URINARY PEPTIDE-BASED PREDICTION OF PROGRESSION FROM CHRONIC KIDNEY DISEASE STAGE 2 TO 3

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Introduction

In the context of clinical management of chronic kidney disease (CKD), prediction of disease progression is crucial. In 2014, the European Medicines Agency (EMA) (1) proposed that the primary efficacy endpoints for compound testing should be the prevention or delay of renal function decline defined as either time to occurrence or incidence rate of CKD stage 3. In this study, we compared the previously established urinary multipeptide CKD273 classifier (2) to baseline albuminuria and baseline eGFR for the prediction of change from CKD class 2 to 3.

Material and Methods

Patients data

The cohort consisted of 1642 patients with CKD 2, of which urine samples were previously analysed by capillary electrophoresis coupled to mass spectrometry (CE-MS). The CE-MS data from human urine were selected from the Human Urinary Proteome Database (3).

During 3 years of follow up, 242 patients progressed to CKD stage 3 and above.

Table 1 Cohort design. All parameters are expressed as mean SD.

Cohort summary			
Number of patients	N=1642		
Follow-up (months)	56±10.6		
Mean of datapoints	5±3.8		
Gender (female/male)	(816/826)		
Age	48.6±14.5		
Urinary albumin (mg/L)	15±69.0		
	Baseline data	Follow-up after 3 years	
CKD stages	CKD 2 N=1642	CKD 2 N=1400	CKD 2 to 3 N=242
Mean of eGFR	74±7.8	72.7±7.3	55.4±4.3

Statistical analysis

The primary endpoint defined by the time to reach CKD class 3 during 3 years of follow- up was addressed by a Kaplan-Meier analysis. Receiver operating characteristic (ROC) plots, Kaplan Meier analysis, logistic regression analysis were performed by the MedCalc Software version 12.1.0.0 [www.medcalc.be]. To address the contribution of the CKD273 classifier to baseline covariates, we calculated the net reclassification index (NRI) and the integrated discrimination improvement (IDI).

Results

1. Risk and multivariable-adjusted risk for CKD class changes. Figure 1A and **1B** showed a Kaplan Meier analysis performed to determine the hazard ratio to predict a class changes from CKD class 2 to 3.

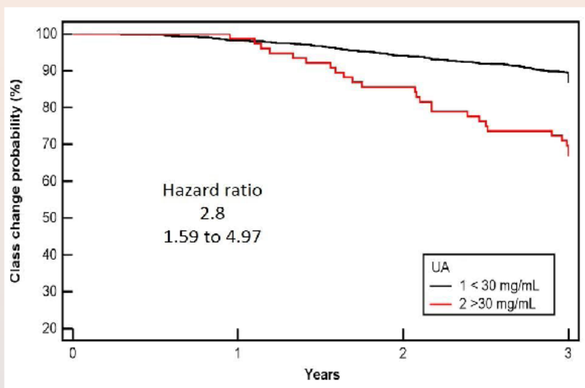

Figure 1A

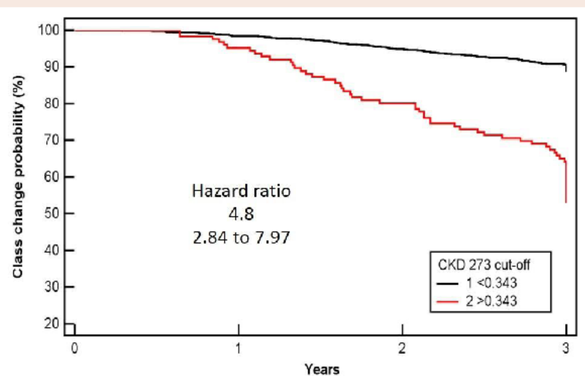

Figure 1B

The prognostic utility of baseline values of baseline urinary albumin (**Fig 1A**, risk group >30mg/mL) was compared with the CKD273 classifier (**Fig 1B,** risk group >0.343 established cut-off).

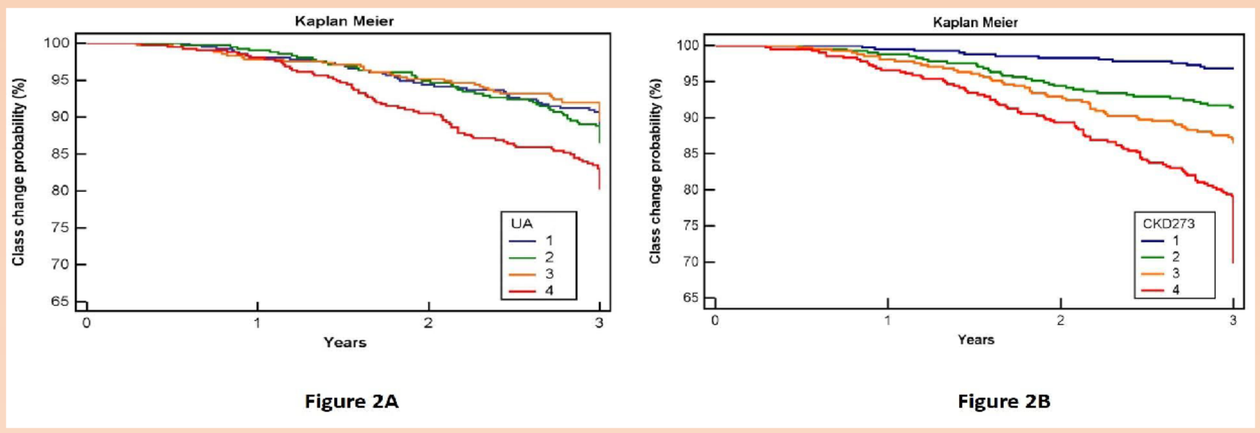

Figure 2A Figure 2B

The same comparison was made dividing the cohort in quartiles on the basis of both urinary albumin values and CKD 273 classifier score (**Fig 2A** and **2B**, respectively). While based on the urinary albumin the first three quartiles do not display any significance in the risk of progression (P>0.05), it is observed that the CKD 273 classifier can better stratify the CKD patients in terms of the risk of progression (P<0.05 for all the four curves).

2. Contribution of the CKD273 classifier to the baseline covariates for prediction of CKD class change

A significant improvement (P <0.0001) in the prediction of CKD class change was obtained by the combination of baseline eGFR, baseline albuminuria and the CKD273 classifier, compared to the validated risk factor–based model of the combination of baseline eGFR and albuminuria. These results are supported by the **net reclassification index (NRI)** (0.24 0.04 P<0.0001) and the **integrated discrimination improvement (IDI)** (0.06 0.009 P<0.0001).

3. Individual urinary peptides predicting class changes

We performed *de-novo* statistical analysis to determine differences in abundance of urinary peptides specifically predicting the CKD 2 to 3 and 4 class changes.

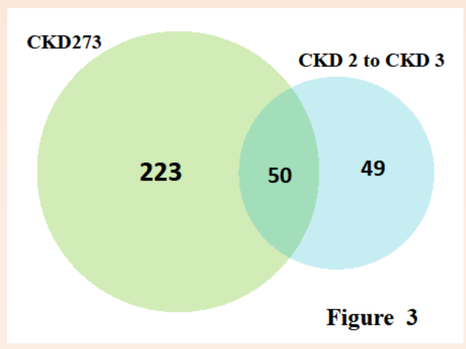

Figure 3

Figure 3 shows the peptide overlapping with the CKD 273 classifier. 50 peptides were already included in the classifier. 94% displayed reduced urinary abundance in patients progressing to CKD 3. The majority of these overlapping peptides were fragments of the alpha chain of type I collagen (67%).

The newly identified peptides associated to CKD class change are directly or indirectly related to the extracellular matrix (ECM) and inflammation processes

- **<u>Peptides with decreased abundance:</u> peptides associated with ECM**

-80% are collagen fragments derived from collagen alpha-1(II) chain, collagen alpha-1(II) chain, collagen alpha-2(I) chain, collagen alpha-3(IV) chain, collagen alpha-1(XI) chain, collagen alpha-1(XIV) chain, collagen alpha-1(XVI) chain, collagen alpha-1(XXIV) chain.

-Microtubule associated protein 6 (MAP6), which stabilizes the microtubule formation.

- **<u>Peptides with increased abundance</u>: peptides associated with Fibrosis**

-Fibrinogen fragments indicated chronic renal damage.

-Mucin-1 subunit alpha (MUC1) caused autosomal dominant tubulointerstitial disease renal fibrosis.

Conclusions

- ❑A novel approach for clinical assessment of CKD was established using the CKD273 classifier to predict progression of CKD from CKD stage 2 to CKD stage 3 or higher.
- ❑A patient with a CKD273 score above the predefined cut-off (0.343, Good *et al.* 2010) has a significantly higher probability to display a class change from CKD II to CKD III or higher and outperforms urinary albumin in the stratification of CKD patients.
- ❑The addition of CKD 273 classifier to the individual use of baseline eGFR and baseline albuminuria significantly improved the prediction of class change.
- ❑New potential biomarkers need further validation.

References:
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(2) Good DM, Zurbig P, Argiles A, Bauer HW, Behrens G, Coon JJ, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol Cell Proteomics 2010.
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