Metabolomics to predict progression in chronic kidney disease

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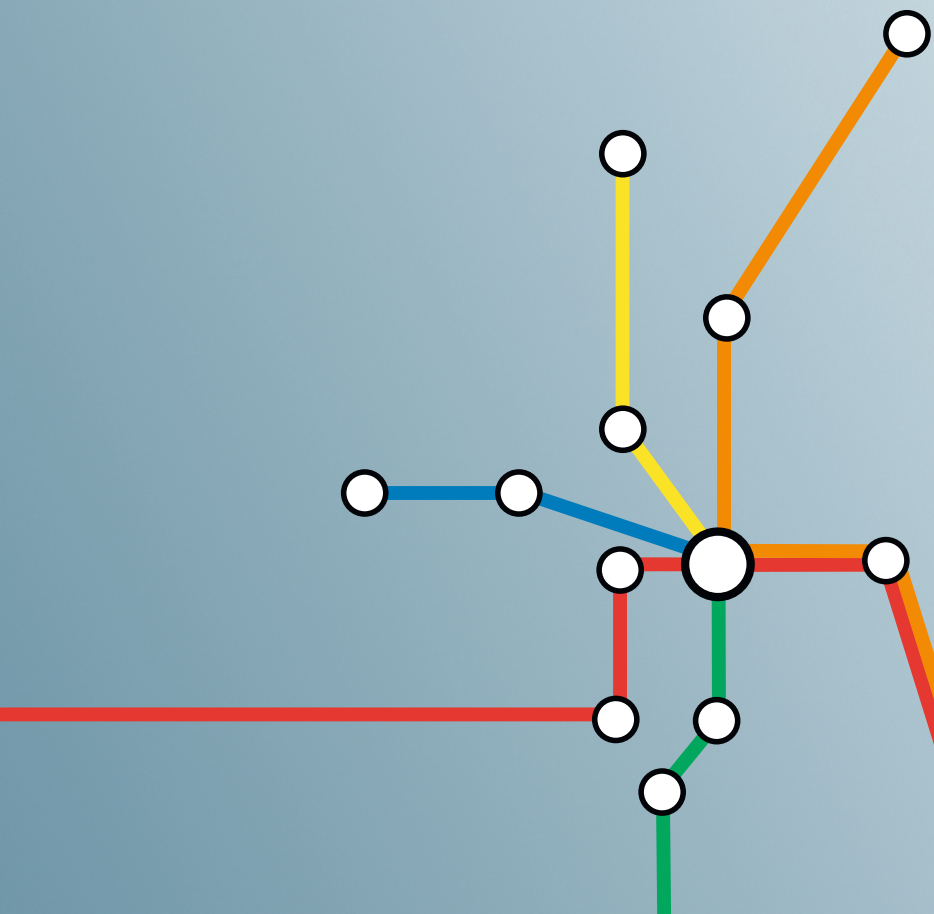

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Summary and general discussion

The challenges in detecting chronic kidney disease (CKD) and predicting future disease course make it difficult to select patients who require treatment aimed at slowing down the rate of renal function decline. Identification of alternative biomarkers is an important unmet clinical need to improve current risk assessment strategies and patients' prognosis. A surrogate biomarker ideally fulfils the following features to be of clinical relevance: 1) sensitively and quantitatively reflect disease severity, 2) robustly predict disease progression, in particular prior to eGFR decline, 3) easily to obtain, and relatively inexpensive to measure, 4) allow repeated measurements during a follow-up period, and 5) rapidly respond to an intervention for assessing efficacy, which could also provide prognostic insights. Several sources including urine and blood have been used for biomarker research. Analysis of urine is in particular of interest because it can accumulate and tolerate major differences resulting in larger percentages of changes in composition.[1] Furthermore, the wide availability and non-invasive nature of urine sampling also make it an ideal source.

In this thesis we explored surrogate biomarkers in a specific type of chronic renal disease, namely autosomal dominant polycystic kidney disease (ADPKD). ADPKD is the most common inherited kidney disease.[2] A well-defined, large, homogeneous cohort of patients with different stages of CKD with a standardized, longitudinal follow-up was available for biomarker research. In **chapter 1** a general introduction was given, discussing the need of prognostic biomarkers in CKD, the limitations of current biomarkers for this purpose and the potential of novel biomarker platforms for risk stratification.

Urinary biomarkers for risk stratification in CKD

In CKD various urinary biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), monocyte chemoattractant protein-1 (MCP-1) and beta-2 microglobulin (β2M), were studied as potential candidates for predicting disease progression.[3,4] These biomarkers have been evaluated in many CKD etiologies, but also in acute kidney injury (AKI). In **chapter 2** we provided an overview of urinary biomarkers for predicting disease progression in CKD patients with ADPKD that have been studied prior to our research. This narrative review showed that there are two prognostic panels of markers, a biomarker score based on β2M and MCP-1 and a urinary peptidomic profile, which hold promise to support patient risk assessment. To date, none of these biomarkers are routinely used in clinical practice. We concluded that the role of urinary biomarkers in chronic renal disease remains unclear and is therefore still an important area to research.

Two novel markers, the cell cycle arrest markers tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7), have been incorporated in a clinical diagnostic test for AKI.[5] Since AKI is an important risk factor

for CKD, urinary biomarkers of AKI might also be of interest in chronic renal disease. In **chapter 3** we addressed the potential of these tubular markers in CKD patients with ADPKD, since the pathophysiology of ADPKD involves tubular dysfunction. We hypothesized that in ADPKD progressive cyst growth and compression of surrounding tissue could cause a 'snowball' effect with multiple repetitive acute ischemic events, which may result in changes in urinary TIMP-2 and/or IGFBP7. The markers were studied separately and after multiplication ([TIMP-2] x [IGFBP7], according to the NephroCheck test). Baseline urinary TIMP-2 and IGFBP7 levels were not higher and tended to be lower in this cohort of ADPKD patients compared with healthy controls. Levels did not differ across the subsequent stages of CKD. Because urine is more diluted in later-stage CKD, we corrected for this potential confounder. However, after correcting TIMP-2 and IGFBP7 for osmolality, levels still did not differ across the stages of CKD. Furthermore, multivariable regression analyses showed no association between eGFR and both tubular markers and their product. The same was true for height-adjusted total kidney volume (htTKV). In conclusion, urinary TIMP-2 and IGFBP7 levels did not correlate with the degree of disease progression in CKD patients with ADPKD and lack potential for risk stratification.

Alternative prognostic biomarkers

Given that a panel of markers could improve risk stratification in kidney disease more effectively than a single biomarker, the metabolomics approach is especially valuable. In **chapter 4** and **5** we studied the potential of urinary metabolic profiling in chronic renal disease using a targeted, quantitative nuclear magnetic resonance (NMR)-based metabolomics strategy. Compared with mass spectrometry (MS), NMR offers unsurpassed analytical reproducibility.[6,7] In **chapter 4** we quantified twenty-nine urinary metabolites in baseline samples from chronic renal disease patients with ADPKD with various stages of CKD. The NMR work-flow that has been built is specified and largely automated, making it possible to reliably and accurately measure and quantify a large group of urine metabolites in large patient cohorts.[8] To explore whether these metabolites associate with renal function, we applied an automatic optimization procedure to evaluate all possible combinations of the quantified metabolites and select the statistically best performing combination of metabolites for the model, considering the interaction between metabolites. We identified an optimal subset of four urinary metabolites (myo-inositol, 3-hydroxyisovalerate, ADMA and creatinine) that strongly associated with the actual estimated glomerular filtration rate (eGFR). To predict disease progression, we evaluated whether the set of twenty-nine urinary metabolites associate with annual change in eGFR. Applying the same model optimization routine as used for baseline eGFR, we found that the ratio of baseline urinary alanine over citrate was the strongest combination that associated with the subsequent rate of eGFR decline over time. Patients with a more rapid decline in renal function showed a higher

baseline urinary alanine/citrate ratio than those with a slower decline in eGFR. Furthermore, this metabolite ratio showed additional value beyond conventional risk markers including htTKV. These results were validated in a separate cohort of patients with ADPKD. We concluded that urinary metabolic profiling is a promising tool for risk stratification in CKD. In **chapter 5** we further explored the robustness of this platform. In a separate cohort of ADPKD patients, we quantified fifty-five urinary metabolites and ratios (thirty-eight urinary metabolites and seventeen physiologically relevant metabolite ratios). We used the same model optimization routine to find the best subset of metabolite predictors for distinguishing fast from slow progressors and found that an optimal combination consisted of two urinary metabolites (betaine and phenylacetylglycine) and one metabolite ratio (alanine/citrate). When measured in a single urine sample, the prognostic performance of the metabolite model to predict fast progressive disease was comparable to that of a model including the currently more established risk marker htTKV. Combining htTKV and the metabolite model improved the predictive value beyond that of single predictors. To evaluate whether changes in urinary metabolites over a 3-year follow-up period differ between fast and slow progressors, we used, for every metabolite and metabolite ratio, an individual two factor (time and progression) repeated measure ANOVA model. Longitudinal analyses showed an increase in the myoinositol/citrate ratio in fast progressors over time, while no significant change was found in those with slow progressive disease. This study demonstrated that a single-timepoint urinary metabolite profile offers a promising, straightforward approach for risk stratification in chronic renal disease.

In addition to risk stratification, the 'perfect' biomarker ideally fulfills additional features to be of clinical relevance including responding to an intervention for assessing therapy efficacy and providing prognostic insights in a disease. The DIPAK 1 trial showed that the somatostatin analogue lanreotide reduced height-adjusted total liver volume (htTLV) in comparison with standard care in patients with ADPKD.[9] Polycystic liver disease (PLD) is an important extra-renal manifestation of ADPKD. In situations of intrahepatic bile acid accumulation such as cholestasis, increased bile acid (BA) levels have cytotoxic effects leading to cholangiocyte cell damage.[10] Excess urinary elimination of BAs in turn may contribute to tubular epithelial injury.[11,12] In **chapter 6** we determined BAs as markers of liver and renal disease progression in relation to lanreotide efficacy. Using liquid chromatography-mass spectrometry (LC-MS/MS) we quantified eleven bile acids in serum and six in urine from ADPKD patients with and without PLD who had participated in the DIPAK 1 trial. We hypothesized that serum BA levels rise in PLD, and that somatostatin analogues might lower these levels due to their therapeutic effect on liver growth rate. We furthermore hypothesized that urinary BA excretion is increased in PLD patients which might contribute to the advancement of renal disease. Baseline serum levels of taurine- and glycine-conjugated BAs were associated with liver

volume. Lanreotide reduced BA levels. However, this reduction was not associated with a change in liver volume. We cannot assess causality in this study. Whether the reduction in serum BAs lies in a causal pathway of the beneficial effect of lanreotide on liver growth remains unknown. In this study, urine BA levels did not correlate with renal disease progression.

In conclusion, this thesis shows that risk stratification in kidney disease could be improved by combining biomarkers into panels. Urinary metabolic profiling added predictive value on top of conventional risk markers in chronic renal disease.

Future perspectives

It remains a challenge to identify a panel of biomarkers to fulfill all the relevant features in the clinical management of patients with progressive (chronic) renal disease. In each chapter in this thesis the main study strengths and limitations were acknowledged. Briefly, all studies were conducted in a phenotypically well-defined, large, homogeneous cohort of patients with standardized follow-up data. Furthermore, an established platform was used for biomarker assessment. NMR spectrometry offers reliable analytical reproducibility and only minimal required sample pre-treatment. It allows measurement and quantification of many metabolites in a large number of samples. A limitation of the research described in this thesis includes the limited follow-up period. The use of hard study endpoints, such as end-stage renal disease or doubling of serum creatinine, was therefore not feasible. Such outcomes strengthen the reliability and performance for predicting disease progression. In addition, we used a targeted instead of an explorative approach to select and quantify urine metabolites and used a methodology which might be less sensitive than some other analytical techniques including MS. Future studies might focus on overcoming the limitations of current biomarkers and exploring other potential characteristics of biomarkers.

First, the prognostic performance of biomarkers should be validated in external cohorts. Instead of the discovery of the next novel biomarker, in-depth analysis of currently available data is desirable and may impact patient care. Combining metabolic markers, current urinary biomarkers and conventional clinical markers into a multi-marker panel and performing a comparative analysis in one cohort is critical to refine the search to the 'perfect' biomarker panel. To overcome current study limitations, prospective longitudinal studies in large populations with a long follow-up period and a broad range of patient characteristics, CKD etiologies and rates of renal disease progression would be valuable.

Second, future research may focus on the role of biomarkers in improving our understanding of the pathophysiology of kidney disease. Is the biomarker specific to a

particular type of kidney disease, or does it reflect a more general form of damage. The urinary metabolites that we identified may play a role in chronic renal dysfunction in general and not be specific to ADPKD, but this remains unknown. Most urinary markers are not specifically intended for diagnostic purposes, but rather for staging of disease and for making a prognosis. However, research that aims to better understand the physiology of biomarkers could contribute to a better understanding of the mechanisms that underly and drive disease progression; mechanisms which are mostly multifactorial and only partially understood.[13] A single biomarker is insufficient to address the need for dissecting the mechanisms underlying disease progression. A holistic approach such as metabolomics may offer significant benefits. Metabolomics provides an analytical framework for evaluating a panel of markers, which is crucial in the study of a multifactorial disorder like CKD. Additionally, metabolomics may offer valuable insights into the pathophysiological mechanisms involved in renal disease. Recent data also acknowledged and confirmed the added value of metabolomics in CKD.[14,15] The two metabolite ratios we identified to be most important in renal disease progression both included urinary citrate as their denominator. This strengthens its well-documented role as a marker of chronic renal dysfunction. Besides, citrate and myoinositol, another prognostic metabolite that we identified, are commonly reported in literature in the context of chronic renal disease. A recent study strengthens these findings and reports a set of urinary metabolites including citrate for predicting the progression of CKD.[16] The study design of our research was not suitable for an unequivocal causal or mechanistic interpretation of the identified urinary metabolites. The potential of metabolomics in future research might be to unravel the role of specific metabolites in pathophysiology of renal diseases to improve the understanding in mechanisms underlying disease progression. In this thesis we used a targeted approach in which a known set of metabolites was quantified. In future research using an explorative approach with a sensitive analytical technique could be of added value.

Last, a future role of biomarkers in clinical practice would be to use them to follow-up on therapeutic efficacy or better, to use them to predict a response to treatment early on, so as to tailor therapy to an individual. Personalized medicine will be increasingly relevant, especially with the advent of potential renoprotective therapies. Markers should reliably distinguish a specific patient that will respond to treatment from non-responders. Previously, several studies reported data on approaches to explore this need. The Parameter Response Efficacy (PRE) score, an algorithm based on short-term drug-induced changes in multiple markers, was developed to predict the long-term effect of a drug on cardiovascular and renal outcomes. It included various clinical and laboratory markers including blood pressure and the urine albumin-creatinine ratio. The score has been validated in several studies.[17-19] However, data on its role at an

individual level is lacking. Another score that has been studied is the CKD273-classifier. A specific pattern of 273 protein fragments was identified which was used for the prediction of CKD progression.[20-22] The potential value of this score in the context of response to treatment was determined in various smaller studies.[23,24] A recent large study used in silico evaluation to examine the predictive value of three urinary peptidomic classifiers, CKD273, HF2 and CAD160, in 5585 subjects in response to in silico treatment. This biomarker-guided approach determines the risk of CKD onset and progression. In case of increased risk the impact of commonly used interventions, including antidiabetic and antihypertensive drugs, on the proteomic classifiers could be modulated based on the data.[24] A shortcoming of this approach is that the study was based on in silico intervention only, however, since it provides information on the future impact of therapy, it might be a next step towards personalized medical care and individualized therapy. Additionally, if the likelihood of a favorable response to therapy can be predicted with a biomarker panel, this panel could also be used to select an optimal patient population for clinical trials.

In conclusion, there remains a clinical gap for sensitive and specific non-invasive biomarkers that enhance patient management in chronic renal disease. A holistic 'omics' approach offers promising potential for advancing the study of renal diseases.

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