Urinary proteomic biomarkers, a powerful tool for prognosis of Autosomal dominant polycystic kidney disease progression

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Background:

Autosomal dominant polycystic kidney disease (ADPKD) accounts for about 5% of patients with end stage renal disease (ESRD). Now that specific treatment options likely become available in the near future, prediction of disease course would be of utmost importance to select high risk patients for treatment.

We have previously identified ADPKD-specific patterns of urine peptide excretion that differentiate ADPKD from control patients and correlate with disease severity, but we have not been able to predict disease course over the short term so far. Here, we used extended follow up time and a hard endpoint and identified a set of urinary peptides that predicted ESRD, thus allowing early detection of patients at high risk for progression.

Materials and Methods:

Urine proteome analysis:

We used capillary electrophoresis online coupled with mass spectrometry (CE-MS) to analyze the low molecular weight urinary proteome of morning urine samples. This method allows to **simultaneously** quantify > 1.000 peptides in a single urine sample with high degree of reproducibility. Sequence identification of relevant was attempted using LC-MS.

CE-MS instrumental setup
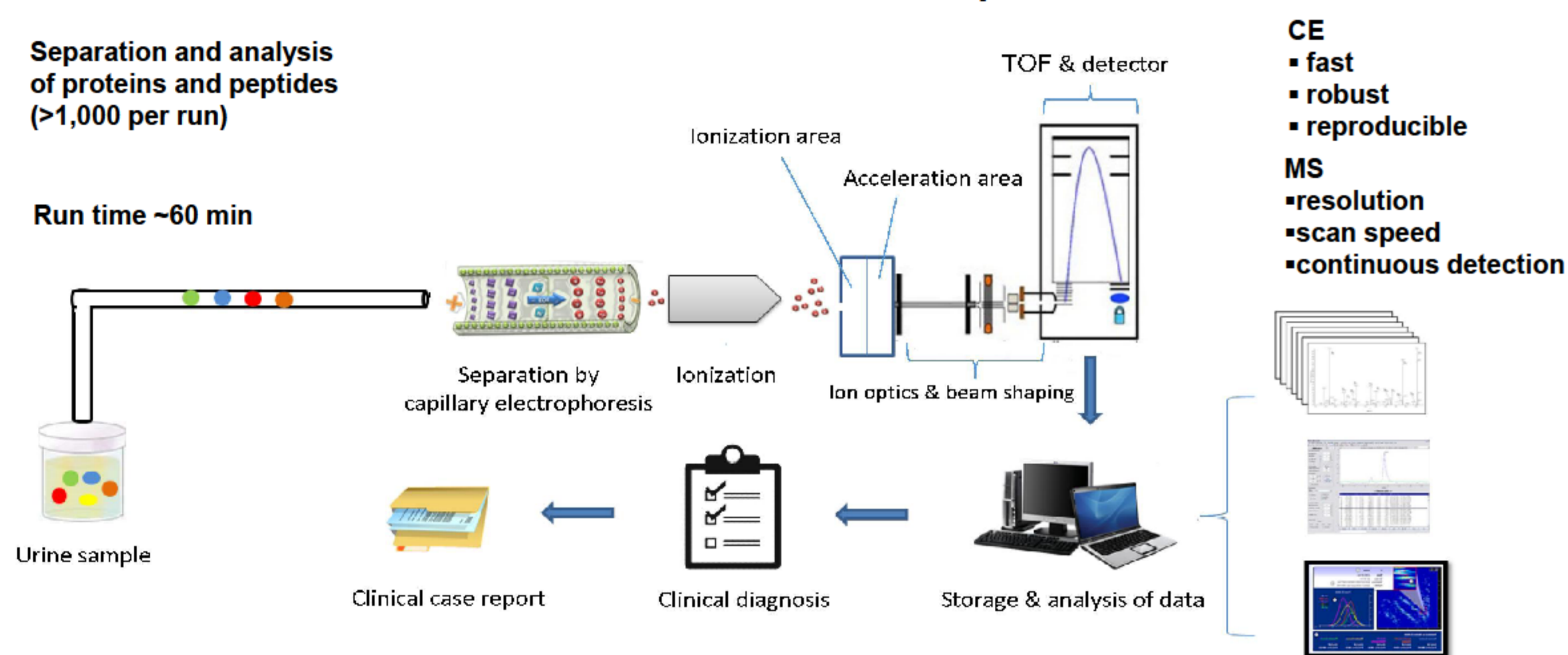
On-line coupling of CE for peptide m/z separation and ESI-TOF-MS for mass detection. CE-MS coupling uses a coaxial sheath-flow system. The setting allows sensitive and fast mapping of low-molecular weight urinary proteomes. Spectra of identified polypeptide peaks are transduced to peptide lists for comparative analysis and support vector machine (SVM) learning to generate multimarker models.

Patient cohort and clinical assessment:

The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) cohort includes 241 patients with ADPKD, age 15-45 years and creatinine clearance >70 ml/min at baseline. Patients were followed with serum creatinine, iothalamate GFR, and MRI total kidney volume (TKV) measurement at baseline, year 1, 2, 3 (CRISP I), 6 and 8 (CRISP II) and many of the patients continue to be followed up with follow up time of up to 13 years.

Statistical analysis:

To identify markers associated with risk of progression, we compared baseline urine samples of patients reaching ESRD during follow up with those fulfilling the following criteria: (1) no ESRD during follow up; (2) >7 years follow up time available; (3) negative GFR slope of no more than 3ml/min/year; (4) age matched. Two thirds of both cases and controls were used as a derivation cohort. Their urinary peptidomes were compared to each other and significantly different peptides were combined into a support-vector-machine based model to differentiate cases from controls. The model score was then validated in the remaining one third of ADPKD ESRD and control patients.

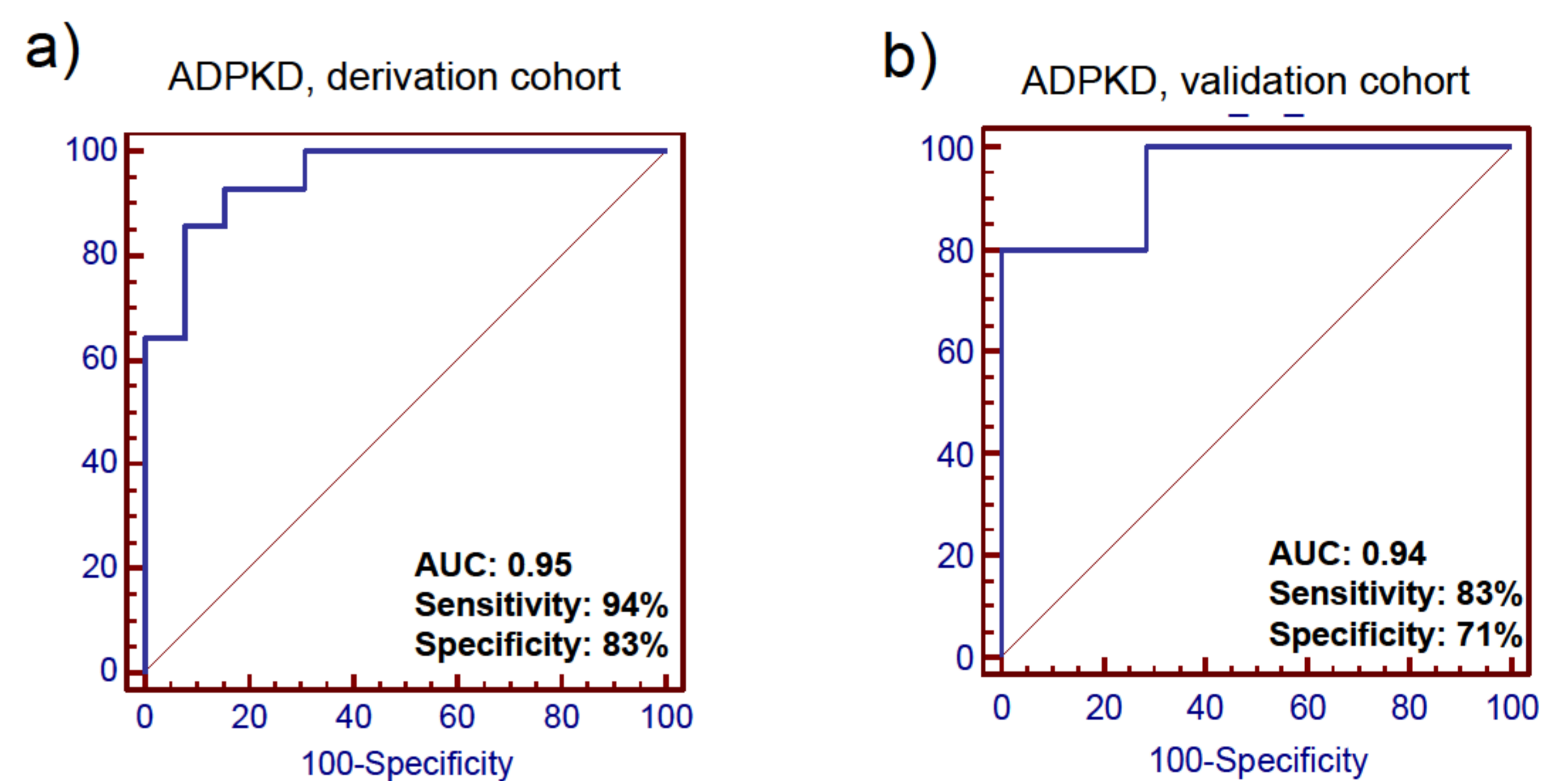### Clinical proteome data processing system
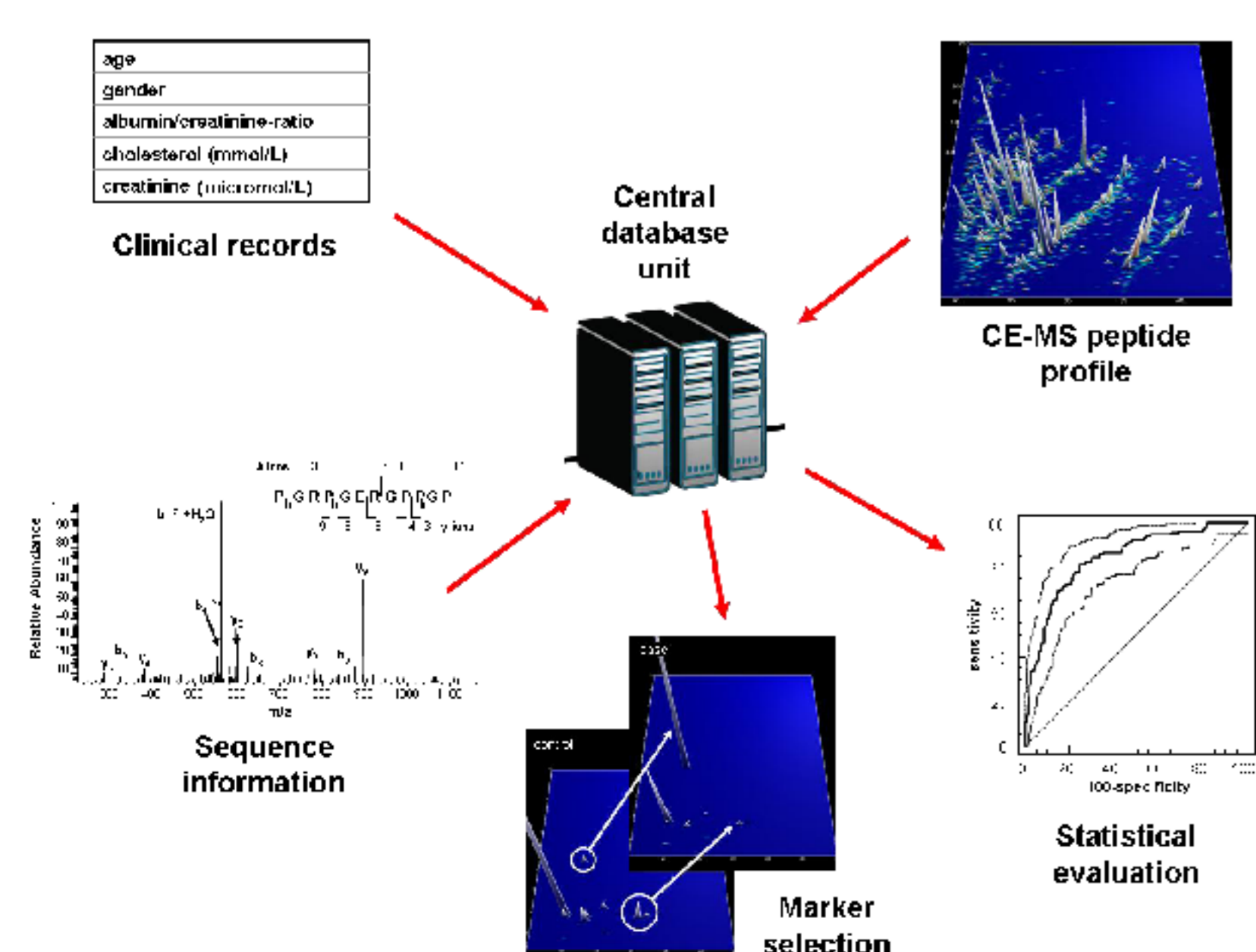
Storage and retrieval of CE-MS peptide spectra, peptide sequences and patient clinical records in a central database for appropriate sample selection and comparative proteome analysis.

Results:

Of 241 ADPKD patients with an available baseline urine sample, 28 reached ESRD over a follow up time of up to 13 years.

We selected 14 ADPKD patients with progression towards ESRD and 39 ADPKD patients with slow disease progression as development cohort for a prognostic biomarker model (Figure 1a).

A multidimensional marker model based on 16 urinary peptides enabled the prediction of ESRD in validation cohort (n=19; 5 with ESRD with age <40 at baseline and 14 without ESRD during follow up with age <24 at baseline) with an AUC of 0.94 (95% CI 0.73-0.99). Sensitivity and specificity were 83% and 71%, respectively, at a predetermined cut-off level (Figure 1b) .For further validation, we applied the proteomic model to young ADPKD patients (<24 years of age) to predict their progression to ESRD (i.e GFR change from baseline to year 8). In this group the model showed moderate correlation with GFR slope $R^2=0.29$ (Figure 2).

Figure 1: Receiver operating characteristics curve for prediction of ESRD

a) ADPKD, derivation cohort — AUC: 0.95, Sensitivity: 94%, Specificity: 83%

b) ADPKD, validation cohort — AUC: 0.94, Sensitivity: 83%, Specificity: 71%

Receiver operating characteristics (ROC) curves for the prediction of end stage renal disease (ESRD) in the derivation cohort and validation cohort of ADPKD patients.
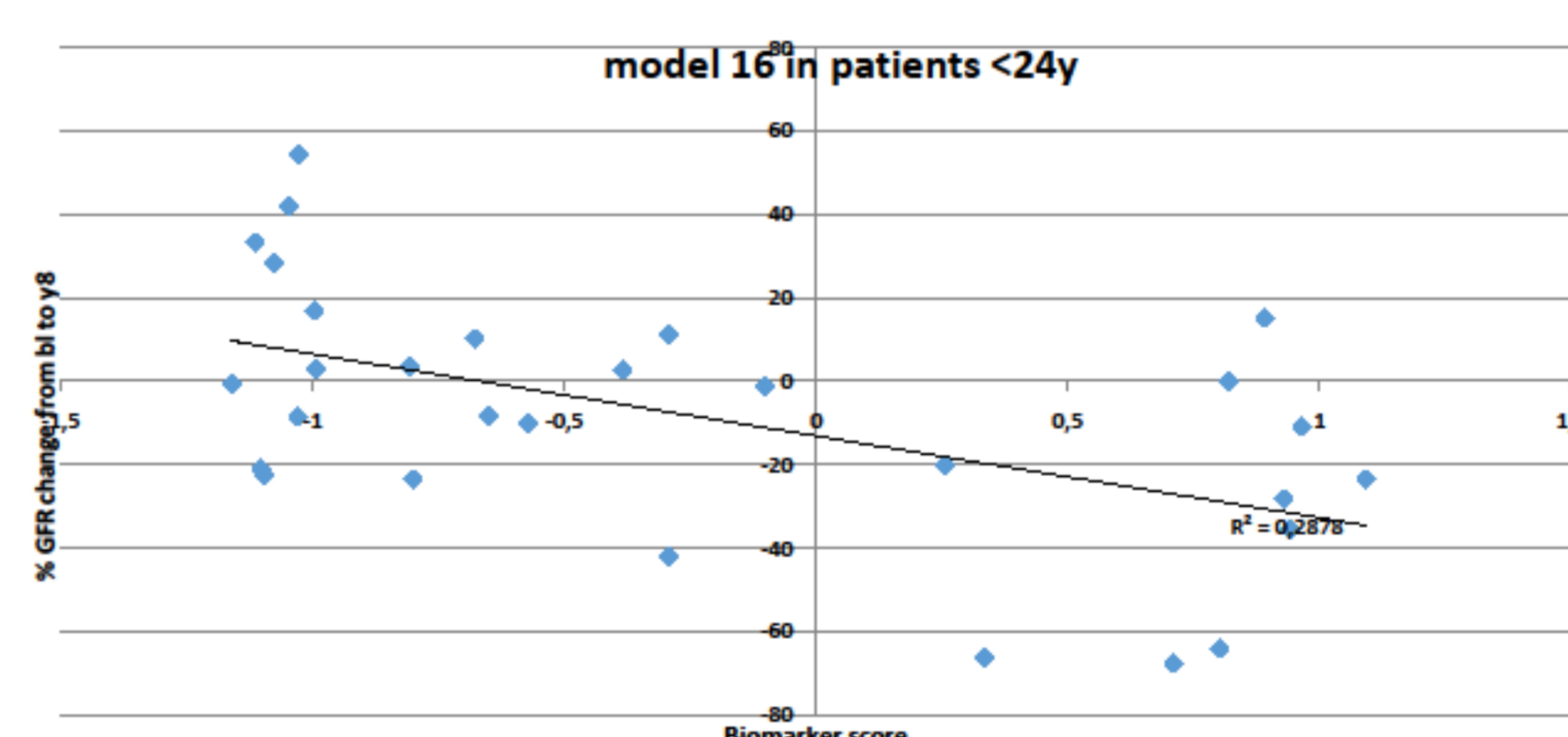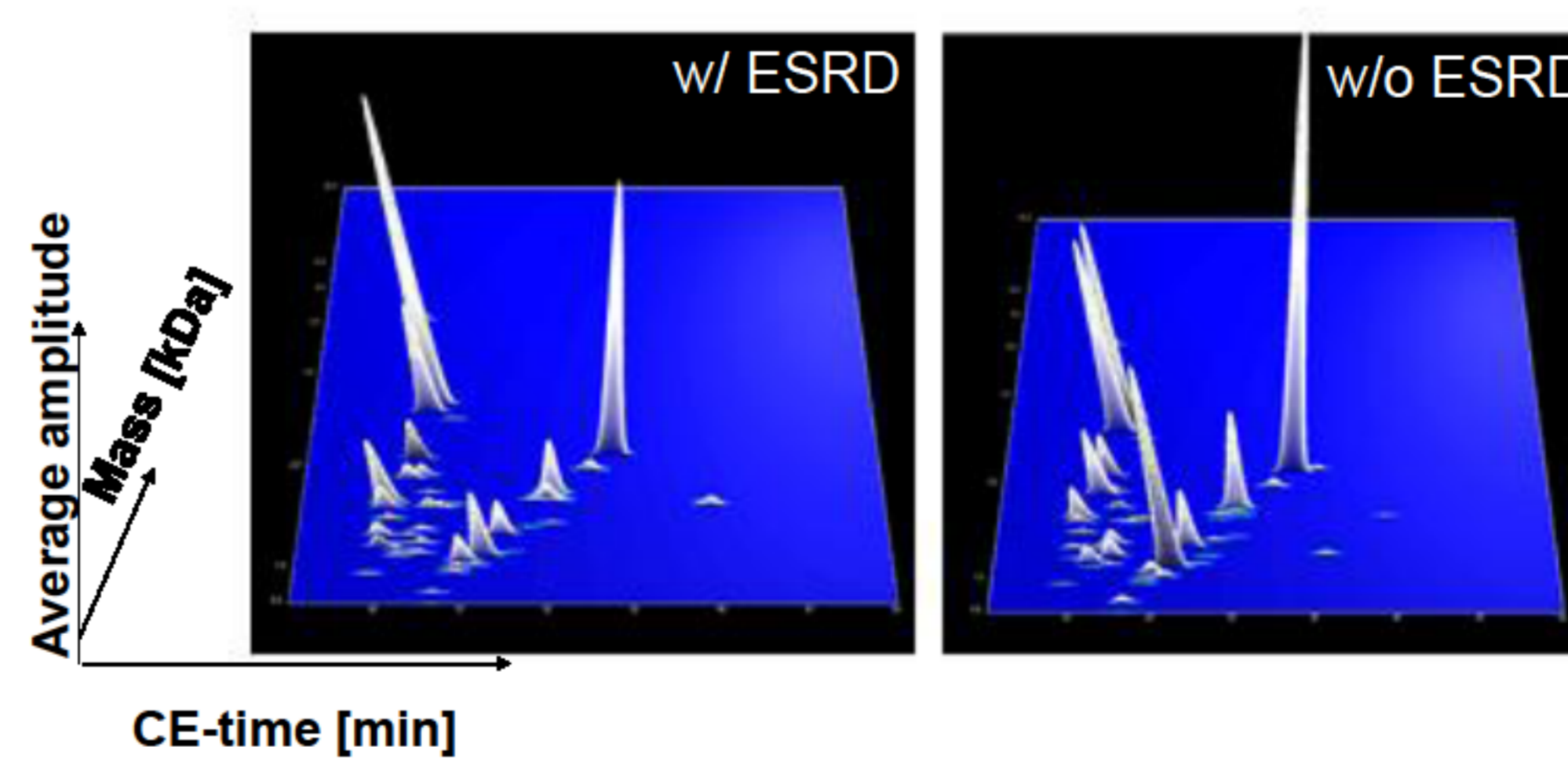

model 16 in patients <24y

Figure 2. Performance of the model in young ADPKD patients

CE-MS profiling of ADPKD patients with and without ESRD

w/ ESRD   w/o ESRD

Compiled CE-MS spectra of peptides included in the urine marker panels. CE migration time (x-axis, min) is plotted against log molecular weight (y-axis, kDa). Mean signal intensity is given as peak height (in arbitrary units).

12 of the 16 urinary peptides included in the model could be identified by sequencing. Most of them were collagen-derived fragments. These suggest structural changes to the extra cellular matrix (ECM) during cysts formation. Other peptides included in the model were fragments of fibrinogen alpha chain, agrin, antithrombin-III and alpha-1-microglobulin.

Conclusion:

We have identified a small group of urinary peptide markers that apply additional prognostic information besides the determination of the slope of glomerular filtration rate and the total kidney volume in order to improve prediction of ESRD in ADPKD patients.