2.91 (1.86, 4.55)§	1.56 (1.26, 1.93)§	1.49 (1.13, 1.96)†
SF	1.74 (1.38, 2.19)§	3.97 (2.32, 6.78)§	1.83 (1.46, 2.29)§	1.53 (1.17, 2.01)†

eGFR, estimated glomerular filtration rate calculated according to the MDRD or CKD-EPI formulas, as described in references [16] and [17], respectively. Frequencies of the eGFR stages according to the MDRD and CKD-EPI are given in the Results section. Odds ratios, given with 95% CI, express the risk associated with a 1–SD increase in the proteomic biomarkers. All associations were adjusted for mean arterial pressure, waist-to-hip ratio, smoking, log γ-glutamyltransferase (index of alcohol intake), total-to-HDL cholesterol ratio, blood glucose, log 24-h albuminuria, and use of diuretics, vasodilators (calcium channel blockers and α-blockers) and inhibitors of the renin–angiotensin system (β-blockers, angiotensin-converting-enzyme inhibitors and angiotensin receptor blockers). Significance of odds ratios.
$^{*}P \leq 0.05$.
$^{\dagger}P \leq 0.01$.
$^{\ddagger}P \leq 0.001$.
$^{\S}P \leq 0.0001$.

Table 4. Multivariable-adjusted association of renal function changes with baseline urinary proteomic biomarkers

Biomarker measured at baseline	Serum Creatinine	eGFR (MDRD)	eGFR (CKD-EPI)
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
CKD273	0.07 (−1.14, 1.27)	−0.34 (−1.09, 0.41)	−0.27 (−1.05, 0.50)
HF1	0.96 (−0.23, 2.15)	−1.02 (−1.75, −0.29)†	−0.95 (−1.71, −0.20)*
HF2	1.25 (0.01, 2.49)*	−1.34 (−2.09, −0.60)‡	−1.27 (−2.05, −0.50)†
SF	1.03 (−0.23, 2.29)	−1.18 (−1.94, −0.43)†	−1.11 (−1.90, −0.32)†

eGFR, estimated glomerular filtration rate calculated according to the MDRD or CKD-EPI formulas, as described in reference [16] and [17], respectively. Change in renal function was computed as follow-up minus baseline value. Estimates given with 95% CI, express the change in renal function associated with a 1–SD increase in the explanatory variables derived from the urinary proteome. SDs were 0.38 for CKD273; 0.91 for HF1; 0.59 for HF2; and 0.96 for SF. All associations were adjusted for baseline renal function and log urinary albumin, log-transformed follow-up time, mean arterial pressure, waist-to-hip ratio, smoking, blood glucose, log γ-glutamyltransferase, and total-to-HDL cholesterol ratio. Associations with changes in serum creatinine and creatinine clearance were additionally adjusted for sex, age and body mass index. Significance of the associations.
$^{*}P \leq 0.05$.
$^{\dagger}P \leq 0.01$.
$^{\ddagger}P \leq 0.001$.
$^{\S}P \leq 0.0001$.

CKD273 did not predict change in renal function or progression across stages of eGFR. Second, the published literature focused on CKD273 [5, 6, 8, 9]. However, CKD is a forerunner of heart failure and decline of left ventricular performance leads to pre-renal dysfunction [22, 23]. We therefore assessed two multi-dimensional classifiers associated with left ventricular dysfunction. Third, we combined CKD273, HF1 and HF2 into SF. However, in longitudinal analyses, the biomarker combining

Table 5. Multivariable-adjusted hazard ratios predicting changes in CKD stages in relation to the baseline urinary proteome

Endpoint	Rate (%)	Hazard ratios (95% CI)			
		CKD273	HF1	HF2	SF
MDRD					
Stage 2 → ≥3	49/456 (10.7)	1.29 (0.94, 1.77)	1.39 (1.02, 1.89)*	1.57 (1.17, 2.11)†	1.52 (1.13, 2.05)†
Stage ≤2 → ≥3	50/579 (8.64)	1.30 (0.94, 1.78)	1.45 (1.06, 1.98)*	1.71 (1.29, 2.26)‡	1.62 (1.21, 2.17)†
CKD-EPI					
Stage 2 → ≥3	54/388 (13.9)	1.18 (0.87, 1.59)	1.38 (1.03, 1.84)*	1.41 (1.06, 1.88)*	1.41 (1.06, 1.88)*
Stage ≤2 → ≥3	54/580 (9.31)	1.33 (0.98, 1.81)	1.53 (1.13, 2.05)†	1.58 (1.20, 2.08)†	1.61 (1.22, 2.13)‡

eGFR, estimated glomerular filtration rate calculated according to the MDRD or CKD-EPI formulas, as described in reference [16] and [17], respectively. Frequencies of the eGFR stages according to the MDRD and CKD-EPI are given in the Results section. Rate is the number of endpoints divided by number of participants at risk (%). Hazard ratios, given with 95% CI, express the increase in risk associated with a 1–SD increase in the explanatory variables derived from the urinary proteome. SDs were 0.38 for CKD273; 0.91 for HF1; 0.59 for HF2; and 0.96 for SF. All hazard ratios were adjusted for baseline values of mean arterial pressure, waist-to-hip ratio, smoking, blood glucose, log γ-glutamyltransferase, total-to-HDL cholesterol ratio and log 24-h albuminuria. Significance of the hazard ratios.

*$P \leq 0.05$.

†$P \leq 0.01$.

‡$P \leq 0.001$.

§$P \leq 0.0001$.

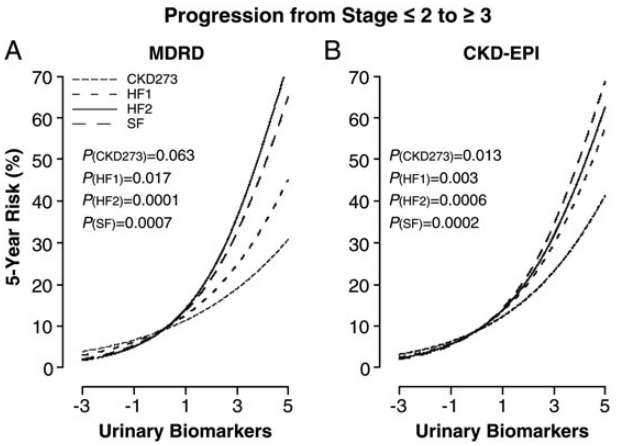

FIGURE 2: Five-year risk of progressing from CKD Stage ≤2 to stage ≥3 in 621 participants. CKD stages were based on the MDRD (A) or CKD-EPI (B) formula, as described in references [16] and [17]. To plot risk functions simultaneously for four biomarkers, we expressed the scores in units of standard deviation of the respective distributions. The risks were adjusted for baseline values of mean arterial pressure, waist-to-hip ratio, smoking, log γ-glutamyltransferase (index of alcohol intake), total-to-HDL cholesterol ratio, blood glucose and log 24-h albuminuria. P-values indicate the significance of the 5-year risks associated with the urinary biomarkers.

information from the three classifiers did not perform better than HF1 and HF2. Fourth, we assessed the incidence of cardiovascular and cardiac events in relation to the urinary proteomic biomarkers and noticed that HF2 and SF were significant predictors of such events, over and beyond covariables including baseline eGFR and 24-h urinary albuminuria.

Our current results reinforce the concept that dysfunction of the kidney and the left ventricle often co-exist [22–24]. Haemodynamic and non-haemodynamic mechanisms underpin the two-way interaction between the kidney and the heart [22, 23]. A decline in left ventricular systolic function and cardiac output activates the sympathetic nervous system and the renin–angiotensin system. The ensuing sodium and water retention and expansion of the circulating volume, although maintaining renal perfusion, increase afterload and can aggravate left ventricular dysfunction [22–24]. Left ventricular diastolic dysfunction with preserved ejection fraction may be accompanied with higher pressure in the central venous system [25], which in turn may increase renal intratubular pressure and reduce ultrafiltation pressure, thereby reducing glomerular filtration [26]. The drivers of the non-haemodynamic cardiorenal connection are the renin–angiotensin system, sympathetic nervous tone, inflammation and the balance between nitric oxide and reactive oxygen species [22]. Our study did not directly address the interaction between renal and left ventricular function, but might point to an avenue worthy of further research in predominantly asymptomatic people.

The present study must be interpreted within the context of some potential limitations. First, we determined proteinuria only at baseline and not at follow-up. However, in our view, 24-h albuminuria reflects microcirculatory organ damage rather than renal function. At baseline, this measure of target organ damage increased with all urinary proteomic biomarkers. In the Olmesartan for the Delay or Prevention of Microalbuminuria in Type 2 Diabetes trial, micro-albuminuria developed in 8.2% of the patients in the olmesartan group and in 9.8% in the placebo group [27]. The incidence of microalbuminuria in our population study was presumably only a minor fraction of that observed in the high-risk ROADMAP patients, so that it is unlikely that we missed important information. Second, we lost 176 participants (22.1%) in the follow-up study of renal function. However, at baseline participants followed up and those not reassessed had similar distributions of female sex (49.9 versus 53.4%; P = 0.41), age (50.8 versus 51.8 years; P = 0.52) and previous cardiovascular disease (4.8 versus 6.3%; P = 0.45). Third, the renal function indices were only measured twice. However, Figure 1 shows the expected age-related shift, which excludes confounding by regression-to-the mean. Finally, CKD273 and HF1 shared 24 common peptide fragments, CKD273 and HF2 135 and HF1 and HF2 47. However, only 20 peptides were common to

Table 6. NRI and IDI by adding the baseline urinary proteomic biomarkers to a model including covariables

	IDI			NRI		
	IDI (%)	CI (%)	P-value	NRI (%)	CI (%)	P-value
MDRD						
CKD273	0.82	0.00, 1.63	0.051	17.6	−11.4, 46.5	0.23
HF1	0.95	−0.36, 2.26	0.15	33.8	5.36, 62.2	0.020
HF2	2.54	0.65, 4.44	0.009	50.7	22.5, 78.8	0.0004
SF	2.00	0.17, 3.83	0.032	48.4	20.2, 76.5	0.0008
CKD-EPI						
CKD273	0.65	−0.15, 1.45	0.11	17.5	−10.5, 45.5	0.22
HF1	1.35	0.04, 2.65	0.043	30.8	3.09, 58.5	0.029
HF2	1.82	0.34, 3.30	0.016	46.6	19.3, 73.9	0.0008
SF	2.00	0.17, 3.83	0.032	48.4	20.2, 76.5	0.0008

Controls are participants not progressing from baseline to follow-up beyond CKD Stage 2. Cases are participants progressing from CKD ≤2 to ≥3. Models with and without biomarkers are compared. The reference models include as covariables baseline values of mean arterial pressure, waist-to-hip ratio, smoking, blood glucose, log γ-glutamyltransferase, total-to-HDL cholesterol ratio and log 24-h albuminuria (see Table 5). The IDI is the difference between the discrimination slopes of basic models and basic models extended with a predictor variable. The discrimination slope is the difference in predicted probabilities (%) between cases and controls. The NRI is the sum of the percentages of subjects reclassified correctly as cases and controls.

CKD273, HF1 and HF2. Moreover, the signal strength of shared polypeptides differed between classifiers, suggesting that the classifiers provided complimentary rather than duplicate diagnostic information. Supplementary data, Table S5 illustrates the differences in signal intensity for 20 polypeptides common to CKD273, HF1 and HF2.

CONCLUSION

The urinary proteome refines the diagnosis of already existing or progressing renal dysfunction. Our current findings extend previous reports including patients with CKD or diabetes mellitus [5, 6, 8, 9] to the general population, thereby providing a proof of concept in people at low risk. However, further studies are required to port our observation to clinical practice. Prospective studies in other populations should confirm our current findings and randomized clinical trials should demonstrate that the urinary proteome changes in parallel with the response to treatment. Having these research goals materialized would be a major step forward in view of the high prevalence of CKD, which in several countries currently affects over 10% of the adult population [28, 29].

SUPPLEMENTARY MATERIAL

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

T.K., P.Z. and H.M. are employees of Mosaiques-Diagnostics GmbH. None of the other authors declares a conflict of interest.

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Adiponectin receptor and adiponectin signaling in human tissue among patients with end-stage renal disease

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ABSTRACT

Background. Adiponectin plasma levels in chronic kidney disease (CKD) are two to three times higher than in individuals with normal kidney function. Despite adiponectin's anti-diabetic, anti-inflammatory and anti-atherogenic properties, patients with CKD have insulin resistance, systemic inflammation and accelerated atherogenesis. Hence, although adiponectin production is increased by adipose tissue in end-stage renal disease (ESRD), it is unclear if its effects on metabolism remain intact.

Methods. To determine if there is adiponectin resistance in ESRD, we measured tissue levels of adiponectin receptor-1 (AdipoR1) and adiponectin downstream effectors in ESRD patients compared with normal kidney function controls. Blood and tissue samples were obtained from participants at the time of kidney transplantation or kidney donation. A follow-up blood sample was obtained 3–6 months after transplantation.