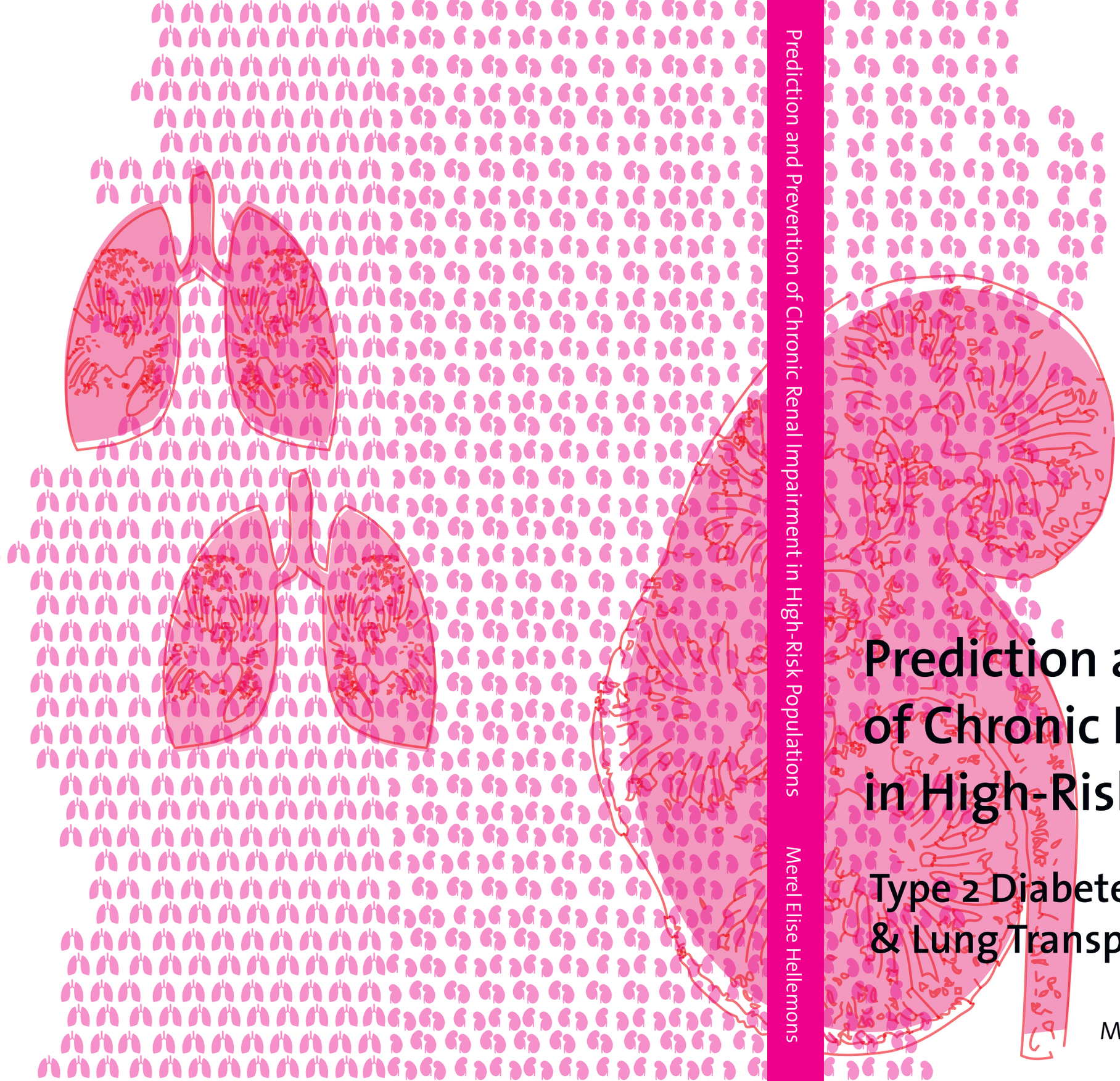
Prediction and prevention of chronic renal impairment in high-risk populations

Hellemons, Merel Elise



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Merel Elise Hellemons

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M.E. Hellemons
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- Type 2 diabetes mellitus, hypertension & lung transplantation -
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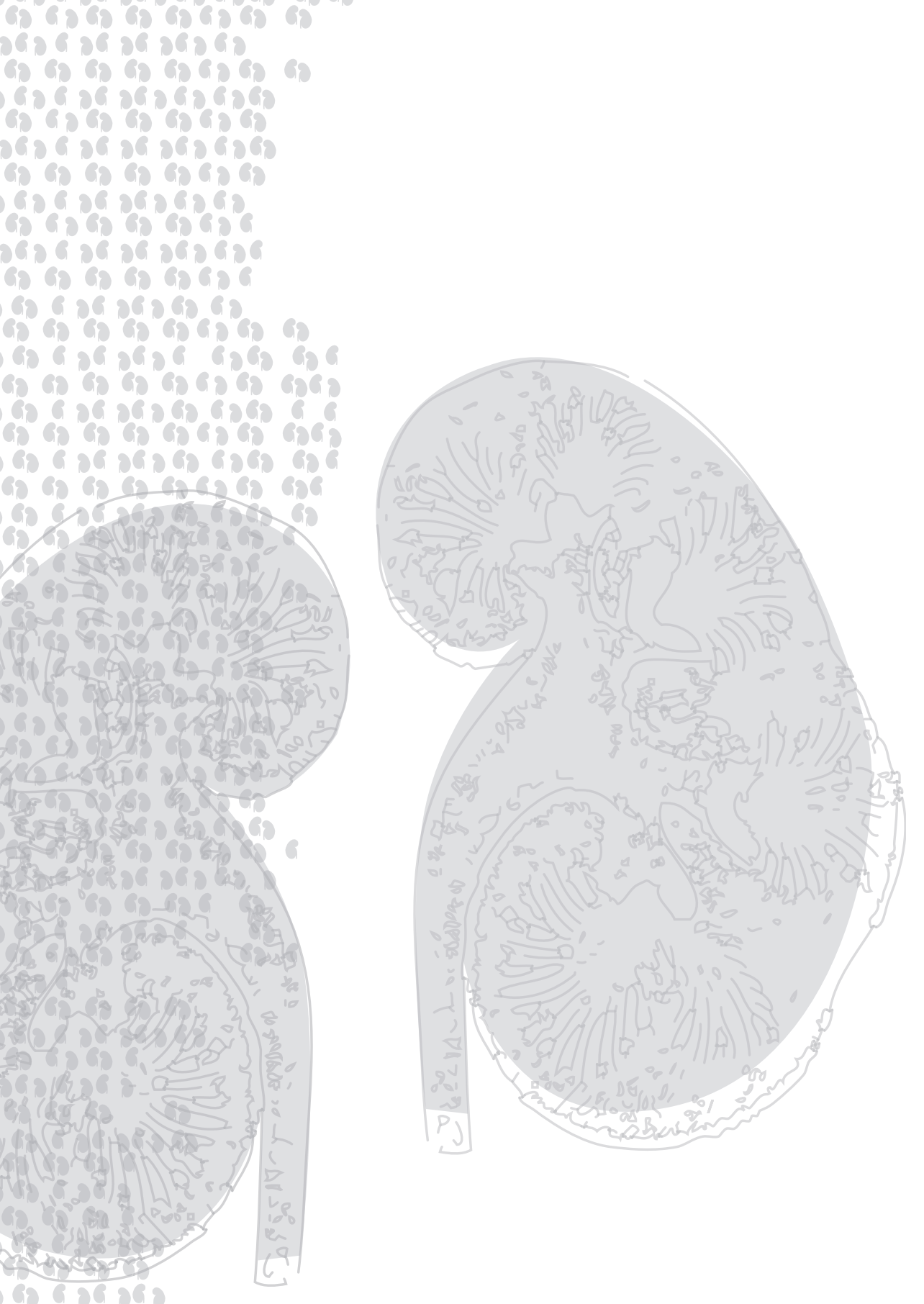


1

Introduction and Aims of the Thesis

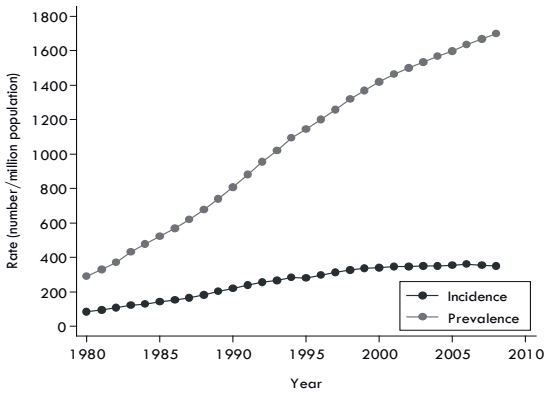
Introduction

Chronic Renal Impairment - Scope of the problem

Chronic renal impairment is a global public health concern. Over the past decades the prevalence of chronic renal impairment has steadily risen in the entire world.[1-4] In the NHANES population survey performed between 1999 and 2004 it was estimated that 26 million adults suffered from impaired renal function, which is approximately 13% of the adult US population,[5] while in the prior NHANES survey (1988-1994) this was only 10% of the adult US population. This indicates an increase of 3% in 10 years time. These high and steeply increasing numbers are also seen in other parts of the world, although accurate data are limited.[6-8]

Chronic renal impairment may eventually lead to end stage renal disease (ESRD). Along with the prevalence of chronic renal impairment, there has been a dramatic increase in the prevalence of ESRD over the last decades (figure 1).[2,9] In the US alone, in 2008, approximately 480.000 people (~1700 patients per million population) received renal replacement therapy for ESRD - consisting of either dialysis or renal transplantation - and new renal replacement therapy was started in 353 patients per million population. Incidence rates are approximately three times higher in the United States than in Europe, where per million population 113 new patients required renal replacement therapy.[10] Whereas there has been a continuous increase in the incidence of ESRD, this increase has leveled off over recent years. This observation is suggestive for increased awareness of chronic renal impairment and improved therapy.[11]

Figure 1 | Incidence and prevalence of end-stage renal disease



Incidence and prevalence of end-stage renal disease (ESRD) in the US in 2008 (Adapted from: US renal data system 2010 annual data report.[9]

ESRD necessitates renal replacement therapy, which poses a severe burden to the patients and their environment, and has a profound impact on the costs of healthcare.[12-14] Moreover, chronic renal impairment, but particularly ESRD is associated with increased risk of (cardiovascular) mortality. For these reasons prevention or postponement ESRD is of utmost importance.

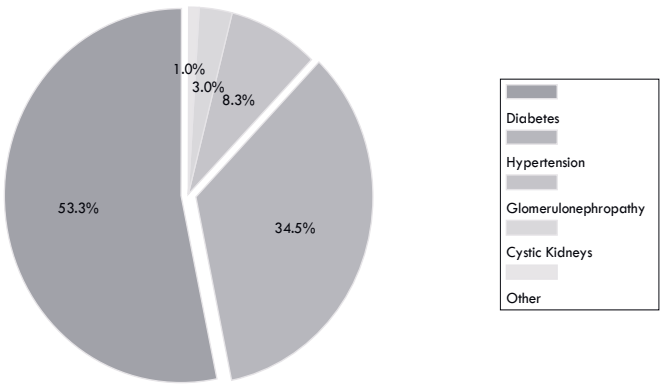
High-Risk populations

One important reason for the increasing prevalence of chronic renal impairment is aging of the population, but this is not the only reason for the increase. Especially the increasing prevalences of important risk factors for chronic renal impairment in the population such as type 2 diabetes and hypertension amount substantially to the total increase in the prevalence of renal impairment.[15,16] Apart from patients with diabetes and patients with hypertension, there are patient groups with an ever higher a priori risk for chronic renal impairment, such as solid organ recipients. However, because these groups are much smaller in size, they do not contribute as substantially to the total prevalence of chronic renal impairment as diabetes and hypertension do.

Type 2 diabetes and hypertension

It is estimated that ~40% of patients with diabetes develop renal impairment, manifested as decline in glomerular function, development of increased urinary albumin excretion or both.[17-19] Despite implementation of better screening procedures and new development

Figure 2| Incidence of ESRD per primary diagnosis



Incidence of end-stage renal disease (ESRD) per primary diagnosis in the US in 2008 (Adapted from: US renal data system 2010 annual data report).[9]

and implementation of preventive treatment strategies, in the US, the prevalence of diabetes increased from 6.0% in NHANES 1988-1994 to 9.4% in NHANES 2005-2008.[20] This is alarming, as diabetes is now already responsible for more than 43% of patients developing ESRD.

Hypertension is another leading risk factor for development of chronic renal insufficiency in general, and as a co-morbid factor in patients with diabetes.[21] In 2000, hypertension affected 29 to 31% of the adult US population, which translates into 58 to 65 million people, compared to 43.2 million people in 1991.[16] After diabetes, hypertension is the second most common risk factor for ESRD, responsible for more 28% of cases (Figure 2). Registry data from Europe, Canada, Australia and Japan show a similar increasing role for diabetes and hypertension as causes of ESRD.[10,11]

Lung Transplantation

A patient population with even higher renal risk than those with diabetes or hypertension, is the group of lung transplant recipients. This group does not contribute substantially to the total of patients with chronic renal impairment because worldwide "only"~2800 lung transplantations are performed annually. However, due to the large surgical procedure and the sustained use of nephrotoxic immunosuppressive medication the incidence of chronic renal impairment in this group is very high: over 90% of lung transplant recipients suffer renal function decline after transplantation and more than 15% of the recipients have severe renal impairment 5 years after lung transplantation. Overall, 5-10% of all lung transplant recipients may eventually reach ESRD, despite healthy renal function at time of transplantation.

Monitoring renal function

The symptoms of worsening kidney function are unspecific and patients therefore are frequently unaware of their condition. Nevertheless, it is important to monitor chronic renal impairment - especially in those at increased risk – because of the increased cardio-renal risk.

Glomerular Filtration Rate (GFR) is accepted as the best overall measure of renal function and represents the overall filtering capacity of the kidneys.[19] GFR can be measured as the plasma clearance of ideal filtration markers, such as radiolabelled 125I-Iothalamate.[20] However, these GFR measurements with ideal filtration markers are complex, demanding and expensive.

Other methods estimate renal function by using serum creatinine as a proxy for renal function and are much less demanding and expensive, but also have important limitations because certain patient-related factors affect creatinine generation and excretion. Serum

creatinine-based GFR equations are superior to using serum creatinine alone as an index for renal function because these equations take into account certain patient-specific biometric indices that affect creatinine generation and excretion (age, gender, race, weight). Nevertheless, the equations also have important shortcomings. They frequently underestimate gold-standard measured GFR (mGFR), especially in the high- and high normal range, and lack sensitivity in detecting progressive renal function loss.[21-23]
Another important marker of renal impairment is an increased urinary albumin excretion (albuminuria). An increase in albuminuria has been linked to subsequent faster decline in renal function.[24,25] In addition, albuminuria is an important risk marker for end-stage renal disease, cardiovascular disease and mortality. Unfortunately, albuminuria is highly variable within and between patients and is not an alternative to renal function measurements, since not all patients with declining renal function develop albuminuria. Nevertheless, albuminuria measurement is very valuable and widely used as a screening tool in populations at increased risk for renal impairment.

The ideal method to monitor renal function and to assess renal risk depends on the population under study and the a-priori risk for renal impairment. Measuring renal function using gold-standard techniques is only feasible in relatively small, selected patient populations with a very high a-priori risk. For the majority of patients the serum creatinine-based estimations of renal function and screening of albuminuria are generally used to assess renal function and renal risk, despite the obvious shortcomings, because the methods are easier to use in clinical practice, less demanding for the patient and less expensive.

Prediction & Prevention

Whereas overall patients with type 2 diabetes and hypertension have increased risk for chronic renal impairment, not all patients in these high-risk groups will eventually develop renal impairment. Moreover, the rate of renal function decline varies substantially between individuals.[29] Apart from the renal function and albuminuria, many important risk factors for progression of chronic renal impairment have been identified, - i.e. hypertension, hyperglycemia, dyslipidemia, smoking and obesity.[21,30-33] However, these key risk factors are unable to adequately discriminate between those patients that will and will not develop progressive chronic renal impairment. The earlier we detect patients at risk, the more important the assessment of individual prognosis is. Currently, risk stratification of individual patient prognosis remains a huge challenge.
Biomarkers may play an important role in overcoming this challenge. A biomarker is "a biological characteristic that is objectively measured and evaluated as an indicator

of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention without necessarily being causally related to the clinical endpoint".[34] A clinically useful biomarker could allow the early diagnosis of chronic renal impairment, the assessment of the future prognosis and potentially even the response to therapy. Earlier detection of those with an increased chance of renal impairment or fast-progressive course could thus help us reduce the incidence of chronic renal impairment through selective preventive treatments for patients with the highest risk.[35-37]

Aims of the thesis

In this thesis several aspects regarding prediction and prevention of chronic renal impairment in high-risk populations were investigated.

Part I: Type 2 Diabetes and Hypertension

In **part I,** we examine prediction of chronic renal impairment in patients with type 2 diabetes and hypertension. This research was conducted within the Systems Biology towards Novel Chronic Kidney Disease Diagnosis and Treatment (SysKID) consortium. A major aim of the SysKID consortium is to identify persons at risk of developing chronic renal impairment in the context of diabetes and hypertension.

Increased albuminuria is one of the earliest signs of nephropathy in diabetes and is associated with increased risk for development of end-stage renal and cardiovascular disease. Early identification of patients at risk for worsening albuminuria allows for start or intensification of treatment which may prevent future complications. Many biomarkers for the prediction of transition between the stages of albuminuria have been published in the last two decades. In **chapter 2** we systematically review the validity of biomarkers for nephropathy in type 2 diabetes.

According to current knowledge leakage of excess albumin is a sign of endothelial damage and vascular dysfunction. In **chapter 3 and 4** we assess whether the micro-vascular damage marker Growth-Differentiation Factor-15 (GDF-15) and micronecrosis marker high-sensitivity Troponin T (hs-TnT) respectively, precede and have additive predictive value in prediction of transition between the stages of albuminuria in patients with type 2 diabetes and patients with hypertension.

For the prevention of renal complications in patients with diabetes, agents blocking the Renin-Angiotensin-Aldosterone System (RAAS) are first choice antihypertensives, as these agents not only lower blood pressure but also lower albuminuria. Current guidelines

recommend dose-titration of RAAS-blockade on blood pressure response without taking responses in albuminuria into account. It is unclear whether the response in blood pressure, albuminuria, or their combination should be considered a target for reno-protective therapy. In **chapter 5** we assess whether the initial treatment responses in albuminuria and blood pressure are associated with long-term renal outcome and may thus be used in risk management strategies in type 2 diabetes patients with microalbuminuria and hypertension.

For clinical practice, guidelines have been developed regarding screening and treatment of albuminuria in high-risk patients based on the current clinical evidence. It remains however unclear how well these guidelines are applied in practice. Therefore, in **chapter 6** we assessed the adherence to guidelines for albuminuria screening and treatment in patients with type 2 diabetes in a large primary care cohort.

Part II: Lung transplantation

In **part II** we examine chronic renal impairment in lung transplantation (LTx) recipients. Chronic renal impairment is an important clinical problem after lung transplantation. Due to changing acceptance policies, lung transplantation recipients are increasingly older and have a higher proportion of 'co-morbidities', thus increasing expected renal risk. Meanwhile, increased awareness and improved treatment may have counterbalanced the increase in risk factors. In **chapter 7** we assessed the changes in renal risk factors in the LTx population and the incidence of renal function impairment after LTx over time.

Besides the high prevalence of chronic renal impairment, also the prevalence of former smoking is relatively high in LTx recipients. We wondered whether, even after smoking-cessation, smoking history could be relevant for morbidity after LTx, in particular chronic renal impairment because of the high prevalences of both chronic renal impairment and former smoking. In **chapter 8** we assessed the impact of former smoking on the incidence of chronic renal impairment and mortality after LTx.

Lastly, in our cohort, GFR was monitored after LTx using ^{125}-Iothalamate measurements. Whereas this method is the gold standard, it is invasive, demanding and costly and is therefore not commonly used in most centers to monitor renal function over time. Other centers mostly use the more easily available creatinine-based GFR estimations. However, these estimations have never been validated in LTx recipients and are likely not very accurate in this population, because of the distinct characteristics of the LTx recipients. In **chapter 9** we aimed to assess how well the more easily available renal function equations correlate with gold standard GFR in LTx recipients and to assess the factors of systematic bias.

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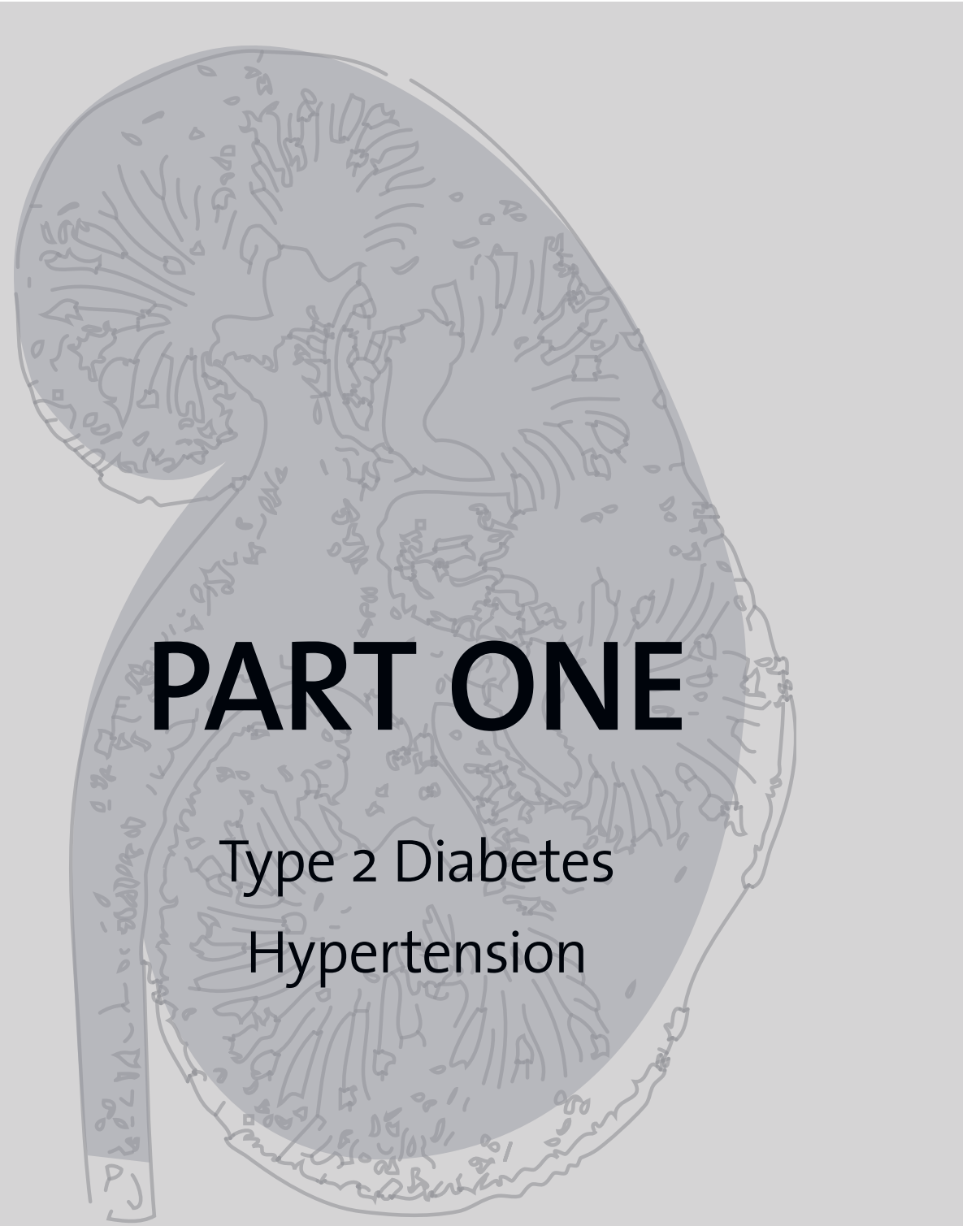
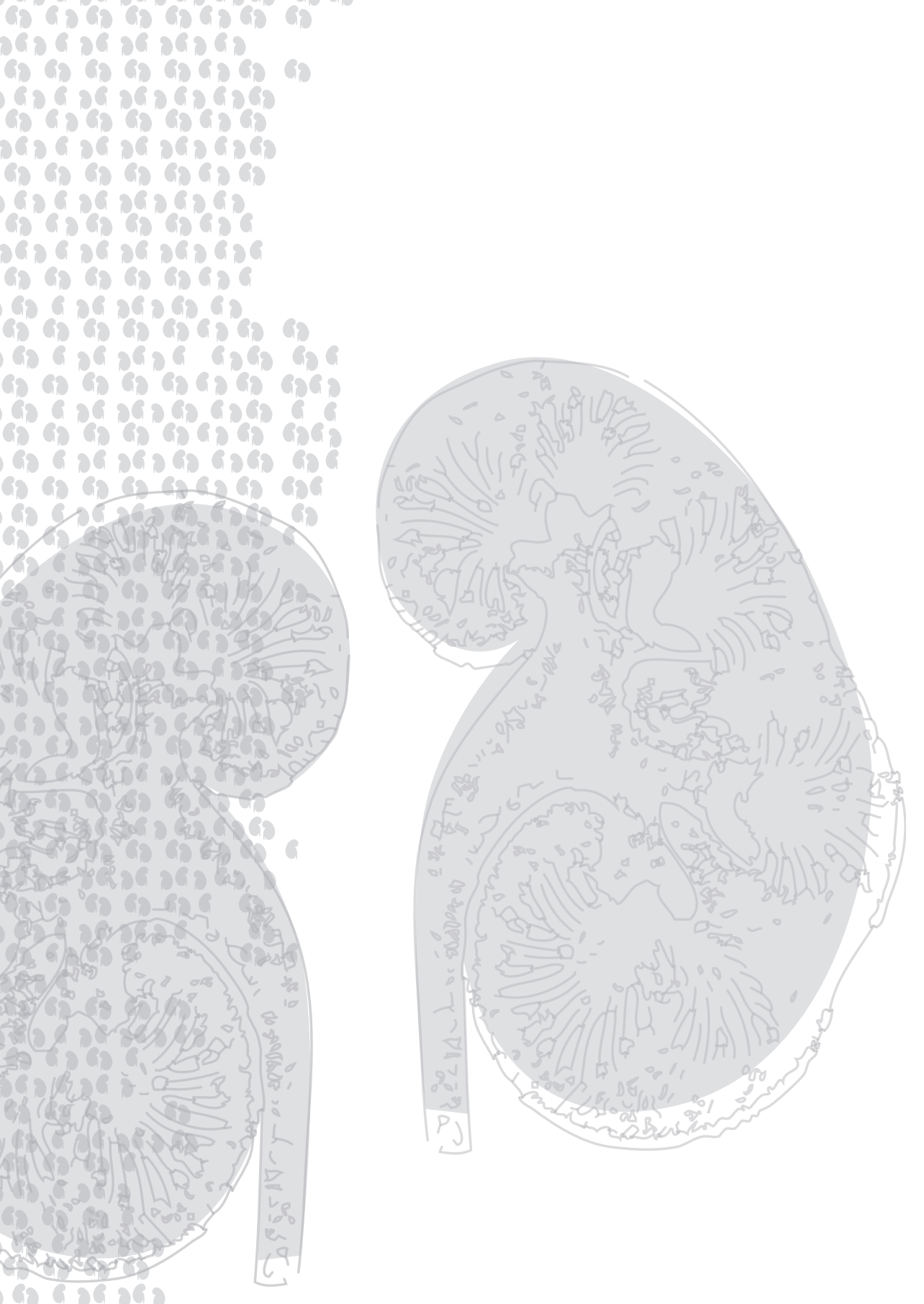


PART ONE

Type 2 Diabetes
Hypertension



2

Validity of Biomarkers Predicting Onset or Progression of Nephropathy in Patients with Type 2 Diabetes; A Systematic Review

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Abstract

Background: Novel biomarkers predicting onset or progression of nephropathy in patients with type 2 diabetes have been recently identified. We performed a systematic review to assess the validity of biomarkers predicting onset or progression of nephropathy in patients with type 2 diabetes in longitudinal studies.

Methods: The methodological quality of the studies was scored using Standards for Reporting of Diagnostic accuracy (STARD) criteria and the independent predictive value of the biomarkers beyond conventional risk factors was scored according to the adjustment for conventional risk factors. Validity of the biomarkers was determined by summarizing the methodological quality and the adjustment score.

Results: We identified 15 studies describing 27 biomarkers. Six studies had sufficient methodological quality. These studies identified 13 valid and significant markers for nephropathy in diabetes: serum interleukin 18, plasma asymmetric dimethylarginine; and urinary ceruloplasmin, immunoglobulin G and transferrin were considered valid markers predicting onset of nephropathy. Plasma asymmetric dimethylarginine, vascular cell adhesion molecule 1, interleukin 6, von Willebrand factor and intercellular cell adhesion molecule 1 were considered valid biomarkers predicting progression of nephropathy. Plasma high-sensitivity C-reactive protein, E-selectin, tissue-type plasminogen activator, von Willebrand factor and triglycerides were considered valid markers predicting onset and progression of nephropathy.

Conclusion: Several novel biomarkers for prediction of nephropathy in diabetes have been published, which can potentially be applied in future clinical practice and research. Because of the heterogeneous quality of biomarker studies in this field, a more rigorous evaluation of these biomarkers and validation in larger trials are advocated.

Introduction

Diabetic nephropathy is the leading cause of chronic renal failure in the USA and Western countries. As such, it is not only associated with considerable morbidity and premature mortality but it also negatively affects patient's quality of life and their social environment, and it poses a significant burden on national healthcare budgets.[1]

Albuminuria is one of the first asymptomatic clinical manifestations of microvascular damage in diabetes.[2] It has been shown that the presence of micro- or macroalbuminuria is associated with progressive renal function loss and an increased risk of cardiovascular disease.[3] Therefore, screening for, and quantification of albuminuria is recommended in all patients with diabetes to identify those who are at risk for long-term complications.[4] In recent years, multiple urinary and serum/plasma biomarkers for the prediction of onset of microalbuminuria and for, progression of nephropathy in patients with micro- and macroalbuminuria have been investigated in patients with Type 2 diabetes.

The term 'biomarker' describes a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention without necessarily being causally related to the clinical endpoint (e.g. Troponin T in myocardial infarction).[5] Biomarkers can be used as diagnostic tools, staging tools, prognostic tools or tools for prediction/monitoring of clinical response to an intervention.[6] For the latter purpose, certain biomarkers are eligible to substitute as clinical endpoints in intervention trials. Such a surrogate marker, also referred to as a surrogate endpoint, is expected to predict clinical benefit as evidence exists that this marker is associated causally with a certain clinical outcome (e.g. elevated blood pressure as a marker for cardiovascular disease). Therefore, only a subset of biomarkers will achieve surrogate marker status.

In this review, we focus on biomarkers for prognosis and risk prediction, which potentially can become surrogate markers in future studies. A more prominent role for biomarkers is proposed in early non-invasive screening and assessing overall renal risk in patients with Type 2 diabetes.[7] In this perspective, biomarkers may be important tools in guiding early and more aggressive therapy in high-risk patients in order to prevent long-term renal complications.

Before a putative biomarker can be applied in clinical practice as a prediction tool, rigorous evaluation is advocated and several criteria should be met.[8-12] First of all, biomarkers should be tested in longitudinal, methodologically well-designed studies with sufficient power in order to ascertain the generalizability of the results.[10] The association between the biomarker and disease should be independent of potential confounders and should add

to risk prediction beyond conventional risk factors.[8,11,12] Lastly, results on biomarkers should be reproduced in other studies to validate the results.[8-10] So far, validity of biomarkers for the prediction of onset or progression of nephropathy in patients with diabetes has not been critically appraised.

The objective of this paper was to systematically assess the validity of biomarkers predicting onset or progression of nephropathy in patients with Type 2 diabetes on the basis of methodological quality of the studies, and by determination of the independent predictive value over conventional risk factors.

Patients and methods

Identification of relevant studies

Relevant studies were identified by searches of Medline via Pubmed, Embase and the Cochrane Library database (Cochrane Central Register of Controlled Trials), with relevant text words and medical subject headings that consisted of the term "biomarker or biological marker" and one of the following terms: "(diabetic) nephropathy", "(non insulin dependent) diabetes mellitus", "microalbuminuria", "albuminuria", "proteinuria", "chronic kidney disease", "diabetes complications". The search was limited to longitudinal studies on humans and adults, published between 1995 and 2010, without language restrictions. We extended our search by reviewing the references from the eligible papers and the papers that cited the eligible papers through Web of Science.

Study selection

Studies were considered eligible if they were longitudinal cohort studies or randomized controlled trials with at least 20 patients, reporting on biomarkers for the prediction of onset or progression of nephropathy in type 2 diabetic patients. We focused on biomarkers that can be measured in urine, plasma or serum. Studies on conventional risk factors/ biomarkers were excluded because we were mainly interested in novel biomarkers that could potentially improve risk prediction beyond conventional risk markers. Therefore, the conventional markers rather served as a basis for quality assessment of the studies (see below). The literature search, data extraction, and scoring were carried out by two reviewers independently by use of a standardized approach (JK, MEH). Any disagreement was resolved by a third reviewer (MR).

Terminology

Diabetic nephropathy is histopathologically characterized by several changes in the kidney, such as nodular glomerulosclerosis, mesangial expansion, basement membrane thickening and interstitial fibrosis. Clinically, diabetic nephropathy is usually a constellation of persistent albuminuria, elevated arterial blood pressure, and decline in kidney function.[13] Changes in albuminuria are considered a hallmark of onset or progression of nephropathy. Albuminuria levels have been categorized into normoalbuminuria (<30 mg/day or <20 µg/min), microalbuminuria (30-300 mg/day or 20-200 µg/min) and macroalbuminuria (>300 mg/day or >200 µg/min). Consequently, studies often report transition in albuminuria class (from normo- to microalbuminuria and from micro- to macroalbuminuria) or doubling of serum creatinine from baseline as indicators of nephropathy onset or progression. In our study onset of nephropathy was defined as the development of microalbuminuria in previously normoalbuminuric patients (early nephropathy). Progression of nephropathy was defined as either the transition from normo- or micro- to macroalbuminuria, a longitudinal change in the extent of albuminuria or doubling of serum creatinine in micro- and macroalbuminuric patients (late nephropathy).

Study analysis

Studies were divided into three groups: studies on biomarkers predicting onset of nephropathy in patients with normoalbuminuria (early nephropathy), studies predicting progression of nephropathy in patients with micro- or macroalbuminuria (late nephropathy), and studies predicting onset and progression of nephropathy in cohorts that included patients with normoalbuminuria and microalbuminuria.

Quality assessment

Studies were assessed for validity using a modified checklist of the STARD criteria (table 1). The STARD initiative developed a set of 25 criteria for reporting of studies of diagnostic accuracy in order to improve the quality.[14] For the purpose of this review, we limited the quality assessment to 11 items, mainly focusing on methodological quality of the study. Items focusing on specific laboratory methods or adverse events of the tests were not included in this review. Study quality was considered as "good" if the score was ≥ 10, "average" if the score was 8-9, "fair" if the score was 6-7, and "poor" if the score was <6.

Adjustment score and biomarker validity score

In order to be clinically useful, biomarkers for onset and progression of nephropathy should have additional predictive value on top of conventional risk factors (age, sex, race,

Table 1 | Methodological quality assessment according to 11 relevant items of the STARD criteria

Section		Criteria	Scoring	Comments
Introduction	1	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	stated=1 not stated=0	Stated in all.
Methods	2	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	stated=1 not stated=0	Stated in all, but two (28,37).
	3	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria/a randomized controlled trial?	consecutive/RCT=1 non-consecutive/not stated=0	Non-consecutive/RCT or not stated in eight (25,2,28,32,34,35,37,38).
	4	Data collection: Was data collection planned before the index test and reference standard were performed (prospective) or after (retrosp ective)?	prospective=1 retrospective=0	Prospective in all, but one (36).
	5	The reference standard and its rationale.	stated=1 not stated=0	Stated in all, but two (36,37).
	6	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	stated=1 not stated=0	Stated in all, but two (25,30).
	7	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	stated=1 not stated=0	Stated in all, but one (34).
	8	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% conf. intervals).	stated=1 not stated=0	Not stated in four.
Results	9	When study was performed, including beginning and end dates of recruitment.	stated=1 not stated=0	Not stated in five (26-29,35).
	10	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	stated=1 not stated=0	Stated in all, but one (28).
	11	Distribution of severity of diasease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	stated=1 not stated=0	Stated in all, but two (33,37).

RCT, randomized controlled trial; STARD, Standards for Reporting of Diagnostic Accuracy.

hypertension, HbA1c, BMI, diabetes duration, hypercholesterolemia, retinopathy, smoking, albuminuria and use of RAAS-inhibitors).[15-24] As a quality assessment we scored whether the studies took conventional risk factors into account. One point was attributed to each of the following conventional factors that was adjusted for: Age and sex, blood pressure/ hypertension and/or use of antihypertensive agents, HbA1c or fasting plasma glucose, BMI, diabetes duration, total cholesterol and/or HDL cholesterol, retinopathy, smoking, use of RAAS-inhibitors, UAER or albumin-to-creatinine ratio (ACR). Race was not applicable because all of the studies were conducted in homogenous Asian, Caucasian or Native American populations. The maximum adjustment score was 10 points. Adjustment was considered "good" if the score was ≥ 9, "average" if the score was 7-8, "fair" if the score was 5-6, and "poor" if the score was <5.

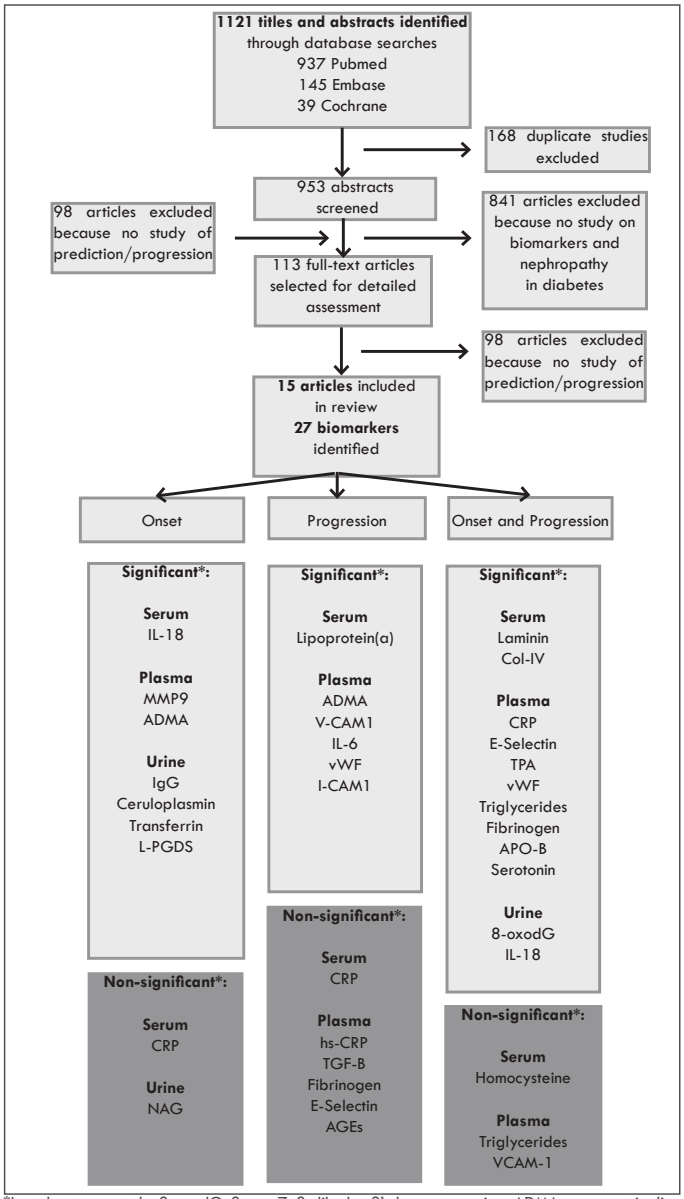
The adjustment score when added to the methodological quality score resulted in a combined biomarker validity score. Biomarkers were considered as valid biomarker candidates if both the methodological quality score and the adjustment score were at least "good" or "average" (biomarker validity score ≥ 15).

Results

The systematic search (performed 1 July 2010) for articles of longitudinal studies on biomarkers for prediction of the onset or progression of nephropathy in patients with type 2 diabetes resulted in 953 non-duplicate articles of which 841 were not on biomarkers other than conventional risk factors for nephropathy in diabetes type 2, and were therefore excluded. We also excluded studies on populations with chronic kidney diseases other than diabetic nephropathy, studies on nephropathy in patients with diabetes type 1, or studies on non-serum and non-urine biomarkers. The remainder of the articles (N=112) were reviewed in full text. Of these, only 14 studies met the inclusion criteria and were included in the present review.[25-38] The main reason for exclusion in this phase was cross-sectional study design. Explosion search (the references of the selected papers) and forward citation search (all studies referring to the selected papers) resulted in identification of one additional paper.[39] A flow-chart demonstrating the study selection process is shown in figure 1.

The 15 identified studies reported on 27 individual biomarkers, of which 19 were serum/ plasma biomarkers, six were urinary biomarkers and IL-18 was measured in both serum and urine (and therefore counted as two individual biomarkers). Four studies reported on prediction of the onset of nephropathy in patients with normoalbuminuria,[25-28] two on progression of nephropathy in micro- or macroalbuminuric patients,[29,39] and seven

Figure 1 | Flow chart of the identification process of eligible studies



*In at least one study. 8-oxodG, 8-oxo-7, 8-dihydro-2'-deoxyguanosine; ADMA, asymmetric dimethylarginine; AGE, advanced glycation end-product; Apo-B, apolipoprotein B; Col-IV, collagen IV; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; ICAM1, intercellular cell adhesion molecule 1; IgG, immunoglobulin G; IL, interleukin; L-PGDS, lipocalin-type D2 synthase; MMP9, matrix metallopeptidase 9; NAG, N-acetylglucosaminidase; TGFb; transforming growth factor b; TPA, tissue-type plasminogen activator; VCAM1, vascular cell adhesion molecule 1; vWF, von Willebrand factor.

reported biomarkers in combined normo- and microalbuminuric patient cohorts (i.e. onset and progression) and thus do not make a distinction between onset and progression.[30-36] Another two studies assessed biomarkers in combined normo- and microalbuminuric patient cohorts and report the results for onset and progression of nephropathy separately.[37,38]

Study characteristics

Study characteristics are shown in table 2. Individual study size ranged from 30 to 1103 patients with a total number of 3529 patients. Mean/median follow-up ranged from 0.5-9.0 years and mean age of the study populations ranged from 51.8 to 67.5 years. All but one study included both men and women in approximately equal proportion.[36] Eleven out of 15 studies were conducted in Asian populations,[25-29,32-34,36-38] one study in a Native American population,[35] and 3 studies in Caucasian populations.[30,31,39] In studies assessing biomarkers for prediction of onset of nephropathy the transition in albuminuria class (i.e. normo- to microalbuminuria) was the endpoint used in all studies. In studies assessing biomarkers for prediction of progression of nephropathy the endpoints used were transition in albuminuria class (i.e. micro- to macroalbuminuria) in microalbuminuric patients in three studies,[37-39] and doubling of serum creatinine in macroalbuminuric patients in one study.[29] In studies of mixed normo-, micro- or macroalbuminuric patients the endpoints used were very heterogeneous and no distinction was made between onset and progression of nephropathy. Therefore, the identified biomarkers are summarized as the separate category "onset and progression". Endpoints used by these studies were change of urinary albumin excretion rate over time (without indication of the baseline urinary albumin excretion rate),[36] the ratio of the urinary albumin excretion rate at baseline and urinary albumin excretion rate at end of follow-up,[34] development of macroalbuminuria in combined normo- and microalbuminuric patients,[31,32,35] transition from either normo- to microalbuminuria or micro- to macroalbuminuria),[33] and transition from normo- to either micro- or macroalbuminuria.[30] Meta-analysis was not performed because of marked heterogeneity in biomarkers analysed, study endpoints, statistical methods used and biomarker cut-off levels.

Methodological quality score, adjustment score, and biomarker validity score

Overall, seven of the 15 studies had good methodological quality, six studies were classified as average and two studies as fair. "Average" to "good" adjustment score corresponding to an adjustment for at least seven of 10 of the conventional risk factors was only present in six studies (table 3). All of these six studies also had good or average methodological quality. The biomarkers from these six studies were considered as valid. These studies identified

Table 2| Characteristics of selected studies stratified for prediction of onset, progression, and onset and progression of nephropathy

Study	Year	Ethnicity	Patients (N)	Men [N (%)]	Normo (N)	Micro (N)	Macro (N)	Age[b]	Type of AM	Follow-up	Endpoint
Onset of nephropathy											
Ebihara (27)	1998	Asian	30	17 (57)	30	0	0	55.5 ±15.5	UAER	4	Normo to micro
Kazumi (26)	1999	Asian	77	50 (52)	77	0	0	56.3 ± 10.6	ACR	2	Normo to micro
Narita (25)	2006	Asian	117	60 (51)	117	0	0	60.4 ± 8.9	ACR	5	Normo to micro
Uehara (28)	2008	Asian	121	-	121	0	0	-	ACR	2	Normo to micro
Araki (38)	2007	Asian	249	130 (52)	173	76[f]	0	61 ± 9	UAER	7 [3-8][c]	Normo to micro
Hanai (39)	2009	Asian	225	144 (64)	183	42[f]	0	64 ± 10	ACR	5.2[d]	Normo to micro
Progression of nephropathy											
Persson (29)	2008	Caucasian	269	184 (68)	0	269	0	57.8 ± 8.5	UAER	2	Micro to macro
Song (30)	2005	Asian	81	36 (44)	0	0	81	59 ± 9.2	UAER	2	Doubling of baseline sCr
Araki (38)	2007	Asian	249	130 (52)	173[f]	76	0	61 ± 9	UAER	7 [3-8][c]	Micro to macro
Hanai (39)	2009	Asian	225	144 (64)	183[f]	42	0	64 ± 10	ACR	5.2[d]	Micro to macro
Onset and progression of nephropathy											
Fukui (37)	2009	Asian	162	162 (100)	-	-	-	63.9 ± 11.1	ACR	2	Longitudinal change in UAER
Bruno (32)	2003	Caucasian	1103	477 (43)	677	426	0	67.5 ± 10.2	UAER	5.3 [0.1-7.9][c]	Normo/micro to macro
Hinokio (34)	2002	Asian	396	190 (48)	115	281	0	51.8 ± 10.3	ACR	5	Normo to micro/micro to macro
Looker (36)	2003	Nat.American	229	-	152	77	0	-	ACR	8.6[e]	Normo/micro to macro
Okazaki (33)	1995	Asian	66	57 (66)	-	-	-	-	Dipstick	3.5 [3-4][c]	Normo/micro to macro
Nakamura (35)	2005	Asian	76	34 (41)	41	31	10	62.5 ± 7.5	UAER	0.5	UAER-post/ UAER-pre ratio
Stehouwer (31)	2002	Caucasian	328	191 (58)	191	92	45	54.3 ± 8.2	UAER	9±2.9[b]	Normo to micro/macro

* Completed follow-up. †Mean ± SD, ‡Median and range, ∫Median, ¶Mean.**In the studies (25) and (26) normo- and microalbuminuric patients were analysed separately. ACR, albumin-to-creatinine ratio; AM, albuminuria measurement; Long, longitudinal; Macro, macroalbuminuric patients; Micro, microalbuminuric patients; Nat.American, native American; Normo, normoalbuminuric patients; sCr, serum creatinine; UAER, urinary albumin excretion rate.

17 valid biomarkers, of which 13 yielded significant results predicting nephropathy. Detailed results of all biomarkers are shown in tables 4, 5 and 6. Results were stratified for studies reporting on biomarkers predicting the onset of nephropathy, the progression of nephropathy, and the onset and progression of nephropathy in combined patient cohorts, respectively.

Biomarkers predicting onset of nephropathy in diabetes (early nephropathy)

Five urinary biomarkers for prediction of the onset of nephropathy in diabetes were evaluated (Table 4).[25-27] Of these, urinary IgG, ceruloplasmin, and transferrin were predictive of nephropathy onset and had highest validity because of average study design and prediction beyond most conventional risk factors. Urinary transferrin was evaluated in two studies of good methodological quality, and it was significantly associated with onset of nephropathy in both.

Four serum/plasma biomarkers for the onset of nephropathy were evaluated. IL-18 (a marker of subclinical inflammation) and asymmetric dimethylarginine (ADMA; a marker of endothelial dysfunction) could be considered as most promising predictors of microalbuminuria, as these were identified in well-designed studies adjusting for (nearly) all conventional risk factors.

Although urinary *N*-acetylglucosaminidase (NAG) and serum C-reactive protein (CRP) were considered valid candidates, results were not significant. The remaining biomarkers [matrix metallopeptidase-9 (MMP9) in plasma and lipocalin-type prostaglandin D2 synthase (L-PGDS) in urine] were not considered valid due to lack of adjustment for conventional risk factors.

Biomarkers predicting progression of nephropathy in diabetes (late nephropathy)

Twelve biomarkers for progression of nephropathy in diabetes were evaluated (Table 5). Of these, nine plasma biomarkers were evaluated by Persson *et al*. in patients from the well-designed randomized control trial 'Irbesartan MicroAlbuminuria Type 2 Diabetes Mellitus in Hypertensive Patients' (IRMA-2).[40] Vascular cell adhesion molecule-1 in serum (sVCAM1), interleukin-6 (IL-6), von Willebrand factor (vWf), and intercellular cell adhesion molecule-1 in serum (sICAM1) were significantly associated with albuminuria progression, whereas high sensitive CRP (hs-CRP), transforming growth factor β (TGF-β), fibrinogen, E-selectin, and advanced glycation end-product (AGE) peptides were not significant. Interestingly, this study also tested the combined z-scores of multiple biomarkers (biomarker panel). The panel of markers of endothelial dysfunction (i.e. sVCAM1, vWF, sICAM1 and E-selectin) was found as an independent predictor of progression, whilst the panel of inflammation markers (i.e. hs-CRP, IL-6 and fibrinogen) was not. The valid biomarker ADMA that was

Table 3 | Adjustment score

Study	UAER	Diabetes Duration	Age & Sex	Hypertension	HbA1c	Smoking	Retinopathy	Lipids	BMI	RAAS -Inhibition	Adjustment score
Araki (38)	■	■	■	■	■	■	■	■	■	■	10
Bruno (32)	□	■	□	●	■	□	□	□	□	□	3
Ebihara (27)	□	□	□	□	□	□	□	□	□	□	0
Fukui (37)	□	■	□	■	■	■	□	■	■	□	6
Hanai (39)	■	■	■	■	■	□	■	■	■	■	9
Hinokio (34)	□	■	□	■	■	□	□	□	□	□	3
Kazumi (26)	■	□	■	■	■	■	□	■	■	□	7
Looker (36)	■	■	■	□	■	□	□	□	□	□	4
Nakamura (35)	■	□	□	□	□	□	□	□	□	□	1
Narita (25)	■	■	■	■	■	■	■	■	■	■	10
Okazaki (33)	□	■	■	■	■	□	□	□	□	□	4
Persson (29)	■	□	■	■	■	■	■	■	□	■	8
Song (30)	■	□	□	■	■	□	□	□	□	□	3
Stehouwer (31)	■	■	■	■	■	■	□	■	■	□	8
Uehara (28)	□	□	□	□	□	□	□	□	□	□	0

■, Criterion is met; □ Criterion is not met.

earlier shown to predict onset of nephropathy also predicted progression of nephropathy.[38] In contrast, IL-18 predicted onset but not progression of nephropathy and hs-CRP neither predicted onset nor progression of nephropathy in the study by Araki *et al.*[37] Whereas the methodological quality of the study on Lipoprotein(a) was good, the study did not address adequate correction for conventional risk factors resulting in low overall biomarker validity.

Biomarkers predicting onset and progression of nephropathy in diabetes

Fourteen biomarkers were evaluated in seven studies in combined cohorts of normo-, micro- and macroalbuminuric patients with variable endpoints (Table 6). Of these studies all but one had average to good methodological quality. However, only the study by Stehouwer *et al.* (assessing six biomarkers) performed adequate adjustment for conventional risk factors, and identified hs-CRP, E-selectin, tissue-type plasminogen activator (TPA), (vWf) and triglycerides, but not sVCAM1, as valid and promising biomarkers.[30]

Table 4| Overview of biomarkers for onset of nephropathy in diabetes

Biomarker	U/P/S	Reference	Meth. Quality Score	Adj. Score	Biomarker Validity Score	Cut-off	Unit	Results	Sensitivity/ specificity
IL-18	Serum	(38)	10	10	20	134.6[a]	ng/L	OR [95%CI]: 3.6 [1.2-10.4]	-
ADMA	Plasma	(39)	11	9	20	0.46 µmol/l[a]	µmol/L	HR [95%CI]: 2.61 [1.06-6.43]	-
IgG	Urine	(25)	9	10	19	-	mg/gCr	OR [95%CI]: 8.99 [3.16-25.6]	0.47/0.91
Ceruloplasmin	Urine	(25)	9	10	19	-	mg/gCr	OR [95%CI]: 4.67 [1.67-13.1]	0.47/0.84
Transferrin	Urine	(25)	9	10	19	-	µg/gCr	OR [95%CI]: 5.52 [1.81-16.8]	0.35/0.91
Transferrin	Urine	(26)	10	7	17	>107 µg/mmolCr[b]	µg/mmolCr	OR [95%CI]: 7.04 [1.02-48.5]	0.56/0.84
MMP9	Plasma	(27)	8	0	8	-	µg/L	P<0.001 (48 months)	-
L-PGDS	Urine	(28)	7	0	7	4.2 mg/gCr	mg/gCr	AUC [95%CI]: 0.759 [0.725-0.791]	-
hs-CRP	Serum	(38)	10	10	20	-	mg/L	Not reported (n.s.)	-
NAG	Urine	(25)	9	10	19	-	U/gCr	Not reported (n.s.)	-

*Median, †Mean ± 2 SD. ADMA, asymmetric dimethylarginine; AUC, area under the curve; CI; confidence interval; Cr, creatinine; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IgG, Immunoglobulin G; IL; interleukin; L-PGDS, lipocalin-type prostaglandin D2 synthase; MMP9, matrix metallopeptidase 9; NAG, N-acetylglucosaminidase; NS, not significant; OR, odds ratio.

Table 5|Overview of biomarkers for progression of nephropathy in diabetes

Biomarker	U/P/S	Reference	Meth. Quality Score	Adj. Score	Biomarker Validity Score	Cut-off	Unit	Results	Sensitivity/ specificity
ADMA	Plasma	(39)	11	9	20	0.51 µmol/l[a]	µmol/L	HR [95%CI]: 7.57 [1.42-40.38], P=0.018	-
sVCAM-1	Plasma	(29)	10	8	18	1 SD change	ng/mL	HR [95%CI]: 2.06 [1.26-3.36], P=0.004	-
IL-6	Plasma	(29)	10	8	18	1 SD change	pg/mL	HR [95%CI]: 1.72 [1.12-2.66], P=0.014	-
vWf	Plasma	(29)	10	8	18	1 SD change	%	HR [95%CI]: 1.69 [1.10-2.59], P=0.016	-
sICAM-1	Plasma	(29)	10	8	18	1 SD change	ng/mL	HR [95%CI]: 1.99 [1.04-3.82], P=0.038	-
Lipoprotein(a)	Serum	(30)	10	3	13	per 10 mg/dL	mg/dL	OR [95%CI]: 1.418 [1.040-1.934],P=0.027	0.93/0.55
hs-CRP	Serum	(38)	10	10	20	Not reported	mg/L	n.s.	-
IL-18	Serum	(38)	10	8	18	1 SD change	mg/L	n.s.	-
hs-CRP	Plasma	(29)	10	10	20	Not reported	ng/L	n.s.	-
TGF-β	Plasma	(29)	10	8	18	1 SD change	ng/mL	n.s.	-
Fibrinogen	Plasma	(29)	10	8	18	1 SD change	g/L	n.s.	-
E-Selectin	Plasma	(29)	10	8	18	1 SD change	ng/mL	n.s.	-
AGEs	Plasma	(29)	10	8	18	1 SD change	%	n.s.	-

*Median, †Mean ± 2 SD. ADMA, asymmetric dimethylarginine; AUC, area under the curve; CI, confidence interval; Cr, creatinine; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IgG, immunoglobulin G; IL, interleukin; L-PGDS, lipocalin-type prostaglandin D2 synthase; MMP9, matrix metallopeptidase 9; NAG, N-acetylglucosaminidase; NS, not significant; OR, odds ratio.

Table 6| Overview of biomarkers for onset and progression of nephropathy in diabetes

Biomarker	U/P/S	Reference	Meth. Quality Score	Adj. Score	Biomarker Validity Score	Cut-off	Unit	Results	Sensitivity/ specificity
CRP	Plasma	(31)	11	8	19	per 1.0 mg/L	mg/L	OR [95%CI]: 1.06 [1.02-1.11]	-
E-SEL	Plasma	(31)	11	8	19	per 10 µg/L	µg/L	OR [95%CI]: 1.08 [1.03-1.13]	-
TPA	Plasma	(31)	11	8	19	per 10 µg/L	µg/L	OR [95%CI]: 1.02 [1.00-1.04]	-
vWf	Plasma	(31)	11	8	19	per 10%	%	OR [95%CI]: 1.05 [1.00-1.10]	-
Triglycerides	Plasma	(31)	11	8	19	per 1.0 mmol/L	mmol/L	OR [95%CI]: 1.10 [1.00-1.20]	-
Fibrinogen	Plasma	(32)	11	3	14	3.49-4.12 (R:<3.00)	g/L	OR [95%CI]: 1.93 [1.18-3.16]	-
APO-B	Plasma	(32)	11	3	14	95-112 (R:<74)	mg/dL	OR [95%CI]: 1.73 [1.05-2.87]	-
Laminin	Serum	(33)	9	4	13	-	U/ml	P<0.01	-
COL-IV	Serum	(33)	9	4	13	-	ng/mL	P<0.05	-
8-oxodG	Urine	(34)	9	3	12	>400 (R:<200)	pmol/kg/day	OR [95%CI]: 2.71 [1.78-3.88]	0.45/0.87
Serotonin	Plasma	(37)	6	6	12	per log unit	ng/mL	β=0.284, P=0.0013	-
IL-18	Urine	(35)	8	1	9	-	pg/ml	r = 0.234, P=0.042	-
IL-18	Serum	(35)	8	1	9	-	pg/ml	r = 0.268, P=0.018	-
VCAM	Plasma	(31)	11	8	19	per 100 µg/L	µg/L	n.s.	-
Triglycerides	Plasma	(32)	11	3	14	-	mmol/L	Not reported (n.s.)	-
Homocysteine	Serum	(36)	9	4	13	per 5 µmol/L (±1SD)	µmol/L	n.s.	-

*Median. ADMA, asymmetric dimethylarginine; AGE, advanced glycation end-product; CI, confidence interval; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; NS, not significant; OR, odds ratio; sICAM1, intercellular cell adhesion molecule 1 in serum; sVCAM1, vascular cell adhesion molecule 1 in serum; TGF-b, transforming growth factor b; vWf, von Willebrand factor.

Discussion

In this systematic review we identified 15 publications on longitudinal studies reporting on 27 candidate biomarkers for the prediction of nephropathy in type 2 diabetes. We scored the methodological quality of the identified studies and we evaluated whether these biomarkers add information on risk prediction. Using this approach we demonstrated that the overall study quality of these studies is in general modest. This not only limits proper assessment of the potential clinical value of the identified biomarkers, it also limits the generalizability and comparability of the results.

Most of the marker molecules identified in this systematic review represent subclinical systemic inflammation (CRP, IL-6, IL-18, fibrinogen), endothelial dysfunction (ADMA, vWF, VCAM, ICAM1, TPA), extracellular matrix synthesis (TGFb1, laminin, collagen type 4) or glomerular and tubular dysfunction (urinary IgG, ceruloplasmin, and transferrin). Due to the clear pathophysiological connection between these molecules and nephropathy in diabetes it is tempting to utilize these biomarkers in clinical practice. However, before these markers can be applied in practice, the clinical applicability of these biomarkers needs to be confirmed in high-quality validation studies.

We would like to highlight several important methodological issues that are relevant for the quality of biomarker research. First, we found that studies of predictive markers frequently calculated odds ratios or relative risks to demonstrate the strength of association between the biomarker and the outcome. However, odds ratios and hazard ratios inaccurately predict the risk for individual subjects as the ratios are only a measure of association between biomarker and outcome, but do not characterize the ability to discriminate between future health or disease.[41]

Another limitation of odds ratios and relative risks is that the size of the ratio depends on the units of measurement.[8] Some form of standardization is necessary, for example by division of a continuous measure by its standard deviation. Moreover, even if very large odds ratios are reported, one cannot conclude that the marker has good predictive power, since each odds ratio could correspond to largely variable true-positive/ false-positive fractions. Hence, instead of using odds ratios, the additive value of a marker in risk prediction should be specified by prediction analyses such as false-positive/true-positive fractions, area under the receiver-operator characteristic curve, the net reclassification improvement (NRI), integrated discrimination improvement (IDI),[42] or the discriminative likelihood ratio (dLR).[43,44] Only one study included in this review reported an area under the ROC-curve,[28] and 4 other studies provided sufficient detail for the calculation of sensitivity and specificity.[25,26,29,33]

A second important issue in biomarker research concerns the validation of results of

a study in other patient populations. Biomarkers only have clinical value if the results are reproducible (external validation). Of the 27 identified biomarkers, the majority was evaluated in only one (longitudinal) study, and only 10 markers (plasma VCAM1, hs-CRP, vWF, fibrinogen, E-selectin, triglycerides, transferrin, serum IL18, homocysteine and urinary transferrin) were analyzed in at least two studies. In nearly all cases results could not be replicated (potentially attributable to limited power).

A last important methodological issue is the heterogeneity of endpoints in some of the studies. Moreover, the methods of albumin assessment and the number of albuminuria measurements were either not stated or highly heterogeneous between the studies. Lastly, the frequently used endpoint "transition in albuminuria class" has crucial limitations, e.g. a patient with a rise in albuminuria from 29 to 31 mg/day is defined as progressor, while a patient who increases from 31 to 299 mg/day is not. Thus, avoiding albuminuria classification itself or introduction of combined relative and absolute changes in albuminuria (e.g. class transition and at least 30% increase) may represent a valid alternative. This also highlights the importance to reach consensus on definitions of endpoints in biomarker research.

Aside from these limitations in study design, several biomarkers showed promising results. For the prediction of onset of nephropathy in diabetes, urinary IgG, ceruloplasmin and transferrin, serum IL-18 and plasma ADMA were most promising. The results on biomarkers for progression of diabetic nephropathy are not conclusive due to differences in adjustment for conventional risk factors.

This review has certain limitations due to its focus on longitudinal studies for prediction of nephropathy and its focus on methodological quality. We were unable to compare the measures of association of the described biomarkers due to marked heterogeneity in study endpoints, statistical methods and different cut-offs. Head-to-head comparison of biomarkers in a well-designed and sufficiently large longitudinal study is most likely the best way to compare biomarkers. Secondly, we did not take into consideration the individual time of follow-up in the individual studies. This is of particular importance in a slow progressive disease such as nephropathy in diabetes.

Based on the status of current biomarker research in this field, we recommend that future research should be directed at both further biomarker discovery and validation of published biomarkers in large well-designed longitudinal studies. Specific prediction analyses should be applied to assess the additive predictive value of novel and published biomarker candidates beyond conventional risk factors.

In the end, all effort of biomarker research should be directed towards the development of a reliable, accurate, reproducible and robust "Diabetic Nephropathy Biomarker Panel" that would compare to the biomarker panel consisting of creatine kinase (CK), CK-MB, Troponin

I and Troponin T currently used in cardiology. Both clinical practice and clinical trials on the efficacy of various treatments on renal disease in type 2 diabetic patients would benefit of such a biomarker panel, thus developing such a biomarker panel would be a major step

Conclusions

The fact that many well-designed studies were not able to confirm the results on certain biomarkers emphasizes the remaining uncertainty of the clinical utility of many of the studied markers despite promising findings. Future research will have to elucidate the true value of the current biomarker candidates for prediction of onset and progression of nephropathy in diabetes. However, current results prevent us from making clear recommendations for clinical practice at this moment.

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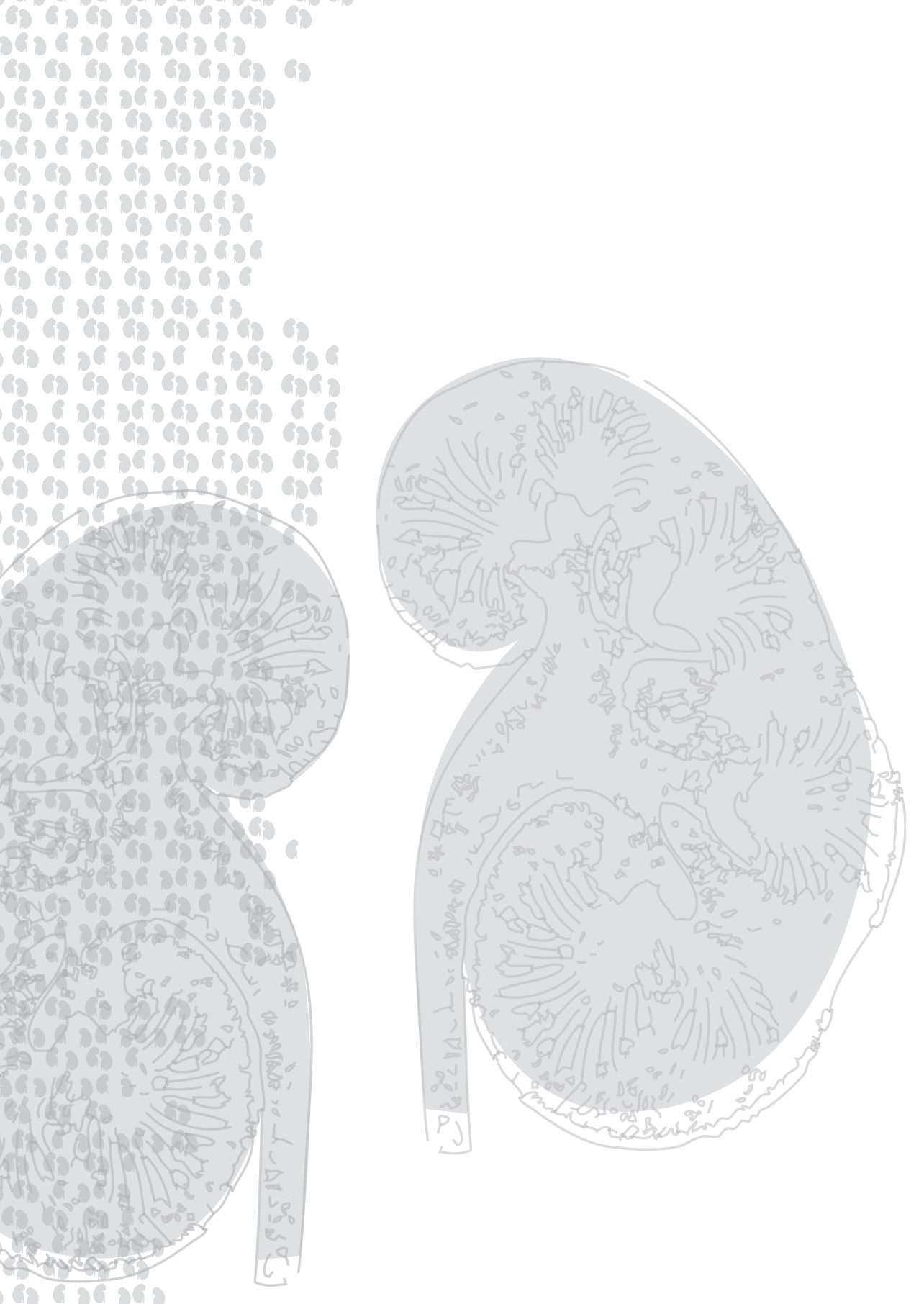

3

Growth-differentiation Factor 15 Predicts Worsening of Albuminuria in Patients with Type 2 Diabetes

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Abstract

Background: Development of micro- or macroalbuminuria is associated with increased risk of cardio-renal complications, particularly in diabetes. For prevention of transition to micro- or macroalbuminuria more accurate prediction-markers on top of classical risk-markers are needed. We studied a new promising marker, Growth-Differentiation Factor-15 (GDF-15), to predict transition to increasing stage of albuminuria in type-2 diabetes (T2DM). In addition, we looked at the GDF-15 potential in non-diabetic subjects with hypertension (HT).

Methods: Cases and controls were selected from the PREVEND-cohort, a large (n=8,592), prospective general population study on the natural course of albuminuria, with >10 years of follow-up and repeated albuminuria-measurements. We found 24 diabetic and 50 hypertensive cases transitioning from normo- to microalbuminuria, and 9 diabetic and 25 hypertensive cases from micro- to macroalbuminuria (average follow-up 2.8 yrs). Controls with stable albuminuria were pair-matched for age, gender, baseline albuminuria status and diabetes-duration. GDF-15 was measured in samples prior to albuminuria-transition.

Results: Prior to transition, GDF-15 was significantly higher in cases with T2DM than in controls (1288[885-1546] vs. 948[660-1016] pg/mL,*P*<0.001). The odds-ratio for transition in albuminuria increased significantly per SD of GDF-15 (2.9 [95%CI 1.1-7.5],*P*=0.03). GDF-15 also improved prediction of albuminuria-transition, with significant increases in C-statistic (from 0.87 to 0.92, *P*=0.03) and integrated-discrimination-improvement (0.148, *P*=0.001). In HT, GDF-15 was also independently associated with transition in albuminuria-stage (2.0[1.1-3.5], *P*=0.02) and improved prediction significantly.

Conclusions: We identified GDF-15 as a clinically valuable marker for predicting transition in albuminuria-stage in T2DM beyond conventional risk markers. These findings were confirmed in non-diabetic hypertensive subjects.

Introduction

The prevalence of chronic kidney disease (CKD) is increasing and has become a major public health challenge.[1] This increase in CKD is largely due to the rapidly expanding epidemic of type 2 diabetes mellitus (T2DM) leading to diabetic nephropathy and ultimately end-stage renal disease (ESRD).[2] Transition to increasing stages of albuminuria (i.e. normo- to microalbuminuria and micro- to macroalbuminuria) is considered a hallmark of progression of renal disease in diabetes.[3] However, once transitioned from normo- to microalbuminuria or to macroalbuminuria, regression of disease is very difficult to achieve. Indeed, recent trials in normo-, micro-, and macroalbuminuric diabetic subjects showed that early intervention (in normoalbuminuric stage) is more effective than late intervention.[4] Early markers that detect those that have an increased risk for developing micro- or macroalbuminuria could thus help to reduce the number of patients at renal risk through selective and appropriate treatment of such patients.[5,6]

Many risk factors have been linked to transition from normo- to micro and micro- to macroalbuminuria, such as hyperglycemia, hypercholesterolemia and hypertension.[7] However, accurate risk stratification remains challenging. Novel biomarkers may help to improve the identification of subjects at risk, as well as improve insight in the underlying pathophysiology of the development of micro- or macroalbuminuria. Whereas several promising novel biomarkers were described in literature, this had not led to improved risk stratification in T2DM.[8]

The lack of well-designed prospective studies that first stored samples of individuals for novel risk marker analyses and then followed the course of albuminuria over time may explain the paucity of knowledge on the prognostic value of novel biomarkers to improve risk stratification. We performed a nested case-control study in the large general population cohort 'Prevention of REnal and Vascular End-stage Disease (PREVEND) to investigate novel biomarkers that may precede and predict the transition in albuminuria.[9] Growth Differentiation Factor-15 (GDF-15), a member of the TGF-β family, is a promising novel biomarker, which has been implicated as predictor for cardiovascular and all-cause mortality.[10-12] Interestingly, it was also associated with renal outcome and a faster decline of eGFR as well as mortality in type 1 diabetic patients with macroalbuminuria.[13] It is unclear whether these findings regarding renal outcome are also applicable to patients with type 2 diabetes. In the current study, we investigated whether circulating GDF-15 levels precede and predict the development of micro- or macroalbuminuria in type 2 diabetic patients. To test whether this is specific to diabetes, we performed a replication study to assess the predictive value of GDF-15 in non-diabetic hypertensive patients.

Patients and methods

The present study was performed as a nested case-control study in subjects participating in the 'Prevention of REnal and Vascular End-stage Disease' (PREVEND) study. This prospective community-based cohort study on the natural course of urinary albumin excretion with serial follow-up measurements was initiated in 1997. Details of the study protocol have been published elsewhere.[9] In short, all inhabitants of the city of Groningen aged 28–75 years were sent a questionnaire and a vial to collect a first-morning-void urine sample. Of these individuals, 40,856 responded (47.8%). From these individuals a cohort consisting of 8,592 subjects was selected (the PREVEND cohort). In this ongoing study participant are invited to visit an outpatient clinic for detailed medical examination at ± 3-year intervals. At each screening round participants fill out questionnaires on demographics, medical history and drug use. Information on drug use is completed with data from community pharmacies, including information on class of antihypertensive medication (ACEi/ARB). At the study visits participants deliver two 24-hour urine collections, blood pressure is measured, anthropometrical measurements are performed, and fasting blood samples are taken.

The PREVEND study was approved by the institutional ethics review board and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

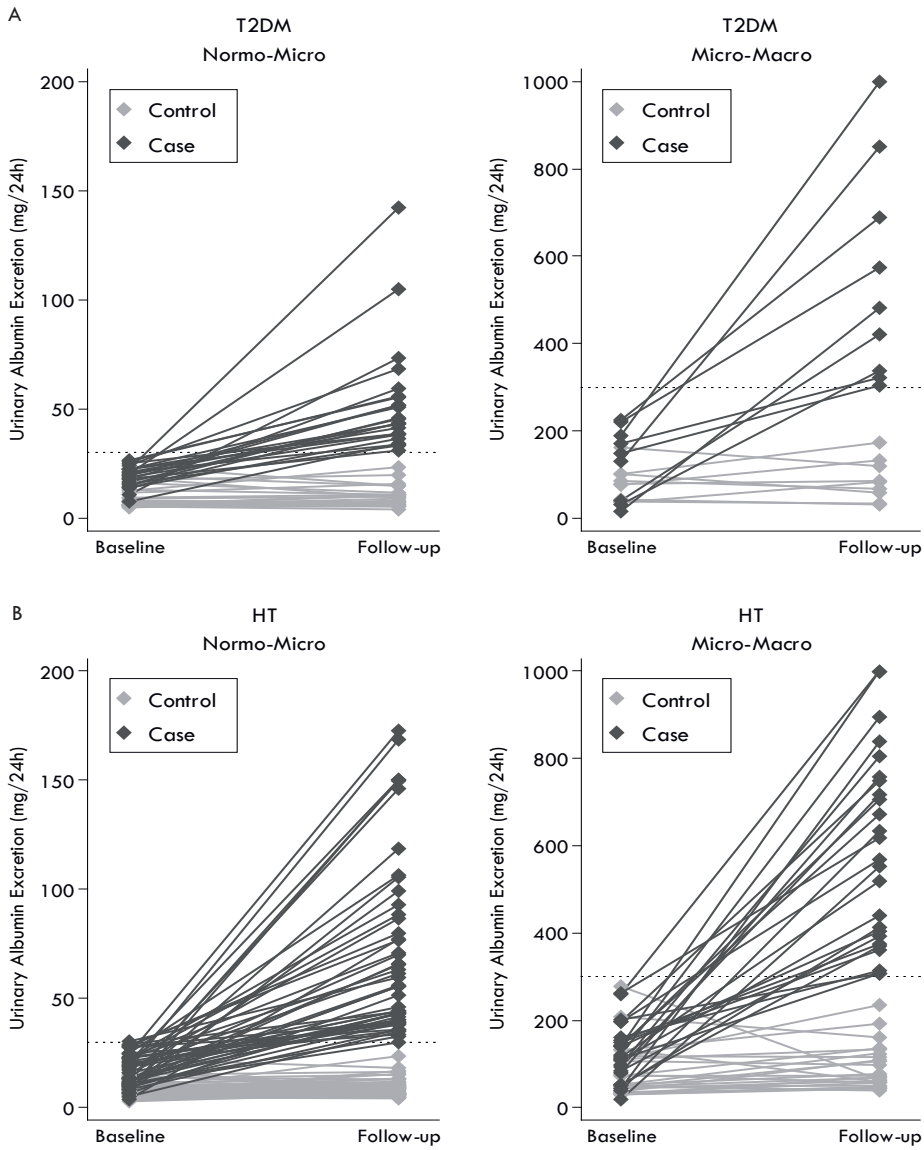
Definitions

Normoalbuminuria was defined as urinary albumin excretion (UAE) <30 mg/24h, microalbuminuria as UAE 30-299 mg/24h and macroalbuminuria as UAE ≥300 mg/24h. Albuminuria status was based on the average of 2 consecutive measurements in 24-hour urine collections. Transition in albuminuria was defined as a transition from normo- to micro- or from micro- to macroalbuminuria with at least 30% increase in urinary albumin excretion from baseline between two consecutive study visits. T2DM was defined as the use of oral anti-diabetic treatment (self-reported or by information retrieved from the regional pharmacy database), a fasting plasma glucose >7.0 mmol/L (126 mg/dL) or non-fasting plasma glucose >11.1 mmol/L (>200 mg/dL). HT was defined as the use of anti-hypertensive treatment or a systolic/diastolic blood pressure > 140/90 mmHg.

Selection of cases and controls

For the present study, we selected patients with type 2 diabetes mellitus (T2DM) and transition from normo- to microalbuminuria or from micro- to macroalbuminuria. As controls, we selected T2DM patients who had persistent normoalbuminuria or

Figure 1 | Individual courses of urinary albumin excretion in progressors and non-progressors in albuminuria (cases and controls)



Individual course of UAE during follow-up for cases (black) and controls (grey) in patients with diabetes (1A) or hypertension (1B) stratified for albuminuria-status at baseline (left: normoalbuminuria; right: microalbuminuria). T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

microalbuminuria throughout the same time interval as cases (Figure 1A). As a secondary cohort we selected similar cases and controls out of all non-diabetic patients with hypertension (Figure 1B). Controls were matched to the cases 1:1 based on diabetes/non-diabetes, age, gender, baseline normo- or microalbuminuria status, and if applicable duration of diabetes. For each subject that showed transition in albuminuria, a matched control was selected that most optimally resembled the case on these combined parameters.

Plasma samples of these patients were used from the visit *prior* to the transition from normo- to microalbuminuria, or micro- to macroalbuminuria (baseline). The use of agents intervening in the Renin-Angiotensin-Aldosterone system (RAAS) (ACEi/ARB) was allowed, but the type of drug and their dose had to remain stable during the study period.

Of the 8592 study participants, 318 had T2DM and 33 of these subjects had unambiguous transition in albuminuria and available samples of sufficient quality. Hypertension (without diabetes) was present in 1178 participants. Of these participants 75 subjects had unambiguous transition in albuminuria and available samples of sufficient quality.

Measurements

Plasma samples were stored at -80°C and all samples underwent 1 freeze-thaw cycle. Measurements were performed blinded and in duplicate. GDF-15 was measured with a novel pre-commercial assay based on the Eclia principle (Roche Diagnostics) with a LLD of 200 pg/mL and intra-individual CV of 6.7 to 9.2%.

Urinary albumin excretion (UAE) is given as the mean of the two 24-hour urinary excretions. Blood pressure was measured twice, in the supine position, every min for 10 min with an automatic device (Dinamap XLModel 9300; Johnson-Johnson Medical, Tampa, FL, USA). eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) study equation, using gender, age, race and serum creatinine.[14]

Urinary albumin concentration was determined by nephelometry (Siemens, Munich, Germany). Concentrations of total cholesterol and plasma glucose were measured using standard methods. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, New York, USA), with intra-assay coefficient of variation of 0.9% and inter-assay coefficient of variation of 2.9%.

Statistical Analysis

Analyses were performed using STATA version 11.2 (StataCorp LP, Lakeway Drive, Texas, USA). A study with 50 patients will provide at least 80% power to detect an odds ratio of 1.5 assuming a type 1 error of 5% and no residual confounding after matching cases and

controls (www.bioconductor.org). Variables with normal distribution are given as mean ± standard deviation and variables with skewed distribution as median [inter quartile range (IQR)]. Variables with skewed distribution were log-transformed for analyses. Graphical methods were used to ascertain normalization of the distribution after transformation. Differences between the cases and controls were tested with paired-samples t-test for continuous variables and chi-squared test on paired proportions for categorical variables. Differences between non-paired groups were tested with independent samples t-test for continuous variables and chi-square test for categorical variables.

To investigate the association between the levels of the markers and transition in albuminuria we used conditional logistic regression because of the paired study design. In multivariable analyses, we adjusted for differences in baseline urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR) between cases and controls as these two markers are important risk factors for transition in albuminuria stage. We tested for interaction between patients who made a transition from normo- to microalbuminuria and those who made a transition from micro- to macroalbuminuria by adding an interaction term for baseline albuminuria-status and GDF-15 in the model.

To assess whether the markers improved risk prediction and discrimination we determined the c-statistic and Integrated Discrimination Improvement (IDI). We calculated the c-statistic (discriminatory ability) based on the most important established risk factors (UAE, eGFR) and compared those to c-statistics after addition of GDF-15 to the established model. Differences in c-statistic were tested with chi-square test.

In addition to the c-statistic, we calculated the integrated discrimination improvement (IDI), another measure of discrimination.[15] The IDI is the difference in discrimination slopes between cases and controls before and after the addition of the biomarker(s) to the model. It assesses the improvement in average sensitivity without sacrificing average specificity.[16] Calculation of the IDI is done by computing average predicted probabilities of the event in cases and controls in models with and without the biomarker(s) and subtracting the values from cases and controls from each other. The increase in difference between cases and controls after addition of the biomarker(s) is the integrated reclassification improvement. The IDI is often more sensitive than the rather conservative c-statistic because when several highly predictive markers are in the model, enormous odds ratios are required to meaningfully increase the c-statistic.[17] For all analyses two-sided P-values <0.05 were considered statistically significant.

Table 1 | Baseline Characteristics for patients with T2DM and non-diabetic patients with HT

	Type 2 Diabetes		Hypertension	
Patient Characteristics	Cases	Controls	Cases	Controls
Number of patients	33	33	75	75
Demographic Characteristics				
Age - years	62.7 ± 9.2	62.8 ± 9.6	66.3 ± 9.8	65.6 ± 8.7
Male gender - n (%)	25 (75.8)	25 (75.8)	53 (70.7)	53 (70.7)
Race - n (%)				
Caucasian	30 (90.9)	31 (93.9)	71 (94.7)	73 (97.3)
Other	3 (9.1)	2 (6.1)	4 (5.3)	2 (2.7)
Clinical Characteristics				
Smoking - n (%)	6 (18.1)	5 (15.1)	18 (24.0)	13 (17.3)
BMI - kg/m2	30.0 ± 6.3	27.8 ± 4.2	28.5 ± 4.5	27.9 ± 4.5
Blood pressure - mmHg				
Systolic Blood Pressure	137 ± 15	134 ± 20	139 ± 19	137 ± 18
Diastolic Blood Pressure	75 ± 9	75 ± 8	77 ± 8	78 ± 9
History of Coronary Heart Disease[$]- n (%)	7 (21.3)	2 (6.1)†	11 (14.9)	9 (12.0)
Follow-up time	2.7 [2.2-3.8]	2.8 [2.3-4.0]	2.8[2.3-4.0]	2.8 [2.1-3.7]
Laboratory Parameters				
Urinary albumin excretion - mg/24h	22 [17-34]	12 [6-40]*	22 [13-83]	11 [7 -37]‡
eGFR - mL/min/1.73m2	76 ± 18	80 ± 17	72 ± 21	69 ± 20
Total Cholesterol - mmol/L	5.0 ± 1.4	5.2 ± 1.4	5.3 ± 1.0	5.4 ± 1.1
Fasting Plasma Glucose - mmol/L	7.6 ± 1.9	7.2 ± 1.2	5.3 ± 0.8	5.1 ± 0.9
Treatment - n (%)				
Antihypertensive drugs	23 (69.7)	11 (33.3)	75 (100)	75 (100)
ACEi/ ARB	16 (48.5)	2 (6.1)*	39 (52.0)	39 (52.0)
Oral antidiabetics	24 (72.7)	24 (72.7)	0 (0)	0 (0)
Lipid lowering drugs	18 (54.6)	7 (21.2)†	27 (36.0)	20 (26.7)
Baseline UAE status - n (%)				
Normoalbuminuria	24 (72.7)	24 (72.7)	50 (66.7)	50 (66.7)
Microalbuminuria	9 (27.3)	9 (27.3)	25 (33.3)	25 (33.3)
Median change in UAE - mg/24h	31 [22-151]	0 [-4 - 3]‡	70 [29-310]	0 [-2 – 8]‡
Median change in UAE - %	164 [106-340]	0 [-23 – 38]	314 [143-668]	5 [-18 – 46]

*Cases vs. controls P < 0.05, † Cases vs. controls P<0.01, ‡ Cases vs. controls P<0.001. Plus-minus values are means ± SD and non-normally distributed variables are median [inter quartile range (IQR)]. $ Self-reported Coronary Heart Disease. T2DM: Type 2 Diabetes Mellitus; HT: Hypertension; BMI: body mass index (weight kg/length m2); eGFR: estimated glomerular filtration rate (modification of diet in renal disease-formula); ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin-2 receptor blockers; UAE: urinary albumin excretion rate. To convert values for serum cholesterol from mmol/L into mg/dL multiply by 38.67, to convert values for fasting plasma glucose from mmol/L into mg/dL multiply by 18 and to convert values for serum creatinine from μmol/L to mg/dL divide by 88.4.

Results

In total, 33 cases with type 2 diabetes mellitus (T2DM) and transition from normo- to microalbuminuria or from micro- to macroalbuminuria were identified. These cases were pair-matched to 33 controls with T2DM and stable albuminuria.
The baseline characteristics are presented in table 1. Mean age of cases was 62.7 years, 25 (75.8%) patients were male and the median urinary albumin excretion rate was 18 [9-44] g/24h. Median follow-up was 2.7 [2.2-3.8] years. Cases had a significantly higher baseline UAE than controls, were more frequently treated with lipid lowering treatment and more often received ACEi/ARB treatment when compared to matched controls. All other parameters were similar. Median follow-up time was 2.7 [2.2-4.0] years.
Change in albuminuria concentrations during follow-up, according to the selection of cases and controls, is shown in figure 1A. Patients with transition in albuminuria (cases) had a median increase in albuminuria of 31 mg/24h (164%) compared to 0 mg/24h (0%) in controls.

GDF-15 in Type 2 Diabetic Patients

The mean concentration of GDF-15 in cases and for controls, prior to transition in albuminuria, is presented in figure 2A. In figure 2B these concentrations are presented separately for transition from normo- to micro and micro- to macro. GDF-15 concentrations were significantly higher in cases vs. controls (1288 [885-1546] vs. 948 [660-1016] pg/mL, P<0.001). The concentrations were lower in normoalbuminuric cases and controls than in microalbuminuric cases and controls (910 [737-1162] vs. 1008 [763-1470], *P*=0.03).
The odd ratio for transition in albuminuria was 3.58 [95%CI 1.51-8.47] (per standard deviation increment in GDF-15) (table 2) and 2.87 [95%CI 1.10-7.53] after adjustment for baseline albuminuria and eGFR. There was no statistical significant difference between the odds for transition from normo- to microalbuminuria and from micro- to macroalbuminuria with each SD increment in GDF-15 (P for interaction=0.55). GDF-15 significantly improved the c-statistic on top of a baseline model consisting of UAE and eGFR (increase from 0.87 to 0.92, *P*=0.03). GDF-15 also improved the Integrated Discrimination Index (IDI) (0.148, *P*=0.001), indicating that GDF-15 improved discrimination between cases and controls beyond baseline UAE and eGFR (table 3).

GDF-15 in non-diabetic Hypertensive Patients

The replication study was a second nested case-control study, which consisted of 75 (non-diabetic) cases with hypertension (HT) and transition to increasing stages of albuminuria that were pair-matched to 75 controls with HT and stable albuminuria (table

1). Characteristics of patients with HT were remarkably similar to patients with T2DM, as was the median follow-up time (2.8 [2.3-3.9] years in HT vs. 2.7 [2.2-4.0] years in T2DM). Cases with HT had a median increase in albuminuria of 70 mg/24h (314%) compared to 0.2 mg/24h (5%) in controls.

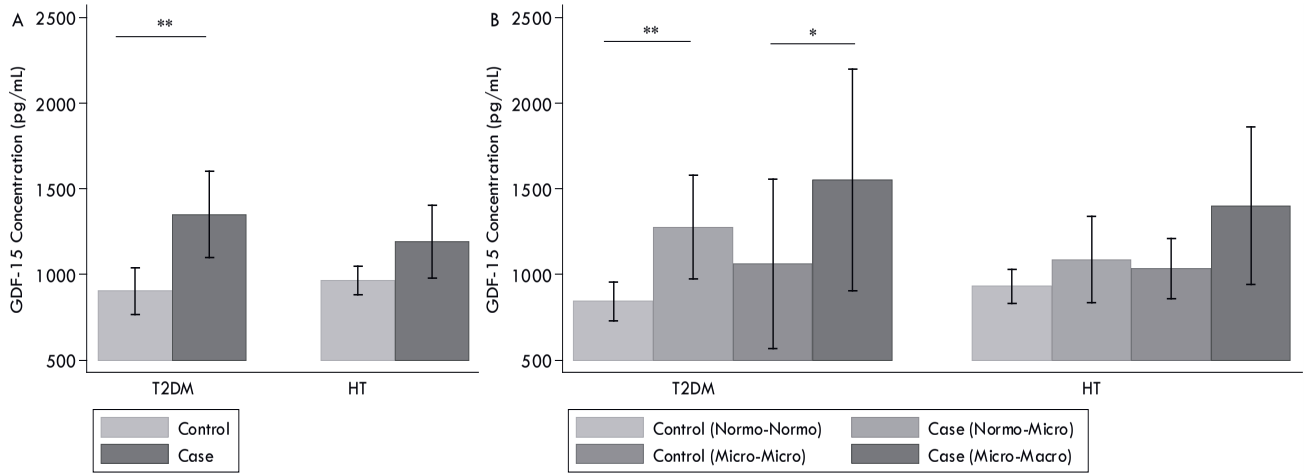
GDF-15 concentration in patients with HT was similar to the concentration in patients with T2DM (910 [738-1210] vs. 992 [799-1347] resp.; *P*=0.63). In cases with HT GDF-15 concentration was borderline significantly higher than in controls (975 [739-1222] vs. 872 [726-1172] pg/mL, *P*=0.09). The odds of transition to micro- or macroalbuminuria was significantly increased per SD increment in GDF-15 (1.96 [95%CI 1.12-3.45]). Also in patients with HT no difference was observed in the odds for transition from normo- to microalbuminuria or micro- to macroalbuminuria with each increment in GDF-15 (p for interaction=0.90). Addition of GDF-15 to the prediction model of transition in albuminuria, consisting of baseline UAE and eGFR, did not result in significant improvement of the c-statistic (from 0.88 to 0.89, *P*=0.8), but significantly improved IDI (0.059, *P*=0.04).

Table 2| Odds ratios for GDF-15 and transition in albuminuria per log-unit increase and per SD increase in the level of the biomarker for patients with T2DM and non-diabetic patients with HT

	Per doubling		Per SD increase		
	Odds ratio	95% Conf. Int.	Odds ratio	95% Conf. Int.	*P*-value
Type 2 Diabetes Mellitus					
GDF15 - Crude	7.66	[1.94-30.14]	3.58	[1.51-8.47]	0.004
GDF15 - Adjusted Model*	5.39	[1.16-25.05]	2.87	[1.10-7.53]	0.03
Hypertension					
GDF15 - Crude	1.58	[0.92-2.73]	1.33	[0.95-1.88]	0.10
GDF15 - Adjusted Model*	2.94	[1.20-7.20]	1.96	[1.12-3.45]	0.02

Values were calculated with conditional logistic regression analyses. *Established model: prediction model based on urinary albumin excretion and estimated glomerular filtration rate. The biomarkers were modelled as continuous (log-transformed) variables. P values are for comparison of the models with the baseline model. GDF-15: Growth-differentation factor-15; T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

Figure 2| GDF-15 levels in progressors and non-progressors in albuminuria (cases and controls) for patients with T2DM and non-diabetic patients with HT



GDF-15 levels (pg/mL) (mean ± SE) in progressors and non-progressors in albuminuria for T2DM and HT; 2B: GDF-15 levels (pg/mL) (mean ± SE) in progressors and non-progressors in albuminuria for T2DM and hypertension stratified for baseline albuminuria (normoalbuminuria or microalbuminuria); * P<0.05, ** P<0.001. Abbreviations: GDF-15: Growth-differentiation factor-15; T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

Discussion

To the best of our knowledge, this is the first study to indicate that GDF-15 precedes and predicts the development of micro- or macroalbuminuria in patients with type 2 diabetes mellitus. GDF-15 was not only independently associated with transition in albuminuria during a follow-up of ~3 years, it also improved discrimination between patients who did and did not develop micro or macroalbuminuria, beyond conventional risk markers. These findings were extended and replicated in a cohort of non-diabetic hypertensive patients.

Previous studies mainly implicated GDF-15 as predictor for cardiovascular events and all-cause mortality in patients with previous myocardial infarction.[10-12] The only previous study focusing on GDF-15 and renal outcome was performed by Lajer et. al. in patients with type I diabetes mellitus and macroalbuminuria. In this observational study, higher GDF-15 levels were associated with a faster decline of eGFR and higher risk of development of end-stage renal disease.[13] Our study extends these latter findings to earlier stages of renal disease during which appropriate intervention is most beneficial.

Despite a growing body of literature demonstrating that GDF-15 is a valuable biomarker for cardiovascular disease, relatively little is known on the pathophysiological role of GDF-15. Its expression is markedly increased in response to injury in various tissues, including heart and kidney, and is believed to be a protective factor by reducing apoptosis and influencing cellular proliferation.[18-20] Higher levels of GDF-15 may thus indicate tissue damage. As endothelial cells appear to be a prominent source of GDF-15, the circulating levels of GDF-15 most likely represent generalized endothelial and microvascular damage.[21] The relation of GDF-15 with micro-vasculature may explain the link with micro- or macroalbuminuria, as increased albuminuria supposedly reflects established microvascular damage in the renal and peripheral vasculature.[22] Since our cases and controls were at the same stage of albuminuria (either normo- or microalbuminuria) at time of GDF-15 measurement, GDF-15 seems to already be increased before microalbuminuria becomes detectable. Our finding that GDF-15 also contributed in identifying subjects at risk for transition in albuminuria stage in a non-diabetic hypertensive cohort implies that GDF-15 is not specifically related to diabetes. Future research will be necessary to delineate the exact pathophysiological role of GDF-15 in diabetes or hypertension and to assess its relation with albuminuria.

Traditional risk factors associated with transition in albuminuria are age, gender, dyslipidemia, insulin sensitivity, hyperglycemia, duration of diabetes, increased blood pressure, duration of hypertension, body mass index, and smoking.[7,23] Despite identification of these important risk factors for transition in albuminuria stage, the ability to stratify subjects at risk is still limited. The current data, if confirmed, raise the possibility of identification of subjects at risk even when traditional risk factors do not indicate risk,

facilitating early identification of those with an increased chance of developing micro- or macro- albuminuria. Because early intervention is more effective than late intervention,[4] preventive treatment could thus reduce the risk of renal and cardiovascular events in patients with T2DM.[5,6] Our findings also applied to non-diabetic hypertensive patients, in whom albuminuria is also strongly associated with renal and cardiovascular events.[24,25]
We incorporated prediction analyses in the present study to determine GDF-15 could add to risk prediction in individual subjects beyond traditional risk markers. Whereas the c-static is most commonly used, the c-statistic is not very sensitive in detecting small but meaningful contributions of biomarkers in correctly classifying individuals. Hardly any improvement of the c-statistic can be reached when the model already includes one or several important risk markers.[17] Yet, we found improvement in C-statistic in our study in T2DM. Because of the insensitivity of the C-statistic, other measures of risk classification such as the IDI have recently been proposed.[15,16] In our analyses, we also found significant improvement in IDI for GDF-15, both in patients with T2DM and non-diabetic subjects with hypertension.
The availability of patient samples from the large general population cohort PREVEND was a unique feature of this study. Because this study followed the natural course of albuminuria by performing repeated measurements of albuminuria for more than 10 years, we were able to measure GDF-15 in samples prior to the transition in albuminuria, whereas many studies have a cross-sectional design and test biomarkers in patients with established increased albuminuria. Other strengths include the well-defined phenotype of the population, with albuminuria status based on two consecutive 24-hour urine collections, the rigorous definitions for transition in albuminuria stage, and the availability of samples that had never been thawed. Because of these strict criteria and the limited number of patients with diabetes in this general population cohort we were only able to obtain 33 valid cases.
A limitation of this study was that there was a modest difference in baseline albuminuria. This small baseline difference in albuminuria was inherent to the design of the study, as the level of albuminuria in itself is related to transition to increasing stages of albuminuria and we matched by albuminuria-stage rather than by albuminuria level. Adjustment for baseline albuminuria showed that the association of GDF-15 with albuminuria transition was independent of baseline albuminuria. A second limitation is the fact that the nested case-control design of the study may overestimate the true predictive capacity of the models because of the relative high event rate in case-control studies.[26] Absolute values of the c-statistic should therefore be interpreted with caution. The improvement of the c-statistic and the level of significance, however are unaffected by case-control design of studies. Lastly, whereas these results are promising and could be confirmed in non-diabetic

hypertensive patients, replication of the current results in other studies, preferably large prospective cohort studies, is warranted before final conclusions can be reached.

Conclusions

We identified GDF-15 as a marker for prediction of transition in albuminuria in type 2 diabetic subjects. GDF-15 moreover had significant additive value on top conventional risk markers in the prediction of albuminuria-transition. These findings were confirmed in non-diabetic hypertensive subjects. If these findings prove to be replicable in other studies, GDF-15 might be a valuable marker for individual risk stratification, facilitating start or intensification of treatment in high risk patients in order to prevent or delay the progression of nephropathy.

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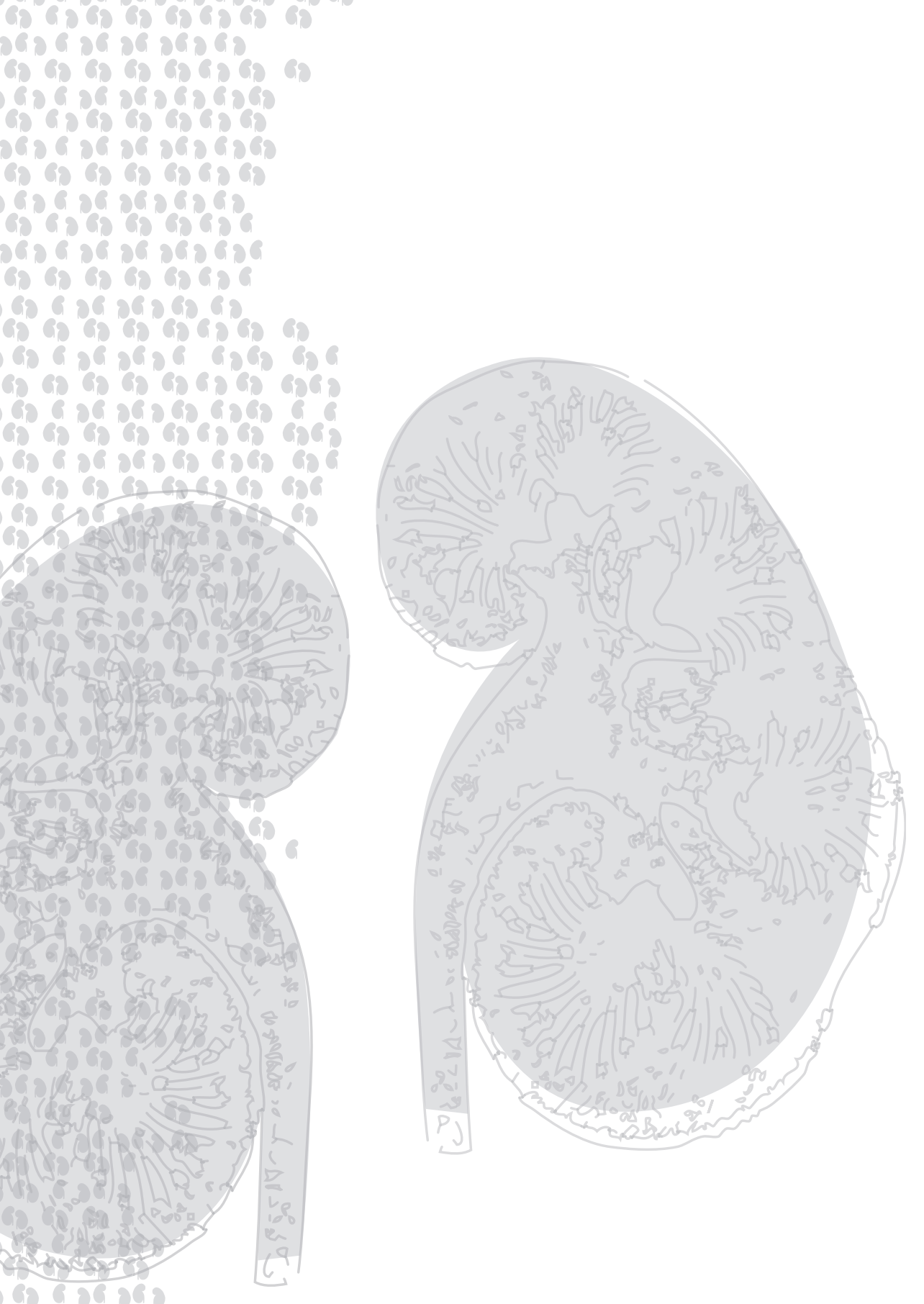

4

High-sensitivity Troponin-T Predicts Worsening of Albuminuria in Hypertension; Results of a Nested Case-Control Study with Confirmation in Diabetes

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Submitted

Abstract

Background: Hypertension is an important cause of end-stage renal disease. Development of microalbuminuria is the first clinical sign of renal dysfunction, progressing to macroalbuminuria and eventually resulting in ESRD. Markers that could predict the development of micro- or macroalbuminuria beyond traditional risk markers would allow for earlier intervention and better prevention of ESRD. We investigated in a case-control study whether circulating levels of the micronecrosis marker high-sensitivity TroponinT (hs-TnT) add to predicting the development of micro- or macroalbuminuria in hypertensive patients (HT) and performed a replication study in type 2 diabetic individuals (T2DM).

Methods: Cases and controls were extracted from a large (N=8.592) general population cohort with long-term follow-up and repeated measurement of albuminuria (PREVEND-study). Cases were defined by transition in albuminuria stage, i.e. from normo- to micro- or from micro- to macro-albuminuria (average follow-up 2.8 yrs). Controls with stable albuminuria were pair-matched for age, gender, and albuminuria-status. Hs-TnT was measured at baseline in 75 case/control-pairs with HT and 33 case/control-pairs with T2DM.

Results: Prior to transition in albuminuria, hs-TnT was higher in cases than in controls (6.6 [3.1-11.6] vs. 5.3 [2.9-8.6] pg/mL, *P*=0.05). The odds for transition in albuminuria increased significantly per SD increase in hs-TnT (2.2[1.2-4.3], *P*=0.02). In addition, hs-TnT improved prediction of albuminuria transition, with significant increases in integrated-discrimination-improvement (IDI) of 0.048, *P*=0.02. Similar results were found in the T2DM case-control cohort.

Conclusions: We identified hs-TnT as an independent marker predicting the transition in albuminuria-stage in HT beyond conventional risk-markers. These findings were confirmed in subjects with T2DM.

Introduction

An increasing number of individuals is recognized to have chronic kidney disease (CKD), with hypertensive nephropathy second only to diabetes as a leading cause of progressive CKD.[1] Microalbuminuria, starting from levels of 30 mg/day and on, is an established and important risk factor of hypertensive renal disease with progressive CKD.[2-4] Especially levels of albuminuria >300 mg/day are associated with fast-progressive CKD and ultimately end-stage renal disease that requires dialysis or renal transplantation.[2-4]

It is obvious that effective therapeutic strategies for progressive CKD should include prevention of micro- or macroalbuminuria.[1] Early markers that detect those that have an increased chance for developing micro- or macroalbuminuria could thus help us reduce the number of patients at renal risk through selective preventive treatments of such patients.[5,6]

Many risk factors have been linked to development of micro- or macroalbuminuria, such as hyperglycemia, hypercholesterolemia and hypertension.[7] However, these risk factors insufficiently discriminate those that will develop increased albuminuria from those that will not. Novel early biomarkers may help to improve the identification of subjects at risk, as well as lead to a better understanding of the underlying pathophysiology of transition in albuminuria.

A lack of well-designed prospective studies that first stored samples of individuals for novel risk marker analyses and then followed the course of albuminuria over time may explain the paucity of knowledge on the value of novel biomarkers to improve risk stratification. The 'Prevention of REnal and Vascular End-stage Disease' (PREVEND) study is a large prospective study on the natural course of albuminuria in the general population, with to date five repeated measurements of albuminuria with 3-year intervals in 8.592 subjects.[8] This cohort offers a unique opportunity to prospectively study the predictive power of biomarkers for morbidity and/or mortality events.

The micronecrosis marker high-sensitivity Troponin T (hs-TnT) is increasingly recognized as a strong predictive marker for vascular events.[9-13] Interestingly, hs-TNT appeared to be an independent risk factor prediction the progression of diabetic nephropathy in patients with type 1 diabetes.[14] We aimed to investigate whether hs-TNT levels precede and predict the development of micro- or macroalbuminuria in subjects with hypertension and to compare and confirm findings in subjects with type 2 diabetes (T2DM). We therefore performed a nested case-control study in the PREVEND-cohort.

Methods

The present study was performed as a nested case-control study in subjects participating in the Prevention of REnal and Vascular End-stage Disease (PREVEND) study. This prospective community-based cohort study with serial follow-up measurements was initiated in 1997 to investigate the natural course of urinary albumin excretion and its relation to renal and cardiovascular disease in a predominantly Caucasian population. Details of the study protocol have been published elsewhere.[8] In short, all inhabitants of the city of Groningen aged 28–75 years were sent a questionnaire and a vial to collect a first-morning-void urine sample. Of these individuals, 40 856 responded (47.8%). From these individuals a cohort consisting of 8592 subjects was selected (the PREVEND cohort). In this ongoing study participant are invited to visit an outpatient clinic for detailed medical examination at ± 3-year intervals. At each screening round participants fill out questionnaires on demographics, medical history and drug use. Information on drug use is completed with data from community pharmacies, including information on class of antihypertensive medication (ACEi/ARB). At the study visits participants deliver two 24-hour urine collections, blood pressure is measured, anthropometrical measurements are performed, and fasting blood samples are taken.

The PREVEND study was approved by the institutional ethics review board and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

Definitions

Normoalbuminuria was defined as urinary albumin excretion (UAE) <30 mg/24h, microalbuminuria as UAE 30-299 mg/24h and macroalbuminuria as UAE ≥300 mg/24h. Albuminuria status was based on the average of 2 consecutive measurements in 24-hour urine collections. Transition in albuminuria was defined as a transition from normo- to micro- or from micro- to macroalbuminuria with at least 30% increase in urinary albumin excretion from baseline between two consecutive study visits. HT was defined as the use of anti-hypertensive treatment or a systolic/diastolic blood pressure > 140/90 mmHg. T2DM was defined as the use of oral anti-diabetic treatment (self-reported or by information retrieved from the regional pharmacy database), a fasting plasma glucose >7.0 mmol/L (126 mg/dL) or non-fasting plasma glucose >11.1 mmol/L (>200 mg/dL).

Selection of cases and controls

For the present study, we selected patients with HT and progression from normo- to microalbuminuria or from micro- to macroalbuminuria, and patients with type 2 diabetes

mellitus (T2DM) and progression in albuminuria-stage as cases. As controls, we selected HT or T2DM patients who had persistent normoalbuminuria or microalbuminuria throughout the same time interval as the selected cases. Controls were matched to the cases 1:1 based on hypertension/diabetes, age, gender, baseline normo- or microalbuminuria status, and duration of diabetes. For each subject that showed transition in albuminuria-stage, a matched control was selected that most optimally resembled the case on these combined parameters. Plasma samples of these patients were used from the visit *prior* to the transition from normo- to microalbuminuria, or micro- to macroalbuminuria (baseline). The use of agents intervening in the Renin-Angiotensin-Aldosterone system (RAAS) (ACEi/ARB) was allowed, but the type of drug and their dose had to remain stable during the study period.

Of the 8592 study participants, hypertension was present in 1178 participants. Of these participants 75 subjects had unambiguous transition in albuminuria and available samples of sufficient quality. Of the 8592 study participants, 318 had T2DM and 33 of these subjects had unambiguous transition in albuminuria and available samples of sufficient quality.

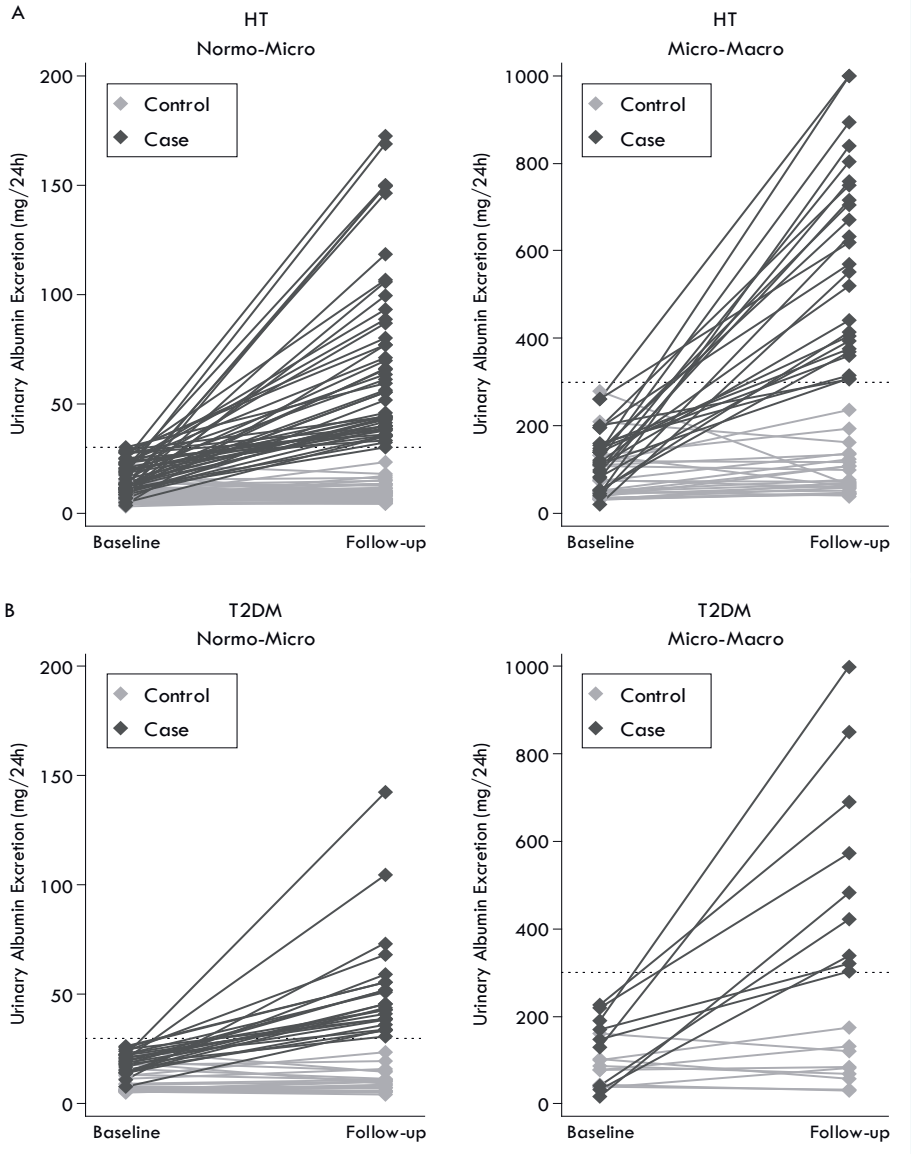
Measurements

Plasma samples were stored at -80°C and all samples underwent 1 freeze-thaw cycle. Measurements were performed blinded and in duplicate. Hs-TnT was measured with a novel highly sensitive assay, Elecsys® high-sensitivity Troponin T (Roche Diagnostics) with a lower limit of detection (LLD) of 3 pg/mL and intra-individual coefficient of variation (CV) of 0.7 to 5.6%. For the purpose of meaningful data-analyses, values below the LLD were conservatively set at a value of 2.9 pg/mL.

Urinary albumin excretion (UAE) is given as the mean of the two 24-hour urinary excretions. Blood pressure was measured twice, in the supine position, every min for 10 min with an automatic device (Dinamap XLModel 9300; Johnson-Johnson Medical, Tampa, FL, USA). eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) study equation, using gender, age, race and serum creatinine.[15]

Urinary albumin concentration was determined by nephelometry (Siemens, Munich, Germany). Concentrations of total cholesterol and plasma glucose were measured using standard methods. Serum creatinine was measured by dry chemistry (Eastman Kodak, Rochester, New York, USA), with intra-assay coefficient of variation of 0.9% and inter-assay coefficient of variation of 2.9%.

Figure 1 | Individual courses of urinary albumin excretion in progressors and non-progressors in albuminuria (cases and controls)



Individual course of UAE during follow-up for cases (black) and controls (grey) in patients with hypertension (1A) or type 2 diabetes (1B) stratified for albuminuria-status at baseline (left: normoalbuminuria; right: microalbuminuria). T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

Statistical Analysis

Analyses were performed using STATA version 11.2 (StataCorp LP, Lakeway Drive, Texas, USA). A study with 50 patients will provide at least 80% power to detect an odds ratio of 1.5 assuming a type 1 error of 5% and no residual confounding after matching cases and controls (www.bioconductor.org). Variables with normal distribution are given as mean ± standard deviation and variables with skewed distribution as median [inter quartile range (IQR)]. Variables with skewed distribution were log-transformed for analyses. Graphical methods were used to ascertain normalization of the distribution after transformation. Differences between the cases and controls were tested with paired-samples t-test for continuous variables and chi-squared test on paired proportions for categorical variables. Differences between non-paired groups were tested with independent samples t-test for continuous variables and chi-square test for categorical variables.

To investigate the association between the levels of the markers and transition in albuminuria we used conditional logistic regression because of the paired study design. In multivariable analyses, we adjusted for the differences in baseline characteristics between the groups: urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR) (the latter only borderline significantly different), both also important risk factors for transition in albuminuria-stage.[16,17] We tested for interaction between patients who made a transition from normo- to microalbuminuria and those who made a transition from micro- to macroalbuminuria by adding an interaction term for baseline albuminuria status and hs-TnT in the model.

To assess whether the marker improved risk prediction and discrimination we determined the c-statistic and Integrated Discrimination Improvement (IDI). We calculated the c-statistic (discriminatory ability) of a model including UAE and eGFR (established model) and compared those to c-statistics after addition of hs-TnT to the established model. Differences in c-statistic were tested with the chi-square test.

In addition to the c-statistic, we calculated the integrated discrimination improvement (IDI) as described by Pencina et al., another measure of discrimination.[18] The IDI is the difference in discrimination slopes between cases and controls before and after the addition of hs-TnT to the model. It assesses the improvement in average sensitivity without sacrificing average specificity. Calculation of the IDI is done by computing average predicted probabilities of the event in cases and controls in models with and without hs-TnT. The prediction probabilities of models with and without hs-TnT are subtracted in cases and controls respectively. The increase in difference between cases and controls after addition of the biomarker is the integrated reclassification improvement. The IDI is often more sensitive than the rather conservative c-statistic because when several highly predictive markers are in the model, enormous odds ratios are required to meaningfully

Table 1 | Baseline Characteristics for patients with T2DM and non-diabetic patients with HT

	Hypertension		Type 2 Diabetes	
Patient Characteristics	Cases	Cases	Cases	Controls
Number of Patients	75	33	33	75
Demographic Characteristics				
Age - years	66.3 ± 9.8	62.7 ± 9.2	62.7 ± 9.2	65.6 ± 8.7
Male Gender - n (%)	53 (70.7)	25 (75.8)	25 (75.8)	53 (70.7)
Race - n (%)				
Caucasian	71 (94.7)	30 (90.9)	30 (90.9)	73 (97.3)
Other	4 (5.3)	3 (9.1)	3 (9.1)	2 (2.7)
Clinical Characteristics				
Smoking - n (%)	18 (24.0)	6 (18.1)	6 (18.1)	13 (17.3)
BMI - kg/m2	28.5 ± 4.5	30.0 ± 6.3	30.0 ± 6.3	27.9 ± 4.5
Blood Pressure - mmHg				
Systolic Blood Pressure	139 ± 19	137 ± 15	137 ± 15	137 ± 18
Diastolic Blood Pressure	77 ± 8	75 ± 9	75 ± 9	78 ± 9
History of Coronary Heart Disease[$]- n (%)	11 (14.9)	7 (21.3)	7 (21.3)	9 (12.0)
Follow-up time - years	2.8[2.3-4.0]	2.7 [2.2-3.8]	2.7 [2.2-3.8]	2.8 [2.1-3.7]
Laboratory Parameters				
Urinary Albumin Excretion - mg/24h	22 [13-83]	22 [17-34]	22 [17-34]	11 [7 -37]‡
eGFR - mL/min/1.73m2	72 ± 21	76 ± 18	76 ± 18	69 ± 20
Total Cholesterol - mmol/L	5.3 ± 1.0	5.0 ± 1.4	5.0 ± 1.4	5.4 ± 1.1
Fasting Plasma Glucose - mmol/L	5.3 ± 0.8	7.6 ± 1.9	7.6 ± 1.9	5.1 ± 0.9
Treatment - n (%)				
Antihypertensive Drugs	75 (100)	23 (69.7)	23 (69.7)	75 (100)
ACEi/ ARB	39 (52.0)	16 (48.5)	16 (48.5)	39 (52.0)
Oral Antidiabetics	0 (0)	24 (72.7)	24 (72.7)	0 (0)
Lipid Lowering Drugs	27 (36.0)	18 (54.6)	18 (54.6)	20 (26.7)
Baseline UAE status - n (%)				
Normoalbuminuria	50 (66.7)	24 (72.7)	24 (72.7)	50 (66.7)
Microalbuminuria	25 (33.3)	9 (27.3)	9 (27.3)	25 (33.3)
Median change in UAE - mg/24h	70 [29-310]	31 [22-151]	31 [22-151]	0 [-2 – 8]‡
Median change in UAE - %	314 [143-668]	164 [106-340]	164 [106-340]	5 [-18 – 46]

*Cases vs. controls P < 0.05, † Cases vs. controls P<0.01, ‡ Cases vs. controls P<0.001. Plus-minus values are means ± SD and non-normally distributed variables are median [inter quartile range (IQR)]. $ Self-reported Coronary Heart Disease. T2DM: Type 2 Diabetes Mellitus; HT: Hypertension; BMI: body mass index (weight kg/length m2); eGFR: estimated glomerular filtration rate (modification of diet in renal disease-formula); ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin-2 receptor blockers; UAE: urinary albumin excretion rate. To convert values for serum cholesterol from mmol/L into mg/dL multiply by 38.67, to convert values for fasting plasma glucose from mmol/L into mg/dL multiply by 18 and to convert values for serum creatinine from µmol/L to mg/dL divide by 88.4.

increase the c-statistic.[19] For all analyses two-sided P-values <0.05 were considered statistically significant.

Results

In total, 75 cases with hypertension (HT) and progression from normo- to microalbuminuria or from micro- to macroalbuminuria were available. These cases were pair-matched to 75 controls with HT and stable albuminuria.

The baseline characteristics of these cases and controls are presented in table 1. Mean age of the patients with HT was 64.9 years, 176 (72%) patients were male, and the median urinary albumin excretion rate was 18 [9-44] g/24h. Cases had a significantly higher baseline UAE than controls. All other parameters were similar. Median follow-up time was 2.8 [2.3-3.9] years. The changes in albuminuria during the follow-up for both cases and controls are shown in figure 1. Patients with transition in albuminuria had a median increase in albuminuria of 70 mg/24h (314%) compared to 0.2 mg/24h (5%) in controls.

Hs-TnT in Hypertensive Patients

The baseline (before albuminuria-stage transition) hs-TnT concentrations are presented in figure 2, stratified by cases and controls and presented separately for transition from normo- to micro and micro- to macro. Hs-TnT concentration was below the lower limit of detection (LLD, 3.0 pg/mL) in in 24 (32%) cases and in 18 (24%) controls (*P*=0.28). Hs-TnT concentrations were higher in cases vs. controls (6.6 [3.1-11.6] vs. 5.3 [2.9-8.6] pg/mL; *P*=0.05), and marker concentrations were lower in normoalbuminuric cases and controls than in microalbuminuric cases and controls (4.4 [2.9-7.8] vs. 5.4 [2.9-11.3], *P*=0.04).

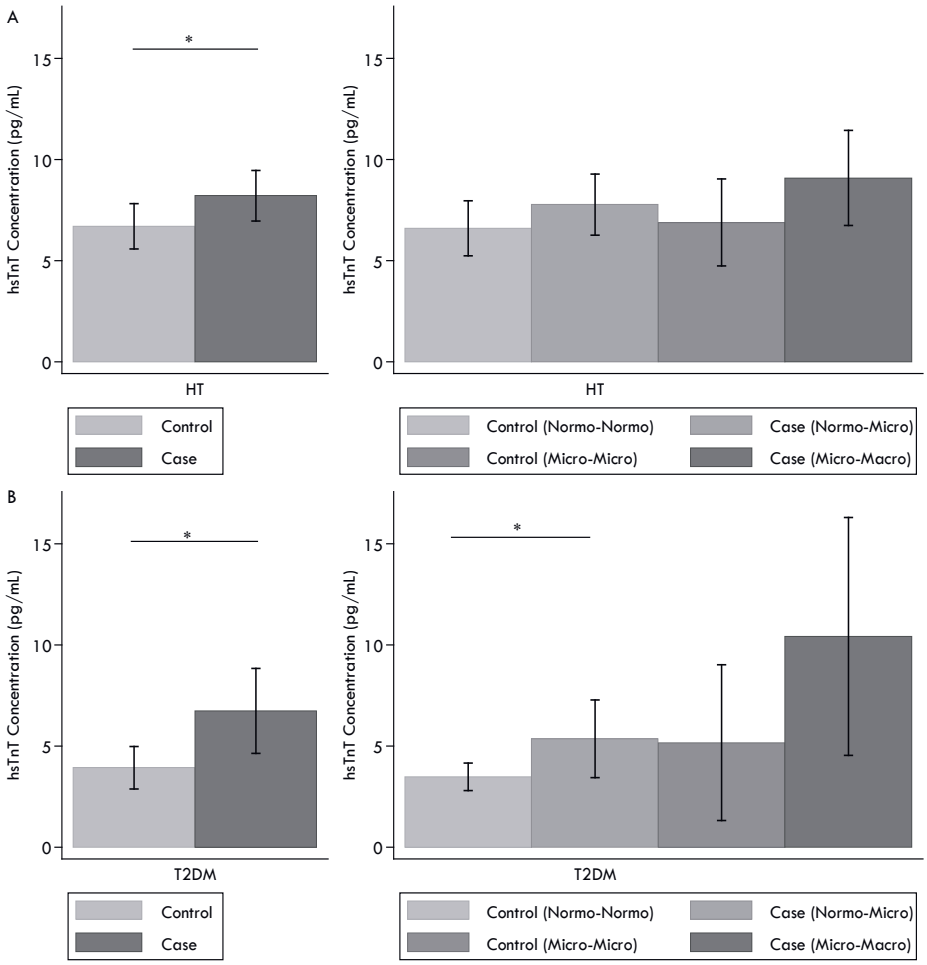
The adjusted odds for transition in albuminuria per standard deviation increment in hs-TnT was significantly increased (2.2 [95%CI 1.2-4.3]. There was no statistically significant difference between the odds for transition from normo- to microalbuminuria and from micro- to macroalbuminuria with each SD increment in hs-TnT (P for interaction=0.58).

We calculated the c-statistic and integrated discrimination improvement (IDI) to test whether the markers had additive value on top of UAE and eGFR in discriminating between patients with and without transition in albuminuria (table 3). Addition of hs-TnT to the baseline model gave non-significant improvement in c-statistic (from 0.88 to 0.91, *P*=0.2), but significant improvement of IDI (0.048, *P*=0.02).

Hs-TnT in Type 2 Diabetic Patients

The replication study was a second nested case-control study, which consisted of 33 cases

Figure 2| Hs-TnT levels in progressors and non-progressors in albuminuria (cases and controls)



Hs-TnT levels (pg/mL) (mean ± SE) in cases and controls and cases and controls stratified for baseline albuminuria for hypertension (upper panels) or type 2 diabetes (lower panels). * P<0.05. T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

with diabetes (T2DM) and progression of albuminuria, which were again pair-matched to 33 controls with T2DM and stable albuminuria. Average follow-up in T2DM patients was 2.7 [2.2-4.0] years, which was very similar to the follow-up in patients with HT. Baseline characteristics are presented in table 1. The patients with T2DM and transition in albuminuria had a median increase in albuminuria of 31 mg/24h (164%) compared to 0 mg/24h (0%) in controls (figure 1).

Mean hs-TnT concentrations were significantly lower in T2DM compared to HT (2.9 [2.9-5.3] vs. 5.5 [2.9-10.4]; P<0.001). Hs-TnT concentration was below the lower limit of detection in 24 (73%) controls and 14 (42%) cases (*P*=0.01). Like in HT, hs-TnT concentrations were higher in cases vs. controls in T2DM (4.0 [2.9-7.2] vs. 2.9 [2.9-3.1] pg/mL; *P*=0.002).
The adjusted odds for transition in albuminuria in T2DM patients per standard deviation increment in hs-TnT was numerically similar to HT, although in T2DM the odds ratio did not reach statistical significance (2.00 [95%CI 0.70-5.68]) (table 2). No difference was observed in the odds for transition from normo- to microalbuminuria or micro- to

Table 2| Odds ratios for hs-TnT and transition in albuminuria-stage per log-unit increase and per SD increase in the level of the biomarker for patients with HT and patient with T2DM

	Per doubling		Per SD increase		
	Odds ratio	95% Conf. Int.	Odds ratio	95% Conf. Int.	*P*-value
Hypertension					
Hs-TnT - Crude	1.51	[0.99-2.32]	1.39	[0.95-2.03]	0.09
Hs-TnT - Adjusted Model*	2.83	[1.23-6.50]	2.24	[1.17-4.31]	0.02
Type 2 Diabetes Mellitus					
Hs-TnT - Crude	5.66	[1.19-26.80]	3.39	[1.28-8.97]	0.01
Hs-TnT - Adjusted Model*	3.51	[0.62-19.90]	2.00	[0.70-5.68]	0.16

Values were calculated with conditional logistic regression analyses. T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

Table 3| C-statistics and integrated discrimination improvements (IDI) for logistic regression models predicting transition in albuminuria for patients with HT and patient with T2DM

	C-statistic	95% Conf. Int.	*P*-value	IDI	95% Conf. Int.	*P*-value
Hypertension						
Established Model*	0.88	[0.82-0.94]	ref	ref		ref
Established Model + hs-TnT	0.91	[0.86-0.95]	0.2	0.048	[0.009-0.086]	0.02
Type 2 Diabetes Mellitus						
Established Model*	0.87	[0.78-0.96]	ref	ref		ref
Established Model + hs-TnT	0.89	[0.81-0.97]	0.13	0.059	[0.001-0.117]	0.04

* Established model: prediction model based on urinary albumin excretion and estimated glomerular filtration rate. The biomarkers were modeled as continuous (log-transformed) variables. P values are for comparison of the models with the baseline model. T2DM: Type 2 Diabetes Mellitus; HT: Hypertension.

macroalbuminuria with each increment in hs-TnT (*P*=0.70 for hs-TnT). Lastly, addition of hs-TnT to the baseline model consisting of UAE and eGFR did not significantly improve the c-statistic (from 0.87 to 0.89, *P*=0.13), but did result in significant increase in IDI (0.059, *P*=0.04).

Discussion

In this study, we found that hs-TNT predicts progression of albuminuria-stage in subjects with hypertension. This predictive property is independent of baseline albuminuria and improves discrimination between cases and controls beyond conventional renal and cardiovascular risk markers. In a smaller sample of cases and controls with T2DM, we confirmed these findings, albeit at a lower offset level of hs-TNT.

Traditional risk factors associated with transition in albuminuria are age, gender, dyslipidemia, hypercholesterolemia, insulin sensitivity, hyperglycemia, duration of diabetes, increased blood pressure, duration of hypertension, body mass index and smoking.[7,20] Despite identification of these important risk factors for transition in albuminuria, the ability to stratify subjects at risk is still limited. The current data, if confirmed by other studies, raise the possibility of identification of subjects at risk even when traditional risk factors do not indicate risk, facilitating early identification of those with an increased chance of developing micro- or macroalbuminuria. Because early intervention is more effective than late intervention,[21] preventive treatment could thus reduce the risk of renal and cardiovascular events in patients with HT and T2DM.[1]

Previous studies mainly implicated hs-TnT as predictor for cardiovascular events and all-cause mortality in various populations,[9-12] and only one study previously reported on hs-TnT and renal outcome in T1DM.[14] TnT has generally been considered a marker for cardiomyocyte necrosis. However, hs-TnT has also been detected in non-cardiac conditions, including renal failure, pulmonary hypertension, pulmonary embolism, sepsis and dialysis, albeit in substantially lower concentrations.[13,22-26] The findings of hs-TnT levels in this low range in conditions other than cardiac ischemia, indicate that even the low levels may confer prognostic information. The exact mechanisms for the release of low levels of TnT remain unclear. Amongst the mechanisms suggested are systemic inflammatory processes and microvascular changes.[27,28] Of note, the baseline levels of hs-TnT were higher in HT than in T2DM, suggesting that increased cardiac work-load or structural remodeling in hypertension might, at least in part, be responsible for the enhanced release of hs-TnT.

Our findings also applied to patients with type 2 diabetes. Transition in albuminuria-stage in type 2 diabetes is considered a hallmark of progression of renal disease and increases

the risk for cardiovascular events.[16,17] Early identification of subjects at risk and appropriate treatment in type 2 diabetic patients is at least of equal importance as in subjects with hypertension. In this respect hs-TnT is a candidate marker that now requires validation in larger cohorts.

Our use of samples of a large prospective cohort study with repeated measurements of albuminuria aiming primarily focusing on albuminuria as pathophysiological phenomenon and predictor of cardiovascular and end-stage renal disease was a unique feature of this study. Other strengths include the well-defined phenotype of the population, with albuminuria status based on two consecutive 24-hour urine collections, the rigorous definitions for transition in albuminuria, and the availability of samples that had never been thawed. Because of these strict criteria and the limited number of patients with diabetes or hypertension in this general population cohort we were only able to obtain 75 cases of HT with progression of albuminuria and 33 cases of T2DM with albuminuria.

A limitation of this study was the modest difference in baseline albuminuria between cases and matched controls. The difference in baseline albuminuria was inherent to the design of the study as the level of albuminuria in itself is related to transition in albuminuria and we did not match subjects on their baseline albuminuria but on albuminuria-stage (i.e. normoalbuminuria or microalbuminuria at baseline). To account for the modest difference in baseline albuminuria we adjusted the regression analyses for the baseline albuminuria level and showed that hsTnT independently contributed in identifying individuals at risk for transition in albuminuria-stage. A second limitation is the fact that the nested case-control design of the study may overestimate the true predictive capacity of the models because of the relative high event rate in case-control studies.[29] Absolute values of the c-statistic should therefore be interpreted with caution. The level of significance of improvement of the c-statistic however, is unaffected by the case-control design of the study. Nevertheless, as with all biomarker studies, replication of the current results in other studies, preferably large prospective cohort studies, is warranted.

Conclusions

We identified hs-TnT as marker for prediction of progression in albuminuria in hypertension. As hs-TnT had significant additive value on top conventional risk markers to predict albuminuria progression, hs-TnT may contribute to the early identification of patients at risk for progression of to hypertensive nephropathy and also for progression to diabetic nephropathy. We performed a confirmation study of our findings in diabetic

patients. If these findings are replicated in other studies it could facilitate studies that test whether start or intensification of treatment in high risk patients will prevent or delay the progression of nephropathy in HT as well as T2DM.

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4 | Chapter

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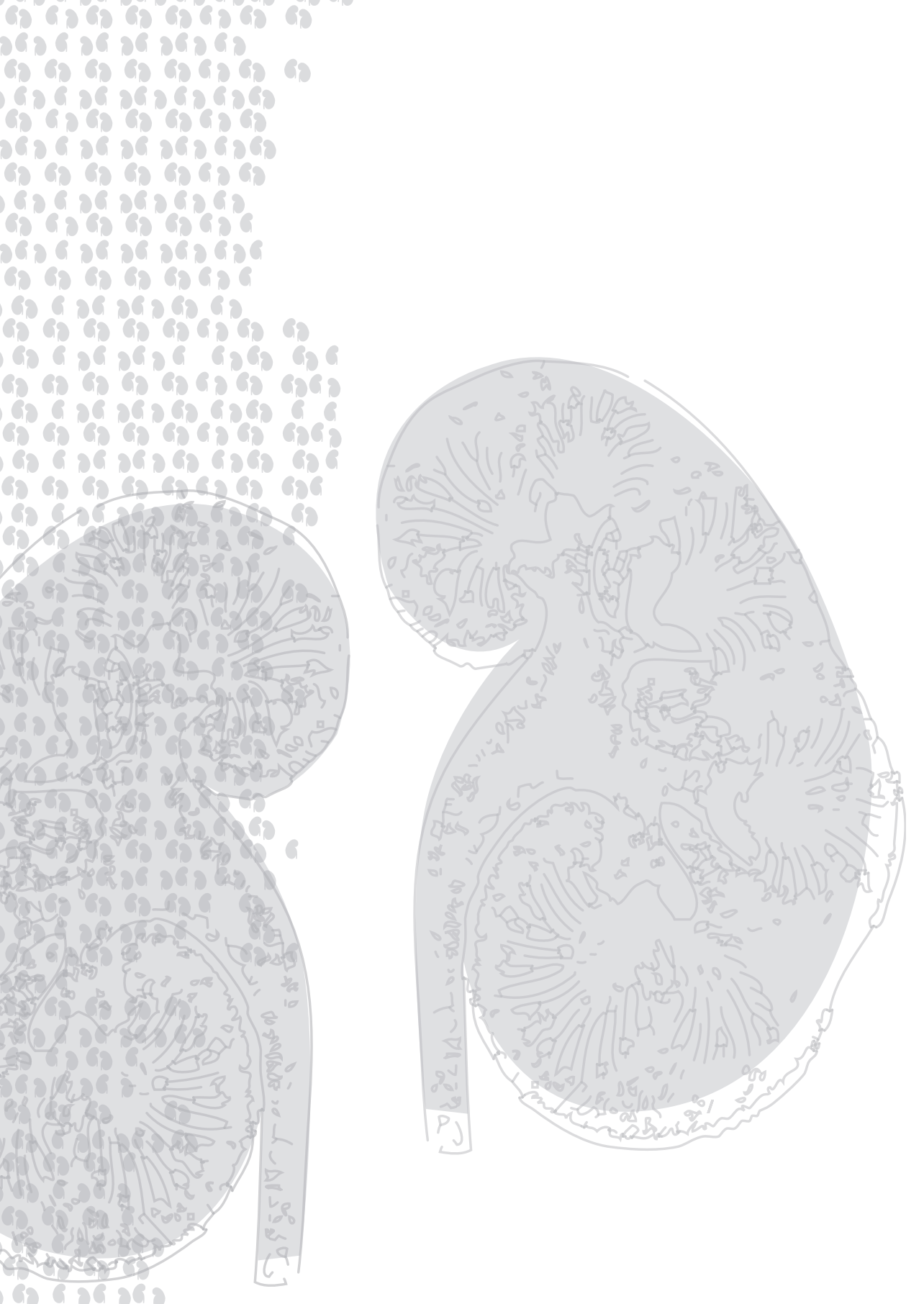

5

Initial Angiotensin Receptor Blockade Induced Decrease in Albuminuria is Associated with Long Term Renal Outcome in Type 2 Diabetic Patients with Microalbuminuria; a Post-hoc Analysis of the IRMA-2 trial

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Abstract

Background: We aimed to investigate the individual impact of initial responses in urinary albumin excretion (UAE) and systolic blood pressure (SBP) to Angiotensin-II-Receptor-Blocker (ARB) treatment on long term renal outcome in patients with type 2 diabetes and microalbuminuria.

Methods: In a post-hoc analysis of the IRMA-2 trial we first assessed the individual variability in UAE and SBP-response (0-6 months) in 531 subjects. Subsequently, we analyzed the individual effect of both response parameters on renal outcome defined as change in estimated glomerular filtration rate (eGFR) during 2 years of follow-up.

Results: The median reductions in UAE and SBP in the population were -18% and -11mmHg respectively. In Irbesartan treated patients, 85 (24.4%) had a robust (>median) reduction in UAE but not in SBP (discordant SBP-response) and 67 (19.3%) had a robust (>median) reduction in SBP but not in UAE (discordant UAE-response). The degree of reduction in UAE was independently associated with the rate of eGFR decline (p=0.0037). SBP showed a similar trend (P=0.087). The relation between a larger UAE-reduction and a slower rate of renal function decline was present in both the cohort with a SBP-change above and below the median.

Conclusions: Within an individual, UAE response to ARB-therapy may be discordant from SBP-response. The initial change in UAE was independently associated with eGFR slope: the more UAE reduction the less eGFR decline, irrespective of the SBP-change. These results suggest that in microalbuminuric patients with type 2 diabetes, UAE should be monitored after initiation of therapy and a separate target for renoprotective therapy.

Introduction

Current treatment strategies in diabetes separately target risk factors for micro- and macro-vascular complications. HbA1c is targeted with anti-diabetic agents, cholesterol levels with statins and blood pressure (BP) with antihypertensive agents. Agents blocking the Renin-Angiotensin-Aldosteron-System (RAAS) are first choice antihypertensives in patients with diabetes as these agents not only lower blood pressure but also lower urinary albumin excretion (UAE), another important renal risk factor.[1,2] Current guidelines recommend dose-titration of RAAS-blockade on BP response to achieve a systolic blood pressure (SBP) below 130 mmHg, without taking the response in UAE into account.[3]
It is known that the initial response in proteinuria during RAAS blockade independently determines renal outcome in patients with diabetes and proteinuria.[4] Moreover, recent studies have illustrated that within an individual, the response in BP is not always paralleled by a response in proteinuria or vice-versa.[5] These so-called discordant responses allow a, albeit retrospective, look at whether the response of BP, proteinuria, or their combination, is the driving parameter for renoprotection. Data in patients with proteinuria have demonstrated that long-term renoprotection is mainly achieved in those patients with an initial fall in proteinuria irrespective of the BP response. Accordingly, this suggests that a treatment approach solely focusing on BP reduction may not be the most efficacious way to achieve renoprotection.[6-8] Whether responses in albuminuria irrespective of BP relate to long-term renoprotection in patients with microalbuminuria have not been published. We therefore performed a post-hoc analysis in the Irbesartan in Patients with Type 2 diabetes and Microalbuminuria (IRMA-2) trial,[9] investigating the variability in initial treatment responses in UAE and SBP in patients with type 2 diabetes and microalbuminuria. Secondly, we aimed to determine the impact of different UAE and SBP responses on renal outcome. This should provide insight as to whether albuminuria should be considered a target for renoprotective therapies in addition to BP in microalbuminuric patients.

Patients and methods

The IRMA 2 study was a 2-year multi-center, randomized, double-blind trial in patients with type 2 diabetes and microalbuminuria comparing Irbesartan (150 or 300 mg once daily) versus placebo on top of conventional antihypertensive treatment. The design of the study has been reported elsewhere.[9] In brief, eligible patients had their antihypertensive agents discontinued during the run-in period and replaced by placebo. After three weeks patients were randomly assigned to receive Irbesartan 150, 300 mg, or matching placebo

once daily. A total of 590 patients were followed for 2 years for the development of overt nephropathy. Patients were seen at month 3, 6, and every 6 months thereafter. Additional blood pressure lowering medication, apart from Angiotensin-Converting Enzyme inhibitors (ACEi) and Angiotensin-II-Receptor Blockers (ARBs), was allowed to reach the target blood pressure of 135/85 mmHg. The study protocol was in accordance with the declaration of Helsinki and was approved by all local institutional review boards. All patients gave written informed consent.

Patients

IRMA-2 enrolled hypertensive patients with type 2 diabetes, ranging in age from 30 to 70 years. All patients had persistent microalbuminuria which was defined as a urinary albumin excretion rate of 20 to 200µg/min in at least two out of three consecutive, sterile, overnight urine samples. The main exclusion criteria were a serum creatinine concentration >1.5mg/dL (133µmol per liter) for men and >1.1mg/dL (97µmol per liter) for women, non-diabetic kidney disease, cancer, life-threatening disease with death expected to occur within two years, and an indication for Angiotensin-converting–enzyme inhibitors (ACEi) or Angiotensin-II–receptor blockers (ARBs).

Measurements

The urinary albumin concentration was determined by nephelometry at a central laboratory.[10] The serum creatinine concentration was determined by Jaffe reaction with the use of a Hoffmann–LaRoche kit.[11] Glomerular filtration rate (eGFR) was estimated with the Modification of Diet in Renal Disease (MDRD) equation.[12] The lowest arterial blood pressure during a 24-hour period (Korotkoff phase I/V) was measured twice in the sitting position after at least 10 minutes of rest.

Urinary albumin excretion and blood pressure response

This post-hoc analysis focuses on the urinary albumin excretion (UAE) and systolic blood pressure (SBP) change from baseline to month 6. A robust decline in UAE or SBP was defined as a decline in UAE or SBP more than the population median. This approach was aimed at identifying subgroups with identical number of patients to increase the power of the analysis while minimizing the risk of bias. UAE change at month 6 for each patient was calculated as 100*log(UAE at 6 months/UAE at baseline). SBP change was calculated as the difference between the month 6 and baseline value. The month 6 values were chosen for two reasons: 1) the treatment effects were considered to be fully present at month 6, and 2) this was the earliest time-point at which most variables of interest were available.

Patients were divided into groups according to the median of UAE and SBP change from baseline to month 6. Patients with both UAE change and SBP change above or below the median were considered to have a concordant response, whereas patients with either a UAE change or SBP change above the median were considered to have a discordant response.

Renal endpoints

Transition from micro to macroalbuminuria (development of overt nephropathy) was the primary efficacy measure in the IRMA-2 trial which was defined as UAE >200µg/min and at least 30% higher than baseline level of UAE on at least two out of three consecutive samples. Since the initial reduction in albuminuria induced by ARB treatment is directly related to the primary efficacy measure (which includes the long term change in albuminuria), we decided to use the course of decline in eGFR from month 6 to end of follow-up as our primary renal endpoint. We looked at development of overt nephropathy in a secondary analysis.

Statistical Analysis

Categorical variables are reported as frequencies and percentages. Variables with normal distribution are presented as mean with standard deviation (SD) and variables with a skewed distribution are presented as median with interquartile range [IQR]. Non-normally distributed variables were log-transformed before analyses. Graphical methods and normality tests were used to ascertain normalization of the distribution after transformation. Differences between groups were tested with Fishers Exact Test for categorical variables and ANOVA for continuous variables, followed, where applicable, by post-hoc Bonferroni correction for multiple testing. A multivariate mixed model with random intercepts and random slopes was used to assess the relationship between the magnitude of UAE and SBP change and the rate of eGFR decline. Such a model calculates renal function decline over time within and between individuals also taking into account the correlation within individuals and time. For exploration of the relationship between the month 6 change in UAE and eGFR decline, the change in UAE was categorized according to quartiles and related to eGFR decline from month 6. The multivariate mixed model included the following baseline covariates: age, gender, log transformed UAE, SBP and DBP, eGFR, HbA1c, duration of diabetes, total cholesterol, smoking, BMI, and treatment allocation. A multivariate Cox-proportional Hazards model was used to assess the relationship between the magnitude of UAE and SBP change from baseline to 6 months and time to development of overt nephropathy from 6 months to the end of follow up. The multivariate Cox-proportional Hazards model included the same covariates

Table 1 | Baseline characteristics of the patients stratified by groups of change in albuminuria and systolic blood pressure from baseline to month 6

Characteristics	Concordant (Negative) UAE < Median* SBP < Median**	Discordant UAE < Median* SBP > Median**	Discordant UAE > Median* SBP < Median**	Concordant (Positive) UAE > Median* SBP > Median**
Number of Patients	153	112	120	146
Changes 0-6 Months				
Median [IQR] change UAE - %	35 [7 to 92]	35 [0 to 85]	-48 [-63 to -33]	-51 [-68 to -37]
Median [IQR] change SBP - mmHg	0 [-7 to 5]	-21 [-28 to -15]	-5 [-9 to 5]	-21 [-28 to -16]
Demographic Characteristics				
Age - yrs	58.3 ± 8.1	58.7 ± 8.6	57.9 ± 7.5	57.1 ± 8.2
Male Sex - n (%)	104 (68.0)	82 (73.2)	77 (64.2)	100 (68.5)
Race - n (%)				
White	148 (96.7)	110 (99.1)	117 (98.3)	141 (96.6)
Nonwhite	5 (3.3)	1 (0.9)	2 (1.7)	2 (1.4)
Clinical Characteristics				
Body Mass Index	29.9 ± 4.2	29.9 ± 4.1	30.4 ± 4.2	30.0 ± 4.2
Known Duration of Diabetes > 5 yrs - n (%)	112 (73.2)	80 (71.4)	82 (68.3)	100 (68.5)
Smoking - n (%)	24 (15.7)	22 (19.6)	20 (16.7)	31 (21.2)
Laboratory Variables				
Glycated Hemoglobin - %	7.4 ± 1.7	7.1 ± 1.6	7.1 ± 1.7	7.2 ± 1.7
Blood Pressure - mmHg				
Systolic	149 ± 13#	158 ± 15	149 ± 13#	158 ± 13
Diastolic	88 ± 8$	92 ± 10	89 ± 8$	92 ± 10
Urinary Albumin Excretion - µg/min	68.8 ± 42.5	56.3 ± 35.1	68.9 ± 41.6	66.1 ± 39.3
eGFR (MDRD) - mL/min	74 ± 14	71 ± 14	70 ± 13	72 ± 13
Cholesterol - mg/dL				
Total	216 ± 41	224 ± 43	230 ± 60	225 ± 43
Low-density Lipoprotein	137 ± 36	141 ± 33	142 ± 53	140 ± 40
High-density Lipoprotein	43 ± 11	44 ± 12	43 ± 12	44 ± 12

Negative concordant indicates no robust (i.e. more than median) response in neither UAE and SBP, positive concordant indicates a robust (i.e. more than median) response in both parameters. UAE, Urinary Albumin Excretion; SBP, Systolic Blood Pressure; eGFR, estimated Glomerular Filtration Rate; MDRD, Modification of Diet in Renal Disease study equation. *Median UAE response was 18% decline, **Median SBP response was 11mmHg decline, # P<0.001 versus patients with UAE < median & SBP > median and patients with UAE > median & SBP > median$ P<0.01 versus patients with UAE < median & SBP > median and patients with UAE > median & SBP > median.

as the above mentioned multivariate mixed model. The initial fall in eGFR after start of treatment may reflect a hemodynamic response and may be associated with long-term renoprotection.[13,14] Since the month 6 change in eGFR was not associated with the initial

change in UAE and SBP we considered the initial eGFR change not a potential confounder in our analyses. Relative risk reductions are described in the text as percentage reductions ([1–hazard ratio]× 100). *P*-value of <0.05 indicated statistical significance. Data were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL) and SAS (SAS Institute, Cary, NC).

Results

Systolic blood pressure and urinary albumin excretion change

A total of 531 out of 590 randomized patients had urinary albumin excretion (UAE) and systolic blood pressure (SBP) measurements available at baseline and at six months post-randomization and were included in this post-hoc analysis. The median responses in UAE and SBP in the population were 18% and 11 mmHg respectively. We subsequently divided the population according the median response in these parameters, defining a robust response as a response more that the population median. The median decline in UAE and SBP in each subgroup is reported in table 1. In Irbesartan treated patients, 24.4% had a robust reduction in UAE but not in SBP (discordant SBP response) and 19.3% had a robust reduction in SBP but not in UAE (discordant UAE response).

The baseline characteristics according to UAE and SBP response are shown in table 2. There were no differences in baseline characteristics except that patients with a robust reduction in SBP, irrespective of the UAE response, had a higher average baseline systolic and diastolic blood pressure compared to patients without robust SBP decline.

Renal outcome

We assessed whether the degree of change in UAE and SBP was associated with a different slope of renal function loss. A larger decrease in UAE during the first 6 months was independently associated with a slower rate of renal function decline during the follow up time (*P*=0.0037; figure 1). A robust decrease in SBP showed a similar trend of a slower rate of long-term eGFR decline, but was not significant (*P*=0.087).

The rate of eGFR decline according to combined change in UAE and SBP and adjusted for other risk variables is presented in figure 2A. A robust UAE reduction resulted in a slower rate of eGFR decline, also in those patients who did not have a robust SBP reduction. The combination of a robust response in UAE and SBP resulted in the lowest rate of progressive renal function loss.

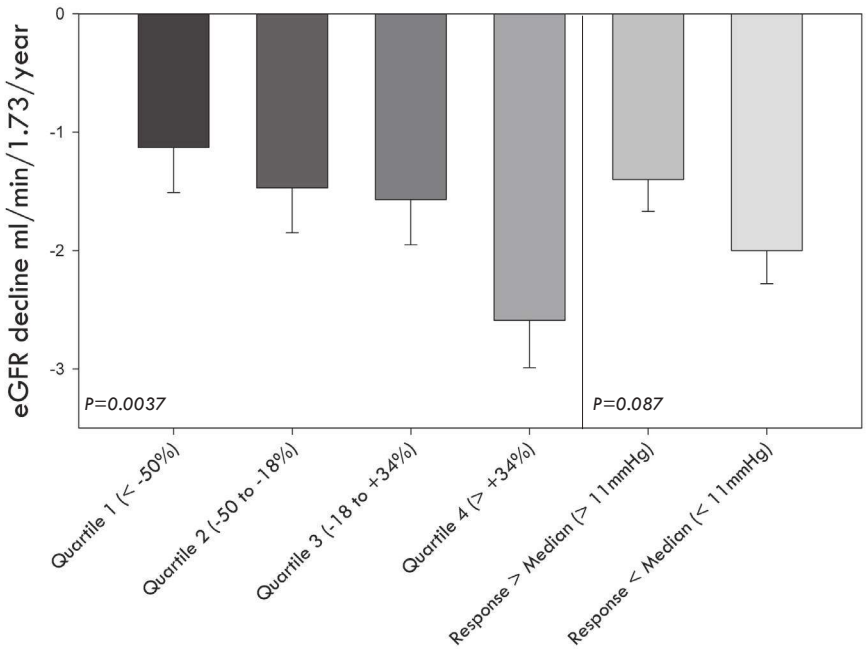
For completeness, we determined the impact of changes in UAE and SBP on the risk for development of overt nephropathy in a secondary analysis. It should be reminded that the initial change in UAE is directly related to the development of overt nephropathy. The

Table 2| Distribution of the Irbesartan and conventional treatment group (number of patients and % of total in parentheses) according to change in albuminuria and systolic blood pressure from baseline to month 6

	Albuminuria Response > Median (Reduction >18%)			Albuminuria Response < Median (Reduction < 18%)			Total (%)
	Quartile 1 (< -50%)	Quartile 2 (-50% to -18%)	Total (%)	Quartile 3 (-18 to +34%)	Quartile 4 (> +34%)	Total (%)	
Irbesartan (N=353)			(%)				
SBP response > median (Reduction > 11mmHg)	69 (19.5)	56 (15.9)	35.4	35 (9.9)	33 (9.3)	19.3	**55**
SBP response < median (Reduction < 11mmHg)	40 (11.3)	46 (13)	24.4	45 (12.7)	29 (8.2)	21.0	**45**
Total (%)			**60**			**40**	
Conventional Treatment (N=178)							
SBP Response > median (Reduction >11mmHg)	7 (3.9)	17 (9.6)	13.5	21 (11.8)	22 (12.4)	24.2	**38**
SBP Response < median (Reduction < 11mmHg)	16 (9.0)	14 (7.9)	16.9	32 (18.0)	49 (27.5)	45.5	**62**
Total (%)			**30**			**70**	

Median UAE response was 18% decline, Median SBP response was 11mmHg decline.

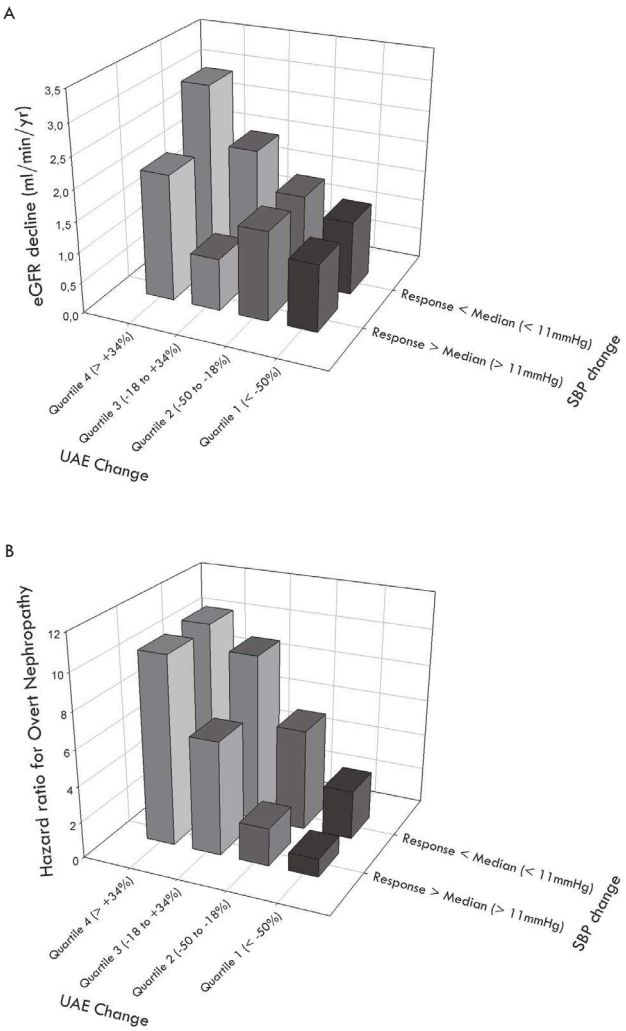
Figure 1| Long-term annual decline in estimated GFR from 6-24 months, per quartile urinary albumin excretion change from baseline to month 6



Long-term annual decline in estimated GFR from 6-24 months, per quartile urinary albumin excretion change from baseline to month 6 (P=0.0037) and per group of systolic blood pressure change from baseline to month 6 (divided over the median change) (P=0.087) in 531 type 2 diabetic patients with microalbuminuria.

risk reduction for development of overt nephropathy was 44%(95%CI 39 to 59%; *P*<0.001) and 9%(95%CI 19 to +2%; *P*=0.098) per 50% reduction in UAE and 5 mmHg SBP reduction respectively. The risk for development of overt nephropathy according to combined change in UAE and SBP and adjusted for other risk variables is presented in figure 2B.

Figure 2| **Renal outcome for groups of urinary albumin excretion and systolic blood pressure change**



A: Decline in eGFR from 6-24 months for groups of urinary albumin excretion and systolic blood pressure change, in 531 type 2 diabetic patients with microalbuminuria. B: Hazard ratios for overt nephropathy from 6-24 months for groups of urinary albumin excretion and systolic blood pressure change, in 531 type 2 diabetic patients with microalbuminuria.

Discussion

The results of this study show that the response to ARB therapy varies for both UAE and SBP even within the microalbuminuric hypertensive patient. The rate of long-term renal function decline showed a clear dependence on the initial response in UAE irrespective of SBP response. The results of this microalbuminuria study confirm a previous study reporting on proteinuria in which similar individual variations in response to ARB (Losartan) were observed, and in which the reduction in proteinuria was independently associated with renal outcome in type 2 diabetic patients with nephropathy.[5] Combining the results of that study and the current study, we conclude that monitoring therapy-induced changes in UAE in individual diabetic patients is important in addition to monitoring blood pressure, since therapy induced changes in both parameters do not run in parallel and both parameters were independently associated with the effectiveness to achieve renal protection.

The reduction in albuminuria achieved during the initial months of RAAS blockade is a critical step to achieve renoprotection. Trials conducted in populations with and without diabetes showed that agents intervening in the RAAS confer additional renoprotection beyond other antihypertensive regimens. Although in most trials BP control was slightly better in the RAAS-treatment arm, the clinical benefit exceeded that what could be attributed to improved BP control.[9,15,16] In addition, the reduction in UAE during the initial months of therapy is the most important determinant of long-term renoprotection. This observation was initially made in diabetic renal disease by Rossing et al.[17] and in non-diabetic renal disease by Apperloo et al.[14] investigating long term eGFR decline, and later on confirmed in analyses from large randomized controlled trials looking at hard renal endpoints.[4,5,7,18] It should be noted that the aforementioned observations are derived from studies enrolling patients with macroalbuminuria (UAE >300 mg/day) and/or eGFR levels below 60ml/min/1.73m^2. Importantly, the results of the present study extend these findings to the patient population with levels of UAE within the microalbuminuric range (UAE 30–300 mg/day) and eGFR levels above 60ml/min/1.73m^2.

An important question is whether changes in albuminuria can be used as a surrogate endpoint in clinical trials. The distinct advantage is that trials with surrogate endpoints require fewer patients, require shorter follow-up, are less expensive and facilitate drug development. To obtain surrogacy status definitive evidence is required demonstrating that the surrogate endpoint is causally related to the clinical endpoint. It has been pointed out that the evidence for albuminuria as a surrogate endpoint is reasonably robust in patients with diabetes and macroalbuminuria but limited data are available in patients with UAE.[19,20] This study is the first to show that even in the low albuminuria range the

initial anti-albuminuric response to ARB treatment is an important independent indicator of renoprotection. This suggests that also in patients with microalbuminuria, albuminuria may be a potential candidate as a surrogate endpoint.

Prospective randomized controlled trials will be necessary to obtain definitive evidence that an approach of targeting UAE confers renoprotection within the microalbuminuria range. These trials should be designed to compare the long-term clinical effect of different predefined UAE targets. Such a design isolates the role of UAE as an independent target for therapy and establishes the clinical relevance of targeting UAE for renal or cardiovascular protection. In this respect, a recent study by Ruggenenti et al. in diabetic and non-diabetic nephropathies compared the efficacy of a treatment strategy specifically targeting UAE with a historical cohort targeting only blood pressure.[21] The results showed that targeting UAE is feasible and translates into substantial risk reductions for End Stage Renal Disease (ESRD). Interestingly, again the reduction in UAE was the only variable in multivariate analyses that was associated with a lower risk of ESRD. The obvious limitation is that the comparisons published in this report were not randomized. The ROAD study is the only randomized controlled trial testing a treatment strategy specifically targeting proteinuria. This trial showed that optimal anti-proteinuric dosages of RAAS blockade is feasible and resulted in a substantially larger reduction in proteinuria and slower rate of renal function decline in non-diabetic patients.[22] Prospective studies confirming these results in diabetic patients with microalbuminuria and proteinuria are needed.

One can only speculate about possible mechanisms underlying the discordant blood pressure and UAE responses. One possibility is that clinical blood pressure and overnight albumin excretion measurements are subject to large random variability and thus do not accurately reflect true UAE and BP. However, patients allocated to Irbesartan more often have a robust decline in UAE compared with patients treated with conventional treatment only (60 vs. 30%, table 1.) Hence, a clear difference in discordant response pattern can be deduced, indicating that the ARB treatment responses are not solely due to random variability.

Another possible explanation for the discordant treatment responses is that the intra-individual discordance in UAE and SBP responses is accounted for by differences in systemic and local RAAS-activity or differences in the extent of tissue penetration of RAAS-blockade. It is hypothesized that the UAE response depends on the extent of intra-renal RAAS-blockade while the SBP response depends on systemic vasculature RAAS-inhibition. In support of this hypothesis, pre-clinical studies have shown that inhibition of extra-renal RAAS plays an important role in mediating blood pressure control.[23] However, further research is needed to elucidate the exact mechanisms.

It is noteworthy that this is a post-hoc analysis of clinical trial data and the results are no longer based on randomized comparisons. Although we adjusted for a large range of potential confounders, unmeasured confounding may have influenced our results. The results can therefore only be interpreted as hypothesis generating.

Conclusions

Our data show that ARB induced responses in blood pressure and urinary albumin excretion are discordant within a large proportion of patients. This underscores the recommendation of treatment guidelines of diabetes associations to regularly assess both blood pressure and urinary albumin excretion in individual patients with diabetes. Importantly, the response in urinary albumin excretion individually determined renal outcome, regardless of the blood pressure response. This implies that renoprotective strategies in microalbuminuric patients with type 2 diabetes should not only target blood pressure but also urinary albumin excretion.

Acknowledgments

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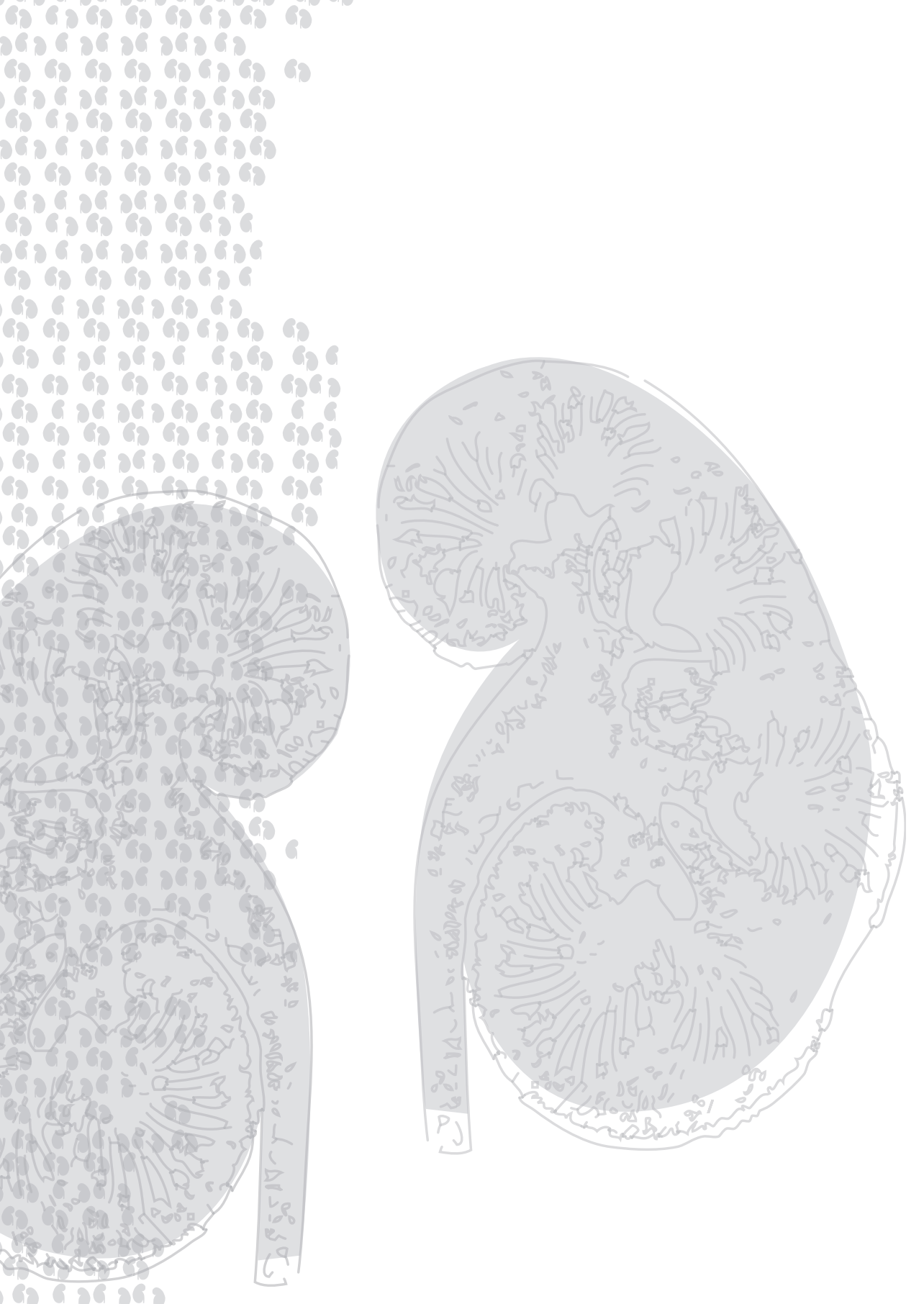

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Albuminuria Screening and Treatment in Type 2 Diabetes in Primary Care; Observational Study of the GIANTT Cohort

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Abstract

Background: Failure of diagnosing and treatment of albuminuria plays a role in high morbidity and mortality in type 2 diabetes (T2DM). We evaluated guideline adherence and factors associated with albuminuria screening and treatment in T2DM patients in primary care.

Methods: Guidelines recommend annual measurement of albuminuria and if increased appropriate treatment with Renin-Angiotensin-Aldosterone-System (RAAS)-blockers. We performed a cohort study of T2DM patients managed by 182 Dutch general practitioners (Groningen Initiative to Analyse Type 2 diabetes Treatment database), and evaluated guideline adherence in the years 2007-2009. We assessed whether demographic, clinical or organizational factors determined guideline adherence with multivariable multilevel-analyses.

Results: Data were available for 14,120 T2DM patients (47.6% male, mean age 67.3 ±11.7 years, median diabetes duration 6 [IQR: 3-10] years). Albumin-creatinine-ratio (ACR) was measured in 45.2% in 2007, 57.4% in 2008, and 56.8% in 2009. Only 23.7% of patients were measured every year and 21.4% was never measured. ACR was more often measured in patients below 75 years, with a previous ACR-measurement, using anti-diabetic medication and receiving additional care by a diabetes support facility. RAAS treatment was prescribed to 78.4% patients with prevalent micro/macroalbuminuria, 66.5% in incident micro/macroalbuminuria, 58.2% in normoalbuminuria and 52.1% of those without ACR measurements. In those not treated with RAAS-blockers, it was initiated in 14.3, 12.8, 3.0 and 2.3%, respectively. Presence of micro/macroalbuminuria, blood pressure and treatment with antihypertensive medication were determinants of RAAS-treatment initiation.

Conclusions: Guideline implementation regarding management of albuminuria in T2DM patients in primary care should be further improved.

Introduction

Type 2 diabetes mellitus (T2DM) is an increasing public health problem due to the ageing population, increasing trend in obesity and changes in lifestyle. In parallel, the costs associated with diabetes care and the disease burden for patients are increasing.[1] One of the major complications of diabetes is diabetic nephropathy. Microalbuminuria is one of the earliest signs of diabetic nephropathy, and is associated with increased risk for end-stage renal disease, cardiovascular disease and all-cause mortality.[2,3] Agents blocking the Renin-Angiotensin-Aldosterone System (RAAS) lower albuminuria and prevent worsening of albuminuria beyond their blood-pressure lowering effect.[4-6] Because of the risks associated with increased albuminuria, clinical guidelines recommend screening for albuminuria in patients with T2DM with sufficient life expectancy.[7,8] In patients with T2DM and confirmed elevated albuminuria RAAS-blockers are the preferred treatment option irrespective of concomitant blood pressure levels.[7,8]

However, despite these therapeutic options, morbidity and mortality is still high in type 2 diabetes. This could be partly due to the fact that the current interventional protocol or strategies do not sufficiently take care of all the risk in these patients. Alternatively, the implementation of such strategies in practice may be partly failing. Discrepancies have been reported between recommended diabetes care by clinical guidelines and observed diabetes care in practice.[9] Much attention has been paid to the quality of risk factor monitoring and treatment of conventional risk factors for diabetic renal and cardiovascular complications, such as blood glucose, blood pressure and cholesterol levels.[10-18] Few studies have focused on the quality of screening for and treatment of elevated albuminuria,[19-22] even though this is among the strongest cardio-renal risk factors.[23]

Therefore, our aim was to assess adherence to clinical guideline recommendations with respect to albuminuria management in Dutch primary care patients with T2DM, and to identify demographic, clinical or organizational factors associated with guideline adherence.

Patients and Methods

Study population and setting

For this observational study, we used data from patients with type 2 diabetes obtained from 182 general practitioners (150 practices) that collaborated in the Groningen Initiative to ANalyse Type 2 diabetes Treatment (GIANTT) project. GIANTT provides quality assessments for most general practitioners (GPs) in Groningen province, The Netherlands.[11]

The included practices covered a total population of over 500,000 persons in 2008. The patient population for this study consisted of all patients who had been diagnosed with type 2 diabetes for at least 1 year on January 1st 2007, were still alive on July 1st 2010, did not leave the practice before July 2010, did not object to the data collection (<1%), and were primarily managed by their GP (n=14,120). In our study region, a regional diabetes facility offers support to GPs. Patients can be referred to this facility for laboratory tests and physical examination. The results are reported back to the GPs who remain responsible for further management and treatment decisions. Patients who were primarily managed by a specialist for their diabetes, as indicated by the GP, were excluded from this study (n=2,933).

Data collection

We used data on demographic characteristics, organizational factors, clinical characteristics, laboratory parameters and prescribed medication from the GIANTT database. This database contains anonymized data extracted from structured tables and free text parts of electronic medical records using an automated and validated method.[24] For research with anonymous medical record data no ethics committee approval is required in The Netherlands.

Guideline Recommendations

The Dutch primary care guideline recommends yearly albuminuria screening in all diabetes patients with sufficient life expectancy (at least 10 years).[8] For the yearly albuminuria-screening, measurement of albumin-creatinine-ratio (ACR) is favoured over urinary albumin concentration.[25] If the measurement indicates the presence of micro- or macroalbuminuria this has to be confirmed by a repeat measurement within the next few months. According the guideline, all patients with confirmed micro- or macroalbuminuria should be prescribed RAAS-treatment (Angiotensin-Converting-Enzyme Inhibitors (ACEi)/ Angiotensin II Receptor Blockers (ARB) even if their blood pressure readings are in the normal range. For the treatment of hypertension in patients with normoalbuminuria, thiazide-diuretics are first-choice antihypertensives, followed by RAAS-treatment.

Definitions

Patients' ACR-values were classified as normoalbuminuria, microalbuminuria or macroalbuminuria. Microalbuminuria was defined according gender specific cut-offs, being ACR > 2.5 mg/mmol in men and > 3.5 mg/mmol in women.[26] Macroalbuminuria was defined as ACR > 25 mg/mmol in men and > 35 mg/mmol in women. We clustered patients with micro and macroalbuminuria for analyses on guideline adherence because

the evaluated guideline recommendations are equal for micro- and macroalbuminuria. These patients are all denoted as having increased albuminuria.

Based on the ultimate ACR measurement in the period 2007-2009 (index measurement) patients were classified into patients without any measurements, patients with normoalbuminuria and patients with increased albuminuria. The patients with increased albuminuria were subdivided into patients with incident increased albuminuria and patients with prevalent increased albuminuria. Based on the prior ACR measurement, patients with repeated increased albuminuria were denoted as having prevalent increased albuminuria.

Treatment with RAAS-blockers was defined as any prescription of an agent intervening in the RAAS-system in the year before the index ACR measurement. A repeat ACR measurement was defined as a second measurement of ACR within 100 days from the index measurement. Initiation of RAAS-treatment was defined as start of RAAS-treatment within 100 days after the index ACR measurement or within 100 days of the repeat measurement of ACR after at least 1 year without prescription of any RAAS-treatment.

Determinants of guideline adherence

As potential patient-related determinants of guideline adherence we considered demographic characteristics (age >75 years, gender), organizational factors (additional care by a diabetes support facility), clinical characteristics (known duration of diabetes, having ACR measurements in the preceding 12 months, prior ACR status (unknown, normoalbuminuria, increased albuminuria), body mass index, systolic and diastolic blood pressure), laboratory parameters (glycosylated haemoglobin, serum creatinine, estimated glomerular filtration rate according to Modification of Diet in Renal Disease (MDRD)-study equation,[27] low-density lipoprotein, high-density lipoprotein) and medication (none, use of oral antidiabetics, use of oral antidiabetics and insulin and use of antihypertensive agents in the 12 months before the index ACR measurement, number of antihypertensives used, use of RAAS). For the clinical and laboratory parameters we used the most recent value in the 12 months before the index ACR measurement if available and the most recent value in the 12 months before a random date generated according to a similar date distribution otherwise.

Statistical Analysis

We performed the analyses with STATA version 11.2 (STATACorp LP, Texas, USA). We calculated in what proportion of patients an ACR measurement was recorded in each calendar year. Differences in patient characteristics between patients with and without ACR measurement in 2009 were compared with students' t-test for continuous variables

and chi-squared test for categorical variables. For all groups of patients we calculated what proportion of patients received RAAS-treatment and in what proportion RAAS-treatment was initiated and, if applicable, ACR measurement was repeated. The descriptive analyses

Table 1 | Baseline characteristics overall and stratified by ACR measurement

	Registration Degree	Overall	ACR measured	ACR not measured	
	N (%)	N=14120	8025 (56.8%)	6095 (43.2%)	*P*-value
Demographic Characteristics					
Age – years	14120 (100)	67.3±11.7	66.5±11.3	67.7±12.5	<0.001
Age >75 years	14120 (100)	4107 (29.1)	2166 (27.0)	1941 (31.9)	<0.001
Male sex	14120 (100)	6722 (47.6)	3948 (49.2)	2774 (45.5)	<0.001
Organizational Factors					
Support care by diabetes facility	14120 (100)	4825 (34.2)	3120 (38.9)	1705 (28.0)	<0.001
Clinical Characteristics					
Known duration of diabetes – years	14120 (100)	6 [3-10]	6 [3-10]	6 [3-9]	<0.001
Body Mass Index – kg/m2	9902 (70)	30.2±5.5	30.2±5.5	30.2±5.6	ns
ACR measured previous 12 months	14120 (100)	8111 (57.4)	5621 (70.0)	2490 (40.9)	<0.001
Blood Pressure - mmHg					
Systolic	12381 (88)	143+19	143±19	142±20	<0.001
Diastolic	12381 (88)	79±10	79±10	78±10	<0.001
Laboratory Parameters					
Glycosylated hemoglobin - %	12803 (91)	7.0±1.0	7.0±0.9	6.9±1.0	<0.001
Serum Creatinine - mmol/L	12223 (87)	93±30	92±26	94±35	<0.001
eGFR (MDRD) - ml/min	12223 (87)	60±18	61±17	60±18	ns
Cholesterol - mg/dl					
Low-density lipoprotein	11751 (83)	2.4±0.9	2.3±0.9	2.5±0.9	<0.001
High-density lipoprotein	11793 (84)	1.2±0.3	1.2±0.3	1.2±0.3	ns
Albumin to creatinine ratio - mg/mmol	8025 (57)	1.2 [0.5-2.7]	1.2 [0.5-2.7]	-	n/a
Medication					
Diabetes Regulating medication	14120 (100)	12037 (85.3)	7175 (89.4)	4862 (79.8)	<0.001
Oral antidiabetics	14120 (100)	11165 (79.1)	6753 (84.2)	4412 (72.4)	<0.001
Insulin	14120 (100)	2768 (19.6)	1539 (19.2)	1229 (20.2)	<0.001
Blood pressure regulating medication	14120 (100)	10882 (77.1)	6356 (79.2)	4526 (74.3)	
Number of antihypertensives	14120 (100)	2 [1-2]	2 [1-2]	2 [0-2]	ns
RAAS-treatment	14120 (100)	8479 (60.0)	5028 (62.7)	3441 (56.5)	<0.001
Beta-blockers	14120 (100)	5642 (40.0)	3271 (40.8)	2371 (38.9)	<0.001
Calcium antagonists	14120 (100)	3088 (21.9)	1819 (22.7)	1269 (20.8)	<0.001
Diuretics	14120 (100)	6607 (46.8)	3831 (47.7)	2776 (45.5)	<0.001
Other	14120 (100)	319 (2.3)	181 (2.3)	138 (2.3)	ns

Data are presented as n(%) or mean±sd or median[iqr]. Abbreviations: ACR: Albumin-to-creatinine ratio; eGFR estimated Glomerular Filtration Rate; MDRD: Modification of Diet in Renal Disease study equation; ACEi: Angiotensin-Converting-Enzyme Inhibitors; ARB: Angiotensin II Receptor Blockers.

are also presented stratified by age below and over 75 years, and repeated using longer time periods in which the ACR measurement could be documented or the RAAS-treatment could be initiated. Multilevel analyses clustering patients by GP were used to assess which patient level factors were associated with ACR measurement. We determined whether random slopes significantly improved model fit with the likelihood ratio test and used a forward selection procedure adding variables to the baseline model (containing age and sex) one at the time, based on the Wald-statistic. To allow analyses with all available patients and to minimize bias, we used multiple imputation of missing values. We used the same multilevel modeling strategy to assess which patient level factors were associated with RAAS-treatment initiation in all patients, including increased albuminuria (increased vs. not increased or not determined) as determinant, and in four subgroups divided by albuminuria status (without ACR measurement, with normoalbuminuria, with incident and with prevalent increased albuminuria). For all analyses we considered a P-value <0.05 statistically significant.

Results

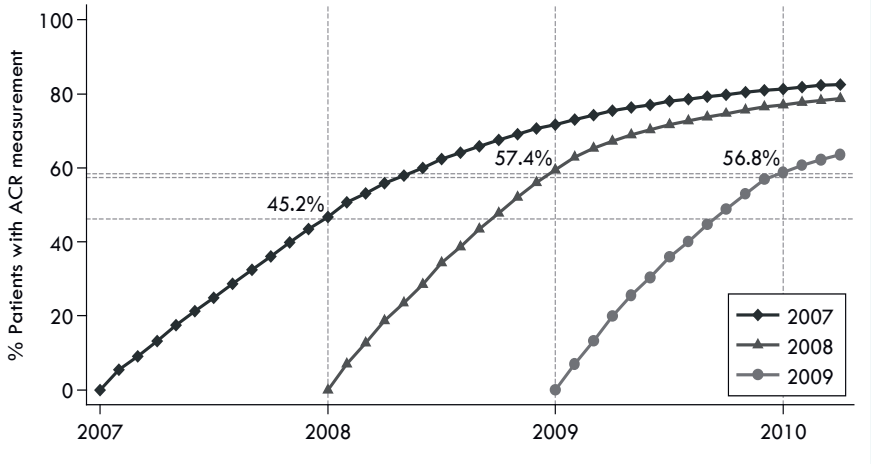
Data between 2007 and 2010 were available from 182 general practitioners with a total of 14,120 patients with T2DM. Of the 14,120 patients, 47.6% were male, the mean age was 67.3±11.7 years and the median duration of diabetes was 6 [IQR 3-10] years (Table 1).

ACR-measurement

ACR was measured at least once in 6,377 patients (45.2%) in 2007, in 8,111 patients (57.4%) in 2008, and in 8,025 patients (56.8%) in 2009. Extending the interval from 12 to 15 months increased these percentages to 53.9%, 65.0% and 61.5% respectively (figure 1). In the 3-year period between 2007 and 2010, 11,106 patients (78.7%) had at least one measurement, 3,351 patients (23.7%) had at least one ACR measurement all three consecutive years, and 3,014 patients (21.4%) did not have any ACR measurement.

ACR was more frequently measured in patients below than above 75 years (58.9 vs. 52.7% in 2009, p<0.001), and in patients receiving care at the diabetes support facility as compared to receiving routine diabetes care in the GP practice (64.7% vs. 52.8%, p<0.001). Differences in characteristics between patient with and without ACR measurement are shown in table 1. In a multivariable multilevel model (Table 2), the most important determinants of ACR measurement were age, previous ACR measurement, additional care by the diabetes support facility, and use of antidiabetic medication.

Figure 1 | ACR Measurement distribution over time between 2007 and 2010 for each year independently



Horizontal dotted lines indicate the percentage of patients with at least one ACR measurement from beginning of 2007, 2008 and 2009 respectively. The population selected was stable (N=141 20) throughout this time-interval, the percentage of patients with at least one ACR measurement was calculated for each year independently.

Prevalence of increased albuminuria

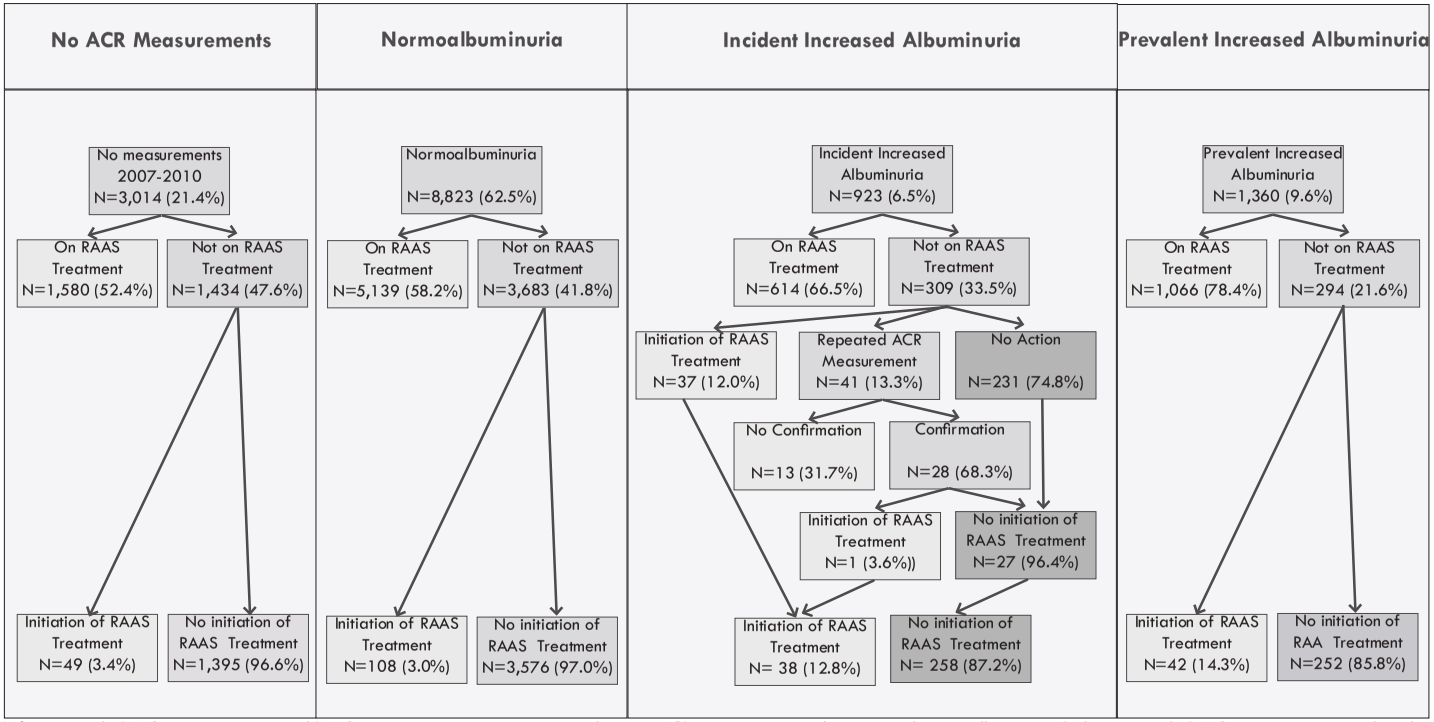
Looking at the most recent ACR measurement before 2010 (N=11,106) 8,823 patients (79.4%) had normoalbuminuria, 1,970 patients (17.7%) had microalbuminuria, and 313 patients (2.8%) had macroalbuminuria. The guideline makes a distinction between actions needed for incident and prevalent increased albuminuria and we therefore used the before-last measurement to distinguish between incident and prevalent increased albuminuria. Of the patients with increased albuminuria (presence of micro- or macroalbuminuria, N=2283) 1,360 patients (59.6%) also had increased albuminuria on the prior ACR measurement, indicating prevalent increased albuminuria. 923 patients (40.4 %) did not have increased albuminuria on the prior ACR measurement or did not have a prior measurement, indicating incident increased albuminuria.

Repeat measurements and RAAS-treatment

Of patients with prevalent increased albuminuria 78.4% received RAAS-treatment in the year up to the index measurement, compared to 66.5% of patients with incident increased albuminuria, 58.2% of patients with normoalbuminuria and 52.4% of patients without any ACR measurements.

In patients with incident and prevalent increased albuminuria not receiving RAAS-treatment, the guidelines recommend further action. The appropriate steps differ

Figure 1 | Flowchart RAAS-treatment and RAAS-treatment initiation



Left two panels: RAAS-treatment status and RAAS-treatment initiation in patients without any ACR measurements and patients with normoalbuminuria. Right two panels: RAAS-treatment status and guideline implementation in patients with incident increased albuminuria and patients with prevalent increased albuminuria respectively. Dark gray colour indicates failure to adhere to guideline. Abbreviations: RAAS: Renin-Angiotensin-Aldosterone system.

between patients with incident increased albuminuria and patients with prevalent increased albuminuria (Figure 2).

Of the 294 patients with prevalent increased albuminuria not on RAAS-treatment, RAAS-treatment was initiated in 42 patients (14.3%) (Figure 2).

In patients with incident increased albuminuria, the albuminuria status merits confirmation by a repeat measurement. Of the 309 patients with incident increased albuminuria not on RAAS-treatment, ACR measurement was repeated in 41 (13.3%) patients, and in 37 (12.0%) patients RAAS-treatment was initiated without confirmatory ACR measurement. In 231 (74.8%) patients no action was undertaken. Of the 41 confirmatory measurements, in 28 (68.3%) patients increased albuminuria status was confirmed and in 1 of these patients RAAS-treatment was subsequently initiated (Figure 2). Overall, RAAS-treatment was initiated in 38 patients (12.3%) with incident increased albuminuria. In total, appropriate action (i.e. repeat measurement performed and/or RAAS-treatment initiation) was undertaken in 51 patients (16.5%). In comparison, in patients with normoalbuminuria not receiving RAAS-treatment, treatment was initiated in 108 patients (3.0%) after the most recent ACR measurement, and in patients without any ACR measurements RAAS-treatment was initiated in 33 patients (2.3%). RAAS-treatment initiation was thus more common in patients with increased albuminuria vs. patients without increased albuminuria (13.6% vs. 2.8%, P<0.001)

Extending the interval for initiation of RAAS-treatment from 100 days after the index ACR measurement to 200 days of the index measurement, did not substantially increase the number of new RAAS-prescriptions in patients with increased albuminuria (data not shown).

The proportion of patients receiving RAAS-treatment was similar in patients with increased albuminuria below and above 75 years (73.3 vs. 72.6%, P=0.13), and appropriate action in patients with increased albuminuria was undertaken in similar numbers of patients 15.0% vs. 13.3% (P=0.34) below and above 75 years respectively.

Factors determining RAAS-treatment initiation

In the overall multivariate analysis, increased albuminuria status, blood pressure and number of antihypertensives (other than those intervening in the RAAS) that were already used were important determinants of RAAS-treatment initiation (Table 2). Looking at the subgroups, we found that amongst patients without any ACR measurement and patients with normoalbuminuria the most important determinants of RAAS-treatment initiation were blood pressure and/or number of antihypertensive agents that were already used (Table 2). In patient with increased albuminuria, the number of antihypertensive agents appeared to be a determinant next to presence of increased albuminuria.

Table 2| Determinants of ACR measurement and RAAS-treatment Initiation

	ACR Measurement			RAAS-treatment Initiation			RAAS-treatment Initiation - per group							
							No ACR Measurement		Normo-Albumuria		Incident Increased Albuminuria		Prevalent Increased Albuminuria	
Determinants	Coef.	95% CI	P-value	Coef.	95% CI	P-value	Coef.	P-value	Coef.	P-value	Coef.	P-value	Coef.	P-value
Demographic Characteristics														
Age >75 years	0.83	[0.76-0.91]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Female sex - n (%)	0.89	[0.82-0.96]	0.004	0.63	[0.47-0.85]	0.002	-	-	-	-	-	-	-	-
Organizational Factors														
Support care by diabetes facility	1.82	[1.57-2.09]	<0.001	-	-	-	-	-	0.51	0.008	-	-	-	-
Clinical Characteristics														
Known duration of diabetes - yrs	0.98	[0.98-0.99]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Body Mass Index – kg/m2	-	-	-	1.03	[1.00-1.07]	0.03	-	-	-	-	-	-	-	-
ACR measured previous 12 months (yes/no)	3.65	[3.28-4.01]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Blood Pressure - per 10 mmHg														
Systolic	-	-	-	1.20	[1.09-1.31]	<0.001	-	-	1.48	<0.001	-	-	-	-
Diastolic	-	-	-	1.21	[1.01-1.45]	0.04	-	-	-	-	-	-	-	-
Laboratory Parameters														
Glycosylated hemoglobin - %	1.09	[1.04-1.13]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Serum Creatinine - mmol/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-
eGFR (MDRD) - ml/min	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cholesterol - mg/dl														
Low-density lipoprotein	0.87	[0.83-0.91]	<0.001	-	-	-	-	-	-	-	-	-	-	-
High-density lipoprotein	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Increased Albuminuria (no or unknown/ yes)	n/a	n/a	n/a	5.16	[3.77-7.05]	<0.001	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Medication														
Diabetes Regulating medication														
None (reference)	1.00	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Oral antidiabetics	1.69	[1.51-1.91]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Oral antidiabetics and insulin	1.36	[1.17-1.57]	<0.001	-	-	-	-	-	-	-	-	-	-	-
Number of antihypertensives														
None (reference)	ref	ref	ref	1.00	ref	ref	1.00	ref	ref	ref	1.00	ref	1.00	ref
One antihypertensive*	-	-	-	1.59	[1.11-2.28]	0.010	2.38	0.08	2.17	0.57	3.98	0.11	1.12	0.02
Two or more antihypertensives*	-	-	-	2.70	[1.89-3.85]	<0.001	2.80	0.02	3.20	<0.001	9.50	0.007	3.01	<0.001

Determinants of ACR measurement, determinants of RAAS-treatment initiation overall, and determinants of RAAS-treatment initiation split out for patients with normoalbuminuria, patients with incident increased albuminuria and patients with prevalent increased albuminuria not on RAAS-treatment. These models take differences between practices into account. *Other than RAAS-blockers.

Discussion

An ACR measurement was observed in less than 60% of T2DM patients in Dutch primary care in 2009, even in patients below 75 years of age. ACR measurement was more common in patients with earlier ACR measurements, more concomitant medication use, and receiving additional care by a diabetes support facility. Patients with increased albuminuria were frequently treated with RAAS-treatment (78.4% in prevalent albuminuria and 66.5% in incident albuminuria). In patients with increased albuminuria not on RAAS-treatment the actions considered appropriate according to the guideline were applied in only a small proportion of patients (<15%).

Albuminuria screening was lower than anticipated based on published screening rates of other renal/cardiovascular risk factors.[10-12] Although the proportion of patients being screened increased when the interval was extended beyond 12 months, as proposed in a recent study,[22] this was a marginal increase leaving more than a third of patients not being screened in 2009. Also, limiting the patients to those who have a longer life expectation did not substantially increase the ACR screening rate. One possible reason for the low screening rates could be that patients did not frequently visit their GP or even avoided care, although this is not plausible as 3 monthly visits are common for patients with T2DM in The Netherlands. In addition, more than 90% of the included patients had their HbA1c checked at least once yearly in our study period and the number of care avoiders in this cohort is estimated to be less than ~5% on the basis of other available visit data and measurements.[11] It is therefore unlikely that care avoidance contributed considerably to the low proportion of ACR screening. Another reason, brought forward by some GPs, are logistical issues regarding albuminuria screening (i.e. instruction first morning void, requirement of specific urine cups, inadequate patient recall system). This may explain why additional care by a diabetes support facility was an important determinant of ACR measurement, as they may be more attentive of laboratory procedures and providing necessary patient instruction and thus may be better equipped to provide optimal diabetes care. In further support of this, additional support by diabetes care facilities has previously been shown to contribute to improve hypertension management in diabetes.[28]

Of the patients who had prevalent or incident micro/macroalbuminuria, a substantial proportion already received RAAS-treatment. It is unclear to what extent RAAS-treatment was prescribed to these patients because of increased albuminuria or because of (previous) uncontrolled blood pressure. Given the fact that RAAS-treatment was also common in patients without any ACR measurements and patients with normoalbuminuria, it is tempting to assume that in many patients the main indication for RAAS-treatment was uncontrolled blood pressure rather than increased albuminuria. This suggestion is

further supported by our finding that blood pressure and number of antihypertensive drugs used (other than RAAS) were determinants of RAAS-treatment initiation in patients with unknown albuminuria or normoalbuminuria. Furthermore, RAAS-treatment was also more likely to be initiated in patients with increased albuminuria when they already used more antihypertensive drugs, suggesting that initiation of RAAS-treatment in these patients is also partly depending on pre-existing high blood pressure. This may indicate that establishment of increased albuminuria in itself is insufficiently recognized as an indication for starting RAAS-treatment.

Across all groups the proportion of patients that was started on RAAS-treatment was highest in patients with prevalent increased albuminuria followed by patients with incident increased albuminuria. Increased albuminuria was an important trigger for RAAS-treatment initiation. Nevertheless, the proportion of patients that was prescribed RAAS after increased albuminuria measurement was disappointingly low (~13%).

How does the Dutch guideline and clinical practice relate to international guidelines and practice? The Dutch primary care guideline differs in some aspects from the standards of medical care by the American Diabetes Association (ADA).[7] The main discrepancy between the Dutch primary care guideline and the ADA guideline is that RAAS-treatment is only recommended in patients with increased albuminuria, whereas the ADA guideline recommends RAAS-treatment in all hypertensive patients with T2DM irrespective of their albuminuria status. This may to some extent limit the direct extrapolation of our results, although similar problems regarding attention for increased albuminuria may occur in other primary care settings.

With respect to screening for albuminuria and its treatment in other parts of the world, a study from the UK reported on the quality of diabetes care and presented data on albuminuria screening and treatment thereof.[9] In this population 75% of patients with T2DM were screened for increased albuminuria in a 15 months period in 2006 which was also lower as compared to other risk factors. Of patients with a diagnosis of micro/macroalbuminuria 86.4% was treated with an ACEi or ARB but no information was presented on subsequent RAAS-treatment initiation. Both the proportion of patients screened and the proportion of patients treated with RAAS-treatment were modestly higher in the UK-setting than we observed in the Dutch setting. The higher proportion of screened patients in the UK study can partly be explained by the longer study period (15 as opposed to 12 months) and the measurement in 'high quality data' general practices. Also, the higher rates of screening and treatment in the UK may be positively influenced by the implementation of the quality and outcomes framework in 2004.[9] In our study, we observed improvement in albuminuria screening in 2008-2009 compared to 2007. As the current guideline dates from 2006, this improvement illustrates that new guideline

recommendations may take time and extra effort before they are fully implemented in practice.[29,30]

It is widely recognized that there can be many barriers and incentives contributing to the implementation of guidelines in practice.[31] We looked at several external factors, including patient characteristics and organizational factors. However, barriers at the level of the health care professional may also be important. There can be cognitive and attitudinal barriers which may explain inertia.[31] A questionnaire among European general practitioners (GPs) showed such internal barriers with respect to albuminuria guideline implementation. A repeat ACR test in T2DM patients with incident microalbuminuria was deemed necessary by only 45-77% (depending of the country) GPs. Even more worrisome was that only 23-50% of GPs would prescribe RAAS-treatment in a T2DM patient with confirmed microalbuminuria. These findings, together with our data, show that awareness and implementation of guidelines regarding albuminuria screening and treatment in primary care need to be improved.[32]

A limitation of this study is that we looked at the most recent ACR measurement and the subsequent actions, whereas some patients may have a history of previous events or outcomes which may influence these actions. To take this into account, we included a wide range of potential patient-related determinants when assessing guideline adherence. Another limitation is that we only looked at new prescriptions of RAAS-treatment and not at changes in RAAS-treatment such as dose-adjustment. This may underestimate the proportion of patient in whom action is undertaken regarding RAAS-treatment on the basis of the ACR measurement. For assessing guideline adherence in our study, however, these patients were considered to be adequately managed. An important strength of this study is that the data reflect a large longitudinally followed group of T2DM patients treated in primary care. All collected data were registered during regular care and this process was unaffected by this study. Data collection and registration have been validated in prior studies underscoring the robustness of the results.[24]

Conclusions

In the primary care setting, the adherence to guidelines with respect to albuminuria screening and treatment was modest and should be further improved in order to accomplish optimal risk management in patients with type 2 diabetes. It is well recognized that albuminuria is one of the strongest cardio-renal risk markers, and early screening and appropriate treatment have the potential to substantially reduce the risk of cardio-renal complications. Nevertheless, in comparison to the management of other risk factors

in T2DM albuminuria receives little attention. Better support systems for regular ACR measurement and more attention for albuminuria as a risk factor beyond blood pressure may improve albuminuria screening and treatment in primary care.

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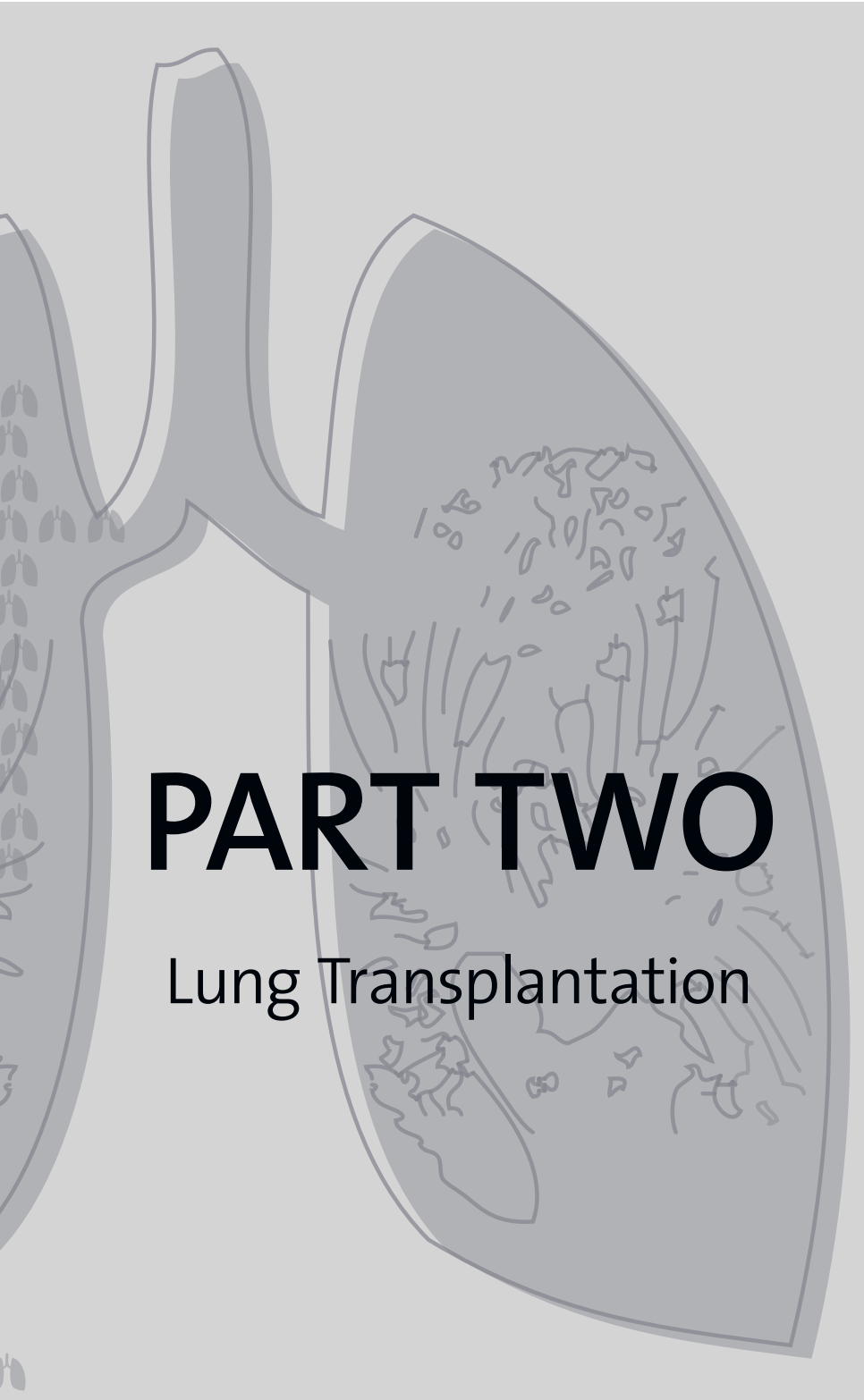
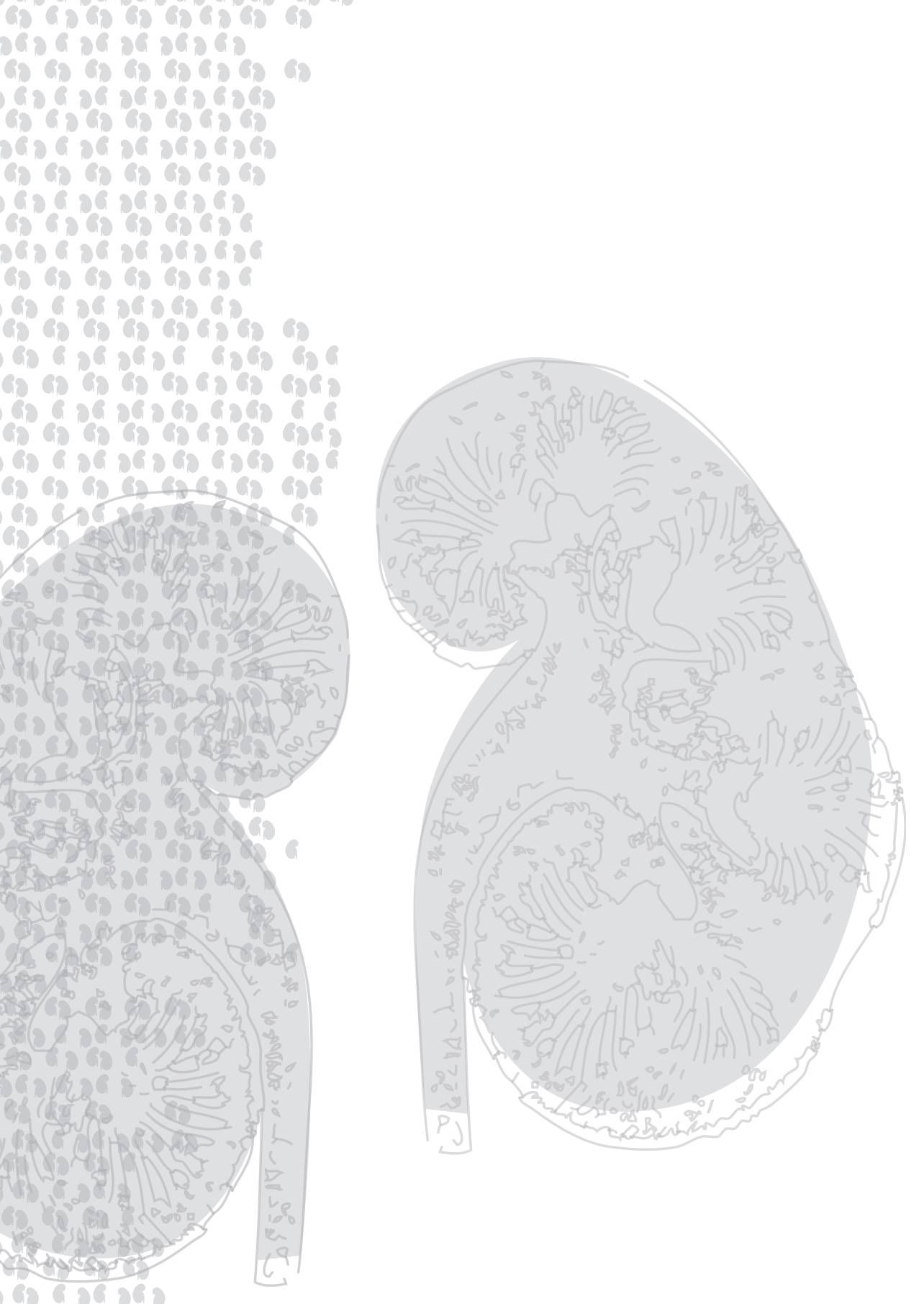


PART TWO

Lung Transplantation



7

Incidence of Impaired Renal Function after Lung Transplantation

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Abstract

Background: Impaired renal function is a frequent complication after lung transplantation (LTx). Since the early days of LTx, recipient eligibility criteria slowly became less strict, while treatment regimens evolved. These developments may have had opposing effects on the risk for impaired renal function. We aimed to study changes in recipient characteristics in conjunction with incidence of impaired renal function in consecutive series of lung transplant recipients (LTRs).

Methods: 340 Adult LTRs (mean age 45±12, 50.3% male, median follow-up 3.4[1.0-7.1] yrs) were divided into 4 consecutive patient series in time: 1990-1996 (n=93), 1997-2001 (n=79), 2002-2005 (n=89), 2006-2008 (n=79). Primary endpoint was cumulative incidence of doubling of serum creatinine (DSC), taking into account the competing risk of death. Measured GFR (mGFR, ^{125}I-Iothalamate) was assessed as a secondary endpoint.

Results: Mean age at transplantation (*P*=0.001), prevalence of hypertension (*P*=0.005), packyears of former smoking (*P*=0.001) and BMI (*P*=0.05) increased across the consecutive series. Cumulative incidence of DSC at 24 months after LTx was 43%, 37%, 35% and 29% resp. in the consecutive series (*P*=0.01). Despite higher prevalence of renal risk factors, there was lower adjusted risk of DSC in the consecutive series, with resp. HRs[95%CI] of 0.62[0.34-1.15], 0.50[0.25-0.98] and 0.31[0.154-0.67] compared to the series of 1990-1996. In line, mGFR at 24 months after LTx was 51±17, 53±17, 57±21 and 63±21ml/min/1.73m^2 in the consecutive series (*P*=0.002).

Conclusions: Despite higher prevalence of renal risk factors in more recently transplanted patients, renal outcome after LTx has improved over time. Nevertheless, impaired renal function remains a frequent complication after LTx.

Introduction

Impaired renal function is a common complication after lung transplantation (LTx).[1-3] Renal function declines progressively in the majority (>90%) of lung transplant recipients (LTRs).[2] At five years after LTx, cumulative incidence of chronic renal failure has been reported to be more than 15%.[2,4] A large proportion (~50%) of these patients ultimately progresses to end stage renal disease (ESRD), requiring renal-replacement therapy.[2,4,5] Since its introduction more than 20 years ago, the field of lung transplantation has evolved considerably. Due to changing acceptance policies LTRs are increasingly older and have a a higher proportion of 'co-morbidities'.[6] Yet, patient and graft survival after LTx have gradually improved.[7,8] The increasing age, higher prevalence of co-morbidities and the longer overall survival may all be suspected to adversely impact on incidence of impaired renal function and ESRD after LTx.[9] These factors may, however, be opposed by a "learning curve" in management of LTRs and decreased nephrotoxicity of the novel immunosuppressive strategies.[2,10,11] Whether these developments over time have affected the risk for renal function impairment after LTx has not been systematically assessed. In the current study therefore, we evaluated the changes in population characteristics that occurred over time in consecutively transplanted series of LTx patients, as well as the incidence of renal function impairment after LTx in these patients, in a large single-center cohort .

Patients and methods

In total 348 lung and 22 heart-lung/lung-liver transplantations were performed between 1990 and 2008 at the University Medical Center Groningen (UMCG), the Netherlands. We excluded 17 pediatric and 13 re-transplantations, leaving 340 primary adult lung or heart-lung transplantations to be included in the present study. Patients were divided into four consecutive patient series with similar numbers of patients: 1990-1996, 1997-2001, 2002-2005 and 2006-2008.
Clinical information on all individuals was obtained at baseline (before transplantation) and post-transplantation during follow-up visits. There was no loss of follow-up. From all patients consent for the use of their data was obtained prior to transplantation.

Data collection

The following parameters were collected for all patients: recipient gender, age of the recipient at transplantation, pulmonary diagnosis, type of transplantation, intra-operative use of cardiopulmonary bypass, immunosuppressive regimen, history of smoking, number

of packyears, diabetes pre-transplantation (antidiabetic treatment), hypertension pre-transplantation (anti-hypertensive treatment), body-mass-index (BMI) , 24-hour creatinine excretion and measures of renal function.

Measures of renal function

Renal function was assessed at baseline and regularly during follow up by serum creatinine (SCr). We also assessed true GFR at baseline and with intervals during follow-up by means of the ^{125}I-Iothalamate and ^{131}I-Hippuran infusion method.[12-14] This method is considered a gold standard method for GFR measurement and used for the development of novel renal function equations.[15] The ^{125}I-Iothalamate and ^{131}I-Hippuran clearances are calculated as $(U^*V)/P_{iot}$ and $(I^*V)/P_{hipp}$,respectively. U*V represents urinary excretion of the tracer; I*V, the infusion rate of the tracer, which equals clearance from plasma during steady state. GFR is calculated as the urinary clearance of ^{125}I-Iothalamate, corrected for voiding errors: $(U^*V/P)_{corr}$. Height and weight were measured in every patient before investigation. Body surface area (BSA) was calculated as $0.007184 \cdot \text{weight}^{0.425} \cdot \text{length}^{0.725}$, and GFR was expressed per 1.73 m^2 of BSA.[16]

Endpoints

Outcome measure for this study was doubling of serum creatinine (DSC). This is an acknowledged endpoint in clinical studies on chronic renal function impairment, including LTx.[4,17-19] Accordingly DSC was defined as the first SCr value twice the baseline value, confirmed by a similar second value at least four weeks after the initial doubling. Baseline SCr was the mean of available measurements in the month prior to transplantation. SCr was measured at all outpatient visits during follow-up. Measured glomerular filtration rate (mGFR) was used as a secondary outcome. From 2004 onwards, mGFR measurements were performed with lower frequency, allowing less resolution in time, and incomplete data in the more recently transplanted patients. We therefore refrained from using mGFR as primary outcome variable for our study.

Medication

Immunosuppressive protocols used over time are described in more detail elsewhere.[20]
In short, induction consisted of rATG (Thymoglobulin; Sanofi Pasteur, Lyon, France) until 2001 and Basiliximab induction thereafter (Simulect, Novartis Pharma, Basel, Switzerland). Maintenance immunosuppression consisted of a calcineurin inhibitor (CNI) based immunosuppressive regimen combined with Prednisolone and Azathioprine (Immuran; GlaxoSmithKline, Brentford, UK) or Mycophenolate Mofetil (Cellcept; Roche, Basel, Switzerland).

Cyclosporine (Neoral; Novartis Pharma, Basel, Switzerland) was the CNI used until 2001 (target levels: whole blood trough levels 400ng/mL initially, tapered to 150 ng/mL within the first three weeks), tacrolimus (Tacrolimus, Prograft; Astellas Pharma, Staines, UK) was the CNI used from 2001 onwards (target levels: 20ng/mL during the first three weeks, 15ng/mL until the third month and 10-12ng/mL thereafter and from 2004 until present target levels 15-18ng/mL during the first three weeks, 12-15ng/mL until the third month, and 10-12ng/mL thereafter).

All patients furthermore received P.Jiroveci prophylaxis (co-trimoxazole), Herpes prophylaxis (aciclovir) and from 2001 all patients at risk received standard CMV–prophylaxis (ganciclovir).

Statistical analysis

Results were analyzed with SPSS version 18.0 and STATA version 11.0. Continuous variables are reported as means with standard deviations, and categorical variables as frequencies with percentages. Recipient characteristics are shown according to the four transplant series. Categorical characteristics of the groups were compared with Chi square test, and continuous characteristics were compared with ANOVA for linear trend. Results were considered significant at two-sided $P<0.05$.

Follow-up was measured from date of transplantation. Patients were censored at date of last follow-up. Death was regarded as a competing endpoint for DSC and cumulative incidence of DSC was calculated taking the competing risk of death into account. Strata of the consecutive patient series were compared with the Gray's log-rank test for competing risks data. We used a cause-specific hazards model for the multivariable regression analyses to model the risk of DSC in the different time-series.[21] In the multivariable analyses, we adjusted for demographics, significant differences between patient series and established renal risk factors in LTRs (age, sex, pulmonary diagnosis, type of transplantation, use of cardiopulmonary bypass, hypertension, diabetes, packyears of smoking, serum creatinine, BMI, and creatinine excretion). In addition, we performed a secondary analysis looking at the consecutive pharmacologic eras (Csa, Tac and Tac-low). Hazard ratios (HR) are reported with 95% confidence interval [CI].

Table 1 | Baseline patient characteristics per series of lung transplant recipients

	1990-1996	1997-2001	2002-2005	2006-2008	*P-value*
Number of Patients	93	79	89	79	
Age at Transplantation - years	42.9 ± 11.1	44.0 ± 11.1	46.0 ± 12.4	48.9 ± 12.7	*0.001*
Male Sex - n (%)	55 (59.1)	35 (44.3)	48 (53.9)	33 (41.8)	*0.08*
Pulmonary Diagnosis - n (%)					*0.004*
Chronic Obstructive Pulmonary Disease	16 (17.2)	14 (17.7)	21 (23.6)	28 (35.4)	
α-1-Antitrypsine Deficiency	30 (32.3)	18 (22.8)	13 (14.6)	8 (10.1)	
Cystic Fibrosis	15 (16.1)	21 (24.1)	19 (21.3)	12 (15.2)	
Primary Pulmonary Hypertension	8 (8.6)	3 (3.8)	5 (5.6)	2 (2.5)	
Secondary Pulmonary Hypertension	6 (6.5)	6 (7.6)	6 (6.7)	5 (6.3)	
Bronchiectasis	9 (9.7)	4 (5.1)	6 (6.7)	2 (2.5)	
Pulmonary Fibrosis	9 (9.3)	15 (18.1)	17 (16.8)	19 (21.3)	
Other	0 (0)	0 (0)	3 (3.4)	4 (5.1)	
Type of Transplantation - n (%)					*0.10*
Unilateral	18 (19.4)	14 (17.7)	20 (22.5)	24 (30.4)	
Bilateral	74 (79.6)	58 (73.4)	62 (69.7)	50 (63.6)	
Combined organ	1 (1.1)	7 (8.9)	7 (7.9)	5 (6.3)	
Cardiopulmonary Bypass - n (%)	69 (74.2)	48 (60.8)	53 (59.6)	34 (40.5)	*<0.001*
Immunosuppressive Regimen - n (%)					*0.001*
Cyclosporin	93 (100)	63 (79.7)	-	-	
Tacrolimus	-	16 (20.3)	38 (42.7)	-	
Tacrolimus-low	-	-	51 (57.3)	79 (100)	
History of Smoking - n (%)	54 (60.7)	42 (54.5)	47 (53.4)	49 (63.6)	*0.30*
Number of packyears	17 ± 14	17 ± 11	22 ± 14	26 ± 17	*0.001*
Hypertension - n (%)	8 (8.7)	12 (15.2)	23 (25.8)	21 (26.6)	*0.005*
Diabetes Mellitus - n (%)	9 (9.8)	6 (7.6)	7 (7.9)	10 (12.7)	*0.67*
Body Mass Index (BMI - kg/m^2)	21.8 ± 3.4	21.7 ± 3.4	22.1 ± 3.7	22.9 ± 4.1	*0.05*
Creatinine Excretion (mmol/24h)	11.0 ± 3.3	9.0 ± 4.3	8.8 ± 4.1	8.9 ± 3.0	*0.001*
Renal Function Measurements					
Pre-transplantation sCr - µmol/L	100 ± 23	96 ± 20	101 ± 21	97 ± 24	*0.12*
Estimated GFR (MDRD) - $ml/min/1.73m^2$	68 ± 20	68 ± 22	64 ± 18	73 ± 25	*0.38*
Estimated GFR (CKD-EPI) - $ml/min/1.73m^2$	76 ± 21	75 ± 22	71 ± 20	78 ± 23	0.79
Glomerular Filtration R ate (GFR) - $ml/min/1.73m^2$	100 ± 22	105 ± 26	94 ± 24	95 ± 25	*0.05*

Abbreviations: MDRD: Modification of diet in renal disease study equation, CKD-EPI: Chronic Kidney Disease–Epidemiology Collaboration equation.

Results

Baseline characteristics for the four consecutive transplant series are shown in table 1. Mean recipient age at transplantation increased from 42.9±11.1 yrs between 1990 and 1996 to 48.9±12.7 yrs between 2006 and 2008 (*P*=0.001). Primary diagnosis gradually shifted from α 1-antitrypsin deficiency as largest group towards chronic obstructive pulmonary disease and pulmonary fibrosis as largest groups (*P*=0.004). The proportion of other pulmonary diagnoses as well as the type of transplantations remained relatively constant over time. The use of cardiopulmonary bypass decreased over time. Importantly, CsA was replaced by Tac as the standard calcineurin inhibitor (CNI) in 2001.

Concerning renal risk factors, prevalence of hypertension before transplantation increased from 8±9% between 1990 and 1996 to 21±276% between 2006 and 2008 (*P*=0.005), and also the number of LTRs with a history of heavy smoking gradually increased over time, with average number of packyears of former smoking increasing from 17±14 yrs to 26±17 yrs (*P*=0.001). Prevalence of diabetes remained stable. Interestingly, recipient BMI increased in the consecutive series from 21.8±3.4 to 22.9±4.1 kg/m^2 (*P*=0.05) whereas 24-hour creatinine excretion, a measure of total muscle mass,[22] decreased in the consecutive series over time from 11.0±3.3 to 8.9±3.0 mmol/24h (*P*<0.001).

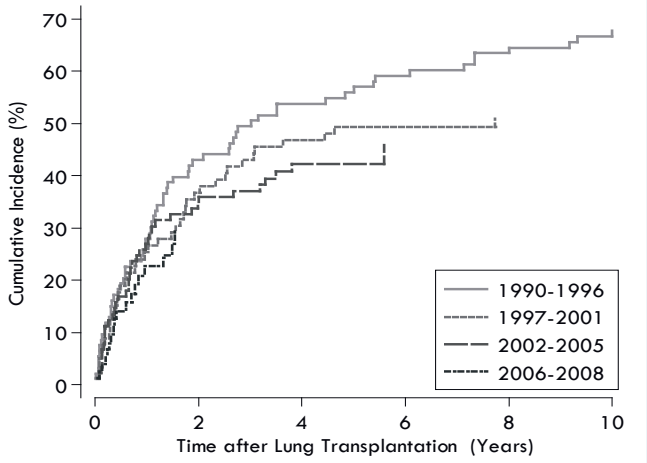
Baseline serum creatinine (SCr) and eGFR assessed by the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease–Epidemiology Collaboration (CKD-EPI) equations were not different between the consecutive series in time. However, the more accurate mGFR shows that both equations underestimate mGFR and that baseline renal function decreased in the consecutive series in time from 100±22 to 95±25 ml/min/1.73m^2 (*P*=0.05). The lower baseline mGFR over time corresponds to the higher age of the LTRs and the increased prevalence of renal risk factors. Survival and cumulative incidence of bronchiolitis obliterans syndrome were at least equal in the consecutive series of LTRs (data not shown).

The overall cumulative incidence of doubling of serum creatinine (DSC) was 27.6% at 1 year after LTx. For the four respective time-series, the cumulative incidence of DSC at 1 year after LTx was 28.0%, 26.6%, 27.0% and 22.7% (*P*=0.01). For the series 1990-1996, 1997-2001 and 2002-2005 overall cumulative incidence of DSC 48 months after LTx was 46.7% and the cumulative incidences for the respective series were 53.8, 46.8 and 42.2% (figure 1, *P*=0.01).

In univariable regression analysis, the most recent cohort (2006-2008) was associated with lowest risk for DSC (HR[95%CI]=0.48[0.29-0.82]), compared to the cohort 1990-1996 (reference) and series 1996-2001 and 2002-2005 were associated with intermediate

risk (HRs of 0.70[0.47-1.03], and 0.61[0.41-0.91] resp.) (table 2). In multivariable regression analyses with adjustment for age, sex, type of LTx, pulmonary diagnosis, use of cardiopulmonary bypass, hypertension, diabetes, packyears, serum creatinine, BMI and creatinine excretion, the HRs compared to cohort 1990-1996 were 0.62[0.34-1.15], 0.50[0.25-0.98] and 0.31[0.15-0.67] for the series of 1997-2001, 2002-2005 and 2006-2008 respectively.

Figure 1 | Cumulative incidence of doubling of serum creatinine per series of lung transplant recipients



Cumulative incidence of doubling of serum creatinine after lung transplantation for the consecutive time-series of lung transplant recipients. Numbers of patients still at risk for DSC at the various time points after LTx are indicated below the graph.

Table 2 | Cause-specific hazards regression for DSC

	Model 1	Model 2
Transplant Series	HR [95%CI]	HR (95%CI)
1990-1996	1.00 (reference)	1.00 (reference)
1997-2001	0.70 [0.47-1.03]	0.62 [0.33-1.13]
2002-2005	0.61 [0.41-0.91]	0.50 [0.25-0.98]
2006-2008	0.48 [0.29-0.82]	0.31 [0.15-0.67]

Model 1: Crude model. Model 2: Adjusted for potential confounders: Age, sex, pulmonary diagnosis, type of transplantation, hypertension, diabetes, packyears of former smoking, serum creatinine, BMI, creatinine excretion and use of cardiopulmonary bypass. HR: Hazard Ratio, CI: Confidence Interval.

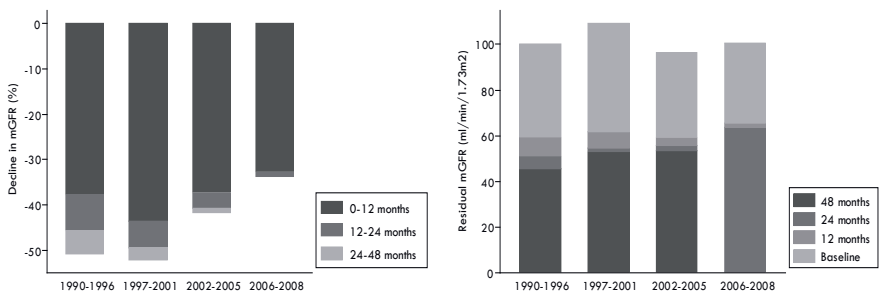
In a secondary analysis looking at the consecutive pharmacological eras (CsA, Tac and Tac-low respectively), respective cumulative incidences of DSC were 27.6, 27.8 and 23.0% (*P*=0.04) at 12 months after LTx, 41.0, 35.2 and 31.4% (*P*=0.04) at 24 months after LTx and 50.6, 44.4 and 39.1% at 48 months after LTx (*P*=0.04). In a multivariable adjusted Cox-regression analysis, HRs for DSC were 0.63[0.33-1.22] and 0.46[0.26-0.81] for Tac and Tac-low respectively, compared to CsA. We found no significant difference between low versus high dose Tac (HR=0.72 [0.36-1.45], *P*=0.36).

As a secondary outcome we analyzed decline in mGFR as well as residual mGFR at 24 and 48 months after LTx (48 months follow-up was not available for the cohort 2006-2008) (figure 2). For the consecutive transplant series mean decline in mGFR was 46, 49, 42 and 30% respectively (*P*=0.005) at 24 months after LTx and 51, 49 and 42% respectively (*P*=0.07) at 48 months after LTx. The long-term renal function decline seems to be less pronounced in the more recent transplant series (figure 2). For the four consecutive transplant series the mean residual mGFR was 51±17, 53±17, 57±21 and 63±21 ml/min/1.73m^2 respectively (*P*=0.002) at 24 months after LTx and 45±18, 52±18, 53±17 (*P*=0.02) at 48 months after LTx.

End-stage Renal Disease (ESRD)

A total of 16 patients progressed to ESRD. In the four consecutive time series, numbers were 11 (11.8% during 6.8[1.6-12.6] years of follow-up), 1 (1.3% during 7.1[0.8-9.0] years), 4 (4.5% during 4.1[3.0-5.5] years) and 0 (0% during 1.5[0.4-2.1] years) respectively. By default, follow-up is longest the earliest time series. Together with the low absolute number of ESRD, this renders it virtually impossible to perform meaningful statistical analyses and draw reliable conclusions on a possible change in the incidence of ESRD over time. We therefore refrained from analyses on ESRD for this manuscript.

Figure 2 | Decline in mGFR and residual mGFR after lung transplantation



Left panel: percentage mGFR decline from baseline for the stacked time-intervals 0-12 months, 12-24 months and 24-48 months, per series of LTRs. Right panel: Residual mGFR before transplantation and 12, 24 and 48 months after Lung Transplantation per series of LTRs. Follow-up of 48 months was not available for cohort 2006-2008.

Discussion

In this study we assessed the change in patient characteristics of consecutive series of LTRs between 1990 and 2008 in our center, and the course of renal function after LTx. First, patient characteristics changed considerably over time, resulting in a higher prevalence of risk factors in the more recently transplanted patients. Nevertheless, the two-year cumulative incidence of DSC decreased in the consecutive transplant series and the risk for DSC was lower in each consecutive series. Thus, renal outcome has ameliorated over time despite a worse renal risk profile at baseline. These results are further supported by the lower mGFR decline and higher residual mGFR at all time points after LTx in the more recent series of LTRs.

The overall cumulative incidences of DSC found in this study of 37% at 2 years after LTx and 47% at 4 years after LTx were in agreement with the cumulative incidence of 53% at 4 years after transplantation in a small study with 45 patients in Italy[21] and cumulative incidences of 43% at 2 years and 53% at 5 years after LTx in a larger study with 219 patients in the US.[4] The characteristics of our population compare well to the population in these two studies with respect to age, pulmonary diagnoses, BMI, prevalence of diabetes, and renal function at baseline. These data support the generalizability of our single center data to other populations. Yet, differences were also present, mainly the proportion of bilateral transplantations, the prevalence of hypertension and the use of Tacrolimus, which were all higher in our population.

The current results confirm our earlier observation of a non-linear decline in renal function over time, with a large initial drop that gradually levels off thereafter, in an early subgroup of our LTx cohort.[23] Despite the decreasing incidence in DSC over time, the marked non-linearity of the decline was present in all patient series (figure 1). Interestingly, we found no difference in baseline GFR estimated with the MDRD and CKD-EPI equation, whereas a difference was observed in baseline mGFR measured with ^{125}I-Iothalamate. In addition, both equations substantially underestimated GFR. The discrepancy between eGFR and mGFR may relate to the observed difference in creatinine excretion, which is a measure of muscle mass. This again stresses the limitations of creatinine-based GFR estimation in a population as LTRs with large variation in muscle mass and muscle mass changes after LTx.[24] Despite the lower baseline GFR in the consecutive series, we did not only find a significantly lower percentage of GFR decline over time, but also a higher residual GFR during follow-up in the more recent series.

One of the factors that has likely played a role in the amelioration of renal function impairment after LTx over time is the switch from CsA to Tac as standard calcineurin inhibitor in 2001. It has previously been shown in other solid organ transplant populations

that Tac, in the dosages used, was less nephrotoxic than CsA.[2] In LTRs, results of studies comparing nephrotoxicity of CsA and Tac have been inconclusive, possibly due to these weaknesses in study design. One study defined CNI use use during >80% of the post-transplant course, and others looked at initial CNI regimen.[2,10,11] These study designs rendered the studies susceptible to bias because deterioration of renal function is often one of the reasons for change of one CNI-regimen into another.[25] Our findings provide support for the assumption that also in LTRs Tac could be less detrimental on renal function than CsA.

Other factors that may have influenced the reduced incidence of renal function impairment are the evolvement of immunosuppressive regimens, improved surgical techniques and post-operative management, increased awareness about renal function impairment and increased experience in the management of LTRs and renal function impairment in LTRs.

The increased awareness about renal function impairment may also be exemplified by how use of CNIs and blood pressure management evolved. During the era in which cyclosporine was used as CNI, targeted levels were maintained, even in case of worsening renal function. When, however, Tacrolimus appeared to be a more potent CNI, gradually lower trough levels were accepted in case of worsening renal function. For blood pressure management, parallel changes occurred. In the early 90's the targeted blood pressure was <160/95, and this was gradually lowered towards the contemporary target of <140/90. In addition, use of ACE-inhibitors in case of worsening renal function increased over time, which are currently first choice antihypertensive agents.

A limitation of this study was that it was an observational and single-center analysis. This could limit the generalizability of our finding to other LTx populations. Nevertheless, the similarity with previously published literature on cumulative incidence of DSC is remarkable. Another limitation of this study is the use of a creatinine-based endpoint. However, DSC is the most frequently used endpoint in research on this topic, facilitating comparison between studies.[11,19] Our results are strengthened by the parallel findings with the gold standard ^{125}I-Iothalamate GFR measurements.[13] Another strength of this study was the large and well-characterized cohort of LTRs, including all patients transplanted in our center since the start of the program, without any loss to follow-up.

Conclusions

Lung transplant recipient characteristics changed over the last two decades, with higher prevalence of renal risk factors and worse overall condition of lung transplant recipients. Yet, prevalence of renal function loss decreased across consecutive series of a large

cohort of lung transplant recipients. Despite the improvements in overall renal outcome, impaired renal function remains an important complication after lung transplantation and exposition of the lung transplant recipients to unnecessary additional renal risks remains to be avoided as much as possible.

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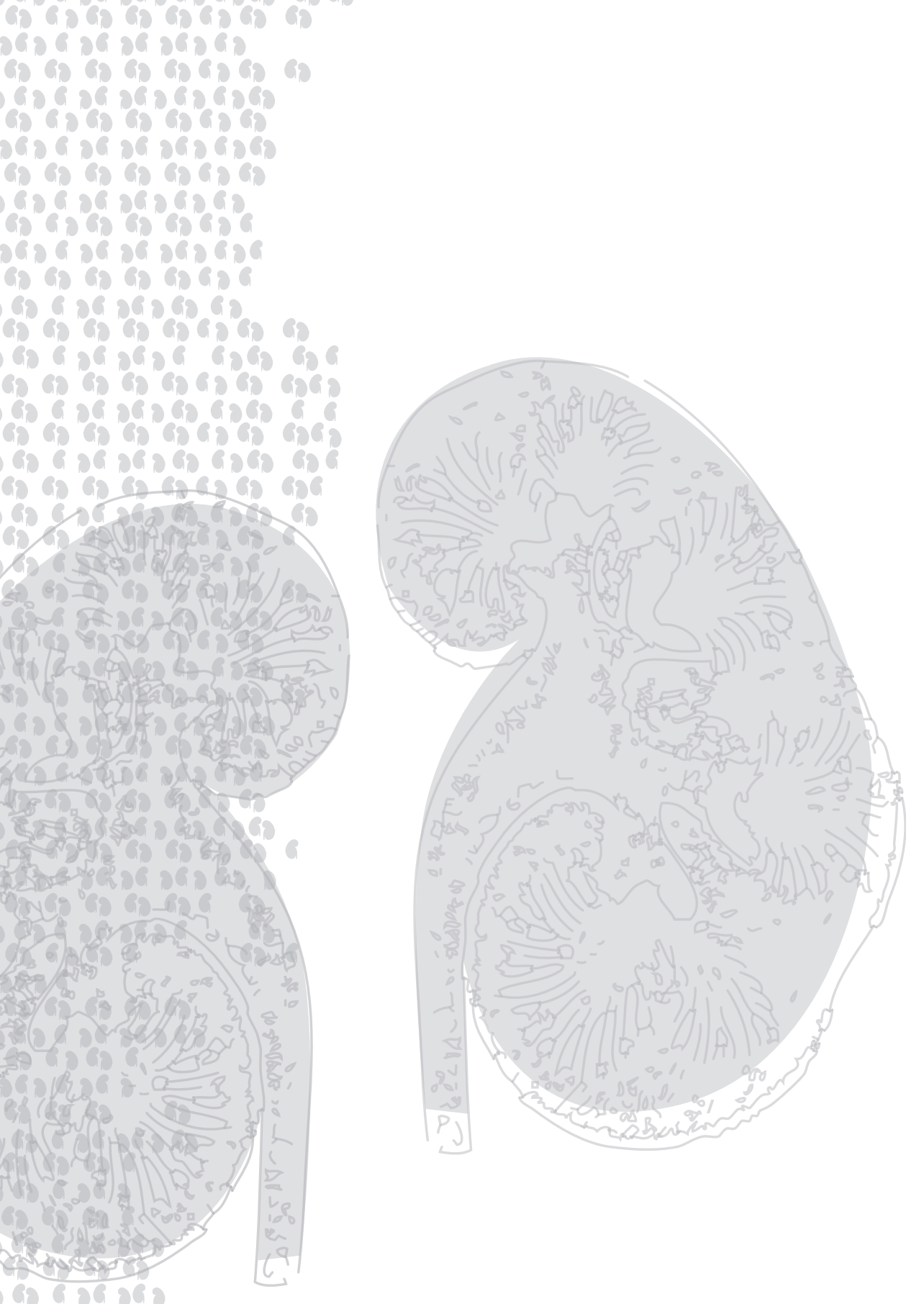

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Former Smoking is a Risk Factor for Chronic Kidney Disease after Lung Transplantation

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Abstract

Background: Chronic kidney disease (CKD) is a common complication after lung transplantation (LTx). Smoking is a risk factor for many diseases, including chronic kidney disease (CKD). Smoking cessation for >6 months is required for LTx-enlistment. However, the impact of smoking history on CKD development after LTx remains unclear. We investigated the effect of former smoking on CKD and mortality after LTx.

Methods: CKD was based on glomerular filtration rate (GFR) (^{125}I-iothalamate-measurements). GFR was measured before and repeatedly after LTx.

Results: 134 recipients never smoked and 192 recipients previously smoked for a median of 17.5 pack years. At 5 yrs after LTx, overall cumulative incidences of CKD-III, CKD-IV and death were 68.5, 16.3 and 34.6% respectively. Compared to never smokers, former smokers had a higher risk for CKD-III (hazard ratio (HR) - 95%-confidence interval [95%CI]=1.69 [1.27-2.24]) and CKD-IV (HR=1.90 [1.11-3.27]), but not for mortality (HR=0.99[0.71-1.38]). Adjustment for potential confounders did not change results.

Conclusions: Despite cessation, smoking history remained a risk factor for CKD in LTx recipients. Considering the increasing acceptance for LTx of older recipients with lower baseline renal function and an extensive smoking history, our data suggest that the problem of post-LTx CKD may increase in the future.

Introduction

Chronic kidney disease (CKD) is a serious complication after lung transplantation (LTx). In most LTx-recipients renal function deteriorates progressively, often resulting in CKD.[1] Progression from CKD to end-stage renal disease (ESRD) currently develops in between 3 and 10% of LTx recipients.[2-4] Recipient age and life expectancy after LTx are increasing, so it is likely that the a priori risk for CKD after LTx is increasing concomitantly, with possible consequences for the number of LTx recipients that develop ESRD on the long term.[5]
Approximately 60% of LTx recipients have a history of smoking.[6] This is the highest rate reported among recipients of solid organs: former smokers account for 51-54% of renal-transplant recipients,[7,8] 42-50% of liver-transplant recipients,[9] and 45% of heart-transplant recipients.[5] Moreover, many former smokers amongst LTx recipients smoked heavily, as around 40-45% of them undergo LTx because of end-stage pulmonary emphysema, which is largely attributable to heavy smoking.[6] Pulmonary emphysema, cardiovascular disease and lung cancer are well-known complications of smoking.[10-12] It may be less well-known that smoking is also a risk factor for CKD.[13, 14] We wondered whether, even after smoking cessation, smoking history could be relevant for morbidity after LTx, in particular CKD, because of the high prevalence of both CKD and *former* smoking in LTx recipients.
Transplant centers, including our own, commonly require that patients have stopped smoking for at least six months before being enlisted for LTx.[15,16] In the current study, we analyzed the potential association between smoking history before LTx and development of CKD after LTx in a large single center cohort of LTx recipients. In addition, we analyzed the impact of past smoking on mortality and causes of death after LTx.

Patients and methods

A total of 370 lung transplantations, of which 22 heart-lung transplantations were performed at the University Medical Center of Groningen (UMCG), the Netherlands between 1990 and 2008. Pediatric transplantations (n=17) and re-transplantations (n=13) were excluded. Quantitative data on smoking history were obtained from patient records. We excluded 14 patients of the remaining 340 patients from further analyses because of lack of data on smoking history, leaving a total of 326 patients eligible for evaluation. Further clinical information of all individuals was gathered at baseline and during follow-up. Consent for the use of patient data was obtained from all patients prior to transplantation.

Smoking history

Smoking cessation for at least 6 months is a requirement for LTx enlistment. Returning to active smoking after LTx is very strongly disapproved and occurs, to the best of our knowledge, only exceptionally. Smoking history prior to transplantation was quantified by number of pack years. One pack year is defined as 20 cigarettes (one pack) smoked per day for one year. Patients were categorized into never and former smokers and the latter were further categorized according to number of pack years: 1-10, 11-25 and >25 pack years.

GFR measurements

Glomerular filtration rate (GFR) measurements were performed by constant infusion of radiolabelled tracers ^{125}I-Iothalamate and ^{131}I-Hippuran as described before.[17-19] This method is considered a gold standard method for GFR measurement, has a day-to-day coefficient of variation of 2.5%[19] and is used for the development and validation of novel renal function equations.[20] Height and weight were measured in every patient before every GFR measurement. Body surface area (BSA) was calculated as $0.007184 * \text{weight}^{0.425} * \text{length}^{0.725}$,[21] and GFR was expressed per 1.73 m^2 of BSA. GFR measurements were performed routinely at outpatient basis, before transplantation and at regular time-intervals after transplantation.

Endpoints of the study

Primary endpoints of the study were CKD-III and CKD-IV. CKD-III and CKD-IV were defined as GFR<60 ml/min/1.73m^2, and GFR<30 ml/min/1.73m^2 respectively, according to the cut-off values in the NKF K/DOQI guidelines.[22]

We used GFR measurements by radiolabelled Iothalamate for the definition of CKD because in LTx recipients creatinine-based GFR estimations are biased by the large variation in muscle mass.[23] For the primary analyses, definition of CKD was based on the first GFR measurement below the cut-off values defining the stages of CKD. We did this because beyond 2 years after LTx we measured GFR with 2-year intervals. Consequently, a proportion of patients with one measurement below the CKD-threshold lacked follow-up until a confirmatory measurement. Defining CKD based on at least 2 measurements below the CKD-threshold would result in underestimation of true cumulative incidence of CKD. Moreover, in patients that had a GFR measurement below the cut-off, only 2.4% the second GFR measurements were inconsistent with the former measurements. This assured us that use of this definition for endpoints was appropriate. However, to consent with the NKF K/DOQI guidelines that indicate CKD should be confirmed by a second measurement >90 days later, we performed secondary analyses with the endpoints defined as such. We furthermore performed a secondary analysis with doubling of serum

creatinine (DSC) as alternative renal endpoint to check consistency across renal endpoints. DSC was defined as a doubling of serum creatinine, confirmed by a similar value >90 days after the initial doubling.

ESRD was defined as the requirement of renal replacement therapy. We recorded occurrence of ESRD and death until January 2010. Causes of death were coded according to the International Classification of Diseases (ICD-9).

Immunosuppressive Regimens

Immunosuppressive protocols used over time are described in more detail elsewhere.[24] In short, maintenance immunosuppression consists of a calcineurin inhibitor (CNI) based immunosuppressive regimen, combined with steroids and azathioprine (Immuran; GlaxoSmithKline, Brentford, United Kingdom). Cyclosporine (Neoral; Novartis, Basel, Switzerland) was the CNI used until 2001. The targeted whole blood trough level of CsA was initially 400 µg/L, tapering to 150 µg/L within the first three weeks. Tacrolimus (Prograft; Astellas, Staines, United Kingdom) was the CNI used from 2001 onwards. Between 2001-2004 target trough levels were 20 µg/L during the first three weeks, 15 µg/L until the third month and 10-12 µg/l thereafter. Lower peri-operative tacrolimus trough levels were targeted from 2004: 15-18 µg/L during the first three weeks, 12-15 µg/L until the third month, and 10-12 µg/L thereafter. We further decreased target levels to 8-10 µg/L in the case of EBV-reactivation.[24]

Prednisolone dose was 0.2 mg/kg/d until the third month and 0.1 mg/kg/d thereafter. Azathioprine dose was 1.5-3 mg/kg/d. Induction consisted of ATG (Thymoglobulin; Sanofi-Pasteur, Lyon, France) until 2001 and Basiliximab induction thereafter (Simulect; Novartis, Basel, Switzerland). We switched some patients from Azathioprine to Mycophenolate Mofetil (Cellcept; Roche, Basel, Switzerland). Patients received P. Jiroveci prophylaxis, Herpes prophylaxis and CMV-prophylaxis (at-risk patients only, from 2001 onwards).

Statistical analysis

Data were analyzed with SPSS version 18.0 (IMB, New York, USA), STATA version 11.2 (STATACorp LP, Texas, USA) and R version 2.12.0 (CRAN, Wien, Austria). The data were obtained from pre-existent databases and from patient chart review. Categorical variables are reported as frequencies and percentages. Variables with normal distribution are presented as mean with standard deviation (SD) and variables with a skewed distribution are presented as median with interquartile range [IQR].

Recipient characteristics are shown according to categories of smoking habits prior to transplantation. We divided patients into never smokers and former smokers and subdivided the latter category into three categories according to number of pack

years. Differences between groups were tested by one-way ANOVA for linear effects for continuous variables and Chi Squared-test for categorical variables. We performed adjustment for multiple comparisons over time using Bonferroni correction.

CKD-III, CKD-IV and all-cause mortality were endpoints in this study. Cumulative incidences of CKD-III and CKD-IV were calculated for never smokers and groups of former smokers using competing risk analysis. Differences between smoking exposure groups were tested with Gray's test for competing risks data.[25,26] We calculated population attributable risk as (proportion exposed*((adjusted hazard ratio-1)/adjusted hazard ratio)*100%.[27] The association of former smoking with CKD-III and IV was modeled with Cox cause-specific hazards regression,[28] and the association with mortality with standard Cox proportional hazards regression. Variables that we selected as covariates in multivariable analyses were patient demographics, variables that were different between the smoking exposure groups at baseline and variables previously found to be risk factors with renal risk after LTx.[29,30] Linearity of continuous covariates was assessed using multivariable fractional polynomials. Optimal model-fit for GFR was obtained by a 1000/GFR transformation, whereas age, BMI and pack years were either best fitted in a linear non-transformed way or did not contribute to the model. We therefore used a 1000/GFR-transformation of GFR in the analyses. To assess dose-dependency, the number of pack years was analyzed as continuous variable.

We performed secondary analyses without the never smokers and stratified for age (< 47 and ≥ 47 years, population median) and for diagnosis (COPD vs. non-COPD) to rule out that (dose) effects were solely based on the differences in characteristics of never smokers and former smokers. Moreover, in further secondary analyses, we repeated the multivariable analyses with CKD-III and CKD-IV confirmed by a second measurement >90 days later and with doubling of serum creatinine, also confirmed by a second measurement >90 days later as alternative renal endpoints. Lastly, as body dimensions may change substantially after LTx we performed a secondary analysis with body surface area as time-dependent variable. Possible interactions between variables were also assessed. Hazard ratios (HR) are reported with 95% confidence interval [95%CI]. Final results were considered significant at a level <0.05.

Table 1 | Baseline patient characteristics of never smokers and former smokers subdivided in categories according to number of pack years

	Never smokers	Former smokers			
	0 Pack years	1-10 Pack years	11-25 Pack years	>25 Pack years	*P-value*
Number of Patients	134	62	74	56	
Pack Years	0 ± 0	6.5 ± 3.0	18.1 ± 4.7	38.7 ± 12.1	
Male Sex - n, %	73 (54.3)	26 (41.9)	40 (54.1)	27 (48.2)	*0.37*
Recipient Age* - years	36.8 ± 12.5	48.5 ± 8.2	50.7 ± 6.9	55.1 ± 5.6	*<0.001*
Race					*0.88*
Whites	131 (99.3)	62 (100)	73 (98.6)	56 (100)	
Blacks	3 (0.7)	0 (0)	1 (1.4)	0 (0)	
Glomerular Filtration Rate - mL/min/1.73m²	108 ± 26	98 ± 18	101 ± 15	93 ± 17	*0.04*
Pre-transplantation SCr - µmol/L	77 ± 19	80 ± 18	79 ± 15	79 ± 16	*0.68*
Body Mass Index (BMI) - kg/m²	21.0 ± 3.5	22.1 ± 3.6	23.3 ± 4.0	23.2 ± 3.2	*<0.001*
Hypertension - n, %	26 (19.5)	15 (24.2)	13 (17.6)	8 (14.3)	*0.59*
Diabetes Mellitus - n, %	23 (17.3)	2 (3.2)	4 (5.4)	2 (3.6)	*0.002*
Pulmonary Diagnosis - n, %					*<0.001*
COPD	4 (3.0)	10 (16.1)	25 (33.8)	39 (69.6)	
α1-antitrypsin-deficiency (AAT)	2 (1.5)	23 (37.1)	31 (41.9)	10 (17.9)	
Cystic Fibrosis (CF)	61 (45.5)	3 (4.8)	1 (1.4)	0 (0)	
Pulmonary Hypertension (PH)	20 (14.9)	9 (14.6)	4 (5.4)	3 (5.4)	
Pulmonary Fibrosis (PF)	28 (20.9)	12 (19.4)	10 (13.5)	4 (7.1)	
Other	19 (14.1)	5 (8.1)	3 (4.1)	0 (0)	
Type of Transplantation - n, %					*<0.001*
Unilateral	21 (15.7)	14 (22.6)	15 (20.3)	19 (33.9)	
Bilateral	100 (74.6)	46 (74.2)	57 (77.0)	36 (64.3)	
Combined organ	13 (9.7)	2 (3.2)	2 (2.7)	1 (1.8)	
Immunosuppressive Regimen - n, %					*0.045*
CsA-based	57 (42.5)	35 (56.5)	39 (52.7)	16 (28.6)	
Tac-based	77 (57.5)	27 (43.5)	35 (47.3)	40 (71.4)	
Transplant Series - n, %					*0.043*
1990-1996	23 (17.2)	10 (16.1)	10 (13.5)	5 (8.9)	
1997-2000	34 (25.4)	24 (38.7)	24 (32.4)	11 (19.6)	
2001-2004	37 (27.6)	16 (25.8)	15 (20.3)	12 (21.4)	
2005-2008	40 (29.9)	12 (29.7)	22 (29.7)	28 (50)	

Recipient age at time of transplantation. **mL/min/1.73m2). sCr: serum creatinine, COPD: Chronic obstructive pulmonary disease, CsA: Cyclosporin, Tac: Tacrolimus. Differences between groups were tested by one-way ANOVA for linear effects.

Results

Quantitative data on previous history of smoking were available in 326 (96%) patients: 134 (41.1%) patients had never smoked and 192 (58.9%) patients had smoked a median [IQR] of 17.5 [10-30] pack years. Baseline characteristics of never smokers and former smokers stratified for number of pack years are shown in table 1.

Overall, the former smokers were older, had higher BMI, more often had pulmonary diagnoses chronic obstructive pulmonary disease (COPD) or α1-antitrypsine-deficiency (as opposed to cystic fibrosis, pulmonary fibrosis or pulmonary hypertension in never smokers), more often underwent unilateral lung transplantation and had lower GFR before transplantation. Comparing subgroups of former smokers the patients with higher numbers of pack years were found to be older, were more often transplanted for COPD and more often received Tacrolimus-based maintenance immunosuppression. A relatively large proportion of heavy former smokers was transplanted in the most recent stratum. This reflects recent changes in acceptance policy for LTx enlistment. Other characteristics were similar for the smoking exposure groups. Cyclosporine and Tacrolimus trough levels were similar for never smokers and all groups of former smokers in all time-intervals during follow-up after transplantation (table 2).

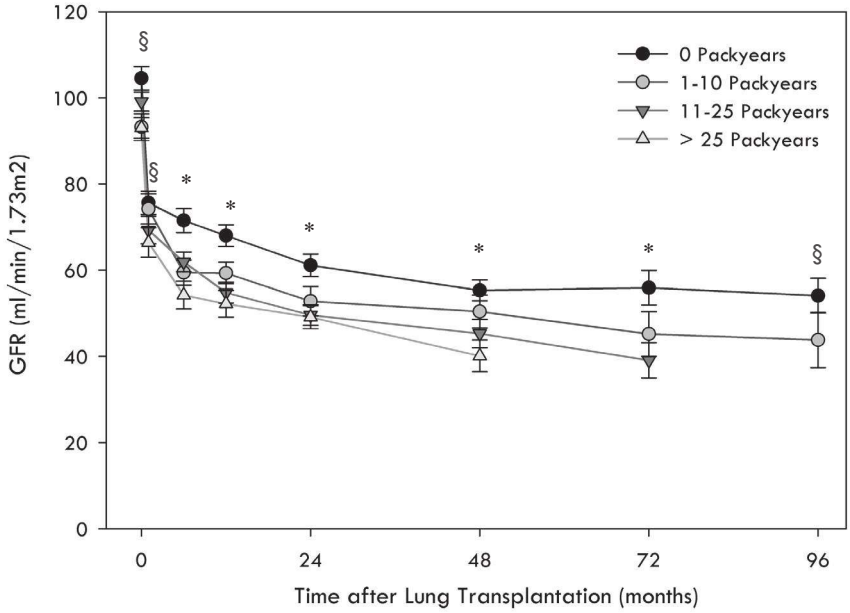
Mean baseline GFR was 102±22 ml/min/1.73m^2. Baseline GFR was higher in never smokers than in former smokers, but this difference did not persist after adjustment of GFR for recipient age. Renal function declined considerably after LTx, on average -43±24% within 2 years after LTx. The decline was particularly steep in the first 6 months after LTx, slowly leveling off beyond 12 months after LTx (figure 1).

Table 2| Median Cyclosporin and Tacrolimus whole blood trough levels (ng/mL) per time interval after lung transplantation according to groups based on number of pack years

Cyclosporin (ng/mL)		**0-1 month**	***N***	**1-6 months**	***N***	**6-24 months**	***N***	**>24 months**	***N***
Never smokers	0 Pack years	293 ± 53	*56*	195 ± 56	*53*	177 ± 41	*45*	182 ± 28	*39*
Former smokers	1-10 Pack years	299 ± 40	*34*	175 ± 25	*32*	166 ± 30	*27*	170 ± 28	*25*
	11-25 Pack years	294 ± 43	*37*	180 ± 25	*31*	175 ± 34	*28*	165 ± 45	*25*
	>25 Pack years	280 ± 35	*16*	180 ± 28	*16*	172 ± 22	*15*	172 ±17	*10*
Tacrolimus (ng/mL)									
Never smokers	0 Pack years	14.9 ± 2.8	*71*	10.9 ± 2.3	*55*	10.2 ± 1.7	*55*	9.8 ± 1.3	*40*
Former smokers	1-10 Pack years	14.8 ± 2.0	*26*	10.8 ± 1.6	*22*	10.7 ± 2.0	*21*	10.1 ± 1.4	*14*
	11-25 Pack years	15.2 ± 2.1	*33*	11.4 ± 1.9	*32*	10.7 ± 2.3	*31*	10.0 ± 1.8	*18*
	>25 Pack years	14.3 ± 1.9	*35*	10.7 ± 1.7	*29*	10.3 ± 1.5	*28*	9.8 ± 1.2	*20*

P > 0.05 for Cyclosporin and Tacrolimus trough levels in never smokers and groups of former smokers at all time-intervals after lung transplantation. Differences between groups were tested by one-way ANOVA for linear effects.

Figure 1 | Glomerular filtration rate after lung transplantation according to number of pack years



Number of Measurements							
0 Pack years	118	83	83	68	50	28	19
1-10 Pack years	56	47	47	32	27	16	10
11-25 Pack years	67	54	58	39	28	20	<10#
>25 Pack years	51	41	39	38	30	<10#	<10#

Means with standard error of GFR relative to body-surface-area per group according to groups based on number of pack years (0 Pack years, 1-10 Pack years, 11-25 Pack years, >25 Pack years). Differences between groups were tested by one-way ANOVA for linear effects. §P<0.05, NS after Bonferroni correction, *P<0.006, significant after Bonferroni correction # Mean estimates not reported for <10 patients.

The slope of GFR decline in the first year after LTx was steeper in former smokers than in never smokers (-34±20 and -25±18% resp., *P*=0.005). Former smokers generally had a significantly lower mean GFR than never smokers from 6 months after LTx onwards. The former smokers with a history of >25 pack years of smoking had lowest mean GFR at all time points.

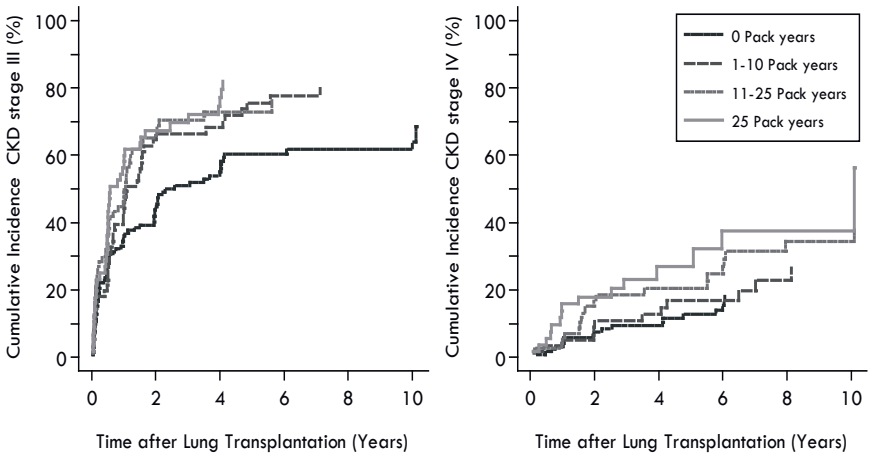
The overall cumulative incidence of CKD-III at 5 years after LTx, taking competing risk of death into account, was 68.5%, with a cumulative incidence of 58.2% in never smokers vs. 74.4% in former smokers (*P*<0.001). This cumulative incidence was 72.6%, 74.7% and 75.8% (*P*<0.001) in former smokers with a history of 1-10, 11-25 and >25 pack years of smoking, respectively. The overall cumulative incidence of CKD-IV at 5 years after LTx, taking competing risk of death into account, was 16.3% with a cumulative incidence of 10.7% in never smokers vs. 20.2% in former smokers (*P*=0.004). This cumulative incidence of CKD-

IV at 5 years after LTx was 15.0%, 20.4% and 26.9% (*P*=0.004) in former smokers with a history of 1-10, 11-25 and >25 pack years of smoking, respectively.

Fifteen (5% of total) patients (mean age at LTx 38±13 years) progressed to ESRD at a mean follow-up of 7.2±4.1 years. Of those patients, 7 had COPD and 7 had cystic fibrosis. Current average follow-up of patients alive is 5.3±4.2 years; hence, the proportion of ESRD might increase with longer follow-up. We refrained from further (multivariable) analyses on determinants of ESRD, because of the small number of patients with ESRD.

In univariable Cox proportional Hazard analyses, former smokers had an increased risk of CKD-III (HR [95%Confidence Interval(CI)]=1.69 [1.27–2.24], *P*=0.001) and CKD-IV (HR=1.90 1.11-3.27], *P*=0.02) compared to never smokers. Among the former smokers, there was an

Figure 2| Cumulative incidences of CKD-III and CKD-IV according to number of pack years



Cumulative incidences of CKD-III (left, (P<0.001)) and CKD-IV (right, (P<0.001)) according to groups based on number of pack years (0 Pack years, 1-10 Pack years, 11-25 Pack years, >25 Pack years). Differences between groups were tested with grays test for competing risks data. *Per 10 pack years. All analyses are multivariable cause-specific hazards regression analyses for pack years and CKD-III and CKD-IV.

increase in risk of CKD-III per group according to number of pack years, with HRs of 1.57 [1.09-2.25], *P*=0.015; 1.65 [1.15-2.36], *P*=0.006 and 1.92 [1.32-2.79], *P*=0.001 for patients with a history of 1-10, 11-25 and >25 pack years of smoking respectively, compared to never smokers. The same was true for CKD-IV, with HRs of 1.30 [0.63-2.70], *P*=0.48; 1.96 [1.04-3.71], *P*=0.04 and 2.78 [1.41-5.46], *P*=0.003 for the respective categories of former smokers compared to never smokers (Figure 2).

In analyses with pack years of smoking as a continuous variable, the HRs per 10 pack years of smoking for CKD-III and-IV were 1.19 [1.09–1.28], *P*<0.001 and 1.40 [1.20-1.62], *P*<0.001 (table 3). In the multivariable analyses this dose-dependent relation remained significant for both CKD-III (HR 1.25 [1.10–1.42], *P*<0.001) and CKD-IV (HR 1.46 [1.17–1.83], *P*<0.001) (table 3). The corresponding adjusted population-attributable risks of former smoking (i.e. the CKD-rate reduction if all patients would have been never smokers) were 11.8% for CKD-III and 18.6% for CKD-IV.

In secondary analyses, the dose-dependent increase in renal risk persisted after exclusion of the never smokers from the analysis (table 4). Results of the analyses stratification by age (<47 and ≥47 years, population median) and diagnosis (COPD vs. non-COPD) are also displayed in table 4. As can be seen, hazard ratios were relatively high in patients < 47 years and in patients with diagnoses other than COPD. To investigate whether the risk was different between stratification groups, we tested for interaction between strata and pack years in the fully adjusted model. There was no significant interaction between age-strata and pack years for CKD stage III (*P*=0.92) and CKD stage IV (*P*=0.81). For diagnosis strata, there was a trend towards interaction for CKD stage III (*P*=0.18) and significant interaction for CKD stage IV (*P*=0.04). These data are consistent with pack years of smoking being a risk factor for CKD both in patients with COPD and in patients without COPD, but highest risk in patients without COPD as primary diagnosis. As a total, the results are consistent and point towards former smoking increasing the risk for CKD.

We found that if analyses were repeated with CKD confirmed by a second value overall, cumulative incidences of CKD-III and CKD-IV after 5 years of follow-up were lower, with cumulative incidences of 57.8 and 10.4% for CKD-III and CKD IV respectively. The results of analyses of smoking as a risk factor for CKD remained essentially unchanged though, with

Table 4 | Secondary analyses stratified by age and pulmonary diagnosis and in former smokers only

		CKD stage III		CKD stage IV	
		HR [95%CI]	*P-value*	HR [95%CI]	*P-value*
Stratified by age					
< 47 years	Pack years*	1.30 [0.88-1.93]	0.19	2.31 [1.01-5.32]	0.05
> 47 years	Pack years*	1.21 [1.05-1.40]	0.008	1.36 [1.07-1.72]	0.04
Statified by diagnosis					
COPD	Pack years*	1.18 [1.02-1.38]	0.03	1.36 [1.07-1.72]	0.01
other than COPD	Pack years*	1.50 [1.11-2.01]	0.008	2.20 [1.17-3.97]	0.01
Former smokers only	Pack years*	1.23 [1.06-1.42]	0.005	1.42 [1.11-1.81]	0.005

*Per 10 pack years. All analyses are multivariable cause-specific hazards regression analyses for pack years and CKD-III and CKD-IV.

Table 3 | Univariable and multivariable cause-specific hazards for CKD-III and CKD-IV

Characteristic	CKD stage III						CKD stage IV					
	Univariable			Multivariable			Univariable			Multivariable		
	HR	[95% CI]	P-value	HR	[95% CI]	P-value	HR	[95% CI]	P-value	HR	[95% CI]	P-value
Pack years	1.19	[1.09-1.28]	<0.001	1.25	[1.10-1.42]	<0.001	1.40	[1.20-1.62]	<0.001	1.46	[1.17-1.83]	<0.001
Male sex	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-
Female sex	0.87	[0.77-0.99]	0.04	0.88	[0.75-1.03]	0.12	1.11	[0.87-1.41]	0.41	1.06	[0.80-1.40]	0.69
Age - years	1.00	[0.99-1.00]	0.97	1.02	[1.00-1.05]	0.03	1.04	[1.01-1.06]	0.005	1.02	[0.98-1.06]	0.47
GFR* - ml/min/1.73m2	1.04	[1.00-1.08]	0.01	1.05	[1.00-1.11]	0.04	1.07	[1.00-1.15]	0.05	1.15	[1.03-1.28]	0.01
BMI - kg/m2	1.03	[0.99-1.07]	0.07	0.97	[0.92-1.02]	0.19	1.05	[0.99-1.11]	0.13	1.01	[0.93-1.09]	0.88
Hypertension	1.16	[0.83-1.61]	0.38	1.03	[0.65-1.63]	0.89	1.35	[0.74-2.44]	0.33	1.37	[0.58-3.27]	0.64
Diabetes Mellitus	0.64	[0.39-1.04]	0.07	0.69	[0.38-1.25]]	0.22	0.58	[0.21-1.58]	0.28	0.64	[0.19-2.20]	0.48
Pulmonary diagnosis												
COPD	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-
α1-antitrypsin-deficiency	0.69	[0.47-1.01]	0.06	0.97	[0.62-1.53]	0.90	0.57	[0.30-1.08]	0.08	0.74	[0.35-1.57]	0.44
Cystic Fibrosis	0.44	[0.29-0.68]	0.001	1.31	[0.63-2.73]	0.47	0.34	[0.15-0.75]	0.008	1.48	[0.41-5.44]	0.55
Pulmonary Hypertension	0.73	[0.46-1.16]	0.18	1.24	[0.62-2.47]	0.54	0.42	[0.16-1.12]	0.08	0.39	[0.09-1.63]	0.19
Pulmonary Fibrosis	0.96	[0.63-1.45]	0.84	1.56	[0.83-2.95]	0.17	0.50	[0.21-1.16]	0.11	0.64	[0.17-2.49]	0.52
Other	0.78	[0.46-1.32]	0.36	1.53	[0.81-2.90]	0.19	0.94	[0.42-2.10]	0.87	1.96	[0.72-5.32]	0.19
Type of transplantation												
Unilateral	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-
Bilateral or combined organ	1.01	[0.74-1.39]	0.93	1.63	[1.08-2.46]	0.02	1.90	[0.91-4.00]	0.09	3.21	[1.24-8.33]	0.02
Immunosuppressive regimen												
CsA-based	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-	1.00	(reference)	-
Tac-based	0.93	[0.64-1.35]	0.69	0.82	[0.44-1.51]	0.51	0.83	[0.49-1.39]	0.47	0.83	[0.29-2.41]	0.73
Transplantation year	1.02	[0.99-1.05[	0.19	1.01	[0.96-1.06]	0.81	0.98	[0.93-1.03]	0.42	0.96	[0.86-1.07]	0.49

*(1000/(GFR)) GFR: Glomerular Filtration Rate, BMI: Body mass index, COPD: Chronic obstructive pulmonary disease, CsA: cyclosporin, Tac: Tacrolimus.

HRs of 1.17 [1.03–1.33], *P*=0.02 and 1.63 [1.25-2.13], *P*<0.001 per 10 pack years of smoking for CKD-III and CKD-IV respectively in the fully adjusted model. Likewise, former smoking was dose-dependently associated with doubling of serum creatinine as renal endpoint, with a HR of 1.28 [1.10-1.49], *P*=0.002 per 10 pack years in the fully adjusted model. Lastly, we performed multivariable analyses with body surface area as a time-dependent variable, yielding HRs of 1.22 [1.08-1.39], *P*=0.002 for CKD-III and 1.48 [1.18-1.85], *P*=0.001 for CKD-IV.

The overall cumulative incidence of all-cause mortality at 5 years after LTx was 34.6%, with a cumulative incidence of 32.9% never smokers vs. 37.2% in former smokers (*P*=0.4). In univariable Cox-regression analysis, former smokers' risk of mortality was similar to that of never smokers (HR = 0.99 [0.71–1.38], *P*=0.9). We found that the main causes of death were graft failure (34.2%, after mean 3.8±3.4 years) and malignancy (17.1%, after a mean of 5.0±4.0 years). Only a small proportion of patients died of cardiovascular causes (6.2%, after a mean of 4.6±4.6 years). Former smokers more often died of malignancy than never smokers did (23% vs. 8.5% resp., *P*=0.03). There were no other significant differences in causes of death between former smokers and never smokers (Table 5).

Table 5 | Causes of death after lung transplantation according to number of pack years

Cause of death	Total N (%)	Never smokers N (%)	Former smokers N (%)	*P-value*
Peri-operative death	17 (11.8)	8 (13.6)	11 (12.6)	
Multi-organ failure	12 (8.3)	8 (13.6)	4 (4.6)	
Infection	19 (13.2)	7 (11.9)	12 (13.8)	
Cardiovascular	9 (6.3)	2 (3.4)	7 (8.0)	
Malignancy	25 (17.4)	5 (8.5)	20 (23)	0.03
Transplant failure	50 (34.7)	25 (42.4)	25 (28.7)	
Other	12 (8.3)	4 (6.8)	8 (9.2)	

Discussion

This study shows for the first time that former smoking is associated with increased risk of CKD-III and CKD-IV after LTx. We found the increased risk of former smoking on CKD to be independent of potential confounders, including baseline GFR, recipient age and pulmonary diagnosis. The association between former smoking and CKD was further supported by evidence of dose-dependency.

The difference in renal function between never and former smokers starts early after transplantation. Whereas both never smokers and former smokers had a steep initial decline in GFR, the initial decline in GFR was more pronounced in former smokers, and even more marked in former smokers with higher number of pack years. After the early rapid decrease in GFR, the rate of decline gradually leveled-off over time, but differences between groups clearly persisted.

Our results suggest that former smoking primes the kidneys for accelerated decline in GFR after LTx. The renal susceptibility to accelerated decline in GFR was not accounted for by adjustment for pre-transplant GFR. Nevertheless, it remains possible that former smokers have pre-existent renal damage. This is not necessarily captured by GFR as kidneys are known to have a certain amount of reserve capacity. This allows kidneys to maintain GFR at a certain level by compensating for damage and/or nephron loss. Consequently, substantial renal damage can be present without any measurable effect on GFR. In addition, it has been demonstrated that intra-renal vascular pathology (myointimal hyperplasia, arteriolar hyalinosis, glomerular sclerosis) in renal biopsies of former smokers is more prominent than in never smokers without an apparent difference in renal function.[31] Thus, renal damage related to prior smoking can be present in the kidney after smoking cessation without being reflected in GFR. Interestingly, it has been observed that renal histological lesions (including myointimal hyperplasia and arteriolar hyalinosis) amplify the nephrotoxic effects of CNIs in renal transplant recipients.[32] Accordingly, renal vascular lesions induced by prior smoking might well be involved in our observations that former smokers are more susceptible to post-transplantation CKD, despite that they no longer smoke. Further research will need to clarify whether these suggested mechanisms indeed play a role in the accelerated decline of GFR in former smokers.

Various mechanisms could underlie the persistence of smoking-associated damage after smoking cessation. Recently it was found that smoking-induced changes in epigenetics of blood platelets can persist for more than 10 years after smoking cessation, showing that distant effects of smoking can last for many years.[33,34] These findings are corroborated by data of persisting increases in risk of smoking-associated conditions long after cessation of smoking, e.g. the risk of lung cancer remains increased 15-fold in men and 9-fold in

women for at least ten years after smoking cessation.[35] Our study is the first to identify former smoking as a risk factor for CKD in lung transplant recipients. The risk was not explained by clinical renal parameters, notwithstanding gold standard renal function measurements. This is of substantial clinical significance, as it means that reliable smoking history could aid in risk-assessment. In a general sense, the association between former smoking and the risk for CKD is very sparsely documented, and so far, data in populations with a high renal risk are lacking altogether.

We did not observe any relationship between former smoking and mortality after LTx, although former smokers more frequently died of malignancy. This would be consistent with the increased risk of malignancy in smokers in the general population. In the general population, smoking is furthermore associated with cardiovascular disease. However, no association between former smoking and cardiovascular death was observed in this study. Importantly, the chances of finding an association between former smoking and cardiovascular disease were small because LTx recipients generally are relatively young and thoroughly screened on cardiovascular status prior to transplantation. They are therefore unlikely to develop cardiovascular disease within several years. Moreover, despite the development of cardiovascular risk factors due to the use of immunosuppressants (hypertension, hyperlipidemia, hyperglycemia etc.), LTx recipients are more likely to die of other transplantation-related causes (such as transplant failure or infection). This is supported by the high mortality due to transplant failure and low numbers of cardiovascular death in our population.

Our study has several limitations. First, several differences in baseline characteristics existed between the never smokers and former smokers. The former smokers more often had pulmonary emphysema and were relatively old, whereas the never smokers often had cystic fibrosis, pulmonary fibrosis or pulmonary hypertension and were relatively young. To ensure that the results of our analyses were less likely to be confounded by these differences, we adjusted for recipient age, gender, baseline GFR, BMI, pulmonary diagnosis, type of transplantation, medication-regimen, hypertension, diabetes and transplantation year. We also performed several sensitivity analyses to check the consistency. Even so, the associations persisted.

In secondary analyses we found that in patients with diagnoses other than COPD, pack years of former smoking was associated with highest risk for CKD. It should be noted that this group contains relatively many younger patients and patients with cystic fibrosis, whom differ in many aspects from patients with COPD (e.g. glomerular hyper filtration making kidneys more susceptible to CNI-toxicity, higher prevalence of diabetes after LTx, higher prevalence of chronic rejection, higher doses of immunosuppression after LTx, all risk factors for CKD). It is possible that the difference between diagnosis strata is due

to residual confounding, however, it is also possible that this reflects a difference in susceptibility of kidneys to the noxious effects of former smoking between the diagnosis groups.

In addition, it is hard to exclude that some patients have resumed smoking after LTx, despite very strong discouragement. Sobering data on smoking resumption after LTx have recently been published, showing that some 11% of patients had resumed smoking – without informing their doctors.[15] Nevertheless, we believe it is unlikely that our conclusions are affected by an undetected sub-cohort that resumed smoking. First of all, others have reported much lower numbers of patients resuming smoking after LTx.[16,36] Also, smoking resumption is more likely after an abstinence period of <6 months. Our center requires an abstinence period of at least 6 months before enlistment. Moreover, a random sample of patients from our population was recently tested for exhaled carbon monoxide levels and we found no evidence for smoking resumption. Last, whereas we cannot fully exclude smoking resumption in a minority of patients, it is unlikely that the presence of a small sub-cohort could lead to the clear dose-dependent effect of former smoking on CKD observed here in the whole cohort. Another limitation of the study is the lack of data on behavior, socio-economic status, presence of other co-morbid conditions and environmental factors. These parameters could be confounding the association between former smoking and CKD. While it would have been appropriate to adjust for these factors, such factors were not documented in the current study and could not be taken into account, resulting in potential unmeasured confounding.

A major strength of our analysis is the use of 125I-Iothalamate GFR measurements for baseline and follow-up. This method is considered a gold standard method for GFR measurement and provides a very reliable measurement.[18] This measurement is superior especially in populations such as LTx, where long-term changes in muscle-mass confound creatinine-based renal function estimates.[23] A comparison of this method with the more widely available eGFR calculations was published in an early subgroup (N=40) from this cohort in 2000,[23] showing that renal function loss is underestimated by creatinine-derived renal function estimates in LTx recipients. Another strength of this study is the availability of serial CNI trough levels in never smokers and groups of former smokers. These levels were similar for never smokers and former smokers, indicating similar CNI exposure and making confounding by CNI toxicity unlikely. Furthermore, this study shows data on a large cohort of LTx recipients with a good representation of all kinds of LTx recipients with long follow-up, without loss to follow-up.

Conclusions

The number of pack years of prior cigarette smoking was dose-dependently associated with an increased risk of both stage-III and stage-IV CKD, but not all cause mortality in a large cohort of LTx patients, independent of potential confounders.
These findings are reason for concern. Waiting times for LTx have continued to increase due to expanding recipient criteria. Likewise, donation criteria have also expanded. Patients as well as organs transplanted are generally in worse condition now than several years ago and LTx recipients are therefore at increased risk of complications after LTx, amongst which CKD.[37] If the association of former smoking and CKD after LTx were confirmed in other studies, it would be relevant to gain further insight in the biology of this association. For the moment, it would seem prudent to consider former smokers at increased renal risk and avoid additional renal risks as much as possible.

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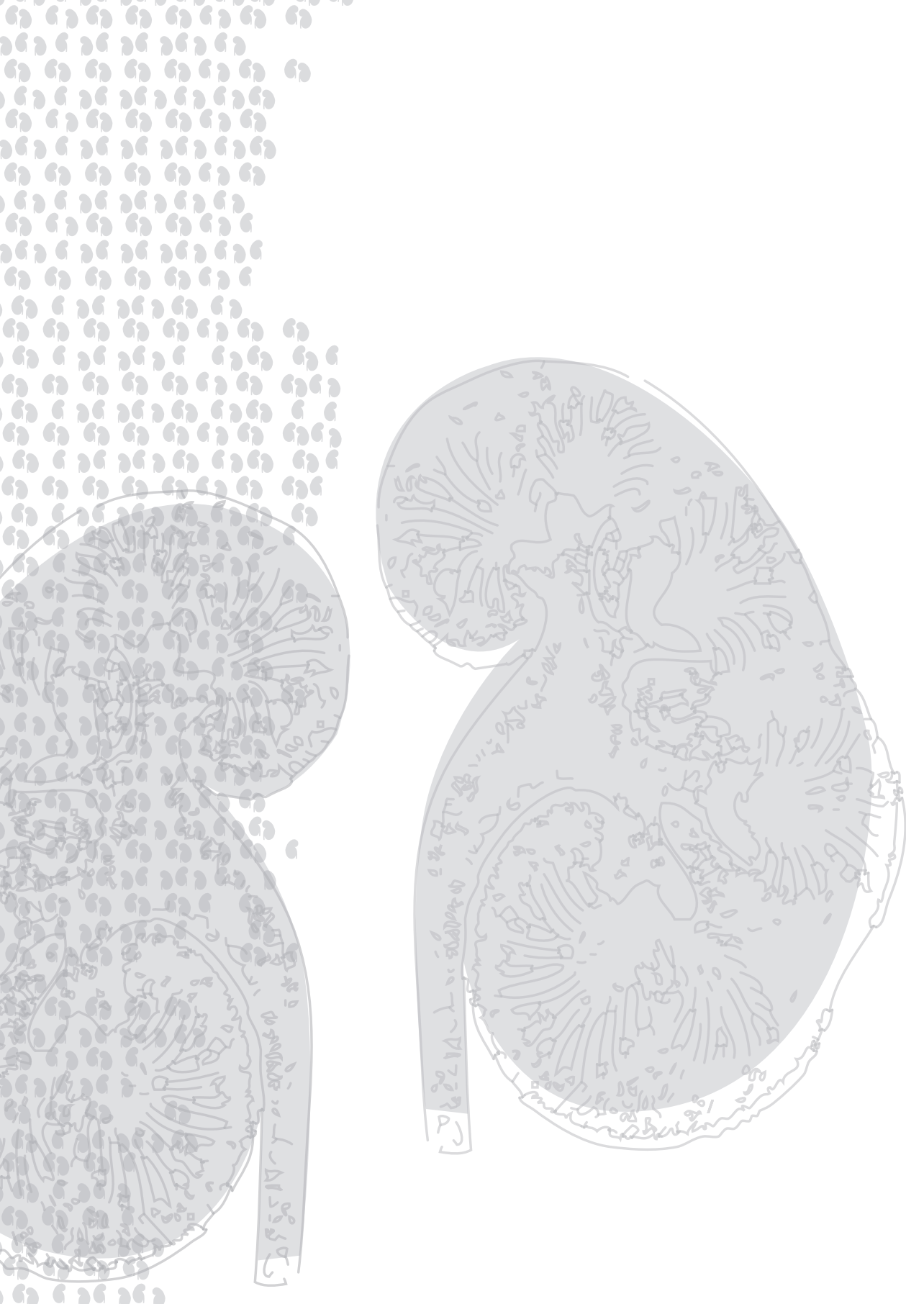

9

Cross-sectional and Longitudinal Performance of Renal Function Equations in Lung Transplantation Recipients

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Submitted

Abstract

Background: Various creatinine-based equations are available to estimate GFR. Lung transplant recipients (LTR) frequently have abnormal body composition that may affect performance of the equations. Therefore, specific validation of the creatinine-based equations in LTR is needed.

Methods: CG, MDRD and CKD-EPI equations for estimated GFR (eGFR) were compared with 2092 assessments of ^{125}Iothalamate-measured GFR (mGFR) in 301 LTR, up to 16 years after transplantation. We assessed performance precision, bias and accuracy), determinants of bias, stability of bias over time and accuracy in monitoring renal function deterioration over time.

Results: CG, MDRD and CKD-EPI underestimated mGFR, with 13.8, 28.8 and 22.7%, respectively. Precision and accuracy were modest. In all equations, bias was larger (i.e. more underestimation) for higher values of mGFR, in female patients and in recipients with cystic fibrosis or emphysema. BMI determined bias of CG and age bias of MDRD. Bias was stable over time. All equations underestimated long-term slope of GFR-decline with ±35% and sensitivity/specificity in detecting fastest progressors was 88-90/68-70%.

Conclusions: The performance of creatinine-based renal function equations is modest in LTR, with average underestimation of GFR, limited precision/accuracy and underestimation of long-term course of GFR. Creatinine-based equations thus have important shortcomings in accurate determination and long-term monitoring of renal function in individual LTR.

Introduction

Progressive renal function deterioration is common after lung transplantation (LTx),[1-3] and requires close monitoring. Glomerular Filtration Rate (GFR) is accepted as the best overall measure of renal function and represents the overall filtering capacity of the kidneys.[4] GFR can be measured as the urinary and plasma clearance of ideal filtration markers, such as radiolabelled ^{125}I-Iothalamate, to give a reliable reflection of renal function.[5]

However, because the GFR measurements with ideal filtration markers are complex, demanding and costly, the use of these methods to monitor renal function in lung transplant recipients (LTR) is often not feasible in clinical practice. Therefore, renal function is generally monitored by serial estimates of GFR using creatinine-based renal function equations. Serum creatinine-based GFR equations are superior to using serum creatinine alone as an index for renal function because these equations take into account certain patient-specific biometrical indices that affect creatinine generation and excretion (age, gender, race, weight). However, the equations have important shortcomings as they frequently underestimate gold standard measured GFR (mGFR), especially in the high- and high-normal range, and lack sensitivity in detecting progressive renal function loss.[6-8] In patients with abnormalities in body composition the estimate is affected even more substantially.[9,10] Of note, renal function equations have been validated almost exclusively for their cross-sectional performance. The sparse data on their longitudinal performance show that renal function loss over time can be underestimated, in some patients substantially.[6,9,11]

LTR frequently have abnormal body composition prior to transplantation and substantial changes in body composition frequently occur after transplantation (e.g. due to improvement of pulmonary status and prolonged steroid use).[12] In addition, renal hemodynamics may be altered due to the pulmonary disease and calcineurin inhibitor use.[13,14] Performance of creatinine-based renal function equations may be hampered by all of these factors. However, little data on performance of the creatinine-based renal function equations are available in this specific group of patients.[13,15] An early study from our group, demonstrated a change in bias during follow up after LTx, hampering the use of estimated GFR for monitoring renal function over time with underestimation of the rate of renal function loss.[13] However, only a small number of patients was analyzed in this study and the duration of follow-up was limited to the first two years after transplantation. Moreover, at the time this study was performed the nowadays most frequently used eGFR equations (abbreviated MDRD and CKD-epi) were not yet available. Therefore, validation of the renal function equations for determination and long term longitudinal monitoring of renal function after LTx is needed.

In the current study, we analyzed how various frequently used creatinine-based renal function equations perform compared to ^{125}I-Iothalamate GFR in a large cohort of LTR, and assessed the determinants of the systematic error. In addition, we assessed longitudinal change of the systematic error and the accuracy in monitoring renal function deterioration over time.

Materials and methods

Between 1990 and 2008 in total 370 lung transplantations and heart-lung transplantations were performed at the University Medical Center of Groningen (UMCG), the Netherlands. We included all adult lung transplant recipients whom had at least 1 measurement of GFR and serum creatinine measurement at the same day for the analysis of performance of the creatinine-based GFR estimations in lung transplantation recipients. Consent for the use of patient data was obtained from all patients prior to transplantation.

GFR measurements

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured simultaneously by constant infusion of radiolabelled tracers ^{125}I-Iothalamate and ^{131}I-Hippuran as described before.[16-18] In short, the method was as follows: after drawing a blank blood sample, the priming solution containing 0.04 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippurate per mL saline) plus an extra of 0.6 MBq of ^{125}I-iothalamate was given, followed by constant infusion at 12 mL/h. To attain stable plasma concentrations of both tracers, a 2 hour stabilization period followed, after which the clearance period started.

Clearances of ^{125}I-Iothalamate and ^{131}I-Hippuran are measured over the next 2 hours and calculated as (U*V)/P and (I*V)/P, respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer and P represents the tracer value in plasma at the end of each clearance period. GFR is calculated from U*V/P of ^{125}I-iothalamate and corrected for voiding errors by multiplying the urinary clearance of ^{125}I-iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-Hippuran. This correction method is based on the fact that, during steady state, the plasma clearance of ^{131}I-Hippuran equals it urinary clearance when urine collection is perfect. This GFR measurement has a day-to-day coefficient of variation of <2.5%[18] and is considered a gold standard method for GFR measurement and used for the development and validation of novel renal function equations.[19]

Serum creatinine was determined in blood samples drawn at the start of the GFR

measurement. Height and weight were measured in every patient before investigation. The GFR measurement procedure was unaltered over the duration of the observation period. Renal function was measured routinely at out-patient basis before transplantation, 1, 6, 12, 18, 24 months after transplantation and every 6-24 months thereafter. For patients screened at another center or patients transplanted with high urgency no pre-LTx measurement was available.

Renal function equations

GFR was estimated using the 3 most frequently used renal function equations. The first creatinine-based renal function equation we used was the Cockcroft-Gault (CG)-formula.[20] The CG-formula was calculated as follows (in ml/min):

*CG = (140-age) * weight/(72 x serum creatinine in mg/dl) * (0.85 if female)*

Secondly, we used the abbreviated, re-expressed, four variable Modification of Diet in Renal Disease-study (MDRD) equation for standardized serum creatinine samples.[21] Because none of the patients were blacks; no correction for ethnicity was applied. MDRD equation was therefore calculated as follows (in ml/min/1.73m^2):

$MDRD = 175 * (\text{serum creatinine in mg/dl})^{-1.154} * (age)^{-0.203} * (0.742 \text{ if female})$

The third equation we used was the more recently developed CKD-EPI equation.[19] This equation is calculated gender-specific and stratified by creatinine levels. It was developed especially to provide a better performance in the mildly impaired, normal and higher range.[22] The following calculations were used (in ml/min/1.73m^2):

$\text{Female with serum creatinine} \leq 0.7 \text{ mg/dL: } GFR = 144 * (0.993)^{age} * (\text{serum creatinine} / 0.7)^{-0.329}$
$\text{Female with serum creatinine} > 0.7 \text{ mg/dL: } GFR = 144 * (0.993)^{age} * (\text{serum creatinine} / 0.7)^{-1.209}$
$\text{Male with serum creatinine} \leq 0.9 \text{ mg/dL: } GFR = 141 * (0.993)^{age} * (\text{serum creatinine} / 0.9)^{-0.4111}$
$\text{Male with serum creatinine} > 0.9 \text{ mg/dL: } GFR = 141 * (0.993)^{age} * (\text{serum creatinine} / 0.9)^{-1.209}$

From here, GFR estimates by these above mentioned equations are referred to as estimated GFR (eGFR). GFR measured by the ^{125}I-Iothalamate method are referred to as measured GFR (mGFR). mGFR and eGFR estimated by the Cockcroft Gault formula were normalized to body surface area (BSA) by dividing the values by 1.73/BSA. BSA was calculated according to the formula of DuBois and DuBois [23] as follows:

$BSA = 0.007184 * \text{weight in kg}^{0.425} * \cdot \text{height in cm}^{0.725}$

Calibration of serum creatinine

Serum creatinine was measured by Jaffé alkaline picrate assay, on the MEGA (Merck KGaA, Darmstadt, Germany) until the 1st March 2006. After this date, serum creatinine was measured by enzymatic assay on the Roche Modular (Roche, Basel, Switzerland). Large differences exist in calibration of the serum creatinine assays across center and within centers over time. Therefore, both methods used in this center were calibrated to the reference standard, i.e. Cleveland Clinic Laboratory measurements, as proposed by Coresh et al.[24]

The calibration procedure was described in more detail elsewhere.[6] In short, 177 samples drawn before 1st March 2006 and 339 samples drawn after 1st March 2006 with a broad range of creatinine values were sent to the Cleveland Laboratory and led to the following calibration equations: Calibrated serum creatinine = [-0.300 + 1.217 * (UMCG Jaffé creatinine values in mg/dL)] for measurements before 1st March 2006 and [0.011 + 1.087 * (UMCG Roche creatinine values in mg/dL)] for measurements after 1st March 2006. CG, MDRD, and CKD-EPI equations were all three calculated with calibrated creatinine values.

Medication

Immunosuppressive protocols used over time are described in more detail elsewhere.[25] In short, induction consisted of rATG (Thymoglobulin; Sanofi Pasteur, Lyon, France) until 2001 and Basiliximab (Simulect, Novartis Pharma, Basel, Switzerland) induction thereafter. Maintenance immunosuppression consisted of a calcineurin inhibitor (CNI) based immunosuppressive regimen combined with Prednisolone and Azathioprine (Immuran; GlaxoSmithKline, Brentford, United Kingdom) or Mycophenolate Mofetil (Cellcept; Roche, Basel, Switzerland).

Cyclosporine (Neoral; Novartis Pharma, Basel, Switzerland) was the CNI used until 2001 (target levels: whole blood trough levels 400ng/mL initially, tapered to 150 ng/mL within the first three weeks), tacrolimus (Tacrolimus, Prograft; Astellas Pharma, Staines, United Kingdom) was the CNI used from 2001 onwards (target levels: 20ng/mL during the first three weeks, 15ng/mL until the third month and 10-12ng/mL thereafter and from 2004 until present target levels 15-18ng/mL during the first three weeks, 12-15ng/mL until the third month, and 10-12ng/mL thereafter).

All patients furthermore received P. Jerovici prophylaxis (co-trimoxazole, 960mg on alternate days) and Herpes prophylaxis (acyclovir) and from 2001 all patients at risk received standard CMV–prophylaxis.

Analysis of performance

Performance of renal function equations compared to mGFR with respect to systematic

and non-systematic error was assessed as proposed by Bostom[26] and Stevens[27] using bias, precision, and accuracy. Bias represents the systematic error and is expressed as the median absolute difference (mGFR – eGFR) and the median percentage (relative) difference ((mGFR – eGFR)/ mGFR * 100).

Precision is a measure of non-systematic error and is expressed by the interquartile range (IQR) and 95% confidence interval (95%CI) of the absolute and relative difference between mGFR and eGFR. Precision is also represented by the overall 'fit' of the equations against the gold standard expressed by the R^2 (the squared correlation coefficient).

Accuracy incorporates both bias and precision. This is reflected by the proportion of subjects with eGFR values within 30% of mGFR (P_{30}), and the root mean squared error (RMSE). The mean squared error (MSE) equals the variance of the errors plus the square mean of the errors and thus incorporates both bias and precision. The RMSE is measured in the same units as the original data and is representative of the size of a typical error.

Statistical analysis

Data were analyzed with STATA version 11.2 (STATACorp, LP, College Station, Texas, USA). Paired sample t-tests were used to analyze differences between mGFR and CG, MDRD, CKD-EPI equations and 24-hour creatinine clearance. To assess systematic error we performed Bland-Altman analyses. Differences in bias between subgroups of potential determinants were tested with analysis of variance (ANOVA). Changes of bias over time as well as multivariable analysis of determinants of bias were tested using multivariable multilevel analyses allowing for random intercept as well as random slope, with bias as dependent variable and potential determinants as independent variables within levels of individual patients. Necessity of a random intercept or random slope was tested with the likelihood ratio test. Individual slopes of GFR were calculated with individual linear regression analysis on the basis of at least 3 measurements >6 months after LTx. Two-sided P-values less than 0.05 indicated statistical significance.

Table 1 | Patient and measurement characteristics

Patient Characteristics		
Number of patients	301	
Male gender - n (%)	145 (48.2)	
Age at time of transplantation - years	45.1 ± 12.0	[18.0-66.7]
Pulmonary diagnosis - n (%)		
Pulmonary Emphysema	135 (44.9)	
Pulmonary Hypertension (PH)	30 (10.0)	
Cystic Fibrosis (CF)	59 (19.6)	
Other	77 (25.6)	
Type of transplantation - n (%)		
Unilateral	66 (21.9)	
Bilateral	223 (74.1)	
Combined organ	12 (4.0)	
Immunosuppressive regimen		
Cyclosporin-based	148 (49.2)	
Tacrolimus-based	153 (50.8)	
Measurement Characteristics		
Total number of GFR measurements	2092	
Pre-transplantation	290	
Post-transplantation	1802	
Average number of GFR Measurements per patient	6.0 ± 4.5	[1-21]
Age at time of measurement - years	47.2 ± 11.5	[18.5-72.4]
Follow-up at time of measurement - years	2.5 ± 3.0	[0-16.0]
Body Mass Index at time of measurement - kg/m2	23.9 ± 4.5	[13.4-45.5]
Renal function Indices		
Glomerular Filtration Rate - ml/min	65 ± 28	[6-199]
Effective Renal Plasma Flow - ml/min	267 ± 97	[27-695]
Filtration Fraction - %	0.24 ± 0.05	[0.10-0.50]

Results

Baseline characteristics

We included 301 adult lung transplantation recipients (LTR) with at least one simultaneous assessment of mGFR and serum creatinine. Patient characteristics are presented in table 1. Of all LTR, 145 (48.2) were male, mean age was 45.1 ± 12.0 years and the major pulmonary diagnosis for lung transplantation (LTx) was emphysema (44.9%). Most patients underwent bilateral lung transplantation (74.1) and 49.2% of the patients received cyclosporine-based immunosuppression.

Performance

In total, there were 2092 mGFR measurements with on average 6 (range 1-21) measurements per LTR. Measurements were performed prior to transplantation (screening, n=290) or up to 16 years after LTx (during follow-up, n=1802). Table 2 presents the performance (bias, precision and accuracy) of the 3 creatinine based GFR equations. The median bias, reflecting systematic error, was relatively large for all three equations and indicated substantial underestimation of mGFR by all three equations. Whereas the absolute bias was larger before transplantation than after transplantation, the bias expressed as a percentage of mGFR was similar before and after LTx. The bias was least pronounced for the CG (-13.8%) equation, most pronounced for the MDRD equation (-28.8 %) and intermediate for the CKD-EPI equation (-22.7%, all three comparisons *P*-value <0.001). In figure 1, Bland-Altman analyses are shown to better observe the bias over the whole range of GFR. It can clearly be appreciated that systematic error increases (i.e. on average more underestimation of mGFR) in the higher range of GFR and that the average systematic error is smaller with the CG equation than with the MDRD and CKD-EPI equations.

The precision, inversely reflecting non-systematic error, is captured by the interquartile ranges and 95% confidence intervals of the absolute and relative bias and by R-square of the relation between mGFR and the individual equations. The CG, MDRD and CKD-EPI equations all had similar association with mGFR (R-squared 0.639, 0.660 and 0.664, resp.). As can be observed in figure 2 the precision was best in the lower ranges of mGFR and gradually decreased in the higher ranges of mGFR for all three equations (larger deviation from the line of identity, dotted line). The curved fitted line in the graphs represents the accuracy of the equation, a measure incorporating both systematic and unsystematic error (i.e. bias and precision). The curved lines show a similar pattern in all three equations with increasing bias and decreasing precision towards the higher ranges of mGFR and very large imprecision in the highest range of mGFR. The imprecision in the highest ranges of mGFR was less for the CKD-EPI equation.

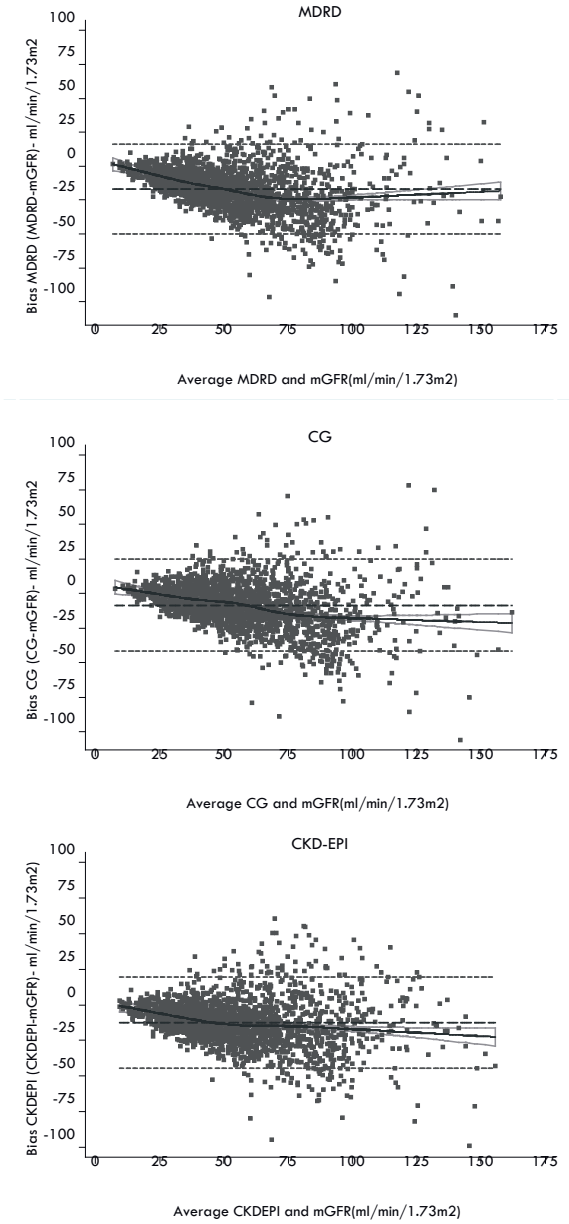
The accuracy is also expressed by the P_{30} (the percentage of estimations within 30% of mGFR) and the root mean squared error (RMSE). The P_{30} was highest for CG (73.1%), lowest for MDRD (49.8%) and intermediate for CKD-EPI (61.5%), whereas the RMSE was comparable for CG, MDRD and CKD-EPI (according to the RSME eGFR was on average within 15.8 to 16.3 ml/min/1.73m2 of mGFR). The different results with these two measures of accuracy is due to the fact that occasional large errors (outliers) weigh heavily in the RMSE but not in de P_{30}.

Table 2 | Bias, precision and accuracy of the renal function estimations in relation to measured GFR

	Mean GFR	Range	Absolute Bias Median [IQR]	95% CI	Relative Bias Median [IQR]	95% CI	P-30	R^2	RMSE
Overall N=2092									
mGFR (reference)	62.4 ± 27.5	5.9-194.9	-	-	-	-	-	-	-
eGFR Cockcroft-Gault	53.3 ± 22.1*	9.2-169.1	-7.7 [-17.6, 1.1]	-39.7, 15.3	-13.8 [-26.9, 2.4]	-41.0, 36.6	73.1	0.64	16.4
eGFR abbreviated MDRD	45.4 ± 22.4*†	7.5-167.1	-15.7 [-26.3, -6.7]	-44.7, 6.9	-28.8 [-39.6, -14.8]	-51.6, 17.2	49.8	0.66	15.9
eGFR CKD-EPI	49.7 ± 24.2*†‡	8.9-139.7	-11.9 [-21.4, -3.4]	-39.9, 13.2	-22.7 [-34.4, -7.2]	-48.0, 25.0	61.5	0.66	15.8
Pre-Transplantation N=290									
mGFR (reference)	98.3 ± 24.8	35.7-194.9	-	-	-	-	-	-	-
eGFR Cockcroft-Gault	75.0 ± 20.1*	27.0-152.1	-23.8 [-36.5, -10.4]	-54.4, 9.1	-24.2 [-35.3, -11.8]	-46.3, 16.3	61.3	0.33	20.2
eGFR abbreviated MDRD	68.4 ± 21.8*†	19.3-167.1	-30.3 [-43.2, -18.2]	-62.3, 6.1	-31.8 [-41.5, -19.1]	-54.1, 5.9	44.7	0.35	19.9
eGFR CKD-EPI	75.1 ± 21.4*‡	20.9-139.7	-23.1 [-36.6, -10.0]	-57.5, 8.4	-25.0 [-34.6, -10.6]	-49.5, 9.1	60.8	0.36	19.7
Post-Transplantation N=1802									
mGFR (reference)	56.3 ± 22.9	5-9-168.8	-	-	-	-	-	-	-
eGFR Cockcroft-Gault	49.8 ± 20.4*	9.2-169.1	-6.1 [-15.0, 2.1]	-29.4, 15.5	-11.8 [-25.1, 4.7]	-40.0, 38.9	75.0	0.62	14.0
eGFR abbreviated MDRD	41.7 ± 20.2*†	7.5-151.8	-14.4 [-23.1, -5.7]	-37.6, 7.1	-28.4 [-39.3, -14.1]	-51.1, 17.9	50.6	0.64	13.7
eGFR CKD-EPI	45.5 ± 22.1*†‡	8.9-136.2	-10.9 [-19.6, -2.6]	-33.2, 13.9	-22.3 [-34.2, -6.5]	-47.9, 26.8	61.6	0.64	13.6

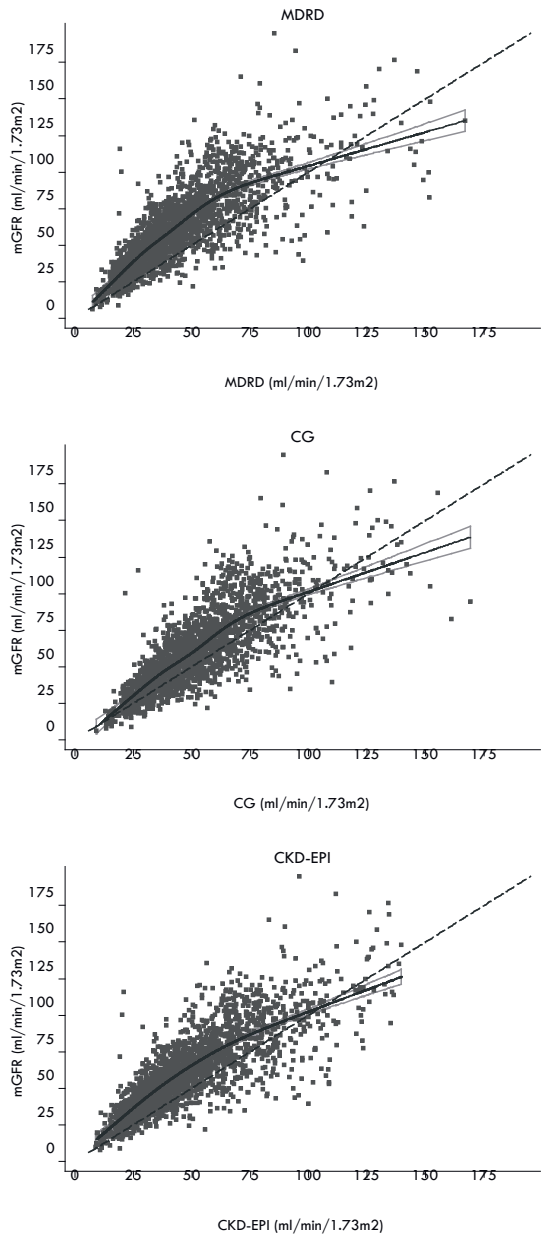
*P< 0.001 compared to mGFR, † P< 0.001 compared to CG, ‡ P< 0.001 compared to MDRD. Absolute bias in ml/min/1.73m^2, relative bias in %.

Figure 1 | Bland-Altman plots of the creatinine-based GFR equations



The differences between measured GFR and estimated GFR plotted against the averages of the two. The dotted lines indicate the mean difference, and the limits of agreement (the mean difference +1.96SD of the difference).

Figure 2| Scatter plots and lowess function with 95% confidence interval fitting the data of the creatinine-based GFR equations



Measured GFR plotted against estimated GFR. Dotted line is the line of identity. Grey curved area is the Lowess function with 95% confidence interval through all data points.

Determinants of bias

We assessed differences in bias across several of the patient characteristics: tertiles of mGFR, tertiles of age, tertiles of BMI, gender and pulmonary diagnosis (figure 3). Mean mGFR for the 3 tertiles was 34.6 (range: 5.9-47.4), 58.4 (range: 47.4-70.4) and 93.2 (range: 70.4-194.8) ml/min/1.73m^2 respectively. For all three equations bias increased significantly per tertile of higher mGFR. Mean age for the 3 tertiles was 41.8 (range: 18.5-44.2), 49.3 (range: 44.3-53.7) and 58.9 (range: 53.7-72.4) years respectively. Only MDRD was slightly affected by age, with least underestimation of mGFR in the oldest patients. Mean BMI for the 3 tertiles was 19.4 (range: 13.4-21.6), 23.3 (range: 21.6-25.1) and 28.9 (range: 28.1-45.5) kg/m^2 respectively. BMI only affected bias of CG with larger underestimation of mGFR in patients with low BMI. Bias of MDRD and CKD-EPI was significantly larger in women and bias of CG and MDRD was larger in patients with cystic fibrosis. Pre-transplantation bias was much larger than post-transplantation; however, this is likely due to difference in mGFR and BMI before and after transplantation.

Longitudinal course of GFR and bias

In figure 4, the longitudinal changes in mGFR, eGFR and relative bias can be appreciated. mGFR initially decreases rapidly after LTx and gradually stabilizes thereafter. At all time points the mGFR was underestimated by all three equations. Relative bias of MDRD and CKD-EPI remained stable throughout the entire follow-up, whereas the relative bias of CG slightly decreased during the first year after LTx and stabilized thereafter.

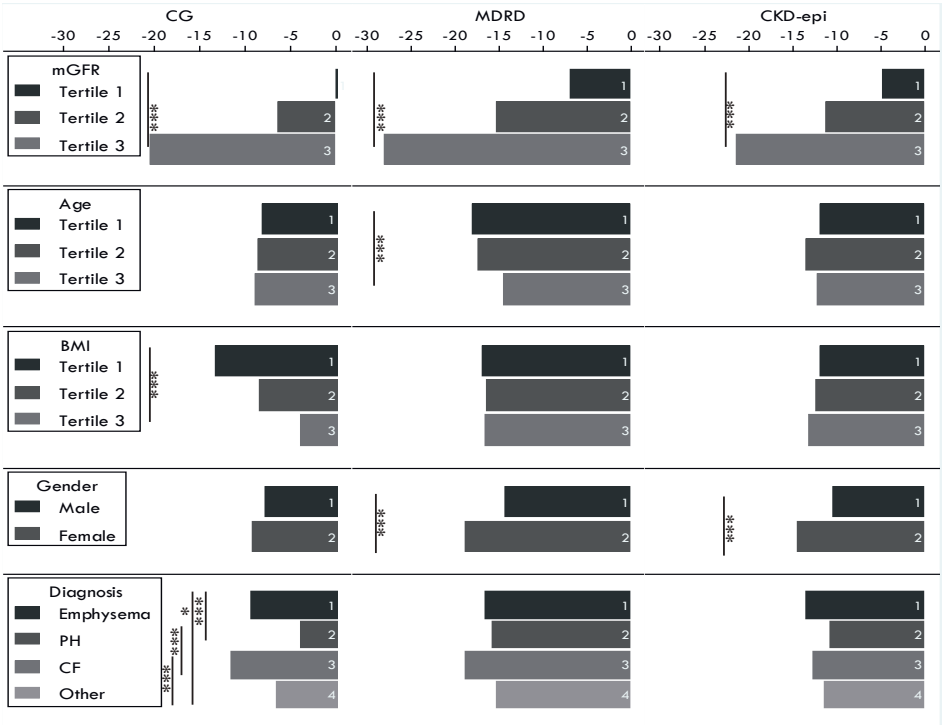
We performed multivariable mixed effects analyses to, firstly, assess stability of bias over time, taking changes in determinants of bias over time into account and, secondly, test whether mGFR age, BMI, gender, and pulmonary diagnosis were independent determinants of bias (table 3). In these multivariable analyses bias was stable over time for all three equations. In all three equations, underestimation of mGFR increased with higher values of mGFR and was larger in female patients. Higher BMI increased bias of the MDRD and CKD-EPI equations, but decreased bias of CG. Higher age increased underestimation of CG, but not MDRD and CKD-EPI equations. Last, pulmonary diagnoses cystic fibrosis and emphysema were associated with significantly larger underestimation of CG, MDRD and CKD-EPI equations (latter borderline significant).

Predictive performance for longitudinal monitoring of renal function

From 6 months after LTx onwards, 220 patients had at least 3 measurements of GFR for the calculation of individual GFR slopes. The median annual mGFR decline was -3.04 [IQR: -4.53, 0.06] (table 4). All creatinine based equations underestimated the annual GFR decline with approximately 35-37% (R^2 of correlation slopes mGFR with slopes eGFR ranged

from 0.63 to 0.65). The 25% patients with fastest mGFR decline had a median annual mGFR decline of -8.61 [-11.37, 6.27]. Also the GFR decline in these patients was substantially underestimated by all three creatinine-based GFR equations; with median eGFR decline of -5.63, -4.74 and -5.43 for the CG, MDRD and CKD-EPI equations respectively (*P*<0.001 for all three equations compared to mGFR). Sensitivity of the renal function equations in detecting the 25% fastest progressors was comparable, with 65.5, 70.9 and 69.1 % for the CG, MDRD and CKD-EPI equations respectively.

Figure 3 | Determinants of bias for the creatinine-based GFR equations



***P<0.001, *P<0.05. Abbreviations: BMI: body mass index; PH: pulmonary hypertension; CF: cystic fibrosis.

Figure 4| Development of mGFR, eGFR and relative bias over time for the creatinine-based GFR equations

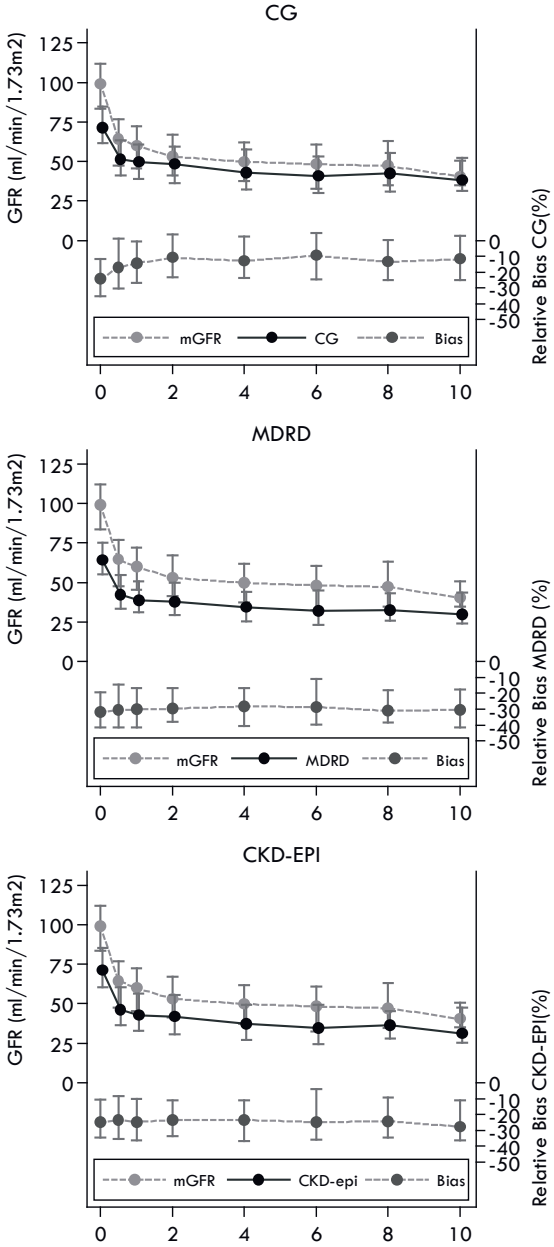


Table 3| Multivariable mixed model of determinants of bias for the creatinine-based GFR equations

	Cockcroft-Gault			Abbreviated MDRD			CKD-EPI		
Determinants of bias	**Coefficient**	**[95% CI]**	***P*-value**	**Coefficient**	**[95% CI]**	***P*-value**	**Coefficient**	**[95% CI]**	***P*-value**
Time after LTx	0.22	[-0.27, 0.72]	0.38	0.09	[-0.34, 0.53]	0.68	-0.11	[-0.59, 0.37]	0.66
mGFR	-0.46	[-0.51, -0.42]	<0.001	-0.28	[-0.33, -0.25]	<0.001	-0.30	[-0.34, -0.26]	<0.001
BMI	0.50	[0.17, 0.83]	0.003	-1.49	[-1.81, -1.18]	<0.001	-1.53	[-1.88, -1.19]	<0.001
Age	-0.56	[-0.77, -0.35]	<0.001	-0.02	[-0.22, 0.19]	0.88	-0.17	[-0.39, -0.05]	0.125
Sex	-4.16	[-7.91, -0.42]	0.03	-10.01	[-13.67, -6.35]	<0.001	-8.69	[-12.53, -4.83]	<0.001
Pulmonary diagnosis									
Pulmonary Emphysema	-5.72	[-10.46, -0.98]	0.02	-5.10	[-9.62, -0.59]	0.03	-5.26	[-10,02, -0.50]	0.03
Pulmonary Hypertension (PH)	-1.87	[-8.77, 5.02]	0.59	-1.46	[-8.00, 5.07]	0.66	-0.56	[-7.46, 6.33]	0.87
Cystic Fibrosis (CF)	-8.13	[-14.97, -1.30]	0.02	-6.51	[-12.99, -0.02]	0.05	-6.68	[-13.53, 0.18]	0.06
Other	ref	ref	ref	ref	ref	ref	ref	ref	ref

Table 4| Slopes of annual GFR decline according to mGFR and creatinine-based equations

	Overall			25% fastest progressors		Correlation		Sensitivity	Specificity
	N	**Median**	**IQR**	**Median**	**IQR**	**R^2**	**95% CI**	**%**	**%**
mGFR (reference)	220	-3.04	[-4.53, 0.06]	-8.61	[-11.37, -6.27]	-	-	-	-
Cockroft-Gault	220	-0.90	[-3.36, 1.59]*	-5.63	[-10.2, -4.13]*	0.63	[0.54-0.70]	65.5	88.5
Abbreviated MDRD	220	-0.64	[-2.76, 1.60]*	-4.74	[-12.67, -3.73]*	0.63	[0.54-0.70]	70.9	90.3
CKD-EPI	220	-0.77	[-3.19, 1.82]*	-5.43	[-14.2, -4.30]*	0.65	[0.57-0.72]	69.1	89.7

Slopes of annual GFR decline according to mGFR and creatinine-based equations from 6 months after lung transplantation onwards (slopes based on at least 3 measurements). * P<0.001 compared to mGFR. No significant differences between CG, MDRD and CKD-EPI.

Discussion

This study evaluated the performance of the CG, MDRD and CKD-EPI equations for accurate determination of renal function as well as long term monitoring of renal function in LTR. Overall, the cross-sectional performance of creatinine-based renal function equations in LTR was modest. All three creatinine-based renal function equations on population level substantially underestimated mGFR both before and after LTx, and precision and accuracy were limited, especially in the high-normal range of GFR. Longitudinally, the long-term course of GFR was substantially underestimated by the creatinine-based renal function equations and the equations lacked sensitivity in detecting the recipients with fast declining renal function. In conclusion, the renal function equations have important shortcomings in accurate determination of renal function and monitoring the course of renal function in LTR.

Cross-sectional Performance

Our study provides the largest analysis on performance of renal function equations in LTR so far. Very limited data on this specific population are available. One study assessed the correlation of the 6-variable MDRD equation with 24-hour creatinine clearance in nearly 600 patients screened before LTx.[28] They found no systematic bias but did find limited precision and accuracy, in particular in the higher range of GFR. It is hard to compare their results to our results because their data were based on 24-hour creatinine clearance, whereas we used gold-standard renal function measurements as reference.

Studies in other transplant populations such as kidney and liver transplant recipients found that renal function equations had a reasonable performance on group level. In renal transplant recipients with average GFR of 55 ml/min, MDRD underestimated GFR slightly with ~5%, and CG overestimated GFR with ~10%.[9] In liver transplant recipients with average GFR of 91 ml/min, both MDRD and CG underestimated GFR with ~10%.[29] No data on CKD-EPI were available. Cautious comparison thus indicates that the bias between the GFR equations and mGFR in LTR is larger than in other transplantation populations. A possible explanation for this difference may be the relative high frequency of abnormal body composition. Other factors that may play a role are the suppression of tubular creatinine secretion due to the general use of trimethoprim,[30-32] which all LTR use in combination with sulfomethoxazol for prevention of P. Jerovici pneumonia, abnormalities in creatinine secretion associated with the DF508 mutation underlying cystic fibrosis,[33] or the use of standardized serum creatinine values in this study.

Longitudinal Performance

Besides the modest cross-sectional performance of the renal function equation in LTR, we found that the longitudinal performance was hampered by substantial underestimation of long-term course of GFR and limited sensitivity/specificity in detecting fast declining renal function. The only study that previously looked at longitudinal performance of renal function equations in LTR is an early study on a subgroup (N=40) of this cohort addressing the first two years after LTx.[13] Similar to the current study it was found that creatinine-based renal function equations underestimate the rate of renal function loss by ~35%.

Underestimation of the rate of renal function loss by renal function equations is not limited to LTR, but has been demonstrated previously in other transplant populations, patients with type II diabetes and non-diabetic patients with chronic kidney disease,[6,8,a9,29] and may be explained by the fact that renal function equations have been validated almost exclusively for their cross-sectional performance. Due to the underestimation of renal function loss, individual patients with fast progressive renal function decline may be missed, despite multiple measurements, long-term follow-up and relative rapidly declining renal function. This warrants caution in the application of the equations to monitor renal function in LTR in clinical practice.

Sources of bias

We found the several important determinants of systematic error. It is well established that there is a larger systematic error in the higher range of GFR. Indeed, the bias was much larger in the high range of GFR, but it was proportionally similar over the whole range. Precision and accuracy, the other two measures of performance, also decreased in the high range. The CKD-EPI equation was specifically developed to have better performance in this range. Our data indicate that the CKD-EPI indeed performs slightly better than the MDRD and CG in the high range.

BMI was a significant determinant of bias of the CG equation, with larger underestimation in patients with low BMI. Several studies in renal transplant recipients and renal patients found that the MDRD and CG equations had opposite effects on the BMI-related error in renal function estimate, with progressive underestimation of renal function at higher BMI by the MDRD and progressive overestimation at higher BMI with CG.[9,34] Our results on multivariate analyses are consistent with these previous findings showing more underestimation at higher BMI by MDRD, less underestimation at higher BMI with CG. Because bias with CG decreased with increasing BMI, bias in LTR decreased in the year after transplantation in LTR that gained weight.

Limitations and strengths

Our study has several limitations. First of all, our data are derived from a single center. Comparability to other center is nevertheless feasible because of the use of calibrated creatinine values to calculate eGFR and the comparison to a robust gold standard measurement that is also used for the development of novel renal function equations and has a low variability (day-to-day coefficient of variation <2.5%). All patients in this study were whites, thus conclusions do not apply to black patients.

The major strengths of this study are the large number (>2000) of measurements by a single standardized method in a population LTR with a large range of GFR, age, BMI and various diagnoses. In addition, there was long-term follow-up after LTx available with multiple measurements per patient, allowing for accurate determination of long-term GFR slopes and long-term performance of the equations.

Conclusions

We found that the performance of creatinine-based renal function equations is modest in LTR, with average underestimation of GFR and limited precision/accuracy. Monitoring renal function over time with the renal function equations is furthermore hampered by the underestimation of long-term course of GFR and limited sensitivity in detecting fast decline of renal function. Creatinine-based renal function equations thus have important shortcomings in accurate determination and long-term monitoring of renal function in individual LTR. Caution is warranted in the application of the equations to monitor renal function in clinical practice.

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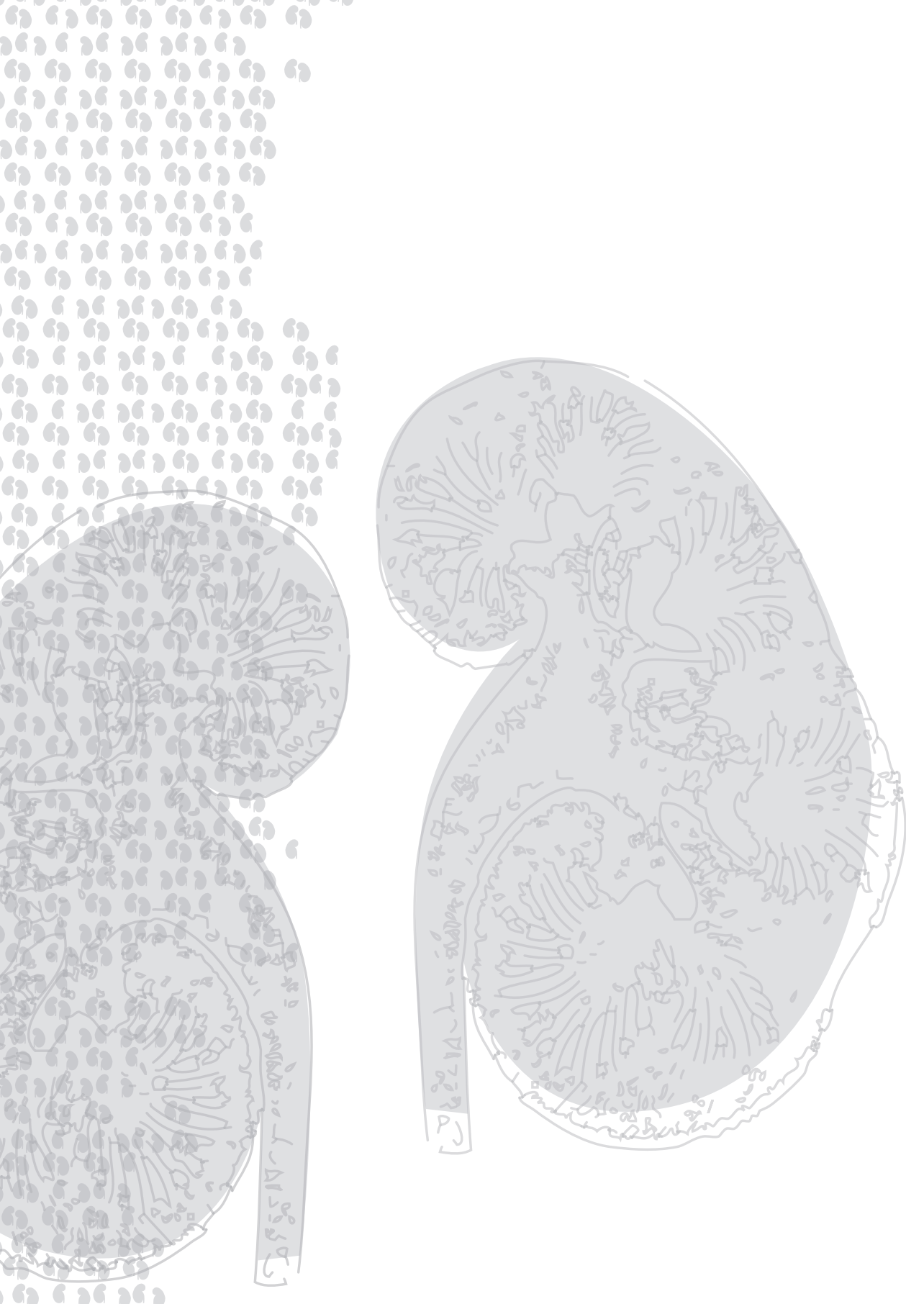

10

Summary and Discussion

Summary and discussion

Part I: Type 2 Diabetes and Hypertension

Chronic renal impairment poses a severe burden on the individual patient as well as on national healthcare systems. In order to institute adequate preventive measures, early identification of patients with fast-progressive renal impairment is of utmost importance. This thesis focused on prediction and prevention of renal impairment in high-risk populations.

In the first part of this thesis we focused on prediction and prevention of renal impairment in type 2 diabetes and hypertension. The first chapters of this part consist of research performed within the sysKID consortium, a European collaboration aiming to identify persons at risk of developing chronic renal impairment. Earlier detection of patients within high-risk groups with an increased risk of renal impairment or fast-progressive course of renal function decline could help reduce the number of patients at renal risk by implementing early preventive efforts, while alleviating the burden of treatment for those with a low risk.[1, 2]

Despite the identification of many important risk factors for progression of chronic renal impairment such as hypertension, hyperglycaemia, dyslipidemia, smoking and obesity,[3-7] risk stratification of individual patients remains a huge challenge. Once renal disease is established, regression of disease is very difficult to achieve. It is therefore imperative to find markers that predict, in early stage of disease, which patients are at risk for chronic renal impairment, in order to timely institute preventive measures. In recent years multiple urinary and serum/plasma biomarkers for the prediction of chronic renal impairment have been proposed, and new biomarkers are continuously being put forward.

In **chapter 2,** we systematically reviewed the validity of biomarkers for renal impairment in type 2 diabetes. We performed a systematic literature search and identified 15 publications on longitudinal studies reporting on 27 candidate biomarkers for the prediction of renal impairment in type 2 diabetes. Based on the methodological quality of the studies, we concluded that the overall study quality of these studies in general is modest. This not only limits proper assessment of the potential clinical value of the identified biomarkers, but it also limits the generalizability and comparability of the results.

We addressed several important methodological issues that were relevant for the quality of the biomarker research. First, we found that studies of predictive markers frequently calculated odds ratios or relative risks to demonstrate the strength of association between

the biomarker and the outcome. Odds ratio's and relative risks are merely a measure of association and this does not necessarily translate into improvement in risk stratification and prediction. We therefore recommended to not only test associations, but also perform separate prediction analyses, such as false-positive/true-positive fractions, c-statistics, net reclassification improvement (NRI), integrated discrimination improvement (IDI)[8] and discriminative likelihood ratio (dLR).[9,10]

A second important issue that we addressed was the lack of validation of results in independent studies, as biomarkers only have clinical value if results are independently reproducible. Based on the status of current biomarker research in this field, we therefore recommended that future research be directed not only at further biomarker discovery, but also at validation of promising biomarkers in large well-designed longitudinal studies. Specific prediction analyses should be applied to assess the additive predictive value of novel and published biomarker candidates beyond conventional risk factors. Future research will have to elucidate the true value of current biomarker candidates for prediction of onset and progression of renal impairment in diabetes. If these biomarkers prove clinically useful in the future, they may get a more prominent role in early non-invasive screening and assessment of overall renal risk in patient groups, guiding early and more aggressive therapy in high risk patients in order to prevent long-term cardio-renal complications.

Transition between the stages of albuminuria (i.e. normo- to microalbuminuria and micro- to macroalbuminuria) is considered a hallmark of progression of renal disease in diabetes, and is associated with fast-progressive renal function decline, end-stage renal disease and also with cardiovascular disease.[11,12] We were therefore interested in novel biomarkers that may precede and predict the transition in albuminuria.

According to current insights leakage of excess albumin is a sign of vascular dysfunction resulting from endothelial damage. Markers of early vascular damage could thus be eligible as markers for transition in albuminuria in diabetes.[13] In **chapters 3 and 4,** we assessed whether the micro-vascular damage marker Growth-Differentiation Factor-15 (GDF-15) and the micronecrosis marker high-sensitivity Troponin T (hs-TnT) respectively, preceded and had additive predictive value in prediction of onset or worsening of albuminuria in patients with type 2 diabetes.

We had the opportunity to investigate these markers using a nested case-control design in the Prevention of REnal and Vascular End-stage Disease (PREVEND) study.[14] A unique feature of our study was that we used patient samples prior to the transition in albuminuria in a longitudinal fashion. This study design allows to test whether the markers under study preceded and predicted the transition in albuminuria. This contrast

most other research in literature in which the biomarker of interest is investigated in a cross-sectional fashion.

The main results of the studies were that both GDF-15 and hs-TnT were independently associated with transition in albuminuria in both an intial cohort and a replication cohort. Furthermore, both markers improved risk stratification between controls and cases with progressive albuminuria in both the initial and replication cohort. The value of GDF-15 and hs-TnT had not previously been demonstrated for renal disease. Regarding GDF-15, previous studies mainly implicated GDF-15 as predictor for cardiovascular events and all-cause mortality. One observation study linked GDF-15 to end-stage renal disease in type 1 diabetes.[15] Likewise, hs-TnT is mostly regarded as marker for acute myocardial infaction.[16] It has also been implicated as marker for later occurrence of cardiovascular and all cause mortality in various populations.[17,18] We now for the first time demonstrated that GDF-15 and hs-TnT may also serve as markers for prediction of progression in albuminuria in hypertensive and type 2 diabetic subjects. The exact underlying mechanisms remain unclear. Potentially, GDF-15 and hs-TnT indicate vascular stress, causing renal as well as cardiovascular damage.

Our results, if confirmed by other studies, raise the possibility of improving the identification of subjects at risk even when traditional risk factors do not indicate risk.

In patients with (established) renal impairment, treatment with antihypertensive agents blocking the Renin-Angiotensin-Aldosteron-System (RAAS) is first-choice treatment to halt further progression of the disease.[1] In large scale randomized clinical trials it has been shown that these agents such as Angiotensin-Converting-Enzyme inhibitors (ACEi), Angiotensin-Receptor-Blockers (ARB) and Direct Renin inhibitors (DRi) do not only lower blood pressure, but also lower urinary albumin excretion and reduce risk of ESRD and mortality in patients with type 2 diabetes and hypertension, compared to treatment with other antihypertensives. RAAS-blockade has similar beneficial effects in diabetic patients without hypertension and in non-diabetic patients.[19-22]

Currently, dose-titration of RAAS blockade is recommended on blood pressure response, without taking responses in albuminuria into account. This is comprehensible, given that these agents are developed and registered as antihypertensive agents. However, recent studies have illustrated that within an individual the response in blood pressure is not always paralleled by a response in proteinuria and vice-versa. It is, however, unclear whether it is the response in blood pressure, in albuminuria, or their combination which is the driving parameter for renoprotection and should be considered a target for reno-protective therapy. In **chapter 5** we assessed the initial treatment responses in albuminuria and blood pressure and the association of the initial treatment responses with long-term

renal outcome in patients with type 2 diabetes and microalbuminuria.
We showed that the response to ARB therapy varies for both albuminuria and blood pressure and that a substantial amount of patients has a discordant treatment response (i.e. a response in either albuminuria or blood pressure). The rate of long-term renal function decline and the risk for transition to overt nephropathy was dependent on the initial response in albuminuria irrespective the blood pressure response.
Whereas it was already clear that the reduction in albuminuria achieved during the initial months of RAAS blockade is a critical step to achieve renoprotection and is the most important determinant of long-term renoprotection, these findings are all based on studies in patients with advanced chronic renal impairment (macroalbuminuria and lower renal function).[23-26] Our results, combined with evidence from studies in patients with more advanced renal disease, suggest that a treatment approach solely focusing on blood pressure reduction may not be the most efficacious way to achieve renoprotection.[27-30]
Monitoring therapy-induced changes in albuminuria in individual diabetic patients is important in addition to monitoring blood pressure, since therapy induced changes in both parameters do not run in parallel and both parameters were independently associated with effectiveness of achieving renal protection.
However, before this finding can be used in risk management strategies, prospective randomized controlled trials will be necessary to obtain definitive evidence that an approach of targeting UAE confers additional renoprotection. From trials in other populations we know that optimal anti-proteinuric dosing of RAAS blockade is feasible and results in a substantially larger reduction in proteinuria and slower rate of renal function decline.[31]

Current guidelines recommend screening for, and treatment of increased albuminuria in all patients with type 2 diabetes for the prevention of long-term cardio-renal complications. Since 2006, the Dutch primary care guideline recommends yearly albuminuria-screening in all diabetes patients with a life-expectancy of at least 10 years.[2] For the yearly albuminuria-screening, measurement of ACR is favored over urinary albumin concentration.[32] If the measurement indicates the presence of micro- or macroalbuminuria, the increased albuminuria has to be confirmed by a repeat measurement within the next few months. In addition, all patients with confirmed micro- or macroalbuminuria should be prescribed RAAS-treatment (Angiotensin-Converting-Enzyme Inhibitors (ACEi)/ Angiotensin II Receptor Blockers (ARB)) even if their blood pressure readings are in the normal range.
Yet, clinical practice does not always immediately follow and comply with recommendations and guidelines. In **chapter 6**, we assessed the adherence to guidelines for albuminuria screening and treatment in patients with type 2 diabetes in a large primary care cohort.

In a cohort of over 14,000 patients with type 2 diabetes, primarily treated by their general practitioner, we found that an ACR-measurement was observed in less than 60% of T2DM patients in Dutch primary care, and that up to one third of patients had never been screened in a three year period from 2007 to 2010. The proportion of patients that was screened for increased urinary albumin excretion was lower than anticipated from looking at screening rates of other risk factors.[33-35] Although the proportion increased when the interval was extended beyond 12 months, as proposed in a recent study,[36] this was a marginal increase leaving more than one third of patients not being screened in 2009. Also, limiting the patients to those who have a longer life expectation did not substantially increase the ACR screening rate.

In our study, we found that cross-sectionally, the proportion of patients receiving RAAS-treatment among patients with prevalent increased albuminuria was highest, followed by patients with incident increased albuminuria. In addition to this, increased albuminuria was a trigger for RAAS-treatment initiation following ACR measurement. Nevertheless, the proportion of patients that was prescribed RAAS following a finding of increased albuminuria was disappointingly low (~13%). It is unclear to what extent RAAS-treatment was prescribed to these patients because of uncontrolled blood pressure rather than increased albuminuria. Given the fact that initiation of RAAS-treatment was also frequently used by patients without any ACR measurement or those with normoalbuminuria, it is tempting to speculate that in many patients the main indication for RAAS-treatment was uncontrolled blood pressure rather than increased albuminuria. This suggestion is further supported by our finding that blood pressure and number of antihypertensives used (other than RAAS) were determinants of RAAS-treatment initiation in patients with unknown albuminuria or normoalbuminuria. This may indicate that establishment of increased albuminuria in itself seems to be insufficiently recognized as an indication for starting RAAS-treatment.

We looked at several external factors, including patient characteristics and organizational factors that may explain the low proportion of ACR measurement and RAAS-treatment initiation. It is widely recognized that there can be many barriers and incentives contributing to the implementation of guidelines in practice.[37] We indeed found barriers at the level of the health care professional. Its explanation may be twofold; on the one hand, according to a study using questionnaires, awareness of the guidelines amongst European GPs was limited and could indeed be improved substantially.[38] On the other hand logistical issues regarding albuminuria screening may play a role. This argument is substantiated by our observation that that additional care provided by a diabetes support facility and previous ACR measurement were important determinants of ACR measurement. The finding that logistical issues play a role in risk factor management is not entirely new. Similar

observations were done previously in the management of hypertension.[39]
Whatever the underlying causes may be, there is room for improvement with regards to screening and treatment of increased urinary albumin excretion in the Dutch primary care setting. It is well recognized that albuminuria is one of the strongest cardio-renal risk markers, and early screening and appropriate treatment have the potential to substantially reduce the risk of cardio-renal complications. Nevertheless, this aspect of risk-reduction in diabetes now receives insufficient attention and this should be brought to the clinicians' attention.

Part II: Lung transplantation

In the second part of this thesis we focused on prediction and prevention of renal impairment in lung transplantation recipients. This patient population has a much higher a priori risk of renal function decline, but due to the relatively small overall number of lung transplantation recipients this group does not comprise a large proportion of all patients with chronic renal impairment. Nonetheless, in this patient group the burden of chronic renal impairment is substantial, with between 5 and 10% of surviving lung transplantation recipients ending up in dialysis.[40-42] Moreover, the renal impairment poses difficulties for effective immuno-suppression to maintain a viable pulmonary graft.[43]
At variance with the populations described in part I, that are managed mainly in general practice, LTx recipients are subject to intensive individual monitoring in specialized centers, that usually also include specialist care. Accordingly, the clinical setting for risk prediction and prevention is highly different from that in diabetes and hypertension.

Several patient and transplantation related factors are established or hypothesized as renal risk factors in lung transplantation recipients, including age, sex, pulmonary diagnosis, type of transplantation, use of cardiopulmonary bypass, hypertension, diabetes, serum creatinine and BMI.[41,44] Since the first lung transplantations more than 20 years ago, the field of lung transplantation has evolved considerably. Due to evolution of acceptance policies, lung transplantation recipients are increasingly old and have an increasing load of 'co-morbidities', adding up to increasing anticipated renal risk. Meanwhile, treatment and management of lung transplantation recipients also evolved, with physicians increasingly becoming aware of the nephrotoxicity of immunosuppression. This "learning curve" may have counterbalanced the increase in renal risk factors in patients accepted for lung transplantation.
In **chapter 7** we described these developments and assessed the changes in renal risk

factors in the lung transplantation population as well as the incidence of renal function impairment after lung transplantation over time. Despite a gradually worsening renal risk profile of the lung transplantation recipient population, we found that renal outcome improved over time. Several factors may have played a role in this improvement. One of the factors that we believe may have played a role in counterbalancing the renal risk is the switch from cyclosporin to tacrolimus as standard calcineurin inhibitor since 2001. This is line with observations that tacrolimus is less nephrotoxic than cyclosporin in other solid organ transplantation populations and experimental studies.
Other factors that may have played a role in the reduction of renal impairment after lung transplantation include improvement of surgical techniques and post-operative management, increased awareness about renal function impairment and increased experience in the management of lung transplantation recipients. The increased awareness and experience in the management of renal impairment have led to institution of preventive measures, such as preventive tapering of immunosuppression or prescription of reno-protective medication in patients with rapidly declining renal function.

We were also interested in other risk factors for renal impairment. We hypothesized that, even after smoking-cessation, smoking history may be relevant for morbidity after LTx, in particular chronic renal impairment, because of the high prevalences of both chronic renal impairment and former smoking. We therefore assessed whether there was an association between smoking history before lung transplantation and renal impairment after lung transplantation.

In **chapter 8,** we showed that former smoking indeed was dose-dependently associated with an increased risk of renal impairment. In addition, former smokers had a lower measured renal function after transplantation compared to never smokers, which was already apparent from early after transplantation and on. Although never smokers were on average younger and had different underlying pulmonary disease, all associations remained after adjustment for these differences in patient characteristics.
Our findings were somewhat in contrast to the general notion that, whereas smoking is an established renal risk factor, this risk diminishes after smoking cessation. This notion has also proven false for other smoking-related conditions, such as lung cancer. It has been shown that the risk of lung cancer remains increased 15-fold in men and 9-fold in women for at least ten years after smoking cessation.[45] Likewise, other sub-clinical effects of smoking remain present for years after cessation.[46, 47]
We believe that former smoking may have primed the kidneys for accelerated decline in GFR after LTx. This would imply there was pre-existent renal damage in former smokers that makes them more susceptible for further CNI-induced renal function loss. Nevertheless,

adjustment for pre-transplantation renal function did not account for the effects found. This seemingly contradictory finding may be explained by the fact that kidneys have a certain amount of reserve capacity: pre-existent damage can be compensated for, and substantial renal damage can be present without any measurable effect on GFR.[48] That this may be the case in former smokers is supported by a renal biopsy study, in which it was shown that renal effects of smoking could be present in former smokers, without an apparent effect on renal function.[49] Pre-existent renal lesions - in combination with diminished renal reserve capacity - may in turn amplify the nephrotoxic effects of CNIs.[50] Accordingly, renal lesions induced by prior smoking might well be involved in our observations that former smokers are more susceptible to post-transplantation renal impairment, despite that they no longer actively smoke.

Interestingly, especially amongst the most recently transplanted lung transplantation recipients there were many former smokers with a history of heavy smoking. This reflects the widening acceptance criteria for lung transplantation that also were mentioned in **chapter 7**. In **chapter 8,** we demonstrated that heavy former smoking was associated with increased renal risk. Nevertheless, we observed a reduction in renal impairment in more recently transplanted recipients. Of course, this is a good development. However, the reduction in renal impairment might even been more pronounced if the amount of heavy former smokers transplanted would have been lower.

Besides the increased renal risk, we could not demonstrate any effect of former smoking on survival after lung transplantation. Importantly, patients are all thoroughly screened prior to transplantation and therefore have a low risk of mortality after transplantation. Therefore, chances were very small to find an association between former smoking and mortality. Despite that we did not observe an association of former smoking with mortality in general, we did find higher mortality due to malignancy in the former smokers (most commonly lung cancer of the native lung), that also gives an indication of the long-term risks associated with smoking. Also from clinical experience, the physicians have the impression that heavy former smokers experience more complications after lung transplantation overall, and thus benefit less in terms of quality of life. All of these suspicions remain to be objectified by further research.

Unfortunately, former smoking is a non-modifiable risk factor and, apart from preventive measures for renal impairment after transplantation, not much can be done to reduce the renal risk in former smokers. The only way to modify the risk associated with former smoking would be to limit the acceptance of heavy former smokers for lung transplantation. Given the absence of an effect on survival, the lack of prediction analyses that show that former smoking is not only associated, but also predicts renal and other

complications and the decreasing burden of renal impairment in this patient group, such measures are currently unjustified.

Last, but not least, monitoring renal function over time is an excellent way to predict future renal risk, and the predictive performance of a slope of repeated renal function measurements over time has shown to be superior to proteinuria.[51] This raises the issue how renal function can best be monitored. Usually, creatinine or creatinine based renal function equations are used to serve that purpose. However, these options suffer from major drawbacks as they frequently underestimate gold-standard measured GFR (mGFR), especially in the high and high-normal range, and lack sensitivity in detecting progressive renal function loss.[52-54]

This is largely a result of the methods and the population in which they were derived. The estimate is affected even more substantially in patients with abnormalities in body composition, because the equations depend strongly on creatinine generation (and thus on body composition).[55,56] Apart from one study comparing creatinine-based renal function equations with 24-hour creatinine excretion,[57] these equations have never been validated in lung transplantation recipients. Because of the distinct characteristics of the lung transplantation recipients, they may not be very accurate in this particular population. As we routinely performed 125-Iothalamate measurements (mGFR) in our outpatient clinical practice, we could test the performance of creatinine based renal function equations that are most commonly used to monitor renal function in lung transplantation recipients. In **chapter 9,** we assessed how well the more easily available renal function equations correlate with gold standard GFR in lung transplantation recipients and assessed the factors of systematic bias.

We found that all three creatinine-based renal function equations that we tested substantially underestimated mGFR on population level (ranging from 12 to 32%, depending on the equation), both before and after lung transplantation. Precision and accuracy were also limited, especially in the high-normal range of GFR. As noted before it is well established that there is a larger deviation between eGFR and mGFR in the high range of GFR. In agreement, we found that the bias was much larger in this range, and that bias expressed as a proportion of mGFR was similar over the whole range of GFR. Other determinants of bias were BMI, age, sex and pulmonary diagnosis (cystic fibrosis and emphysema). The extent to which they bias the equations depended on the equation. In theory, the equations account for age, sex, race and weight, but our findings indicate that in this particular population, age, sex and BMI were insufficiently accounted for.

All creatinine based renal function equations are derived from empirical studies in defined cohorts with certain weights and body compositions.[58-60] In different patients, the same weight is consistent with a wide range of body compositions (muscle mass

to fat ratio) and creatinine generation rates. We expected abnormal body composition, especially reduced muscle mass, of lung transplantation recipients to cause estimates of the creatinine based renal function equations to be biased. However, a low muscle mass would lead to an overestimation of GFR in stead of the underestimation that we found.
A possible explanation for our results is that lung transplant recipients were especially devoid of fat and not so much of muscle mass (abnormal muscle mass to fat ratio). In line with this, patients with cystic fibrosis and pulmonary emphysema had the largest underestimation of mGFR, and these patient groups are particularly devoid of fat. Another issue that may contribute to underestimation of mGFR is that all lung transplantation recipients continuously receive trimethoprim (in combination with sulfomethoxazol) for prevention of P. Jerovici pneumonia after lung transplantation. This drug suppresses tubular creatinine secretion and raises the serum creatinine.[61-63] This leads to lower eGFR, but does not affect mGFR. Likewise, the DF508 mutation underlying cystic fibrosis also suppresses creatinine secretion.[64]
Importantly, also the slope of renal function over time was underestimated by the renal function equations, perhaps inherently to the continuous underestimation of renal function over time. Similar to other patient populations, individual patients with fast-progressive renal function decline may therefore be missed, despite multiple measurements, long-term follow-up and relatively rapidly declining renal function. This lack of sensitivity limits applicability of the renal function equations to monitor renal function in lung transplantation recipients.

As creatinine based estimation of renal function has limitations as shown in **chapter 9,** this also affects the use of creatinine based renal endpoints in research. Commonly, creatinine based eGFR or doubling of serum creatinine are used as endpoints. Whereas these endpoints are flawed to some extent; creatinine based endpoints are easily obtainable, and due to their widespread use facilitate comparison across studies. In **chapter 7,** we also used doubling of serum creatinine as the primary outcome measure and mGFR was used as a secondary outcome. The results using doubling of serum creatinine were paralleled by the results using mGFR. This indicates that despite their limitations, serum creatinine based endpoints have value, and can be used in clinical research, albeit cautiously.

Overall conclusions

In this thesis several aspects regarding prediction and prevention of chronic renal impairment in high-risk populations were investigated. We have looked at prediction of

chronic renal impairment using biomarkers and risk factors, how to improve preventive treatment strategies, and clinical reality.

Whereas the high-risk populations that we've studied face the same threat of chronic renal impairment and prediction and prevention are important in all high-risk populations, several differences have also become apparent. The number of patients with diabetes or hypertension is very large and rapidly increasing. A small proportion these patients will eventually develop end-stage renal disease. These patients are mostly monitored in primary care, until complications become apparent. The number of lung transplantation recipients is much smaller, but the risk of chronic renal impairment they face is higher, the care for lung transplantation recipients is highly specialized and patients are monitored intensively.

Due to these intrinsic differences, the methods for monitoring and treatment differ in certain aspects. In a large high-risk population it is important to limit not only the burden of disease in those with the highest risk, but also to limit the burden of treatment in those with the lowest-risk. In a small very high-risk population, more intensive monitoring and more aggressive treatment are warranted. As chronic renal impairment poses a severe burden on the individual patient, every step towards improving prevention of chronic renal impairment benefits the patients.

10 | Chapter

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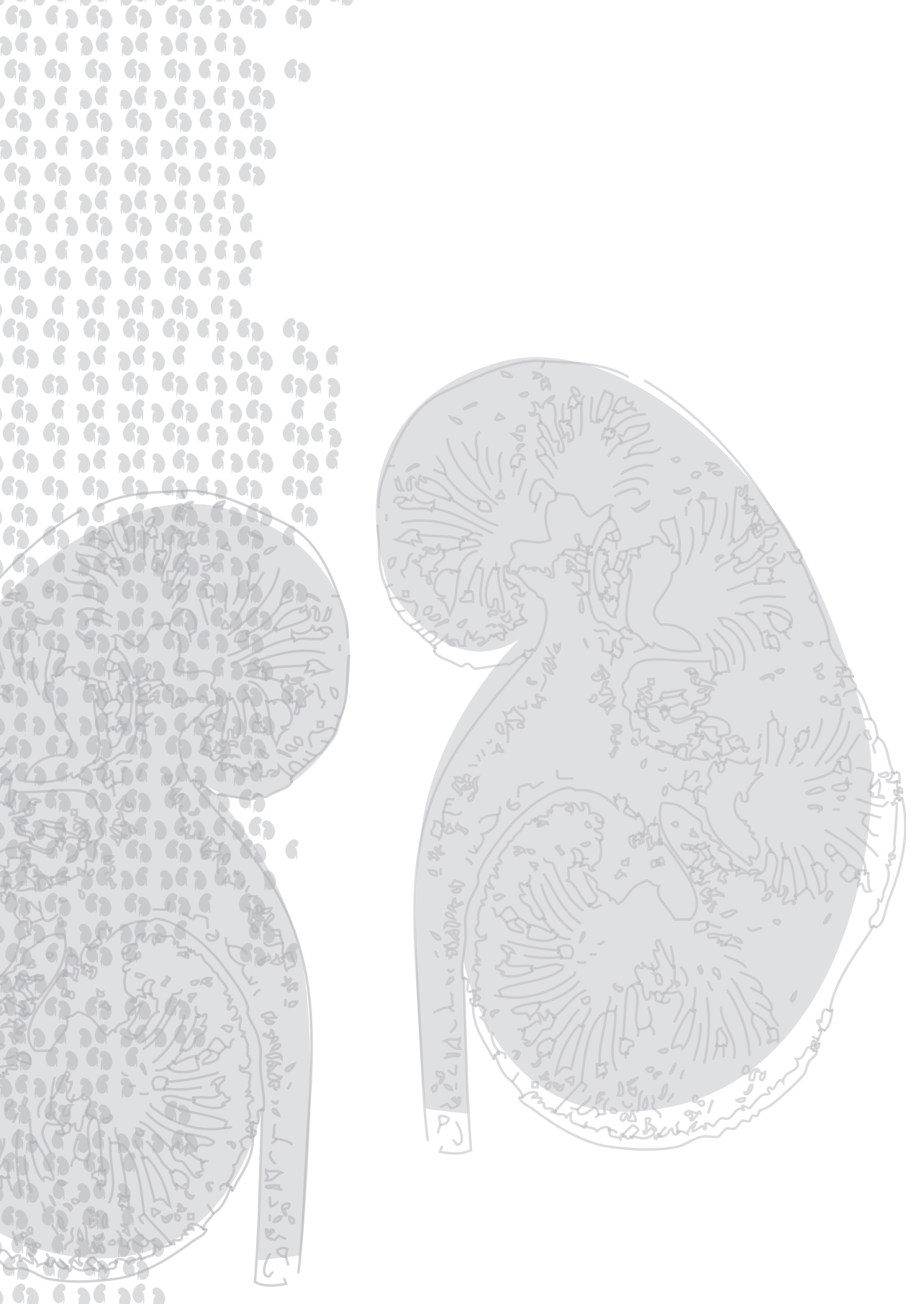


Samenvatting en Discussie

Nederlandse Samenvatting en Discussie

Deel I: Type 2 diabetes en hypertensie

Chronische nierziekten vormen een toenemend wereldwijd gezondheidsprobleem. De belangrijkste oorzaken die hieraan ten grondslag liggen zijn met name de toenemende incidentie van diabetes (ouderdomssuikerziekte) en hypertensie (hoge bloeddruk) door de moderne levensstijl. Patiënten met diabetes en/of hypertensie hebben namelijk een verhoogd risico op onder andere het ontwikkelen van chronische nierziekten. Geschat wordt dat momenteel 10-20% van de bevolking ouder dan 18 momenteel aan enige vorm van nierproblemen lijdt. Hoewel veel mensen in eerste instantie niet doorhebben dat ze lijden aan nierziekten, o.a. eiwit in de urine en versnelde nierfunctieachteruitgang, is het hebben van chronische nierziekten een van de belangrijkste risicofactoren voor het ontstaan van terminaal nierfalen. Nierfalen is ernstig invaliderend omdat de enige behandelingsmogelijkheid niervervangende therapie is, in de vorm van nierdialyse of niertransplantatie. Deze beide behandelingen zijn zeer belastend voor patiënten.
Het hebben van chronische nierziekten, met name in een vergevorderd stadium, brengt daarnaast belangrijke risico's met zich mee op onder andere hart- en vaatziekten en vroegtijdig overlijden. Bovendien wordt de gezondheidszorg fors belast door de stijgende prevalentie van chronische nierziekten en zijn de kosten voor behandeling van chronische nierziekten en aanverwante complicaties erg hoog, met name wat betreft niervervangende therapie bij eindstadium nierfalen.
Als nierziekten vroegtijdig kunnen worden voorspeld of ontdekt, lukt het vaak om het verloop en het ontstaan van complicaties met de juiste behandeling gunstig te beïnvloeden. Vroegtijdige ontdekking is dus erg belangrijk. Dit proefschrift gaat over het voorspellen en voorkómen van chronische nierziekten in patiëntengroepen met een hoog risico op het ontwikkelen van chronische nierziekten.

Het t eerste deel van dit proefschrift gaat over voorspellen en voorkómen van chronische nierziekten bij patiënten met type 2 diabetes en hypertensie. De eerste hoofdstukken maken deel uit van onderzoek wat verricht werd binnen het SysKID consortium, een grootschalig Europees onderzoeksproject wat zich richt op het identificeren van patiënten met een verhoogd risico op het ontwikkelen van chronische nierziekten. Vroegtijdige identificatie van patiënten met een verhoogd risico op chronische nierziekten of een snel progressief verloop van chronische nierziekten kan preventieve maatregelen mogelijk maken, waardoor het risico kan worden gereduceerd. Tegelijkertijd kan bij patiënten die bij

voorbaat een laag risico hebben de last van preventieve maatregelen (zoals behandeling) bespaard blijven.

Hoewel er een grote hoeveelheid risicofactoren bekend is voor het ontstaan of snel progressief verloop van nierziekten - onder andere hoge hypertensie, hyperglycemie (hoge bloedsuikerwaarden), dyslipidemie (verstoorde cholesterolwaarden), roken en overgewicht - , is het nog steeds niet goed mogelijk om patiënten die (ernstige) chronische nierziekten zullen ontwikkelen en patiënten die dat niet zullen ontwikkelen van elkaar te onderscheiden.

Als zich eenmaal een chronische nierziekte heeft ontwikkeld, is het lastig dit met behandeling terug te draaien. Het is daarom belangrijk om parameters te vinden die helpen bij vroegtijdige opsporing van patiënten met een hoog risico om vroegtijdig preventieve maatregelen zoals behandeling in te stellen. Biomarkers zijn "in bloed en urine voorkomende biologische stoffen" waarvan de concentratie bij bepaalde aandoeningen verandert en daardoor een vroegtijdige vaststelling van bepaalde aandoeningen mogelijk kunnen maken. De afgelopen jaren zijn er vele biomarkers beschreven die mogelijk het ontstaan en/of het voortschrijden van chronische nierziekten voorspellen.

In **hoofdstuk 2** hebben we deze biomarkers systematisch op een rijtje gezet met een behulp van literatuuronderzoek en gekeken hoe valide deze markers waren. Hierbij vonden we 15 longitudinale studies waarin één of meerdere biomarkers werden beschreven voor het ontstaan en/of het voortschrijden van chronische nierziekten in patiënten met type 2 diabetes. Helaas bleek dat er tot nu toe weinig biomarkers bekend zijn die een substantiële bijdrage leveren aan de voorspelling van chronische nierziekten. Voor het beoordelen van de validiteit keken we onder andere naar de methodologische kwaliteit van de individuele studies. Over het geheel genomen was de kwaliteit niet meer dan redelijk te noemen. Hierdoor is het moeilijk de waarde van de markers te beoordelen. Door verschillen in studieopzet en uitkomstmaten was het bovendien moeilijk de resultaten te vergelijken en te generaliseren.

Een aantal van de methodologische knelpunten werden uitvoerig bediscussieerd. Een van de zwakke punten is dat veel studies met name kijken naar associaties tussen biomarkers en eindpunten, maar niet in hoeverre een biomarker bijdraagt aan het verbeteren van onderscheidend vermogen tussen patiënten met een hoog en een laag risico, naast de al bekende risicofactoren. We raadden daarom aan om in studies die onderzoek doen naar voorspelling ten minste één vorm van analyse met betrekking tot voorspellend vermogen te verrichten.

Daarnaast viel het op dat resultaten vaak slechts in één studie met goed effect beschreven worden, maar dat er geen andere studies zijn die de resultaten hebben gereproduceerd, danwel dat in andere studies zelfs tegenstrijdige resultaten worden gevonden.

Ons advies is daarom dat er bij onderzoek op het gebied van biomarkers verder onderzoek wordt gedaan naar het ontdekken van nieuwe biomarkers, maar dat validatie van biomarkers ook nagestreefd moet worden. Daarnaast is het nodig om biomarkers te analyseren met betrekking tot voorspellend vermogen. Verder onderzoek zal meer duidelijkheid moeten geven in hoe valide de huidige biomarkers en nieuwe biomarkers zijn. Hopelijk zullen biomarkers in de toekomst een waardevolle bijdrage kunnen leveren.

Albuminurie (eiwituitscheiding in de urine) is een belangrijk kenmerk van chronische nierziekten. Er worden meerdere stadia van albuminurie onderscheiden op basis van de hoeveelheid eiwit die wordt uitgescheiden (respectievelijk normoalbuminurie, microalbuminurie en macroalbuminurie). Transitie van een vroeg stadium naar een later stadium van albuminurie is een belangrijk kenmerk van voortschrijding van chronische nierziekte. Daarnaast is transitie tussen de stadia van albuminurie geassocieerd met snel progressieve nierfunctieachteruitgang en met hart- en vaatziekten.
Volgens de STENO-hypothese is albuminurie een teken van vaatschade, waarbij de binnenbekleding van het bloedvat (het endotheel) beschadigd is en de bloedvaten albumine gaan lekken. Markers die bij dit proces van vaatschade betrokken zijn zouden dus kunnen fungeren als voorspellers van transitie tussen de stadia van albuminurie.
In **hoofdstuk 3 en hoofdstuk 4** keken we naar respectievelijk Growth-Differentation Factor-15 (GDF-15), als marker van endotheelschade, en naar high-sensitivity Troponin T (hs-TnT), als marker van celdood. We onderzochten of deze markers voorafgingen aan een transitie tussen de stadia van albuminurie en of ze bijdroegen aan de voorspelling hiervan. We onderzochten dit in een subgroep van patiënten die meededen aan de 'Prevention of REnal and Vascular End-Stage Disease' (PREVEND) Study, een Gronings bevolkingsonderzoek naar eiwitverlies in de urine voor het voorspellen van nier-, hart- en vaatziekten. We selecteerden patiënten die over de tijd een transitie tussen de stadia van albuminurie doormaakten, en koppelden deze patiënten aan vergelijkbare patiënten met een stabiele albumine uitscheiding in de urine gedurende dezelfde onderzoeksperiode. We namen vervolgens bevroren bloedsamples van deze patiënten aan het begin van de onderzoeksperiode (toen de gekoppelde patiënten hetzelfde albuminurie-stadium hadden) en bepaalden hierin de concentraties van de biomarkers GDF-15 en hs-TnT. Een bijzonder kenmerk van deze studie was dat het longitudinaal van opzet was, terwijl veel studies een eenvoudigere cross-sectionele studieopzet gebruiken. Alleen bij longitudinaal onderzoek kunnen er uitspraken gedaan worden over voorspelling.
In **hoofdstuk 3** lieten we zien dat GDF-15 onafhankelijk geassocieerd was met een transitie tussen de stadia van albuminurie, zowel in een primair cohort van patiënten met type 2 diabetes als in een replicatie cohort van patiënten met hypertensie. Bovendien verbeterde

GDF-15 de discriminatie tussen patiënten met en zonder een transitie tussen de stadia van albuminurie.
In **hoofdstuk 4** lieten we zien dat hs-TnT onafhankelijk geassocieerd was met een transitie tussen de stadia van albuminurie, zowel in een primair cohort van patiënten met hypertensie als in een replicatie cohort van patiënten met type 2 diabetes, hoewel de associatie in het replicatiecohort niet statistisch significant was. Wel verbeterde hs-TnT de discriminatie tussen patiënten met en zonder een transitie tussen de stadia van albuminurie in zowel patiënten met hypertensie als in patiënten met type 2 diabetes.
De waarde van GDF-15 en hs-TnT was nog niet eerder aangetoond voor nierziekten bij patiënten met type 2 diabetes en hypertensie. Eerder waren er al aanwijzingen dat GDF-15 was geassocieerd met het optreden van eindstadium nierfalen in patiënten met type 1 diabetes. Hs-TnT wordt in de dagelijkse praktijk gebruikt als marker voor acute hartinfarcten. Daarnaast zijn deze markers in verschillende studies in verband gebracht met hart- en vaatziekten en sterfte. Als onze bevinding dat GDF-15 en hs-TnT een rol kunnen spelen in voorspellen van progressie van nierziekten kan worden bevestigd in andere studies, zou dit het in toekomst tevens mogelijk kunnen maken vroegtijdig patiënten met een hoog risico op (progressie van) chronische nierziekten op te sporen.

Wat betreft de behandeling van patiënten met chronische nierziekten met albuminurie, heeft behandeling met bloeddrukverlagende middelen die het Renine-Angiotensine-Aldosteron Systeem (RAAS) blokkeren de voorkeur. Dit omdat verschillende internationale klinische studies hebben aangetoond dat zulke middelen niet alleen de bloeddruk verlagen, maar ook de albuminurie verminderen en hiermee het risico op eindstadium nierfalen en sterfte in patiënten met type 2 diabetes en hypertensie verminderen, vergelijken met andere bloeddrukverlagende middelen. RAAS-blokkade heeft vergelijkbare effecten bij patiënten met type 2 diabetes maar zonder hypertensie en patiënten zonder diabetes.
Momenteel wordt de dosis van RAAS-blokkade getitreerd op basis van de bloeddrukverlaging die bereikt wordt, zonder dat daarbij wordt gekeken naar het effect op de albuminurie. Dit is begrijpelijk omdat deze middelen in principe bloeddrukverlagende middelen zijn en ook als zodanig geregistreerd zijn, en het gunstige effect op de albuminurie een later vastgesteld bijkomend voordeel is. Echter, recente studies hebben laten zien dat op individueel niveau een daling van de bloeddruk niet altijd gepaard gaat met een daling van de albuminurie en omgekeerd. Het is echter onduidelijk welke van de twee parameters (de daling in bloeddruk of de daling in albuminurie) of de combinatie van beide het belangrijkst is voor de bescherming van de nier. Daarom bestudeerden we in **hoofdstuk 5** de initiële respons op behandeling met betrekking tot de bloeddruk en de albuminurie, en onderzochten we of de initiële respons op behandeling geassocieerd was

met lange termijn uitkomsten van de nier. We onderzochten dit in een cohort van meer dan 500 patiënten met type 2 diabetes en microalbuminurie (vroegste stadium van eiwit uitscheiding) die meededen met een grote internationale studie naar RAAS-blokkade.
In dit hoofdstuk lieten we zien dat de respons op RAAS-blokkade in de eerste 6 maanden verschillend kan zijn voor bloeddruk en albuminurie en dat een groot gedeelte van de patienten een goede respons had in één van beide parameters, maar niet in de andere parameter (discordante respons). Het risico op transitie van micro- naar macroalbuminurie en de mate van nierfunctieachteruitgang gedurende de 2 jaar na starten van de behandeling waren met name afhankelijk van de respons in albuminurie en dit was onafhankelijk van de respons in bloeddruk.
Het vernieuwende van deze bevindingen is dat dit nog niet eerder aangetoond is in patiënten met een vroeg stadium nierziekte. Onze resultaten vormen samen met resultaten van vergelijkbare studies in patiënten met een vergevorderd stadium nierziekte een belangrijke aanwijzing dat het slechts in overweging nemen van de bloeddrukdaling bij de behandeling met RAAS-blokkade misschien niet de meest effectieve manier is om voortschrijding van nierziekten te voorkómen. Voordat de behandelingsstrategie echter in de praktijk aangepast kan worden zal er eerst bevestigend prospectief en gerandomiseerd onderzoek moeten worden gedaan in deze categorie patiënten.

De richtlijnen voor huisartsen en specialisten raden screening en behandeling van albuminurie aan, met als doel nier-, hart- en vaatziekten te voorkómen bij patiënten met type 2 diabetes. De Nederlandse richtlijn voor huisartsen uit 2006 beveelt bij alle patiënten met type 2 diabetes en voldoende levensverwachting jaarlijkse albuminurie screening aan. Als daarbij albuminurie wordt vastgesteld (bij voorkeur op basis van meerdere metingen) dient er te worden gestart met RAAS-blokkade, onafhankelijk of er ook hypertensie is.
Echter, de praktijk is altijd weerbarstiger dan de ideale situatie volgens de richtlijnen. In **hoofdstuk 6** bekeken we de implementatie van de richtlijn met betrekking tot screening en behandeling van albuminurie bij patiënten met type 2 diabetes in de Nederlandse huisartsenpraktijk. In een groot cohort van meer dan 14,000 patiënten met type 2 diabetes die primair behandeld werden door de huisarts zagen we dat in minder dan 60% een albuminurie meting was gedaan in het jaar 2009, en dat zelfs in één derde van alle patiënten in een periode van 2007 tot 2010 nooit een albuminurie meting was gedaan. De getallen verbeterden weinig als we alleen keken naar patiënten met een ruimere levensverwachting, of als we het interval oprekten van 12 naar 15 maanden.
Wat betreft behandeling met RAAS-blokkade vonden we wel dat patiënten met verhoogde albuminurie vaker werden behandeld met RAAS-blokkade dan patiënten

zonder verhoogde albuminurie en patiënten zonder meting. Ook bleek dat het vinden van een verhoogde albuminurie inderdaad een trigger was voor het starten van RAAS-blokkade, echter het absolute percentage voorschriften van RAAS-blokkade na verhoogde meting was teleurstellend laag (~13%). Bovendien is het niet geheel duidelijk in hoeverre RAAS-blokkade wordt voorgeschreven in verband met verhoogde albuminurie, of dat dit met name samenhangt met verhoogde bloeddruk. Omdat RAAS-blokkade ook vaak werd voorgeschreven bij patiënten zonder verhoogde albuminurie en patiënten zonder albuminurie meting ligt het voor de hand om te speculeren dat met name bloeddrukverhoging de doorslaggevende factor voor het voorschrijven van RAAS-blokkade is. Naast verhoging van albuminurie vonden we bloeddruk inderdaad terug als determinant van voorschrijven van RAAS-blokkade.

Ook wat betreft albuminurie screening verrichtten we een analyse van de determinanten. We vonden andere aanwijzingen dat de logistieke organisatie rondom albuminurie screening mogelijk een rol speelt; zo kregen patiënten die aanvullende begeleiding kregen bij een eerstelijns diabetes-centrum en patiënten die eerder een albuminurie screening hadden gehad vaker een albuminurie screening. Andere studies toonden eerder al aan dat logistiek een rol kan spelen bij de mate van implementatie van richtlijnen.

Al met al konden we op basis van onze observaties concluderen dat er in de huisartsenpraktijk ruimte voor verbetering bestaat met betrekking tot screening en behandeling van patiënten met type 2 diabetes met het oog op risicoreductie van nier-, hart- en vaatziekten. Dit aspect van de diabeteszorg krijgt momenteel onvoldoende aandacht en verdient het om meer onder de aandacht van de huisartsen gebracht te worden.

Deel II: Longtransplantatie

In het tweede deel van dit proefschrift gaat het over voorspellen en voorkómen van chronische nierziekten bij patiënten die een longtransplantatie hebben ondergaan. Deze groep patiënten hebben een nog veel hoger risico op progressieve nierfunctieachteruitgang dan de hoogrisico groepen beschreven in het eerste deel van dit proefschrift, echter, door de veel kleinere groep patiënten waar het om gaat maakt deze groep maar een heel klein deel uit van het alle patiënten met chronische nierziekten.
Desalniettemin, in deze groep patiënten die al veel te maken hebben met ernstige ziekte, zorgen chronische nierziekten ook voor grote ziektelast. Uiteindelijk zal 5-10% van alle patiënten met een longtransplantatie uiteindelijk eindstadium nierfalen ontwikkelen en niervervangende therapie nodig hebben. Daarnaast maken de nierfunctiestoornissen het effectief behandelen met immuunsuppressieve (afweeronderdrukkende) medicamenten ten behoeve van het longtransplantaat lastiger.
Een andere belangrijke tegenstelling tussen patiënten die een longtransplantatie hebben ondergaan en de hoogrisico groepen besproken in deel I van dit proefschrift is dat longtransplantatiepatiënten intensief worden begeleid in gespecialiseerde centra, waar patiënten met type 2 diabetes en hypertensie met name in de eerstelijns gezondheidszorg worden behandeld. Deze andere klinische setting samen met het nog hogere risico maakt dat vele aspecten van voorspellen en voorkómen van nierziekten in deze patiëntengroep anders zijn.

In de literatuur worden meerdere patiëntgebonden en transplantatie gerelateerde factoren beschreven als risicofactoren voor chronische nierziekten in longtransplantatiepatiënten. Dit zijn onder andere leeftijd, geslacht, het chronisch gebruik van immuunsuppressiva die schadelijk zijn voor de nier, onderliggend longlijden, type transplantatie (enkele of dubbele longtransplantatie), het gebruik van de hart-longmachine tijdens de transplantatie, hypertensie, diabetes, roken, de nierfunctie voor de transplantatie en de body-mass index (BMI).
Sinds de eerste longtransplantatie meer dan 20 jaar geleden is er veel gebeurd op het gebied van longtransplantatie. Om te beginnen zijn de criteria voor acceptatie van een ontvanger geleidelijk soepeler geworden, waardoor de patiënten die een longtransplantatie ondergaan steeds ouder zijn geworden en meer bijkomende ziekten hebben naast hun onderliggende longziekte. Deze factoren die veranderd zijn kunnen, zoals hiervoor vermeld, beschouwd worden als risicofactoren voor nierziekten en maken dat de *a priori* kans op nierfunctiestoornissen groter is geworden. Aan de andere kant heeft de medicamenteuze en niet-medicamenteuze behandeling van

longtransplantatiepatiënten een grote ontwikkeling doorgemaakt, onder andere doordat artsen zich steeds meer bewust werden van de gevolgen van de behandeling voor de nier. Deze 'leercurve' zou het toegenomen aantal risicofactoren voor nierziekten in deze patiëntengroep gecompenseerd kunnen hebben.
In **hoofdstuk 7** beschreven we deze ontwikkelingen uitvoerig en gaven we inzicht in de veranderingen in de populatie van longtransplantatiepatiënten sinds het begin van het longtransplantatieprogramma in Groningen. Hoewel het profiel van de longtransplantatiepatiënten geleidelijk ongunstiger is geworden met het oog op nierfunctiestoornissen, zijn de uitkomsten met betrekking tot de nier in de loop der jaren verbeterd. Een aantal factoren zou hierin een rol kunnen hebben gehad. Een van deze factoren in de overgang van ciclosporine naar tacrolimus als belangrijkste immuunsuppressieve middel in 2001. In (dier)experimenteel onderzoek en onderzoek bij andere patiëntengroepen is namelijk de indruk gewekt dat tacrolimus minder schadelijk is voor de nier dan ciclosporine.
Andere factoren die mogelijk hebben bijgedragen aan de verbeterde uitkomsten met betrekking tot nierfunctiestoornissen zijn de verfijning en verbetering van de chirurgische technieken, de zorg rondom de operatie, het toegenomen bewustzijn van het bestaan van nierschade na longtransplantatie en de groeiende ervaring met de behandeling van deze patiënten. De laatste twee factoren hebben ervoor gezorgd dat er tijdig maatregelen worden getroffen om verdere schade te beperken, zoals het preventief verlagen van de dosis immuunsuppressieve medicijnen of het voorschrijven van beschermende medicatie bij patiënten met een snelle nierfunctieachteruitgang.

We waren daarnaast geïnteresseerd welke andere risicofactoren er bestaan voor het ontstaan van nierfunctiestoornissen na longtransplantatie. We vermoedden dat, zelfs na het staken van roken, de voorgeschiedenis met betrekking tot roken mogelijk een belangrijke risicofactor was voor complicaties na longtransplantie, met name chronische nierziekten. Dit omdat roken een belangrijke risicofactor is voor chronische nierziekten en er onder de longtransplantatiepatiënten zowel veel ex-rokers zijn, als er een zeer hoge incidentie van chronische nierziekten is.
In **hoofdstuk 8** onderzochten we daarom of er een associatie was tussen voorheen gerookt hebben en (de tijd tot) het ontstaan van chronische nierziekten bij patiënten die een longtransplantatie ondergaan hebben. We toonden aan dat er inderdaad een dergelijk verband was, waarbij er bovendien een dosiseffect relatie werd gevonden. Al ten tijde van transplantatie hadden de ex-rokers een wat slechtere nierfunctie dan patiënten die nooit gerookt hadden. Aan de andere kant waren de patiënten die nooit gerookt hadden ook gemiddeld jonger en hadden vaak ander onderliggend longlijden. Gecorrigeerd voor

deze verschillen in patiëntkarakteristieken bleef er echter een dosisafhankelijk verband bestaan tussen ex-roken en chronische nierziekten. Hoewel over het algemeen wordt aangenomen dat het schadelijke effect van roken op de nier verdwijnt op het moment van stoppen met roken, blijkt dat in dit geval niet op te gaan. Eerder al bleek dat dit ook niet het geval was voor andere aan roken gerelateerde aandoeningen, zoals het risico op longkanker. Het risico op longkanker blijft namelijk gemiddeld 15 keer verhoogd voor mannen en 9 keer verhoogd voor vrouwen tot in ieder geval 10 jaar na het stoppen met roken.
Het is bekend dat nieren een grote, maar moeilijk meetbare, reserve capaciteit hebben. Hierdoor is het mogelijk dat er pre-existente schade was door het roken, die reeds gecompenseerd was door de reservecapaciteit van de nier, en waardoor de gemeten nierfunctie goed is. Een verklaring voor het verhoogde risico op nierfunctiestoornissen zelfs na het stoppen met roken is daarom dat de nieren al wel beschadigd zijn, maar zonder dat het tot expressie komt in de nierfunctiemeting, en dat ze daardoor gevoeliger zijn voor de schade die wordt toegebracht door de longtransplantatie en de medicatie.

Opvallend genoeg was het percentage ex-rokers die fors gerookt hadden (>25 pack years) het grootst onder de het meest recent getransplanteerde patiënten. Dit is een uitting van de geleidelijk soepeler geworden acceptatie criteria die ook werden genoemd in **hoofdstuk 7**. In **hoofdstuk 8** lieten we vervolgens zien dat ex-roken en in het bijzonder fors ex-roken gepaard ging met een verhoogd risico op chronische nierfunctiestoornissen. Desalniettemin zagen we over het geheel genomen een daling in de incidentie van chronische nierfunctiestoornissen. Aan de ene kant is dit een hele goede ontwikkeling. Echter, als kritische noot kan hierbij worden opgemerkt dat het aantal nierfunctiestoornissen wellicht nog verder was teruggelopen als het aantal ex-rokers die fors hadden gerookt dat was geaccepteerd voor longtransplantatie kleiner was geweest.
Naast het risico op nierfunctiestoornissen hebben we ook gekeken naar het effect van ex-roken op overleving na longtransplantatie. We konden geen effect aantonen. Hierbij is het belangrijk te bedenken dat alle patiënten voorafgaand aan longtransplantatie uitvoerig worden gescreend en daardoor bij voorbaat een laag risico hebben om te overlijden. Om deze reden was de kans erg klein dat we wel een effect van ex-roken op de overleving zouden vinden. Desalniettemin vonden we wél een effect van ex-roken op de kans dat een longtransplantatiepatiënt zou overlijden als gevolg van kwaadaardige kanker (in de meeste gevallen longkanker van de niet-getransplanteerde long), waar toch kwalijke effecten van ex-roken uit naar voren komen. Ook in de kliniek bestaat het idee dat de ex-rokers, met name de ex-rokers die veel hebben gerookt, na longtransplantatie meer complicaties hebben en minder profiteren van de transplantatie met betrekking tot

kwaliteit van leven. Dit blijkt echter vooralsnog niet uit het onderzoek en zal eerst verder geobjectiveerd moeten worden. Helaas is ex-roken een niet-modificeerbare risicofactor en kan er, naast de huidige maatregelen die nu getroffen worden na transplantatie, weinig gedaan worden om het risico op chronische nierfunctiestoornissen in ex-rokers te verlagen. Slechts het niet accepteren van deze patiënten zou de kans op chronische nierfunctiestoornissen verkleinen. Daar er echter geen effect van ex-roken op de overleving na transplantatie wordt gezien in combinatie met het gebrek aan prospectieve gegevens en de dalende incidentie van nierfunctiestoornissen, zijn dergelijke maatregelen op basis van deze argumenten niet verdedigbaar.

Tot slot is het belangrijk om de nierfunctie over de tijd te monitoren. Het verloop van de nierfunctie over de tijd is een goede manier om het toekomstig risico op nierfunctiestoornissen in te schatten, en is dit voorspellend vermogen is zelfs superieur aan het voorspellend vermogen van albuminurie.
Het is echter wel de vraag hoe de nierfunctie het best gemeten kan worden. In de dagelijkse praktijk wordt er veel gebruik gemaakt van serum creatinine concentratie of op serum creatinine gebaseerde formules om nierfunctie te schatten. Er zitten echter belangrijke nadelen aan het schatten van de nierfunctie op basis van het serum creatinine omdat dit vaak de met de gouden-standaard methode gemeten nierfunctie onderschat, met name in de hoog-normale range, en omdat de geschatte nierfunctie vaak relatief insensitief is voor het tijdig herkennen van progressieve nierfunctieachteruitgang.
Dit komt met name door de manier waarop en de patiëntengroep waarin de formules zijn ontwikkeld. Daarnaast schieten de formules nog meer tekort bij patiënten met abnormale lichaamssamenstelling aangezien de serum creatinine concentratie grotendeels wordt bepaald door de hoeveelheid spiermassa. De nierfunctieformules zijn nooit eerder gevalideerd ten opzichte van met de gouden-standaard methode gemeten nierfunctie in longtransplantatiepatiënten. Omdat de longtransplantatiepatiënten in veel gevallen een afwijkende lichaamssamenstelling hebben is het mogelijk dat de formules een afwijking hebben van de geschatte nierfunctie ten opzicht van de gemeten nierfunctie. In Groningen werd bij alle longtransplantatie de nierfunctie na transplantatie vervolgd met 125-iothalamate nierfunctie metingen (gouden-standaard meting, mGFR). In **hoofdstuk 9** hebben de formules vergelijken met de gouden standaard, en keken we welke factoren verantwoordelijk zijn voor de deviatie van de formules.
Er werd een opvallend grote afwijking tussen de geschatte nierfunctie en de gemeten nierfunctie gezien, waarbij de formules gemiddeld een onderschatting gaven van de nierfunctie (van 12-32%, afhankelijk welke formule gebruikt werd) zowel voor als na longtransplantatie. Precisie en accuraatheid waren ook beperkt, en er was een zeer grote

intra-individuele spreiding in de afwijking. De afwijking tussen geschatte en gemeten nierfunctie was groter bij hogere nierfunctie, maar uitgedrukt als percentage was de afwijking over de gehele range gelijk. Andere factoren die verantwoordelijk waren voor afwijkingen waren body-mass index, leeftijd, geslacht en onderliggend longlijden. Dit verschilde per formule. Theoretisch nemen de formules leeftijd, geslacht, ras en gewicht mee in de berekening, maar onze resultaten wijzen erop dat dit onvoldoende tot een goede schatting van de nierfunctie lijdt in deze specifieke patiëntengroep.
Daarnaast viel op dat de helling van de nierfunctieachteruitgang over tijd werd onderschat als er slechts gebruik werd gemaakt van de nierfunctieformules, waarschijnlijk doordat de nierfunctie over de hele range wordt onderschat. Hierdoor worden patiënten met een snelle achteruitgang van de nierfunctie met behulp van de formules niet tijdig opgespoord, ondanks herhaalde metingen. Dit beperkt de toepasbaarheid van de formules bij het vervolgen van de nierfunctie na longtransplantatie. Desalniettemin worden de nierfunctieformules in veel klinieken wel voor dit doel gebruikt omdat het simpelweg duur en zeer belastend voor de patiënten is om de nierfunctie te vervolgen met gouden-standaard metingen.

Omdat de nierfunctieformules op basis van serum creatinine tot een slechte schatting van de nierfunctie leidt, wat we in hoofdstuk 9 hebben laten zien, beperkt dit ook de toepasbaarheid van serum creatinine als uitkomstmaat voor wetenschappelijk onderzoek.

Desondanks worden uitkomstmaten vaak gebruikt in wetenschappelijk onderzoek, ook op gebied van nierfunctie na longtransplantatie. Er zijn ook goede kante aan op serum-creatinine gebaseerde uitkomstmaten: metingen zijn gemakkelijk te verkrijgen, en worden overal gebruikt. Hierdoor kunnen resultaten van studies gemakkelijk vergeleken worden. In **hoofdstuk 7** maakten we ook gebruik van een uitkomstmaat gebaseerd op serum-creatinine (namelijk (tijd tot) verdubbeling van het serum-creatinine). Om de gevonden resultaten te ondersteunen, deden we daarnaast de analyse met gemeten nierfunctie als uitkomstmaat. Omdat de resultaten met beide uitkomstmaten vergelijkbaar waren, blijkt hieruit dat, ondanks de intrinsieke beperkingen van serum creatinine, op creatinine-gebaseerde eindpunten toch van waarde kunnen zijn en voorzichtig als uitkomstmaat gebruikt kunnen worden in wetenschappelijk onderzoek.

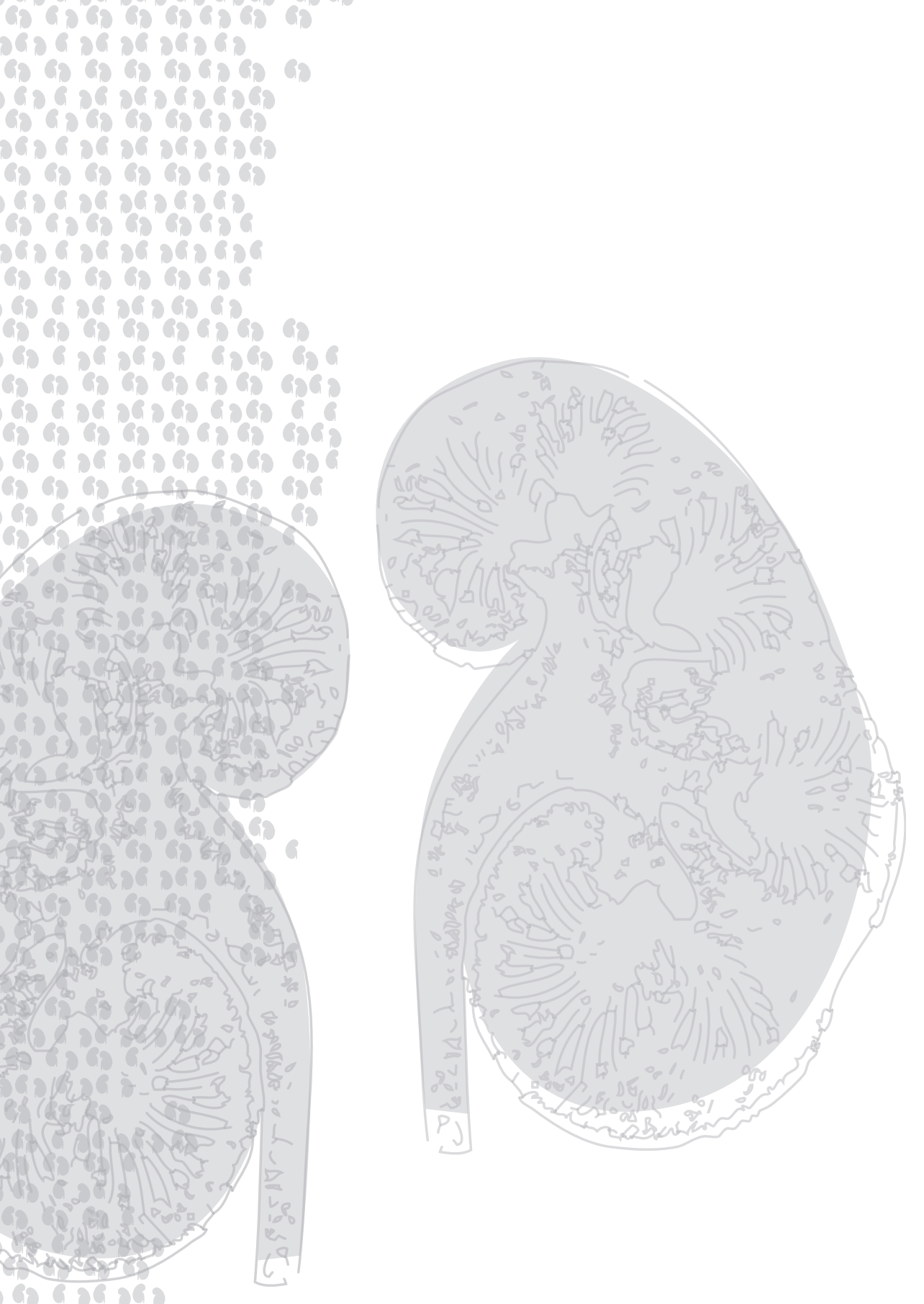
Conclusies

In dit proefschrift zijn diverse aspecten van voorspellen en voorkómen van chronische nierziekten in hoogrisico patiëntengroepen onderzocht. We keken onder andere naar

het voorspellen van chronische nierziekten met behulp van risicofactoren en biomarkers, optimalisatie van strategieën voor het voorkómen van chronische nierziekten en de realiteit van de dagelijkse praktijk.

Hoewel de verschillende patiëntengroepen die we onderzochten als overeenkomst allemaal een verhoogd risico hebben op het ontwikkelen van chronische nierziekten en voorspellen en voorkómen van deze nierziekten belangrijk is, zijn er ook belangrijke verschillen tussen de patiëntengroepen. Het aantal patiënten met type 2 diabetes en hypertensie is erg groot en neemt in snel tempo verder toe. Van al deze patiënten zal uiteindelijk een klein percentage eindstadium nierfalen ontwikkelen. Deze patiënten worden over het algemeen in de eerstelijns zorg begeleid en behandeld, totdat complicaties de kop opsteken. Het aantal longtransplantatiepatiënten is vele malen kleiner, maar het absolute risico op chronische nierziekten daarentegen groter. Daarnaast is de zorg voor deze patiënten zeer gespecialiseerde zorg en worden de patiënten intensief begeleid en behandeld.

Door deze intrinsieke verschillen tussen de patiëntengroepen die aan bod kwamen in dit proefschrift zijn er ook bepaalde verschillen in het monitoren en behandelen. In een grote patiëntengroep met licht verhoogd risico op chronische nierziekten is het niet alleen van belang om de ziektelast van degenen met het hoogste risico zo veel mogelijk te beperken, het is eveneens van belang de last van onnodige behandeling van de degenen met het laagste risico zo veel mogelijk te beperken. In een kleine patiëntengroep met een zeer sterk verhoogd risico op chronische nierziekten zijn meer intensieve monitoring en behandeling op z'n plaats. Omdat chronische nierziekten een zware belasting voor patiënten kunnen vormen is elke stap op weg naar het verbeteren van voorkómen van chronische nierziekten uiteindelijk in het belang van de patiënt.



Dankwoord

Dankwoord Proefschrift

De motivatie vinden om met het dankwoord te beginnen blijkt erg lastig. Het betekent dat het laatste stukje is aangebroken van een project waar het allemaal om draaide de afgelopen jaren, waar ik veel van geleerd heb en waarin ik heb samengewerkt met ontzettend veel leuke, inspirerende, bijzondere en fantastische mensen. Het is jammer te beseffen dat er écht een einde is gekomen aan die tijd. Dit promotietraject was een fantastische leerschool. Het voorliggende resultaat was er niet geweest zonder alle bijdragen en daar wil ik al die genen voor bedanken. In het bijzonder wil ik hier aan aantal mensen noemen die over de tijd waardevolle bijdragen hebben geleverd aan het tot stand komen van dit proefschrift.

De eerste kennismaking met onderzoek was al vroeg tijdens mijn studie. Via via kwamen er wat verschillende projecten op m'n pad, data invoeren vanuit statussen op de niertransplantatie, het nierperfusie-team, experimenten met nierperfusie op het chirurgisch onderzoekslab en vervolgens het eerste onderzoek bij de longtransplantatie. Ik wil iedereen die betrokken was bij mijn eerste kennismaking met wetenschappelijk onderzoek bedanken dat jullie me enthousiast hebben gemaakt.

Mijn eerste onderzoek op gebied van longtransplantatie deed ik met Dr. E.A.M. Verschuuren. Erik, als we afspraken wijdde je altijd eerst een half uur uit over interessante casuïstiek en thoraxfoto´s, voordat we het echt over onderzoek gingen hebben, en tja, dan was de tijd meestal alweer snel voorbij. Het onderzoek doen was vaak met name gezellig en ging niet zo snel, maar uiteindelijk is daardoor misschien wel m´n enthousiasme voor longziekten ontstaan. Desalniettemin was er resultaat: m'n eerste oral presentation op een longtransplantatiecongres in Boston. Daar leerde ik de rest van de vakgroep/ onderzoeksgroep kennen (Wim van der Bij, Michiel Erasmus en Barbara Wagemakers) en ontstond het idee om i.s.m. de afdeling nefrologie te kijken naar nefrologische complicaties na longtransplantatie, een project wat al een tijd op de plank lag.

Dr. W. Van der Bij, Wim, jouw deur stond altijd open (“*Wicht, kom der in”)* voor vragen of gewoon voor een praatje. Met jou ging ik me richten op nierfunctiestoornissen bij longtransplantatiepatiënten. Ik heb je leren kennen als een heel betrokken dokter die ook zeer gewaardeerd wordt door zijn patiënten, droog, gevat, kritisch en met een sterke mening. Je was altijd bereid mee te denken en ik heb je kennis, je mening en je ideeën erg gewaardeerd. Soms kun je je verschrikkelijk opwinden over dingen en een verschrikkelijk humeur hebben (waar ik meestal tijdig voor werd gewaarschuwd door jullie zeer

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Prof. Dr. G.J. Navis, Gerjan, bij het voorstel om eindelijk eens naar de nieren van longtransplantatiepatiënten te kijken was je meteen enthousiast, zoals jij eigenlijk bij alles enorm enthousiast bent. Resultaten vallen nooit tegen, onverwachte resultaten zijn op z'n minst interessant. Zelfs als een stuk al vrijwel helemaal uitgekristalliseerd is heb jij vaak nog nieuwe inzichten of ideeën, wat nieuw licht schijnt op het stuk en het verder verbetert. Je manier van onderzoek doen is erg inspirerend en je bent een goed voorbeeld van *girl power* in het onderzoek. Ik vond het erg leuk om de afgelopen jaren samen met je te werken.

Prof. Dr. D. de Zeeuw, Dick, je hebt me kansen gegeven binnen de vakgroep klinische farmacologie. Je hebt me vaak overdonderd met allerlei plannen, voorstellen en meningen. Toen je me tijdens onze eerste kennismaking vertelde dat ik zou gaan rekenen aan grote datasets had ik vooral weinig idee wat ik kon verwachten. Uiteindelijk ben ik de datasets gaan waarderen en statistiek onverwachts leuk gaan vinden. Ik respecteer je visie en je toewijding en heb veel van je geleerd. Jij krijgt alles voor elkaar wat je in je hoofd hebt. Soms botste dat als ik een andere mening was toegedaan. Soms wist ik niet wat ik met je aan moest, maar uit de verhitte discussies kwamen vaak de mooiste dingen. Uiteindelijk denk ik dat we allebei erg tevreden mogen zijn over het resultaat wat we hebben bereikt en ben ik dankbaar voor de samenwerking.

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The reading committee assessing this thesis consisted of Prof. Dr. G. Mayer, Prof. Dr. H.A.M. Kerstjens and Prof. Dr. H.J.G. Bilo. Your effort is highly appreciated.

I would furthermore like to express my thanks to all the collaborators within SysKID with whom I've had the pleasure of working with and who all strive to make the project a success, in particular Bernd and Gert Mayer, Klaus Weinberger, Ulrika Lundin, Harald Mishak, Joachim Jankowski, Martin de Borst, Paul Perco, Maria Lajer and Peter Rossing. I would also like to thank the co-authors of the post-hoc analysis of IRMA-2 for their fruitful collaboration (Dr. Frederik Persson, Dr. Peter Rossing and Prof. Dr. Hans-Henrik Parving). Ook Prof. Dr. Paul de Jong en Dr. Ron Gansevoort wil ik bedanken voor de toegang tot PREVEND data en samples voor de SysKID discovery study.
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prettig hebben opgevangen en hebben ondersteund in de afronding van dit proefschrift. Binnenkort ben ik er voor de volle 100%!

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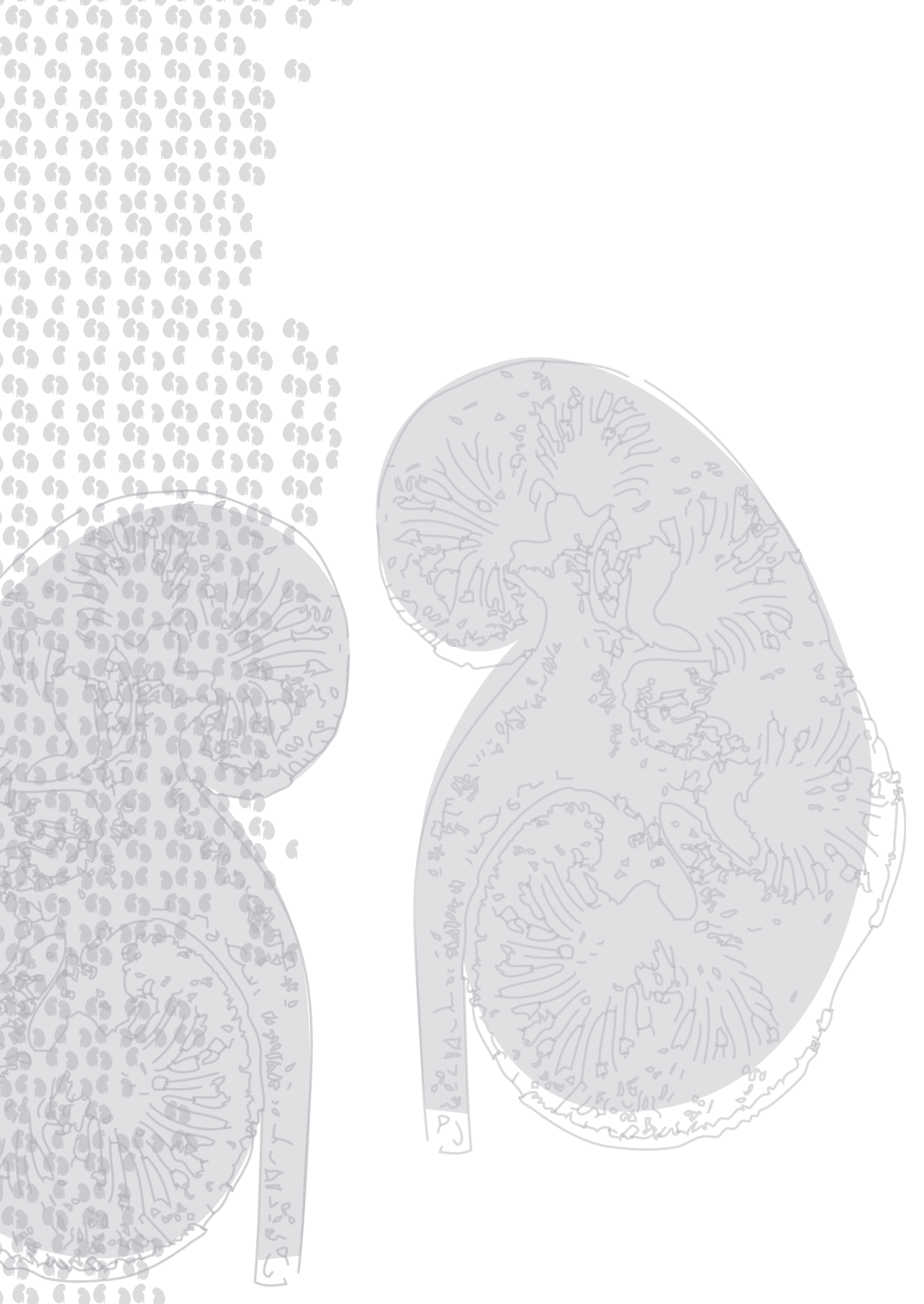
Merel



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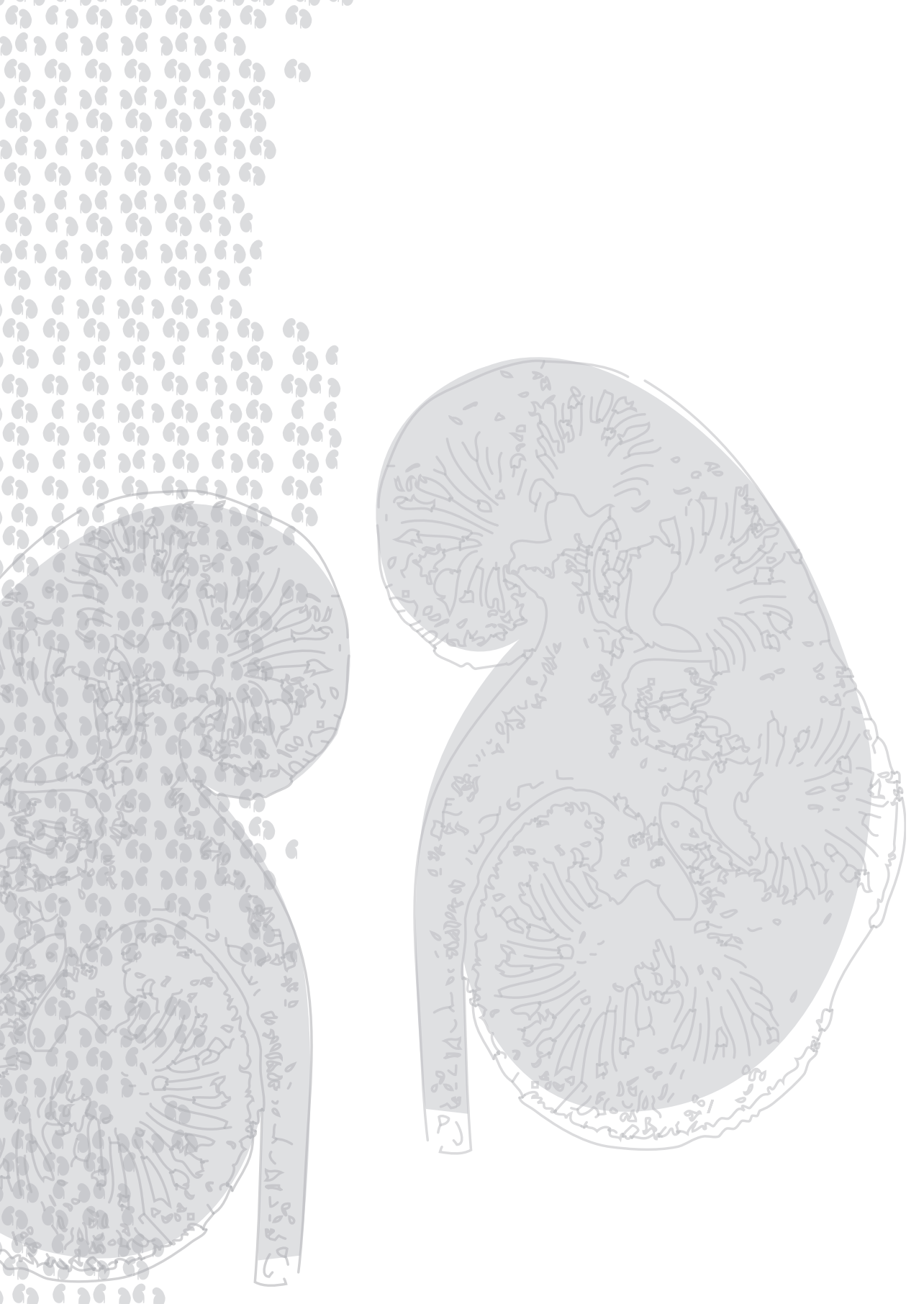

Curriculum Vitae

Curriculum Vitae

Merel is geboren op 4 oktober 1985 in Alkmaar. In 2003 deed zij eindexamen aan het Murmellius Gymnasium in Alkmaar en werd direct ingeloot voor de studie geneeskunde aan de Rijks Universiteit Groningen. Naast haar studie hield ze zich bezig met diverse nevenactiviteiten, zoals de jaarvertegenwoordiging, uittreksels schrijven voor de Joho company, de galacomissie, de co-out dag, de co-raad en buitenlandse stages in Panamá en Peru.

Al tijdens het tweede jaar van de studie kwam ze in aanraking met de wetenschap en deed ze diverse kleine projecten bij de vakgroep niertransplantatie, de vakgroep longtransplantatie, en het chirurgisch onderzoeklab. Ook werkte zij als perfusionist mee aan de nierperfusietrial (NEJM 2009;360:7-19).

Zij liep co-schappen in het Universitair Medisch Centrum in Groningen, het Delftzicht ziekenhuis in Delfzijl en het Sint Elisabeth Hospitaal in Willemstad, Curaçao. Haar wetenschappelijke stage deed ze in het UMCG bij de vakgroep longtransplantatie in samenwerking met de nefrologie. Gezien de ontstane interesse voor de longgeneeskunde liep zij haar keuzecoschap op de afdeling longgeneeskunde van het VU medisch centrum in Amsterdam.

Na haar afstuderen in 2010 besloot ze eerder onderzoek voort te zetten als promotieonderzoek in Groningen, waar dit proefschrift uit voort vloeide. Sinds december 2011 is zij werkzaam als AIOS longziekten in het Spaarne Ziekenhuis in Hoofddorp, en zal eind 2012 met de vooropleiding interne geneeskunde starten in het OLVG in Amsterdam.

Curriculum Vitae