Prediction and monitoring of chronic kidney disease

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Chapter 1
Introduction

Introduction

Chronic kidney disease (CKD) is a leading cause of morbidity across the globe, affecting 10 to 16% of all adults.[1] The most common causes of CKD are hypertension and diabetes mellitus.[2,3] CKD not only leads to end stage renal disease (ESRD), but also leads to cardiovascular disease and mortality, and consequently CKD forms a substantial burden to global health.[4] A problem is that not all patients with CKD will show a decline in kidney function during follow-up. It is therefore necessary to identify factors that can predict whether a patient with CKD is likely to have disease progression, as these patients need close monitoring and treatment to reduce their risk. To this end, biomarkers are commonly used in research and clinical practice. A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenetic processes, or pharmacologic responses to a therapeuting intervention."[5] For CKD, the estimated glomerular filtration rate (eGFR) and presence of increased albuminuria are the most important biomarkers to assess the risk of developing ESRD (Figure 1).[2] Patients in the high risk categories (high albuminuria and/or low eGFR) need treatment to slow kidney function decline, and thus eGFR and albuminuria play a pivotal role in daily nephrology practice.

Prognosis of CKD by GFR and albuminuria category

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/ 1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

Figure 1 Prognosis of CKD by GFR and albuminuria category. Adapted from: KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 3(1), 2013

However, both markers have limitations. Although albuminuria has been shown to have strong predictive power, there is considerable variability in disease progression that is not explained by albuminuria.[6] GFR is usually estimated using an equation that incorporates creatinine, age, gender and race.[7] We know, however, that creatinine is also influenced by non-GFR factors such as muscle mass, which limits the association between eGFR and outcomes.[8] Therefore, there is an ongoing need for new biomarkers that can improve prediction of CKD progression, or that can be used to estimate GFR. Furthermore, eGFR is not only used as a marker to predict clinical end points, clinicians also use eGFR to monitor CKD progression and to evaluate treatment response. In practice, eGFR is therefore not only a biomarker, but it is also used as a surrogate clinical endpoint.

Aims of this thesis

This thesis consists of two parts. Part 1 aims to investigate novel biomarkers that can be used for prediction of CKD progression. Part 2 aims to investigate how eGFR can best be monitored over time, with regards to the choice of assay, methods of sample handling, and choice of biomarker to estimate GFR.

Part 1. Biomarkers for prediction of CKD progression

Serum bicarbonate

As kidney function declines, the capacity of the kidneys to excrete acid reduces, resulting in metabolic acidosis characterized by low serum bicarbonate levels. Earlier studies in patients with CKD showed that low serum bicarbonate is associated with an increased risk of ESRD, as well as all-cause mortality.[9-13] This implies that GFR decline and subsequent metabolic acidosis can in turn cause further GFR decline, thus causing a vicious cycle of increasing kidney damage. For this reason, the current KDIGO guideline (Kidney Disease: Improving Global Outcomes, the global organization that develops evidence based clinical guidelines for kidney diseases) advises bicarbonate suppletion for patients with CKD and serum bicarbonate levels <22 mEq/L.[2] However, this guideline is based on studies that were performed in predominantly non-diabetic individuals, and in clinical practice patients with diabetes form a majority group among patients with CKD. It has been suggested that acid-base metabolism is different in diabetic versus non-diabetic patients at similar level of eGFR. It is therefore unknown whether low serum bicarbonate levels also cause an increased risk of ESRD and mortality in patients with type 2 diabetes. In Chapter 2, we studied the associations of serum bicarbonate with kidney and cardiovascular end points and mortality in a cohort of patients with type 2 diabetes and diabetic kidney disease. We hypothesized that serum bicarbonate has a significant association with kidney and cardiovascular end points and mortality in this population.

Advanced glycation end products and skin autofluorescence

Most biomarkers are measured in blood and/or urine. Many patients therefore have to undergo regular blood draws for disease monitoring and risk prediction. To make disease monitoring more patient friendly, new, non-invasive techniques are under review. One of these non-invasive measurement devices is the AGE-reader. Advanced glycation end products (AGEs) are the end product of the Maillard reaction, which irreversibly glycates proteins. AGE accumulation in the human body has been shown to cause inflammation and tissue damage, and AGE levels are increased in patients with diabetes, cardiovascular disease and kidney disease.[14-24] The AGE reader is an optical device that measures skin autofluorescence (SAF), a validated proxy for skin AGE levels.[25] Previous research has shown that high SAF levels in patients with peripheral artery disease predict cardiovascular events, mortality and limb amputation.[21,23,24] These associations have also been shown in other populations, such as patients with diabetes mellitus, ESRD, and myocardial infarction.[14,26,27] Furthermore, high SAF levels have been associated with progressive eGFR decline in patients with various stages of CKD.[28] We did not know whether SAF is also associated with eGFR decline in a cohort of patients with preserved kidney function. Therefore, in Chapter 3 we investigated the associations of SAF with eGFR decline in patients with peripheral artery disease and preserved kidney function. We hypothesized that increased SAF levels are independently associated with impaired kidney function and diabetes mellitus at baseline and with accelerated kidney function decline during follow-up.

Biomarker panels

As stated above, albuminuria and eGFR are currently the most important biomarkers for prediction of CKD progression. Several other biomarkers have been investigated, but as yet none of them has consistently outperformed albuminuria with regards to prediction of kidney related end points. It is therefore the question whether a single new biomarker will ever outperform the currently established CKD risk factors. CKD is a multifactorial disease, in which progression is determined by various pathophysiological pathways. It may therefore be necessary to use a combination of markers to combine information on all these pathways to obtain an optimal risk prediction equation. A recent example of a successful multi-marker approach to predict disease outcome is the MammaPrint, a 70-gene signature test that can predict whether a patient with early-stage breast cancer is likely to have recurrence of breast cancer after surgery.[29] MammaPrint is now being implemented in clinical practice to decide whether a patients needs chemotherapy after surgery, thus preventing women with low-risk breast cancer from receiving unnecessary chemotherapy. Such a biomarker panel is not yet available for kidney disease. Efforts have been made to create multi-marker risk calculators for patients with CKD. It is generally perceived that those risk calculators were either generated with poor methodology, performed poorly during external validation or are not externally validated at all, and therefore they are not incorporated in daily nephrology practice.[30,31] In Chapter 4, we performed a review of the literature on biomarker panels for prediction of chronic kidney disease progression, specifically in patients with type 2 diabetes. In addition, we made several recommendations for further development of biomarker panels to increase the likelihood of a multi-marker risk calculator for CKD to be incorporated into clinical practice.

Part 2. Biomarkers for monitoring of eGFR decline

Part 2 focuses on the assessment of change in kidney function over time. In epidemiological studies and clinical trials, creatinine is routinely measured from fresh samples by either a Jaffe or an enzymatic assay and then GFR is estimated at each patient visit. 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. 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. Therefore, in the first study of part 2 we investigated the performance of single run versus routine measurement of creatinine, and we compared the performance of eGFR slopes derived from creatinine values assessed by the two different assays.

As reasoned above, estimating GFR based on creatinine is not without limitations, and therefore several new glomerular filtration markers have been proposed for a more accurate estimation. These new markers have only been validated in cross-sectional studies, but not for monitoring of GFR decline.[32,33] This is an essentially different study question, because repeated eGFR measurements can vary over time caused by non-GFR factors instead of actual change in GFR, which can lead to the calculation of inaccurate slopes. Validation of filtration markers and GFR estimation equations is traditionally performed in studies that compare the accuracy of estimated GFR versus the gold standard measured GFR (mGFR). This mGFR is typically obtained by calculating the plasma or urine clearance of an exogenous filtration marker, such as iohexol or iothalamate. However, obtaining mGFR is a time consuming and expensive procedure, which makes it unfeasible for use in large studies that are needed to compare the performance of novel markers for GFR estimation. Therefore we developed a statistical approach to compare eGFR slopes based on either different filtration markers, or based on creatinine determined with different assays, in the absence of mGFR. The goal of our statistical approach was to find the filtration marker or assay that would yield the most reliable eGFR slopes. 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.

Given these considerations, we compared in the following two studies of part 2 of this thesis, three alternative filtration markers and their respective eGFR slopes to creatinine based eGFR slopes. Finally, we performed a brief literature review in which we compare eGFR and mGFR and discuss whether the absence of measurement of mGFR in our studies is a limitation.

Choice of assay in epidemiological studies

In the past, the clinical standard for creatinine measurement was the (modified) Jaffe method. The Jaffe reaction is a colorimetric reaction of creatinine and picrid acid in an alkaline solution. The Jaffe reaction suffers from non-specificity bias because compounds other than creatinine can influence the reaction, such as albumin and glucose.[8] Therefore, this method has been succeeded by enzymatic assays, as chemical interference from other compounds with enzymatic reactions is much lower. However, due to the higher costs of enzymatic assays, the Jaffe method is still used in many laboratories.[34]

To improve the accuracy of creatinine assays, quality control measures have been globally implemented in 2006. At that time isotope dilution mass spectrometry (IDMS)-traceable creatinine control samples were introduced to calibrate the results obtained in a laboratory.[35] However, even with improved calibration, all assays still suffer from drift and day-to-day variability in measurement results.[8,36] For studies with repeated measurements of creatinine, this day-to-day variability can lead to calculation of inaccurate eGFR slopes. For clinical trials, a possible solution could be to perform a single run analysis of all samples per individual at the end of the study. In Chapter 5, we investigated whether measurement eGFR slopes could be improved with two methods: by switching from routine analysis of serum samples to single-run measurement of all samples per individual, or by changing the creatinine assay from Jaffe to enzymatic. To answer this question we used samples of the SUN-MACRO trial, a randomized clinical trial performed in a cohort of patients with type 2 diabetes and diabetic kidney disease.[37,38] We compared the original creatinine results of the SUN-MACRO trial (routinely measured with a Jaffe method) to single run re-measured creatinine, using both a Jaffe and an enzymatic assay. We hypothesized that creatinine measured in a single run with an enzymatic assay would provide the best eGFR slopes, defined as the lowest within- and between individual variability and the highest biological plausibility.

Creatinine versus cystatin C in a population based cohort

Since creatinine, the first choice marker for estimation of eGFR, is not accurate in patients with extremes of muscle mass for their age and gender,[8] cystatin C has been introduced as an alternative filtration marker. Cystatin C is a housekeeping protein (a protein involved in the basic functioning of a cell) produced by all nucleated cells of the human body. In 2009, two GFR estimating equations were introduced by the CKD-EPI (Chronic kidney disease - Epidemiology) consortium for estimating GFR using cystatin C and creatinine plus cystatin C.[32] With these equations cystatin C has been validated as an alternative filtration marker for the estimation of GFR.[2] This validation has only been done 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. 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. In addition, we expanded the analyses of the biological plausibility of the filtration markers, by investigating not only associations with established CKD progression risk factors, but also the associations of eGFR slopes with incidence of cardiovascular disease and all-cause mortality. We hypothesized that the best filtration marker would yield eGFR slopes with the lowest within- and between-individual variability, and the highest biological plausibility, defined

as the strongest associations with CKD progression risk factors, as well with incident cardiovascular events and all-cause mortality.

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

Recently two additional new filtration markers have been introduced: Beta-2-Microglobulin (B2M) and Beta-Trace protein (BTP). Beta-2-Microglobulin (B2M) is a protein component of class I major histocompatibility molecules and is found on the surface of nucleated cells.[39] Beta-Trace protein (BTP) is an glycoprotein produced in the central nervous system.[40] Both markers are produced at a constant rate, freely filtered by the glomerulus and not reabsorbed into the bloodstream after filtration, which makes them suitable candidates to serve as glomerular filtration markers. Recently, the CKD-EPI consortium has developed three new equations estimating GFR from B2M, BTP and B2M plus BTP.[33] In Chapter 7, we performed a head to head comparison of four filtration markers: creatinine, cystatin C, B2M and BTP either alone or when used in combination. As in chapter 5, this study was performed with samples of the SUN-MACRO trial. Since diabetic kidney disease is characterized by rapid eGFR decline, the SUN-MACRO trial provides a population of particular interest to investigate novel filtration markers. We again investigated the within- and between individual variability and biological plausibility of the four marker based eGFR slopes, to find the best marker or combination of markers for monitoring of eGFR decline. We hypothesized that the best filtration marker or combination of markers will yield eGFR slopes with low within- and between-individual variability and high biological plausibility.

mGFR versus eGFR

The studies featured 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. The true GFR is assumed to be the renal clearance of inulin during 24 or 48 hours. Such a long time period of assessment is necessary, because of the circadian rhythm in kidney function.[41] However, it is impossible to incorporate such an expensive and cumbersome procedure in clinical practice. Alternatively, GFR can be estimated with three methods. First, the GFR can be calculated from plasma or urinary clearance of an exogenous marker such as iohexol or iothalamate. In nephrology literature, this is generally defined as the gold standard technique and thus called mGFR. However, as this form of mGFR is typically obtained in a period considerably shorter than 24 hours, it should be realized that actually this mGFR is also just an estimation of true GFR. Second, the GFR can be estimated by measuring the 24 hour urinary clearance of an endogenous marker, such as creatinine or urea. Third, the GFR can be estimated using an equation that incorporates serum creatinine, age, gender and race, such as the CKD-EPI equation.[7] 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.

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