Prediction and monitoring of chronic kidney disease

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Chapter 9

Summary and future perspectives

Summary

Chronic kidney disease (CKD) is defined as impaired kidney function or signs of structural kidney damage, that persist for longer than 3 months and that have implications for health.[1] CKD has a prevalence of approximately 10% in the general population and represents a substantial burden to global health.[2] CKD, and its major causes hypertension and diabetes mellitus, lead to end stage renal disease (ESRD), and can cause cardiovascular disease and mortality.[1,3] Given the large impact CKD has on global health, it is necessary to identify factors that can predict whether a patient with CKD is likely to have disease progression, as these patients need closer monitoring and treatment to reduce their risk. This thesis consists of two parts. Part 1 aimed to investigate novel biomarkers that can be used to predict CKD progression. Part 2 aimed to investigate how estimated glomerular filtration rate (eGFR) can be best monitored over time, with regards to the choice of biomarker to estimate GFR, the choice of assay for that biomarker, and pre-analytic sample handling. In this final chapter the main findings of each chapter are summarized, and implications for future research and clinical practice are addressed.

Part 1. Biomarkers for prediction of CKD progression

Serum bicarbonate

Metabolic acidosis, characterized by low serum bicarbonate levels, is associated with an increased risk of ESRD and mortality in non-diabetic CKD populations.[4-8] It is, however, unknown whether this association is also present in patients with diabetes. In **Chapter 2** we studied the associations of serum bicarbonate with kidney and cardiovascular endpoints and mortality in a cohort of patients with type 2 diabetes and diabetic kidney disease. We found that patients with low serum bicarbonate levels had a higher risk of developing ESRD and a higher mortality risk. However, these associations were dependent on baseline eGFR. These findings contradict earlier studies that did report an independent association of serum bicarbonate with ESRD and mortality.[4-8] The main difference between those studies and our study is that all patients in our study had type 2 diabetes, whereas the other studies predominantly included patients without diabetes. Indeed, when data of other studies were reviewed in more detail, we found that after stratification for diabetes status those studies also showed that low serum bicarbonate levels are not independently associated with ESRD in these subgroups.[5,7,8] The lack of an eGFR-independent association of low serum bicarbonate with ESRD, mortality and cardiovascular end points in our study suggest that in patients with diabetes and CKD, treatment to correct metabolic acidosis may not improve outcome, or should be commenced at a different threshold than the 22 mEq/L as advised by the KDIGO guideline.[1] Therefore, future research should investigate if and when bicarbonate suppletion should be started in patients with CKD and diabetes. Currently four randomized controlled trials are being conducted to assess the benefits and risks of bicarbonate suppletion in patients with CKD, and two trials specifically address patients with diabetes.[9-12] Hopefully the results of these trials will lead to the development of evidence-based guidelines for correction of metabolic acidosis specifically in patients with diabetes and CKD.

Advanced glycation end products and skin autofluorescence

Skin autofluorescence (SAF) is a validated proxy for skin accumulation of advanced glycation end product (AGE). AGEs are irreversibly glycated proteins, and accumulation of AGEs causes inflammation and tissue damage.[13-23] It has already been shown that increased SAF levels are associated with the presence of CKD and diabetes,[21,24] and with progression of CKD in patients with various stages of CKD.[25] It is not yet known whether SAF is also associated with CKD progression in patients with preserved kidney function. In **Chapter 3** we investigated the associations of SAF with diabetes mellitus and eGFR decline in patients with peripheral artery disease and preserved kidney function. We found no association between SAF and eGFR decline, and the association also did not differ between patients with and without diabetes. These results conflict with the findings of the only other study that has investigated the association of SAF and kidney function decline.[25] That study was performed in a Japanese CKD cohort, which found that SAF is associated with kidney function decline in patients with stage 1-5 CKD. We pose two possible explanations for these conflicting results. First, the Japanese cohort did not provide information on baseline cardiovascular comorbidity, and it is known that patients with cardiovascular disease (including peripheral artery disease) have higher SAF levels. Indeed, the mean SAF levels in our study were higher than in the Japanese cohort, suggesting that our cohort had a higher prevalence of cardiovascular comorbidity. It is therefore possible that SAF does not predict kidney function decline in patients who already have high SAF levels due to cardiovascular disease. Second, AGEs are filtered by the glomerulus, and thus AGE levels depend on GFR.[17] It is possible that AGE accumulation due to low GFR only occurs in patients with a GFR below a certain threshold. Our patients had preserved kidney function at baseline, and thus this may be a reason why SAF was not associated with kidney function decline in our study. We conclude from this chapter that SAF is not a good predictor for eGFR decline in patients with preserved kidney function and peripheral artery disease.

Biomarker panels to predict kidney disease progression

Chapters 2 and 3 are both examples of traditional biomarker research in CKD. A single marker is investigated with regards to its association with clinical end points such as ESRD, to determine whether the marker-outcome association is independent of other CKD progression risk factors. Albuminuria and eGFR are currently the most important biomarkers to predict risk of CKD progression. Many other biomarkers have been investigated in studies with similar methods as chapters 2 and 3, but as yet none of them has consistently outperformed albuminuria and eGFR with regards to prediction of kidney-related endpoints. CKD is a multifactorial disease, in which progression is determined by various pathophysiological pathways. It may therefore be necessary to use multiple markers to combine information on all these pathways to obtain an optimal risk prediction equation. In **Chapter 4**, we therefore performed a review of the literature on biomarker panels for prediction of CKD progression, specifically in patients with type 2 diabetes. We analyzed the methodology of nine studies that investigated biomarker panels for prediction of diabetic kidney disease progression,[26-34] based on the criteria presented in Box 1. Overall, we found that all studies represent early phases of the biomarker panel development process, and none assessed the final steps that are necessary to implement a biomarker panel in clinical practice: studying the

biomarker panel in several patient cohorts (clinical utility), and investigating the effect the biomarker panel may have on treatment to improve clinical outcomes. Hence, the conclusion of this chapter is that biomarker panels show promising results for improved prediction of CKD progression, but that the necessary studies to validate these panels for use in clinical practice are lacking. To fill this gap in knowledge we provided key points to improve trial design and methodology for development of biomarker panels in chronic kidney disease.

Box 1 Critical points to assess when reviewing or conducting biomarker studies

Phase of development
- Proof of concept
- Association with hard end points
- Assessment of incremental value
- Clinical utility
- Clinical validation

Population
- Early or late-stage disease

Endpoints
- Surrogate endpoints
- Hard endpoints

Measurement of biomarkers
- Selection of matrix (blood, urine)
- Handling, freezing and storage of samples before analysis
- Assay characteristics
- Cut-off points for biomarker

Statistical analysis
- Sample size and number of events
- Adjustment for covariates (etiologic or predictive)
- Discrimination (AUC, NRI, IDI)
- Calibration (observed and expected event rates)

Part 2. Biomarkers for monitoring of eGFR decline

Creatinine assays

In epidemiological studies and clinical trials, creatinine is routinely measured at each patient visit from fresh samples and GFR is estimated using that creatinine value. Another option could be to store all samples collected during the study, and then perform a single run analysis of all samples per individual at the end of the study under the same analytical circumstances, thereby eliminating the effect of day-to-day variability. On the other hand, using samples that have been stored during prolonged periods of time may induce variability. As yet it is not clear whether single run analysis

for repeated measurement of eGFR yields more reliable eGFR slopes than routine analysis. It is also not known which creatinine assay yields the best eGFR slopes. Two assays are available to measure creatinine: Jaffe and enzymatic. Although Jaffe assays are cheaper, enzymatic assays are considered more reliable due to lower chemical interference by other compounds.[35,36] In **Chapter 5**, using data from the SUN-MACRO trial, we investigated therefore which method would yield the most reliable creatinine-based eGFR slopes in two comparisons: using a Jaffe or an enzymatic assay, and routine or single-run measurement. The most reliable eGFR slope was defined as the slope with the lowest within- and between-individual variability and the highest biological plausibility. Within-individual variability is defined as the eGFR variability over time in a single patient, between-individual variability is defined as the eGFR variability at group level. Biological plausibility of eGFR slopes is defined as the likelihood that an eGFR slope is the result of actual change in GFR. Since patients with a fast eGFR decline have an increased risk of developing subsequent ESRD, it is also biologically plausible that eGFR slopes should be associated with established CKD progression risk factors, such as albuminuria, and conditions associated with ESRD, such as cardiovascular events and mortality. We found that the original Jaffe-based eGFR slopes outperformed the single-run methods with regards to within- and between individual variability and biological plausibility. We found no differences in within- or between-individual variability or biological plausibility between eGFR slopes derived from a single run analysis with either a Jaffe or an enzymatic assay. These were surprising results, as we expected that single run enzymatic creatinine would provide the most reliable slopes. Enzymatic assays have been shown to be superior to Jaffe assays, and single run analysis removes variability caused by day-to-day variability of the assays. We provide two possible explanations for these unexpected results. First, it is possible that prolonged freezing of the samples for approximately 10 years as well as freeze-thaw cycles may have negatively influenced the quality of the samples. Second, the Jaffe method is generally deemed inferior to enzymatic methods to measure creatinine because of chemical interference with several compounds, such as albumin and glucose.[35] However, since these compounds tend to stay at constant levels within patients, it may well be that this does not affect the eGFR slopes calculated from repeated Jaffe-based creatinine measurements. From this chapter we concluded that for monitoring kidney function decline, single run measurement of creatinine does not outperform the use of fresh samples, regardless of the creatinine assay that is used.

Creatinine versus cystatin C in a population based cohort

Creatinine, the most widely used biomarker to estimate GFR, is not accurate in patients with extremes of muscle mass for their age and gender[35]. Therefore cystatin C has been introduced as an alternative filtration marker. The CKD-EPI consortium has developed equations to estimate GFR from creatinine, cystatin C and the combination of creatinine and cystatin C.[37,38] Validation studies of novel filtration markers are traditionally only performed for single measurement of eGFR, but the filtration marker that performs best for single estimations of eGFR may not be the best marker for monitoring change of eGFR. Therefore in **Chapter 6**, we investigated whether creatinine or cystatin C is the best filtration marker for monitoring change of eGFR in a large Dutch population based cohort. Using the same statistical methods as in chapter 5, we found that the within- and between-

individual variability was lowest for $eGFR_{creat}$ slopes. However, $eGFR_{cysc}$ slopes had a significantly stronger association with CKD progression risk factors than $eGFR_{creat}$. Furthermore, we expanded the investigation of biological plausibility by performing Cox regression analyses to find out which marker yielded eGFR slopes with the strongest association with the incidence of cardiovascular disease and mortality. We found that the associations with mortality were similar for all markers, but that only creatinine-based slopes were associated with cardiovascular disease. Two other studies have also investigated the association of creatinine and cystatin C based eGFR slopes with incident cardiovascular events and mortality.[39,40] They too found that the associations of eGFR slopes for each marker with all-cause mortality were similar, but that creatinine-based slopes had a stronger association with cardiovascular disease. We therefore conclude that for monitoring kidney function decline in the general population, creatinine is not consistently outperformed by cystatin C, either alone or in combination with creatinine.

Creatinine, cystatin C, beta-2-microglobulin and beta-trace protein in a diabetic kidney disease cohort

In addition to creatinine and cystatin C, recently two new filtration markers have been introduced: Beta-2-Microglobulin (B2M) and Beta-Trace protein (BTP). Both markers are produced at a constant rate, freely filtered by the glomerulus and not reabsorbed into the bloodstream after filtration, which makes them promising candidates to serve as glomerular filtration markers. The CKD-EPI consortium has developed three new equations to estimate GFR from B2M, BTP and B2M plus BTP.[41] Therefore in **Chapter 7**, we analyzed repeated measures of 4 markers used to estimate GFR: creatinine, cystatin C, B2M and BTP. As in chapter 5, we used data from the SUN-MACRO trial. We aimed to find the best marker or combination of markers to monitor kidney function decline, again using the same statistical methods as in chapter 5. We found that the within- and between- individual variability was lowest for eGFR slopes estimated using BTP, suggesting that more precise eGFR slopes can be obtained with $eGFR_{btp}$. $eGFR_{cysc}$ slopes had the strongest association with CKD progression risk factors and thus the highest biological plausibility. However, this difference in biological plausibility between $eGFR_{cysc}$ and $eGFR_{creat}$ was not significant. Therefore we conclude that none of the novel filtration markers, alone or in combination, consistently outperformed creatinine for the monitoring of kidney function decline. When combining the results of chapter 6 and 7, we thus conclude that there is yet no value to replace creatinine by (combinations of) novel filtration markers.

mGFR versus eGFR and their association with clinical end points

The studies that are described in chapters 5, 6 and 7 all have the limitation that mGFR was not available, due to the costs connected to obtaining repeated mGFR measurements in a large study population. The true GFR is assumed to be the renal clearance of inulin during 24 hours. However, it is impossible to incorporate such an expensive and cumbersome procedure in clinical practice. Alternatively, GFR can be estimated with three methods: calculating the GFR from plasma or urinary clearance of an exogenous marker such as iohexol or iothalamate (mGFR), measuring the 24 hour urinary clearance of an endogenous marker, such as creatinine or urea, and estimating the GFR using an equation that incorporates serum creatinine, age, gender and race, such as the CKD-EPI

equation (eGFR).[42] Although GFR estimated as clearance of an exogenous marker is assumed to be the gold standard, it is yet unclear which of these three methods of estimating GFR, yields a GFR that has the strongest association with hard end points such as end stage renal disease (ESRD) and mortality. In **Chapter 8** we therefore analyzed the currently available literature on the accuracy of the three different methods for estimating GFR, and compared especially mGFR to eGFR with regard to prediction of clinical end points. We found that six studies have investigated and compared the association of eGFR and mGFR with mortality.[39,43-47] The studies yielded varying results. Some studies found eGFR and others mGFR to have stronger associations with ESRD and mortality, whereas others did not find differences between eGFR and mGFR. Overall, these data indicate that mGFR does not consistently outperform mGFR with regard to the association with incidence of ESRD and mortality. A possible explanation for this inconsistency lies in the lack of standardization of the procedure to obtain mGFR. The performance of GFR estimating equations is assessed by comparing it to mGFR. However, mGFR can be obtained with various exogenous filtration markers, and because there is no widespread standardization of the protocols and assays used to obtain mGFR there will always be bias and imprecision amongst the different mGFR markers. This bias will also be present when mGFR is compared to eGFR, and thus a measured difference between eGFR and mGFR cannot be solely attributed to inaccuracy of eGFR. Creatinine based eGFR, on the other hand, is standardized around the globe, with regards to both the assay as well as the GFR estimation equation.[1,48] When these results and considerations are combined with the fact that eGFR can be obtained at a fraction of the costs of measuring GFR, we conclude that eGFR may not be inferior to mGFR after all.

Future perspectives

Box 2 Practical implications of this thesis

- Serum bicarbonate levels do not explain the increased incidence of ESRD and mortality in patients with type 2 diabetes
- Skin autofluorescence cannot be used to predict eGFR decline in patients with peripheral artery disease
- Biomarker panels cannot yet be implemented into nephrology practice due to a lack of validation studies
- Any creatinine assay can be used for monitoring eGFR decline as long as it is calibrated against international standards
- There is yet no reason to replace creatinine for novel makers for the monitoring of eGFR decline
- Procedures to obtain measured GFR need to be standardized in order to improve validation studies of alternative filtration markers

From individual markers to biomarker panels for prediction of CKD progression

The practical implications of this thesis are listed in Box 2. Two main conclusions can be drawn from part 1 of this thesis. First, albuminuria and eGFR are currently used to diagnose and stage CKD in clinical practice, and both are strong predictors for the development of ESRD, and mortality. A novel biomarker has to add predictive performance in addition to these markers to be clinically meaningful. Given the strong predictive performance of albuminuria and eGFR, it will be difficult to find single biomarkers that significantly and clinically meaningfully add to the existing markers. Second, biomarker panels do have the potential to improve prediction of CKD progression beyond albuminuria and eGFR, but the studies conducted to date to develop such biomarker panels towards clinical practice are of insufficient quality. Specifically, studying a biomarker panel in several patient cohorts, and investigating the effect the biomarker panel may have on treatment to improve clinical outcomes are elements in de biomarker validation process that are not often executed. These studies are of vital importance for implementation of a newly developed biomarker panel into clinical practice. If these studies are not conducted, we will never know whether the use of a biomarker panel to guide treatment will actually improve clinical outcomes.

As yet, there is no consensus what the criteria are to assess the value of biomarkers in nephrology. Therefore, there is a need for a guideline for validation of biomarkers and biomarker panels. Existing frameworks, that are used in other areas of medicine, can be used. For example, the framework proposed by Hlatky et al. for the evaluation of biomarkers in cardiology, can be applied to biomarker panel studies in nephrology.[49] Use of such a guideline or framework may help to redirect the focus of researchers from the discovery of new biomarkers towards validation of existing biomarker panels, assessment of their clinical utility, and ultimately implementation in clinical practice.

Biomarker panels can not only be used for improving prediction of CKD progression, but also to evaluate treatment response, making them of interest for improving trial design. If the likelihood of a favorable treatment response can be predicted with a biomarker panel, such a panel could be used to select the optimal population: prediction of adverse reactions to reduce drop-out rates, and prediction of positive drug effects to select a target population for use of a treatment in clinical practice. Two examples of clinical trials that use biomarker panels to select target populations for (investigational) drugs are the PRIORITY (NCT 02040441) and SONAR trial (NCT 01858532). PRIORITY aims to determine the effect of spironolactone versus placebo in delaying the onset of microalbuminuria in patients with type 2 diabetes and normoalbuminuria. The trial selects patients based on a urinary proteomic score. Patients with a positive proteomic score are selected as they are more likely to progress to microalbuminuria and will be randomly assigned to spironolactone or placebo. Patients with a negative proteomic score will be asked to participate in a prospective cohort study.[50] The other example is the SONAR trial. Although SONAR does not evaluate the performance of a specific biomarker score, it uses multiple biomarkers to determine who may benefit from treatment with atrasentan, an endothelin antagonist which is developed for delaying kidney disease progression. SONAR uses an open-label enrichment phase, in which every patient receives atrasentan. Patients can only proceed to the double blind treatment phase if they have a favorable response on a combined safety and efficacy biomarker panel that includes changes in

albuminuria, body weight, creatinine, and brain natriuretic peptide (BNP). Combined, these studies will hopefully show that biomarker panels can indeed identify patients who will most likely have a favorable response to investigational drugs. Additionally, this could lead to a new standard for trial design where biomarker panels consisting of safety and efficacy markers are used to identify the target population for novel drugs to treat CKD. This may not only result in trials with lower drop-out or adverse event rates, but it could also be of help to register new drugs for specific indications, thus aiding clinicians in prescribing the right drug to the right patient. In the era where personalized medicine is emerging, these are important developments.

Monitoring (e)GFR over time

Part 2 of this thesis focused on which marker and assay is best for monitoring eGFR over time. With regard to creatinine assays, we concluded that for monitoring kidney function decline, single run measurement of creatinine from plasma samples that have been stored frozen does not outperform the use of fresh samples, regardless of the creatinine assay that is used. These results suggest that the beneficial effect of less variability caused by laboratory assay induced drift and day-to-day variability on creatinine measurements is not outweighed by the disadvantageous effect of prolonged storage of serum samples and freeze-thaw cycles. As it is already common practice during studies to measure creatinine from fresh samples, our results suggest there is no need to change this practice to single run measurement from frozen samples at the end of a study. In contrast, our finding that eGFR slopes are not affected by the type of creatinine assay can affect future trials. The Jaffe method is still used because it is cheaper than enzymatic assays. Knowing that for repeated measurements of eGFR the choice of creatinine assay does not affect eGFR slopes, implies that robust nephrology research can also be performed in areas with limited research funds.

Chapters 6 and 7 showed that none of the novel markers outperformed creatinine for the monitoring of eGFR over time. Moreover, even combinations of biomarkers did not outperform creatinine. It must however be noted that this only applies to repeated measures of eGFR. For single measurements of eGFR, the combination of creatinine and cystatin C does indeed yield a more accurate estimation of measured GFR.[41] It is possible that just as in the comparison of Jaffe and enzymatic assays for measuring creatinine, the possible (systematic) errors for a point estimate of eGFR does not affect the eGFR slopes.

Four steps have to be taken to definitively select the best marker for eGFR monitoring in the future. First of all, for each marker the eGFR slopes need to be compared to the corresponding mGFR slopes. Second, future studies need to examine which marker or combination of markers yields eGFR slopes with the strongest association with hard end points such as ESRD and mortality. Third, treatment effects on eGFR slopes based on the various filtration markers should be assessed and compared. Fourth, expanding on our earlier call for the development of biomarker panels, GFR estimation equations that add B2M and/or BTP to creatinine and cystatin C need to be developed and rigorously tested in various populations. The marker or combination of markers that yields an eGFR that most closely resembles the true GFR and has least variability due to non-renal influences (i.e. assay variability or biological variability) should be selected. Our results from Chapter 7 suggest that for monitoring eGFR decline, the use of a multi-marker equation does not seem to improve

estimation of eGFR decline. It must however be noted that our study had a relatively short follow-up duration and that hard end points such as ESRD were not available. Therefore testing these markers in different populations and in cohorts with long follow up duration, is still needed to provide a definitive answer on which marker is best for monitoring eGFR. In clinical practice, the most suitable filtration marker may depend on specific patient characteristics. For example, creatinine is not suitable as filtration marker in studies that evaluate treatments that directly affect creatinine production or secretion, such as a low protein diet. In those cases cystatin C or one of the other markers may be a better choice. A combination of filtration markers may also help overcome the limitations of the individual markers.

Measuring GFR with inulin is considered to be the gold standard that each marker and equation for estimating GFR is validated against. However, we showed in chapter 8 that there is considerable analytical variability in mGFR, possibly due to a lack of standardization. All endogenous filtration markers that are used to estimate GFR are validated against mGFR. It is important to note that as long as mGFR is not obtained with a reliable and standardized method, there will always be bias and imprecision in estimated GFR that is not solely attributable to the endogenous filtration marker. Therefore, mGFR measurement needs to be standardized at three levels: standardization regarding the marker used, measurement of the markers in plasma, and standardization of the procedure to measure GFR. As with the choice of filtration marker to estimate GFR, perhaps the preferred procedure to measure GFR can vary depending on the context and indication. Delanaye et al. have recently proposed a pragmatic approach to the use of mGFR in research and clinical practice.[51] They propose that multiple samples need to be drawn at various intervals in situations where mGFR is used as reference method during the development of new GFR estimation equations, whereas a less complicated protocol with a late sample or single sample method can be suitable for epidemiological research or in situations where GFR is a secondary end point.

Conclusion

In part 1 of this thesis we have shown that some selected single biomarkers are not superior predictors of CKD progression when compared to established CKD progression risk markers, such as eGFR and albuminuria. We advise to focus future research on biomarker panels to accomplish more accurate risk prediction for CKD progression. In part 2 of this thesis we have shown that novel filtration markers and even GFR measured with exogenous markers do not consistently outperform creatinine based eGFR to monitor GFR change over time. Therefore there is no reason to replace creatinine, which is currently still the best and cheapest biomarker to monitor change in GFR, by other filtration markers.

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