Renal function estimation:
the implications for clinical practice

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Chapter 1

General introduction, aims and outline of the thesis

The number of patients needing renal replacement therapy is increasing. In the United States the incidence of end stage renal disease (ESRD) increased from approximately 73,035 to 116,395 patients in the period 1995-2009 and is still increasing [1-2]. In the Netherlands in the same period, the number of newly diagnosed patients with ESRD increased from approximately 1280 to 1900 patients [Source: www.renine.nl].
In the nineties of the last century it was recognized that early detection of renal function loss and adequate addressing and treatment of (potentially) damaging factors such as poor lifestyle (high BMI, smoking), hypertension and poor glycemic control was needed in an effort to try to curb the increase in the number of patients developing ESRD. A system classifying renal function loss in different stages would aid in the comprehension of the course, treatment and eventual prevention of progressive renal function loss and its complications over time [3-8]. Until then, patients were often diagnosed late, and in part presented themselves at a moment they already developed symptoms as a consequence of severe renal function loss [8-10]. Moreover, it was acknowledged that not only patients with ESRD but also patients with moderate renal function loss were at increased risk for cardiovascular morbidity and mortality, especially those aged <65 years [11-12]. Depending on the degree of renal function loss, those patients have a 1.5 to 20 fold increased risk for developing cardiovascular disease compared with patients without renal function loss [13]. These findings sparked the introduction of the term chronic kidney disease (CKD) and the development of a classification system with well described definitions.
Primary care physicians have an important task in the early detection of CKD as well as the assessment of cardiovascular risk factors. Moreover, decisions regarding drug-dose adjustments of renally excreted drugs and the eventual initiation of therapies to prevent kidney failure progression may be initiated by primary care physicians [14]. However, primary care physicians were mostly uninformed about renal function loss and insufficiently trained in the interpretation of serum creatinine measurements before the new classification system was proposed [8]. To raise awareness for CKD and to be able to recognize CKD adequately worldwide, a clear definition and classification was needed.

Assessment of glomerular filtration rate

Before the introduction of formulas to estimate GFR in clinical practice, the most commonly used measure of overall GFR was the serum creatinine concentration. Creatinine is a substance produced in the muscles by the non-enzymatic dehydration of creatine. The first time creatinine was proposed as a marker of GFR originates from 1926, when the renal clearance of exogenously administered creatinine was evaluated [15]. Eight years later, the actual measurement of endogenous serum creatinine was realised [16]. However, the drawback of using serum creatinine as a measure of renal function is the hyperbolic relation between serum creatinine and GFR [17]. Moreover, the serum creatinine

concentration is not only determined by glomerular filtration, but also dependent on many other factors (see table 1). These factors, together with the variation coefficient of the assay, and the interperson variability of creatinine production, filtration and secretion results in the conclusion that up to 50% or more of GFR must be lost before serum creatinine values rise above the upper limit of normal [8,18]. An example of the weakness of serum creatinine as a marker of GFR is illustrated by the elderly. In the elderly, muscle mass is usually decreased. When GFR remains unchanged, the decreased creatinine production should lead to lower serum creatinine levels. In the elderly, there is usually evidence of progressive decrease of GFR. As a result serum creatinine values will actually rise to pseudonormal values and be comparable to values in younger persons with a larger muscle mass. Thus, creatinine levels considered normal in a 20-year old may represent major renal function loss in a frail elderly person.

The GFR can be reliably measured by calculating the renal clearance of a substance that is not bound to plasma proteins, freely filtered, not metabolized, and not reabsorbed or secreted by the renal tubules.
Inulin fulfills the prerequisites mentioned above, and is considered the golden standard technique for the measurement of GFR [19-20]. Radioactive markers such as ^{125}I-Iothalamate and ^{99m}Tc-DTPA, provide good alternatives to inulin for measuring GFR. Although these techniques are accurate measures of GFR they are cumbersome, invasive and costly and therefore not suitable for use in daily practice.
A good alternative in daily practice is the creatinine clearance (CCR). The CCR is calculated from the amount of creatinine excreted by the kidneys in 24 hours and the concomitant serum creatinine concentration. The CCR will always provide higher values than true GFR (10-40%), since – as already stated - creatinine is partly eliminated by tubular secretion [21-23]. Therefore, the CCR is considered by many as an imperfect marker of glomerular filtration rate, especially in renal diseases [24]. Moreover, the accuracy of the 24-hour CCR is strongly dependent on the accuracy of the urine collection, especially when urine is collected only once [8]. To improve the accuracy of this method it is recommended to perform a 24-hour urine collection 2-3 times.
The limitations of the abovementioned techniques have stimulated the introduction of formulas for estimating GFR and CCR. These formulas use serum creatinine concentration and take into account several variables influencing the creatinine production (depending on the formula incorporating age, gender, weight and race). In the past decades various formulas have been developed and have become an integral part of daily clinical practice: the Cockcroft-Gault (CG) formula (1976) [25], the Modification of Diet in Renal Disease (MDRD) formula (1999/2002) [18,26] and the Chronic Kidney Disease Epidemiology collaboration equation (CKD-EPI) (2009) [27] formulas. It is

important to know the background of these formulas and their pitfalls to allow correct interpretation of their results. Most of these renal function prediction equations have been developed in populations with specific characteristics, which hamper their use in patients that differ from the population these formulas were developed in. Moreover, these formulas may show substantial variability depending on the technique used to measure serum creatinine [28-32].

Table 1. Factors affecting the serum creatinine concentration [33-36]

- Production
    - Increased creatinine production
        - Increased muscle mass (bodybuilding, great physical effort)
        - Muscle decay (rhabdomyolysis)
        - Meat consumption (stewed meat)
        - Exogenous creatinine sources (creatinine supplements)
    - Decreased creatinine production
        - Decreased muscle mass, e.g. inactivity, amputation, malnutrition
- Excretion
    - Decreased or blocked tubular creatinine excretion
        - Use of medication, e.g. cimetidin (ranitidin and famotidin), trimethoprim, dronedaron.
- Creatinine assays
    - Jaffe assay
        - Increased:
            - Diabetic ketoacidosis
            - Increased glucose
            - Increased serumalbumin
            - Medication: cefoxitine, flucytosine
            - Hemolysis
        - Decreased:
            - Hyperlipidemia
            - hyperbilirubinemia
    - Enzymatic assay
        - The enzymatic assay has less interferences than the Jaffe. Possible interfering substances are: bilirubine, dopamine, ascorbin acid and sarcosin.

GFR prediction equations

The CG formula is a formula that has been developed to estimate the CCR [25]. Apart from age, gender and creatinine, it also contains weight as a variable, since this was considered a crude estimate of muscle mass and therefore also of creatinine 'production'. This equation was developed in a cohort of 249 non-obese male patients with a weight within the 10% range of fat-free body mass. Moreover, the majority of participants had a normal renal function, and the Cockcroft-Gault formula is therefore especially reliable in patients with a CCR >60 ml/min. The correction factor applied for women (0.85) was based on an educated guess [25].

The MDRD has been developed in a training cohort of 1070 and a validation cohort of 558 patients with severe renal function loss (mean GFR 39.8 ml/min), overt proteinuria (>1 g/day urinary protein loss) and all participants were younger than 70 years [18,26]. The body weight was ≥ 80% and ≤ 160% of their standard body weight. The MDRD is indexed for a body surface area of 1.73m^2 (which approximates the BSA of a non-overweight average-sized person). This formula gives inaccurate results when used for people with a normal or slightly reduced eGFR (eGFR >60 ml/min/1.73m^2) [31-32,37-39]. In an attempt to provide better estimates of GFR particularly for values exceeding 60 ml/min/1.73m^2, the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was developed and validated. This formula was developed in a population with a mean GFR of 68 ml/min/1.73m^2 and a mean BMI of 28 kg/m^2. This formula showed to be somewhat more precise and accurate than the MDRD study at higher GFR values leading to less misclassification of CKD [27], although its estimate still may show a considerable deviation from the true value in individual cases [40].

Estimated GFR values are usually applied in guidelines and clinical decisions regarding pharmacovigilance, and referral policies from primary care to nephrology departments also are often made based on these estimates.

CKD-staging: the K/DOQI guidelines

The K/DOQI guidelines are based on such GFR estimates [8]. The GFR estimated by the MDRD formula became one of the two components in the classification scheme of CKD, as developed by the National Kidney Foundation in 2002 [18,26]. These so-called Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines provided a definition of CKD and introduced a uniform staging system (table 1) [8]. The second component was the presence of kidney damage as defined by persistent proteinuria (defined as albuminuria >30 mg/g of urinary creatinine), renal haematuria, or abnormalities in kidney imaging. The diagnosis CKD is made when kidney damage is present according to this definition. In addition, an eGFR value below 60 ml/min/1.73m^2 during ≥ 3 months suffices for the diagnosis CKD.

Since care pathways have been developed for patients with various CKD stages, a proper estimation of GFR is pivotal for good patient care. Since an eGFR <60 ml/min/1.73m^2 apparently is deemed sufficient for the diagnosis of CKD, accuracy of the formulas around this threshold value is of utmost importance. Application of the K/DOQI classification and using MDRD eGFR to the Dutch population, resulted in a high prevalence of CKD in The Netherlands (table 2) [4].

However, there are still many open ends when using GFR estimating equations in daily (clinical) practice; the use of the formulas may give biased results when used in populations that differ from the population in which these formulas originally were developed. Moreover, we need more insight in the differences between the various formulas, the use of threshold values of eGFR in relation to age, the implications of variations in creatinine assays on the prediction formulas, and the role of eGFR on pharmacovigilance.

Stage	Description	GFR (ml/min/1.73m^2)	Prevalence in the Netherlands (%) [41]
1	Normal GFR with evidence of chronic kidney damage *	≥90 *	1.3
2	Mildly decreased GFR with evidence of chronic kidney damage *	60-89 *	3.8
3	Moderately decreased GFR with/without evidence of kidney damage	30-59	5.3
4	Severely decreased GFR with/without evidence of kidney damage	15-29	0.04
5	Kidney failure	<15 or dialysis	<0.04

Table 2 Kidney disease outcomes quality initiative stages [8]. *Other evidence of chronic kidney damage may be: persistent microalbuminuria; persistent proteinuria; persistent haematuria (after exclusion of other causes such as urological disease); structural abnormalities of the kidneys demonstrated on ultrasound scanning or other radiological tests (e.g. polycystic kidney disease, reflux nephropathy); or biopsy-proven chronic glomerulonephritis.

The importance of accurate creatinine measurements

Both Jaffe as well as enzymatic assays are used for routine assessments of creatinine in plasma, serum and urine [42-46]. In the assays for serum and urine creatinine, several substances may (and quite often will) interfere. The Jaffe techniques are among the oldest techniques to measure creatinine and are still often used as a routine method to assess creatinine. Originally the Jaffe reaction is a chemical reaction in which creatinine under alkaline conditions reacts with alkaline picrate; this results in the formation of a red-

orange colored complex that can be detected and quantified [45]. Since its development, several more recent versions of Jaffe assays minimized analytical interferences (such as glucose, uric acid, protein, and ketoacids) by adjusting temperature, calibrator set points, and assay constituents [47-49]. The later developed enzymatic technique, which was less sensitive to analytical interferences, is based on the enzymatic reaction of creatinine with creatinine iminohydrolase [50-52]. This results among others in the formation of ammonia which is quantified by reaction with bromphenol blue. Also for the enzymatic assay, newer and more accurate versions have been developed over time [53-55].

Since the use of formulas to estimate renal function in clinical practice has increased, and the early identification and advices for management of patients with CKD is based on these estimates, focus has been on the consequences of the use of different creatinine measurement procedures among laboratories [56]. Obviously, formulas that are developed in one laboratory can only be reliably used in other laboratories if the assays used in these laboratories report similar values. This was initially not considered. However, some years after the introduction of the MDRD formula, developed from data from the Modification of Diet in Renal Disease Study, it became evident that there was an error when serum creatinine assays were not calibrated with the kinetic Jaffe method as used in the core laboratory in which the formula was developed and validated [57-59.] Especially in the physiologic range, which is important to identify patients with "silent kidney" disease, the calibration bias resulted in larger uncertainty in GFR estimates [41].
Also in trueness verification studies a significant interlaboratory variation was observed and led to the decision that calibration traceability to higher-order reference methods (Isotope-Dilution Mass-Spectrometry (IDMS)) was needed to realize comparable biochemical measurement results in order to produce more accurate renal function estimates [60]. Independent of the method used (enzymatic or Jaffe) or the laboratory that performed the creatinine measurements, this should lead to a better comparability. As a consequence, the MDRD was re-expressed using enzymatic creatinine measurements, and a coefficient appropriate for the new serum creatinine values was applied [30].

Since 1998, it is legally mandatory in the European Union to have calibration traceability in clinical laboratory measurements to higher-order standards (the European in vitro diagnostics (IVD) directive 98/79/EC) [61-62]. Since the development of NIST SRM 967 in 2006, a matrix-based IDMS targeted creatinine standard, all essential elements (i.e. methods, laboratories, and materials) needed to complete the creatinine reference system are in place, as defined by the international Joint Committee on Traceability in Laboratory Medicine (www.bipm.org) [63-64]. Since then, the values assigned by in vitro diagnostic manufacturers to calibrators and control materials supporting routine

measurement procedures have to be methodologically traceable to higher-order reference measurement materials, regardless of the method applied.

In this thesis, different aspects of the use of formulas for estimating GFR in different populations and the impact hereof on referral policies and CKD staging are evaluated. More specifically, our aims were (1) to evaluate the performance of the MDRD and the CKD-EPI equation in a diabetes population and in an obese population, (2) to evaluate whether and how eGFR and albuminuria relate to mortality in a subgroup of elderly patients with T2DM, (3) to assess the consequences of applying age related cut-off values for renal function on the referral policy from primary to secondary care, (4) to evaluate the role of different techniques to measure creatinine (in plasma and urine) on both renal function prediction equations and CCR. In addition, we addressed (5) to evaluate the problem of medication errors in a community based population, and how a medication alert system warning for impaired renal function can improve medication safety.

Outline of the thesis

Several patient- and screening-related factors need to be taken into account when aiming for accurate detection and classification of patients with renal function loss. In **chapter two**, classification of CKD is addressed in a cross-sectional analysis of outpatients of the Maxima Medical Centre Eindhoven with type 2 diabetes mellitus (T2DM) and a known 24-hour CCR. In these patients the performance of the MDRD and the more recently developed CKD-EPI formula was compared. The performance of estimation equations in patients with T2DM was further explored in **chapter three** which focused on the influence of (over)weight on the performance of renal function estimating equations. Since overweight and obesity are prevalent in T2DM and formulas to estimate renal function were originally developed in rather lean populations, this may induce problems in renal function estimation using formulas. Unlike the MDRD and the CKD-EPI, body weight is included as a variable in the CG. Since excess body weight in an overweight and obese population usually comprises adipose tissue and not muscle mass, this formula is thought to overestimate renal function, especially in patients with a body mass index BMI >30 kg/m^2. Therefore, we evaluated the influence of (over)weight on the performance of the MDRD and CKD-EPI equations versus the CG (in the same cohort as was used in chapter two), as well as the implications of using different formulas to estimate renal function on the (mis)classification of chronic kidney disease in this patient group.

Besides patient-related factors, also differences in the assay techniques of creatinine might influence the results of renal function equations and thereby classification of CKD, since serum creatinine is the main component in all equations. In **chapter four and**

five we evaluated the implications of accurate serum creatinine measurements on kidney disease staging. In **chapter four** we discussed this subject from a theoretical point of view, using data from the annual external quality assessment program of the external quality assessment organization in the Netherlands. In **chapter five** the variability between creatinine assays in both plasma and urine and the influence hereof on GFR estimating equations or when calculating the CCR in a real-life population was evaluated.

Although it is well-recognized that CKD is associated with a burden of complications mainly due to cardiovascular comorbidity, the classification and the impact of CKD on cardiovascular morbidity and mortality in different age-groups is still under debate. Age-related cut-off values were suggested, since previous studies have shown that classic cardiovascular risk factors (such as a diminished renal function) possibly have a diminished effect or different consequences when assessed in elderly. In **chapter six** we therefore analyzed the relation between eGFR, albuminuria and mortality in older patients with T2DM.

Quite a few CKD guidelines classify patients independent of their age. However, some guidelines take a different approach, and introduced age-related cut-off values for referral. In **chapter seven** the impact of age-related cut-off values as proposed by the Dutch 'Landelijke Transmurale Afspraak Chronische Nierschade' on referral patterns was compared with the staging system used in the K/DOQI guidelines in which no age-related cut-off values are applied.

Although the MDRD has not been developed in elderly patients and moderate reduction in eGFR (MDRD 45-60 ml/min/1.73m^2) are regularly found in elderly people, the K/DOQI guidelines recommend further diagnostics and treatment when the MDRD is <60 ml/min/1.73m^2. Whether and to what extent renal function loss in the elderly population is pathologic or physiologic is still debated. Although elderly patients may have a diminished renal function that has no consequences for their life expectancy, medication use in elderly patients (especially those with polypharmacy) should be reviewed regularly for potential adverse drug events. Therefore, in **chapter eight** we addressed the problem of increased susceptibility for medication errors in CKD. In this chapter medication errors in patients with an eGFR ≤40 ml/min/1.73m^2 were evaluated in a community based population from a preventive perspective. Finally, in **chapter nine** the different studies in this thesis are discussed. Moreover, some recommendations and future perspectives based on the outcomes of the studies in this thesis are made.

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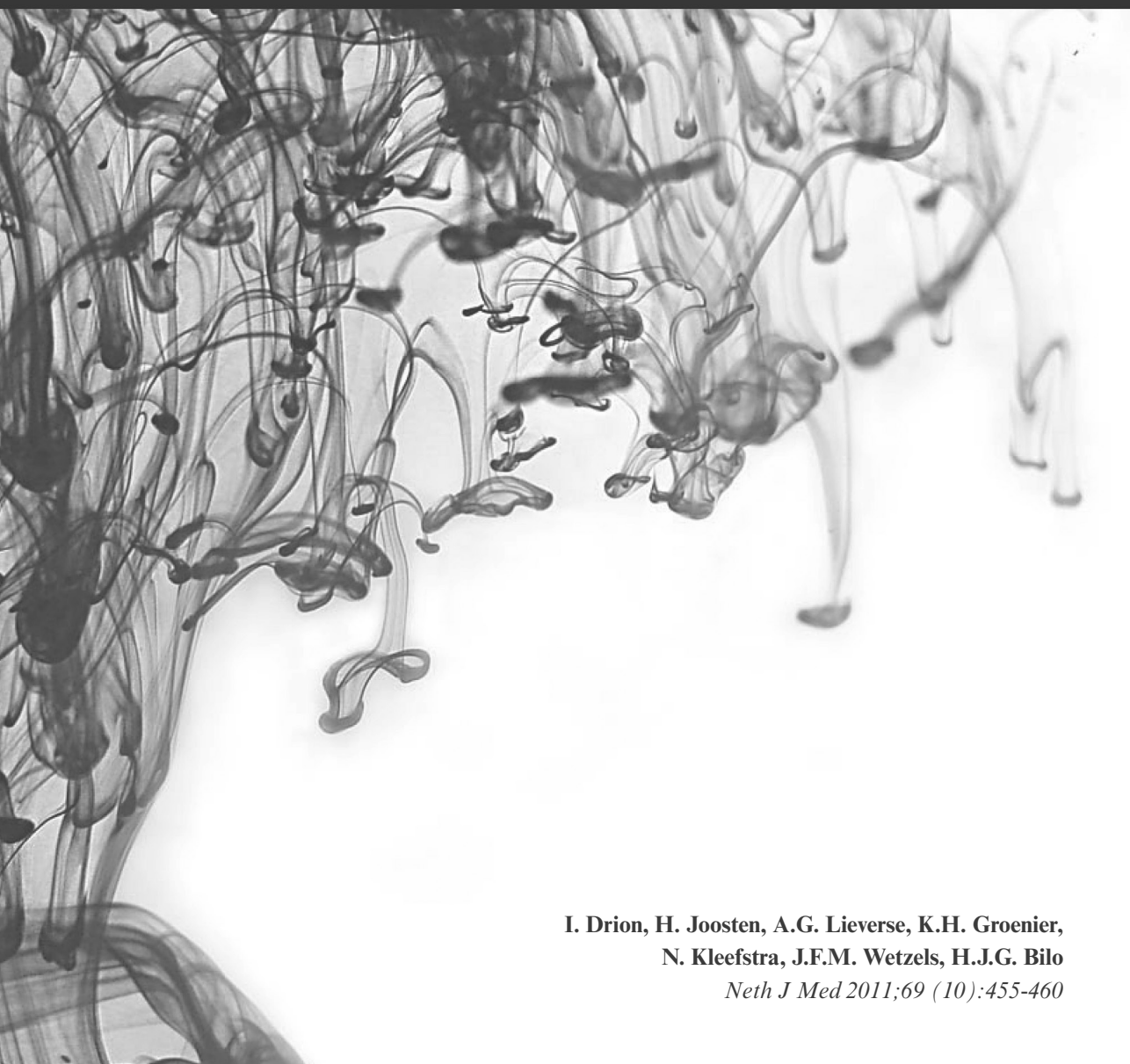
Chapter 2

Equations estimating renal function in patients with diabetes



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Background and objectives Equations to estimate the glomerular filtration rate (GFR) are routinely used to assess renal function. Due to systematic underestimation and limited precision of the current prediction equations, a new prediction equation was presented: the chronic kidney disease collaboration equation (CKD-EPI). We compared the performance of the CKD-EPI and the Modification of Diet in Renal Disease equation (MDRD) and evaluated the implications hereof on chronic kidney disease (CKD) staging in diabetic patients.

Methods This cross-sectional study included 844 diabetic patients with a wide range of age: 18-92 years. Serum creatinine was measured by a traceable enzymatic method and was used to calculate the MDRD and the CKD-EPI. The performance of both renal function estimating equations was examined, by means of the correlation, bias and precision, using the creatinineclearance as a reference method.

Results Correlation between the MDRD, CKD-EPI and the creatinineclearance was 0.75 respectively 0.76. The bias of the MDRD and the CKD-EPI compared with the CCR are -22 (± 26) ml/min/1.73m^2 and -20 (± 26) ml/min/1.73m^2, respectively ($p < 0.01$ for both). When using the CKD-EPI 17.1% of the women was categorized in lower CKD stages as compared with the MDRD. A higher proportion of diabetic patients <65 years were diagnosed with stage 3-5 CKD when using the MDRD; 12.9% versus 10.7%.

Conclusions The MDRD and CKD-EPI are equally imprecise. The CKD-EPI equation gives higher estimates of GFR in young diabetics than the MDRD, leading to a lower CKD prevalence on population level.

Introduction

Renal function testing is routinely performed in various patient populations with a wide range of renal function. Impaired renal function is an independent risk factor for (premature) cardiovascular disease [1]. Several traditional (diabetes mellitus (DM) and hypertension) and non-traditional (among others endothelial dysfunction and oxidative stress) risk factors seem to play an attributable role, but exact mechanisms and interactions remain to be elucidated [1]. Currently, the glomerular filtration rate (GFR) is considered to be the best overall indicator of renal function [2].

Gold standards for assessing GFR, such as renal inulin clearance or isotopic methods are cumbersome and costly and therefore reserved for research settings [3-4]. A less costly and less complex method to measure renal function is the 24-hour creatinine clearance (CCR). This is the most frequently applied method to assess renal function in daily practice, although collecting 24-hour urine samples is time consuming, and the reliability of the outcome is highly dependent on the accuracy of the urine collection [5].

Several prediction formulae for estimating renal function have been developed. The four-variable Modification of Diet in Renal Disease equation (MDRD), is the prediction formula that is most frequently used [2,6]. Its advantages and disadvantages have been extensively debated [7-8]. One of its major disadvantages are its imprecision and its systematic underestimation of GFR, in patients with normal to high normal serum creatinine levels, and the underestimation in women and young people [7,9]. To overcome the aforementioned disadvantages, a new prediction equation, the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was developed [10]. This formula was developed in a population with predominantly young and middle aged people (87% ≤65 years) with an average GFR of 68 ml/min/1.73m^2; 43% was female [10]. Potential complementary covariates such as renal transplant, diabetes and weight were considered, but the final equation used the same variables as the MDRD equation [11]. Therefore, it is not clear whether the CKD-EPI can be applied in all populations. Since an accurate estimate of renal function is important and renal function is frequently assessed in diabetic patients, we wanted to evaluate the performance of the CKD-EPI and the MDRD equations in a large, anthropometrically diverse cohort of diabetic patients.

Materials and methods

This retrospective observational cross-sectional study was conducted at the diabetes outpatient clinic of the Maxima Medical Centre in Eindhoven, The Netherlands. A total of 1097 serum creatinine concentration results from adults, previously diagnosed with

type 1 or type 2 DM, were collected. An anonymous database was created, using data from the "Chipsoft Electronisch Zorg Informatie Systeem" [Chipsoft Electronic Care Information System] (CS-EZIS), the computerized medical record system used at the Maxima Medical Centre. Data collected included 24-hour urinary creatinine (mmol/L), serum creatinine (μmol/L), HbA1c (mmol/mol), weight (kg), height (centimeters), age (years), and gender; all data being collected on the same day, except for the 24-hour urine collection, which was collected in the 24 hours previous to the other measurements. The body mass index (BMI) of each patient was calculated (BMI = weight (kilograms) / height (metres)^2) and added to the database. Ultimately 916 patients remained eligible for inclusion. Two subjects younger than 18 years old and three subjects with a CCR >250 ml/min were excluded, since the MDRD has not been validated in these patient groups. In 176 cases, subjects had collected two 24-hour urine portions during the indicated period. In these cases the mean of the two 24-hour creatinineclearances was used.
Medication details and information on co-morbidities were also not available. Since no information on race was available, all patients were considered to be Caucasian. No formal approval from the Medical Ethics Committee was required, as our data included only anonymous patient characteristics and laboratory data.

Renal function measurements and definitions
The serum creatinine concentration was measured by an enzymatic technique (Modular PA, Roche), and validated by isotope dilution mass spectrometry (IDMS). Renal function was estimated by two different eGFR equations, the MDRD and the CKD-EPI (table 1). 24-hour CCR corrected for body surface area (BSA) was calculated (table 1). The Dubois formula was used to calculate the BSA [12].

Equation	Gender	Serumcreatinine (μmol/L)	eGFR (ml/min/1.73m^2
CKD-EPI	Female	≤62	$144 \times (\text{IDMS creatinine}/88.4/0.7)^{-0.329} \times (0.993)^{age}$
	Female	>62	$144 \times (\text{IDMS creatinine}/88.4/0.7)^{-1.209} \times (0.993)^{age}$
	Male	≤80	$141 \times (\text{IDMS creatinine}/88.4/0.9)^{-0.411} \times (0.993)^{age}$
	Male	>80	$141 \times (\text{IDMS creatinine}/88.4/0.9)^{-1.209} \times (0.993)^{age}$
MDRD	Female	All	$175 \times (\text{IDMS creatinine}/88.4)^{-1.154} \times age^{-0.203} \times 0.742$
	Male	All	$175 \times (\text{IDMS creatinine}/88.4)^{-1.154} \times age^{-0.203}$
Creatinineclearance BSA corrected	All	All	(urine creatinine [mmol/L] x 1000/serum creatinine [μmol/L]) x (24-hour volume urine [ml]/1440) x (1.73m^2/BSA)

Table 1. Renal function prediction equations. CKD-EPI: chronic kidney disease epidemiology equation; MDRD: modification of diet in renal disease formula; BSA: body surface area; IDMS: isotope dilution mass spectrometry.

Statistical analysis

Analyses were performed using SPSS 16.0 (SPSS, Chicago, IL). Q-Q plots and histograms were used to assess normality. Continuous variables are represented as mean (± standard deviation) for the normally distributed values and as a median (interquartile range) for the non-normally distributed variables.

Spearman's coefficient of correlation was calculated to determine the correlation between the CCR and the eGFR calculated by the MDRD and the CKD-EPI formulas. Bland-Altman plots were created; showing the mean of two measurement methods (i.e. CCR and the MDRD / CKD-EPI) against the absolute difference between these two methods. The Krippendorff coefficient, an aggregate measure for method concordance, was calculated (see textbox; 1 meaning perfect concordance and -1 meaning perfect discordance between two methods), since neither maximum correlation nor agreement in accuracy and precision alone will suffice to prove concordance and thus sufficient reproducibility among methods; this requires $\mu_{1=}\mu_2$, $\sigma_1^2=\sigma_2^2$ and $\rho=+1$ (μ_1 being the population mean of the CCR, μ_2 being the population mean of the MDRD or the CKD-EPI, σ_1 and σ_2 being the standard deviation of μ_1 and μ_2 respectively, ρ being the Pearson correlation coefficient between the CCR and the MDRD or the CKD-EPI. The Krippendorff coefficient corresponds to the Bland Altman plot in a similar way as ρ corresponds to the simple scattergram [13], see textbox.

The bias and precision (see textbox) of both formulas were determined. Ultimately we evaluated the classification of patients according to the CKD-EPI or the MDRD equation compared to when the CCR is used to classify patients. Moreover, the prevalence of stage III-V CKD in this diabetic population was evaluated per age group.

Textbox

Bias:	Mean difference between the GFR estimating formula and the creatinineclearance corrected for BSA
Precision:	Standard deviation of the bias
Krippendorff coefficient:	$K= (2 \times \sigma1 \times \sigma2 \times \rho) / (2 \times \sigma1 \times \sigma2 + (\sigma1 - \sigma2)2 + (\mu1 - \mu2))2$

Results

The patient characteristics are presented in table 2. Age ranged from 18-92 years with 53.6% of the population aged under 65 years. The population represented a wide range of renal functions (CCR 11-250 ml/min/1.73m^2). 71 % of the subjects had a CCR >60 ml/min/1.73m^2.

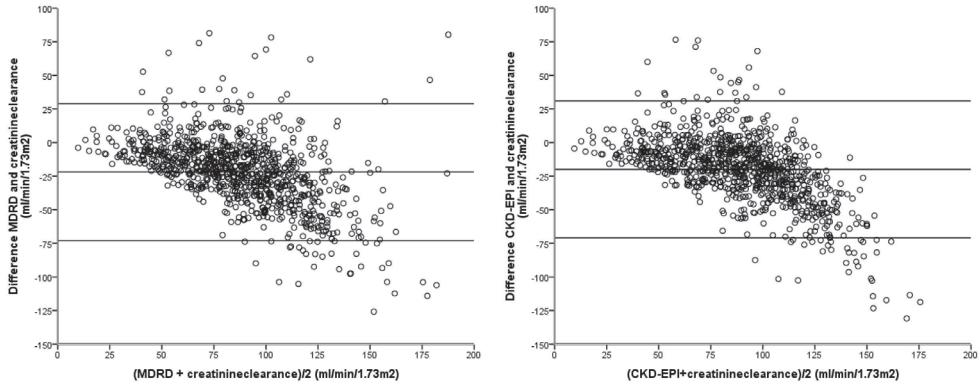
Characteristic	All
n (%)	916
Sex, male (%)	55.3
Age (year)	63 [53, 72]
HbA1c (mmol/mol)	50 [42, 60]
BMI (kg/m^2)	28 [25, 32]
Creatinine (µmol/L)	79 [67, 97]
Creatinine clearance (ml/min/1.73m^2)	96 [70, 123]
MDRD (ml/min/1.73m^2)	77 ± 25
CKD-EPI (ml/min/1.73m^2)	79 ± 24

Table 2. Demographic and clinical characteristics. Data are presented as number (%) or median [interquartile range].

The correlation and Krippendorff coefficient

The correlation was 0.75 and 0.76 between the MDRD respectively the CKD-EPI and the CCR. Figure 1 shows the Bland-Altman plots that evaluate the extend of agreement between the CCR and both GFR estimating equations.

Krippendorff's coefficient, demonstrating the method concordance between both GFR prediction equations and the CCR, was almost equally large for the MDRD and the CKD-EPI: 0.54 respectively 0.57.



Figuur 1. Bland-Altman plots comparing the creatinineclearance and the estimated glomerular filtration rate, calculated by the Modification of Diet in Renal Disease formula or the Chronic Kidney disease Epidemiology Collaboration equation. The upper and the lower horizontal line represent the upper (2 SD) and lower (2 SD) limits of agreement, respectively. The horizontal line in the middle represents the mean difference between the creatinineclearance and the GFR estimating equations.

Bias and precision

The results for the bias and the precision are presented in table 3. The bias of the MDRD and the CKD-EPI compared with the CCR was -22 (± 26) and -20 (± 26) ml/min/1.73m^2,

respectively (p <0.01 for both). Both the MDRD and the CKD-EPI showed a large bias and imprecision in all CCR categories which was most prominent in people with a CCR >90 ml/min/1.73m^2: -53.4 (± 35.2) and -51.4 (± 34.8) ml/min/1.73m^2 for the MDRD and the CKD-EPI, respectively.

Creatinineclearance		**MDRD**		**CKD-EPI**	
(ml/min/1.73m^2)	n	Bias	Precision	Bias	Precision
>90	521	-53.4	35.2	-51.4	34.8
60-90	248	-19.0	18.6	-16.4	18.6
45-59.9	85	-9.4	15.2	-8.5	16.5
30-44.9	44	0.9	20.4	1.2	20.0
<30	18	8.4	21.3	8.7	24.0
All	916	-36.2	35.7	-34.2	35.3

Table 3. Precision of eGFR prediction equations. Precision (ml/min/1.73m^2), defined as the standard deviation of the mean difference between the estimated glomerular filtration rate (estimated by the modification of diet in renal disease formula (MDRD) and the chronic kidney disease epidemiology collaboration equation (CKD-EPI)) and the creatinine clearance, is shown per creatinine clearance stage. CI=confidence interval.

eGFR prediction formulae and staging

Figure 2 represents the eGFR values for both formulas by age category for male (A) and female (B) patients. For both the CKD-EPI and the MDRD a steep decline was observed with aging. When compared with the MDRD, the CKD-EPI gave higher estimates of GFR at young age (≤65 years). In older age, the MDRD and CKD-EPI gave a similar estimation of GFR.

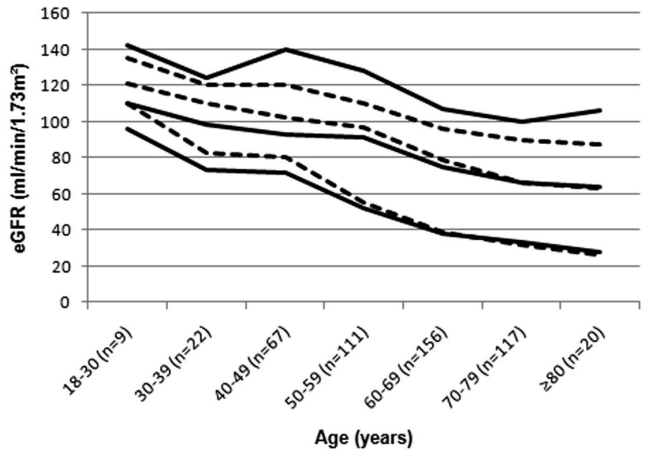


Figure 2. A Men: The dashed lines represent the 5th percentile, the median and the 95th percentile of the CKD-EPI. The black line represents the 5th percentile, the median and the 95th percentile of the MDRD.

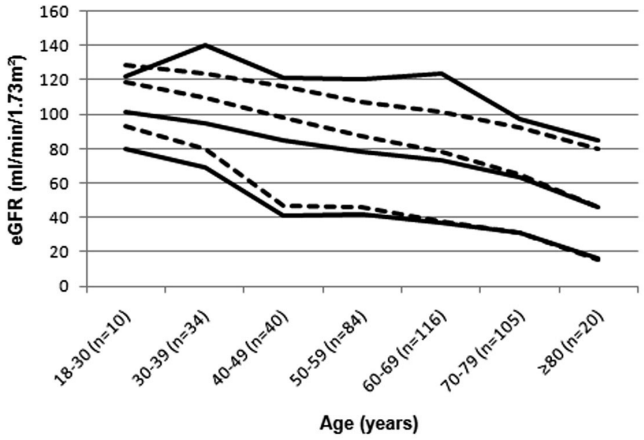


Figure 2. B The dashed lines represent the 5th percentile, the median and the 95th percentile of the CKD-EPI. The black line represents the 5th percentile, the median and the 95th percentile of the MDRD.

The influence on CKD staging using the CKD-EPI or MDRD formula are illustrated in table 4a and 4b, for men and women respectively. Smaller stages than in the KDOQI guidelines are used, to provide a more detailed insight. These tables clearly demonstrate that the CKD-EPI provides higher eGFR values than the MDRD, specifically at higher levels of eGFR and in women (along the total range of renal function). 26.4% of the women were categorized in a lower CKD stage using the CKD-EPI.

MDRD				**CKD-EPI**			
(ml/min/1.73m²)	<30	30-44	45-59	60-74	75-89	>90	Total
<30	**10**						10
30-44	1	**34**					35
45-59		2	**56**	6			64
60-74				**81**	27		108
75-89					**74**	40	114
>90					13	**163**	176
Total	11	36	56	87	114	203	**507**

Table 4a (male). Estimated GFR stage for males using the chronic kidney disease epidemiology collaboration equation (CKD-EPI) or the modification of diet in renal disease formula (MDRD). Numbers represent absolute numbers. Blank cells have no observations.

MDRD				CKD-EPI			
(ml/min/1.73m²)	<30	30-44	45-59	60-74	75-89	>90	Total
<30	**12**	2					14
30-44		**30**	11				41
45-59		1	**51**	12			64
60-74				**60**	38		98
75-89					**50**	45	95
>90					5	**92**	97
Total	12	33	62	72	93	137	**409**

Table 4b (female). Estimated GFR stage for females using the chronic kidney disease epidemiology collaboration equation (CKD-EPI) or the modification of diet in renal disease formula (MDRD). Numbers represent absolute numbers. Blank cells have no observations.

Figure 3 presents the consequence of the introduction of the CKD-EPI on the prevalence of stage III-V CKD. A decline in the number of young people (<65 years) diagnosed with stage III-V is observed, from 12.6 to 10.7%. In the elderly patient category, the numbers of diagnosed patients remains similar using the CKD-EPI or the MDRD.

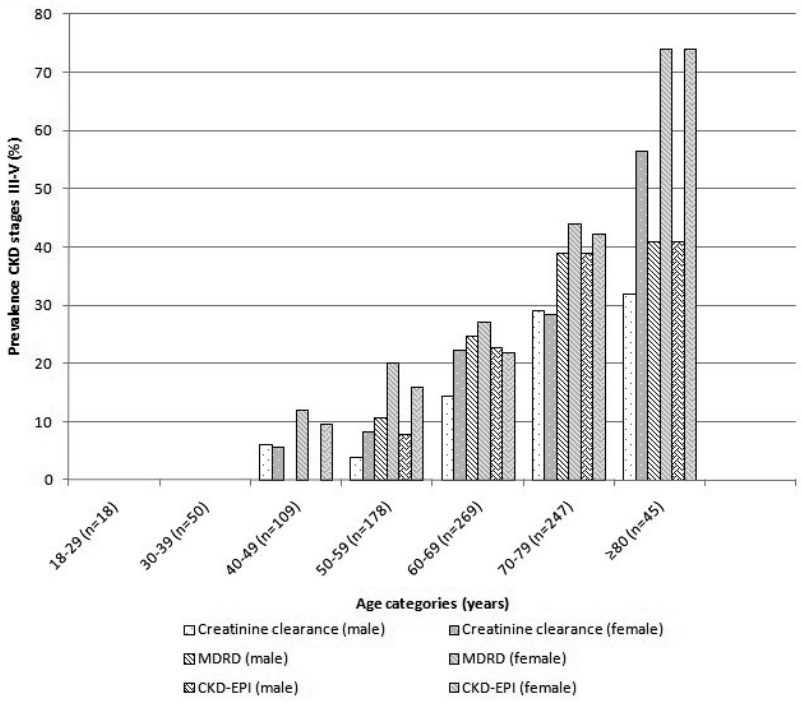


Figure 3. Prevalence of chronic kidney disease (CKD) stage III-V (eGFR <60 ml/min/1.73m²) in Dutch diabetic male and female patients.

Discussion

In this study, we evaluated the performance of the CKD-EPI as a new method of renal function estimation in diabetic patients. When using the CCR as the comparator and using correlation, bias and precision as tools to evaluate the performance of formulas estimating renal function, the CKD-EPI did not show any additional value compared with the MDRD for use in clinical practice. Bias was comparably high for both MDRD and CKD-EPI and both prediction equations had an equal lack of precision; a lack of precision that increased with deteriorating renal function.

The CKD-EPI was developed to overcome the deficiencies of the MDRD equation, such as the lower accuracy when the GFR is >60 ml/min/1.73m^2, and underestimation of eGFR in women and healthy young white men. The proposal of Levey et al. to replace the MDRD with the CKD-EPI formula for routine clinical testing because of its superior accuracy can be disputed, among others in the group of diabetic patients [14]. Although the CKD-EPI performed better than the MDRD in the validation data set when the GFR was >60 ml/min/1.73m^2, its precision remained limited [10]. As this imprecision was seen in all groups of the validation data set, transplant status, diabetes, and weight were selected as predictor variable [11]. The performance of the CKD-EPI did not improve significantly as a result of these attempts to improve the precision of the formula. In spite of these findings, this formula is also recommended to be used in diabetic patients. This lack of precision and the presence of bias has consequences for the correct classification of CKD [14-15].

The performance of the CKD-EPI compared with the MDRD has been sparsely assessed in diabetic patients. [16-17]. In these two recent studies, in which diabetic patients with a good renal function or an impaired renal function, respectively, were assessed (mean measured GFR 102 ± 24 ml/min/1.73m^2 using ^{51}CR-EDTA [16] and 55.4 ± 29 ml/min/1.73m^2 using inulin [17]), it was demonstrated that the CKD-EPI had a substantial larger bias than the MDRD [16-17]. The first study, evaluating the consequences of the bias and imprecision on CKD staging, found that 16% of the study population was misclassified as having CKD [16]. Unfortunately, the authors of the first study [16] did not mention the characteristics of the subgroup that was misclassified.

From studies in the general population with middle-aged people it was shown that using the CKD-EPI equation to estimate GFR reduces the number of patients categorized in CKD stage III-V (eGFR <60 ml/min/1.73m^2) [17-18]. People who had an MDRD-eGFR <60 ml/min/1.73m^2 but were reclassified to 'normal' (no CKD) using the CKD-EPI, had a cardiovascular risk profile similar to the population without evidence of CKD and had no greater expectation of mortality during follow-up. In both studies the individuals

who were reclassified were more often white, women and younger. Those who remained in stage 3a (eGFR <60 and ≥ 45 ml/min/1.73m^2) had a significantly greater burden of diabetes, higher fasting plasma glucose, and higher HbA1c levels.

Based on the results of our study, it can be suggested that the CKD-EPI might lead to underdiagnosing of kidney disease in younger subjects; overall 19.8% is categorised in a lower stage when the CKD-EPI is used. Although the number of patients included in this study is small, there is a trend for young and especially female patients to be re-categorised in a lower CKD stage when the CKD-EPI is used to estimate GFR. Differences in estimated GFR using the CKD-EPI or MDRD were largest in the age category <65 years. The fact that the bias of the CKD-EPI and the MDRD is influenced by age was found previously in a group of potential kidney donors and adult patients who underwent a GFR measurement for clinical reasons, using ^{125}I-iothalamate [19]. It was shown that absolute bias was larger in the younger patient group [19].

From previous studies we know that younger people (18-64 years) have an increased risk of mortality and end stage renal disease at similar levels of GFR (estimated by the MDRD) [20]. Such a finding in relatively young persons requires further evaluation of the patient. The sooner these people are diagnosed as having a reduced renal function, the sooner they can be treated.

Apart from creatinine-based renal function prediction equations, cystatin C is increasingly mentioned as a biomarker that can be used in formulas to predict GFR. Various studies found cystatin C to be a better predictor of GFR than creatinine although other studies found no difference [21-23]. Particularly in patients with muscle loss and in populations where rapid detection of small changes in GFR are important, cystatin C may provide a more accurate estimate of kidney function than serum creatinine [21]. In patients with DM cystatin C appears to be more sensitive than creatinine for the detection of a mild reduction in kidney function [24]. However, whether cystatin C improves medical decision making, leading to more favorable patient outcomes remains to be evaluated in future research [25].

Strengths and limitations

This is one of the few studies evaluating the effect of the CKD-EPI on the classification of CKD in a cohort of patients with diabetes. Recent studies have emphasized the importance of careful calibration of serum creatinine measurements [26]. The fact that a traceable enzymatic serum creatinine technique was used in this study, increases the validity of the study results.

Unfortunately, as we did not have a gold standard to measure GFR, 24-hour CCR was used as measurement. However, since the CCR is still frequently used to asses renal function, comparing the two GFR prediction equations with the 24-hour CCR

is clinically relevant. We did not have data on urinary protein excretion. Therefore we cannot make inferences about the presence of chronic kidney disease (CKD) other than CKD stage III-V in our population. Moreover serum creatinine concentrations were measured only once in the majority of people, so we cannot speculate on chronicity of CKD in this population. Still, estimated GFR based on a single creatinine measurement offers reasonable accuracy for identifying CKD stage III or higher.

Conclusion

The classification of CKD in diabetic patients and the related risk of complications (i.e. cardiovascular morbidity and mortality, acute kidney injury, end stage renal disease) can be facilitated by GFR estimations, as long as one recognizes that the precision of both the MDRD and the CKD-EPI equations is limited. Compared with the MDRD equation, the CKD-EPI equation gives higher estimates of GFR in young diabetic people, leading to a lower prevalence of CKD on population level. Moreover, the performance of the CKD-EPI equation in diabetic patients has to be determined in a study in which a gold standard to measure renal function is used as comparator.

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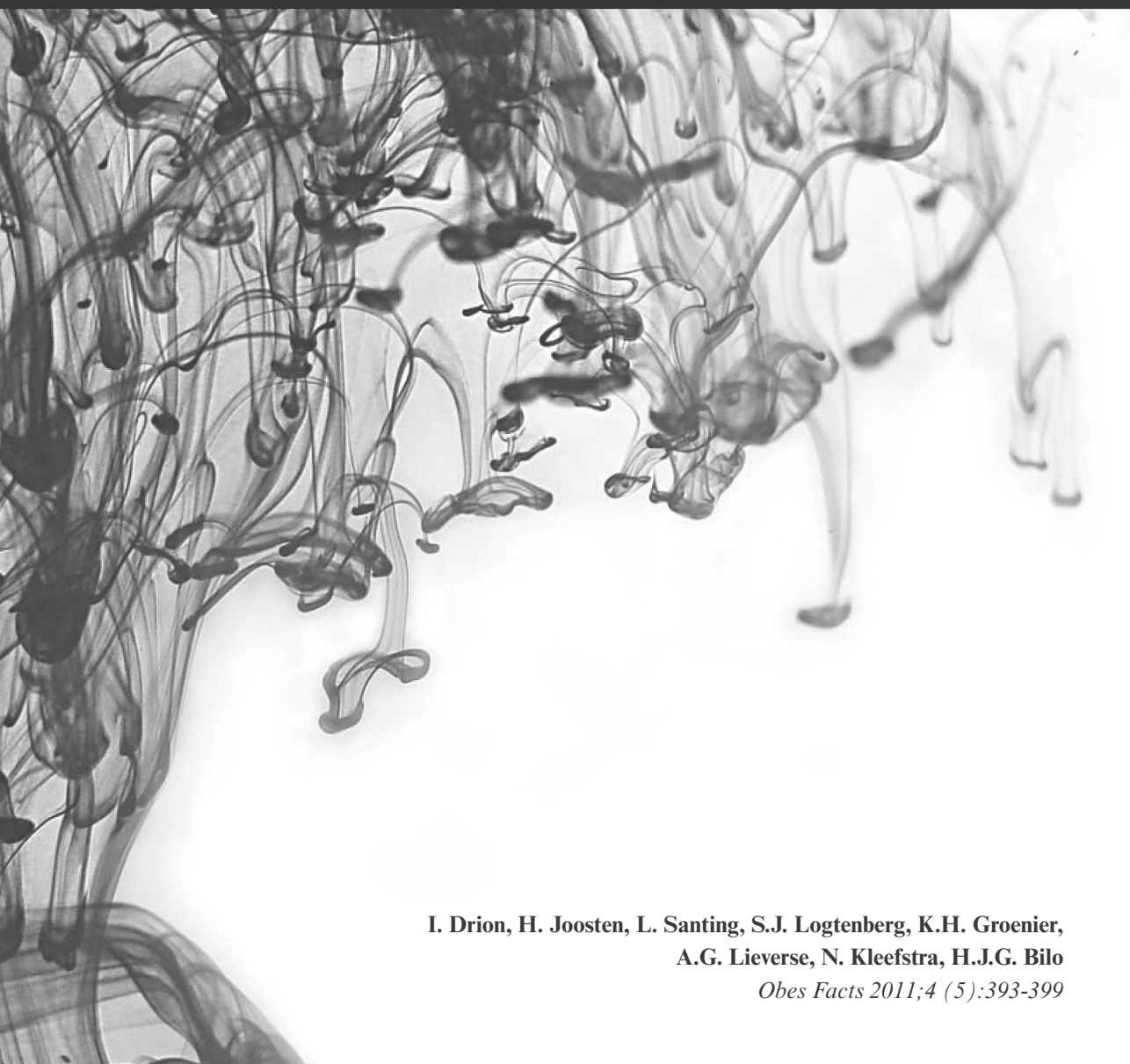
Chapter 3

The Cockcroft-Gault: a better predictor of renal function in an overweight and obese diabetic population



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Background and objectives The performance of the Cockcroft-Gault (CG) equation, the Modification of Diet in Renal Disease (MDRD) formula and the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was evaluated in body mass index (BMI) categories.

Methods In this retrospective cohort study in diabetic patients, creatinine clearance was measured by collecting 24-hour urines. Renal function was estimated by using the CG, MDRD and CKD-EPI. The performance of the equations was evaluated using correlation, Krippendorff's coefficient, bias, precision and accuracy.

Results The bias of the MDRD and CKD-EPI increased from respectively -13.9 ml/min/1.73m^2 and -14.0 ml/min/1.73m^2 (BMI <25 kg/m^2); to -31.7 ml/min/1.73m^2 and -29.6 ml/min/1.73m^2 (BMI >30 kg/m^2). Bias of the CG decreased from -13.4 ml/min (BMI <25 kg/m^2) to -3.2 ml/min (BMI >30 kg/m^2). With an accepted 30% dispersion, CG had the largest accuracy in the overweight and obese group (76.9% and 76.8%, respectively). The MDRD and CKD-EPI had an accuracy of 45.8% and 34.0% (overweight group), respectively, and 51.9% and 37.3% (obese group), respectively.

Discussion All renal function prediction equations are biased when used in overweight or obese diabetic populations with a preserved renal function. The CG provides the best estimate of kidney function. The limitations of renal function prediction equations should be kept in mind when making clinical decisions.

Introduction

Because of the worldwide increasing prevalence of obesity and its associated problems such as diabetes mellitus (DM) and hypertension [1-3], the number of patients with complications such as renal function loss will also increase. Diagnosing renal dysfunction at an early stage is advocated, since early changes in lifestyle and pharmacological interventions can prevent or slow down further progression of renal damage [4-6]. To facilitate early recognition of chronic kidney disease (CKD), the Kidney Disease Outcome Quality Initiative (KDOQI) guidelines were introduced [7]. These guidelines classify CKD based on structural abnormalities, persisting albuminuria and / or haematuria of glomerular origin and an estimated glomerular filtration rate (eGFR) [8-9]. Increased urinary excretion of albumin is an early and sensitive marker of CKD due to DM and hypertension. Numerous studies have shown a strong independent association between the level of urinary protein excretion and the risk of cardiovascular mortality in populations with DM [8,10-11]. Besides albuminuria, eGFR remains the cornerstone for assessment and staging of CKD. Since the use of serum creatinine alone as a measure for renal function is too inaccurate, and inulin, radioactive tracer elements or 24-hour urine collections are either expensive or cumbersome in daily practice, different formulae have been developed in the past decades to estimate the GFR or creatinine clearance (CCR). There is considerable debate regarding the indiscriminate use and the interchangeable results of the 4-variable Modification of Diet in Renal Disease (MDRD) equation [12], the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [13] and the Cockcroft-Gault (CG) equation [14] in overweight and obese patients [15-20].

The MDRD, based on GFR measurements using ^{125}I-Iothalamate, was developed in a relatively young population (subjects <70 years of age) with known renal disease (mean GFR 39.8 ml/min/1.73m^2) and overt proteinuria (>1 gram per day urinary protein loss). The body weight of these subjects was ≥80% and ≤160% of their standard body weight. The MDRD is considered to be reliable in subjects with a GFR ≤60 ml/min/1.73m^2, and is indexed for a body surface area (BSA) of 1.73m^2 (which approximates the BSA of a non-overweight average-sized person) [12].

The CKD-EPI was developed in an attempt to get a better estimate of GFR in values exceeding 60 ml/min/1.73m^2. It was developed in a population with a mean GFR of 68 ml/min/1.73m^2 (indexed for BSA) and a mean BMI of 28 kg/m^2 [13].

The CG is an equation to estimate CCR that was developed in a cohort of largely non-obese male subjects with a wide age range, a weight within the 10%-range of the fat-free body mass and a normal renal function [14]. Therefore, a CG estimate is considered to be especially reliable in CCR levels >60 ml/min. Unlike the MDRD and the CKD-EPI, bodyweight is included as a variable, because it is a crude estimate of muscle mass, and

therefore also of creatinine 'production'. Since excess body weight in an overweight and obese population usually comprises adipose tissue and not muscle mass, this formula is thought to have considerable limitations in this patient category.
Theoretically, the CG will virtually always provide higher results than the MDRD, since the CG equation not only represents the glomerular function but also the tubular function. Furthermore, most adults will have a larger BSA than the standard BSA of 1.73m^2, which is used in the MDRD and CKD-EPI. This means that these differences may lead to misunderstandings, and incorrect interpretation of results. Therefore, we aimed to investigate the influence of (over)weight on the performance of the MDRD and CKD-EPI equations versus the CG equation in diabetic patients, and to analyze the effect on the (mis)classification of chronic kidney disease (CKD).

Methods

Study population
The data for this retrospective, observational, cross-sectional study were collected from May 2005 until December 2006 at the outpatient clinic of the Maxima Medical Center in Eindhoven, the Netherlands. During that period, 1097 24-hour creatinineclearances of adult patients with DM were collected. An anonymous database was created with data abstracted from the 'Chipsoft Electronisch Zorg Informatie Systeem' (CS-EZIS), the computerized medical record system of the Maxima Medical Center. The database thus contained data regarding the 24-hour urinary creatinine, serum creatinine, HbA1c, weight, length, age and sex of each of these patients. In addition, BMI and BSA (BSA = 0.20247 * height (m)$^{0.725}$ * weight (kg)$^{0.425}$) [15] were calculated and added to the database. The patients were divided into three groups, based on their BMI according to the WHO classification: a normal weight group (BMI of 18-24.9 kg/m^2), an overweight group (BMI of 25-29.9 kg/m^2) and an obese group ((BMI ≥30 kg/m^2) *http://www.who.int/mediacentre/factsheets/fs311/en/index.html; accessed 25th May 2011*).
Thirteen patients with a CCR of more than 250 ml/min and two patients who were younger than 18 years old were excluded, as the eGFR prediction equations are not validated in this patient group. In cases in which more than one 24-hour urine sample was performed (n=236) in the indicated period, the most recent sample was used. Ultimately, the database contained complete data for 844 patients.
The population is a mixture of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) patients. The exact numbers of each type are unknown.
Permission from the Medical Ethics Committee was not required, as our data only included anonymized patient characteristics and laboratory data.

Renal function measurement

The enzymatic Roche Modular P method, validated by isotope dilution mass spectrometry (IDMS), was used to measure serum and urinary creatinine. The 4 variable-MDRD and the CKD-EPI were used to estimate GFR (for formulae, see box). CCR was estimated by using the CG equation. In this study, measured CCR was used as a reference value for the renal function. This value was based on a 24-hour urine collection, and calculated using the formula UxV/P (see box), to calculate the 24-hour CCR.

In order to make a better comparison between the estimations of the MDRD and CKD-EPI on the one hand versus the CCR and CG on the other hand, the results of the MDRD and CKD-EPI were recalculated for the individual BSA (using the DuBois formula [15] as mentioned above) of each patient (designated as MDRD-BSA and CKD-EPI-BSA, respectively).

Creatinine clearance
U (creatinine concentration in urine; µmol/ml) * V (Urine volume; ml/min)/ P (creatinine concentration in plasma in (µmol/ml).

Cockroft-Gault equation (ml/min):
1.23 * (140-age)/serum creatinine) * weight (* 0.85 for women).

4-variable MDRD equation (ml/min/1.73m2):
175 * (serum creatinine (µmol/L)/88.4)$^{-1.154}$ * age (years)$^{-0.203}$ (* 0.742 for women).

CKD-EPI (ml/min/1.73m2):
141 * min (serum creatinine (mg/dl)/k,1)^{a} * max (serum creatinine (mg/dl)/k,1)$^{-1.209}$ * 0.993^{age} (* 1.018 for women).

*k is 0.7 for females and 0.9 for males, a is -0.329 for females and -0.411 for males, min indicates the minimum of serum creatinine/k or 1, and max indicates the maximum of serum creatinine/k or 1

Statistical analysis

Data analysis was performed using SPSS version 16.0 (SPPS Inc., Chicago, IL, USA). Demographic data were stratified according to BMI categories, and presented as median (interquartile range, IQR) or mean (standard deviation, SD), depending on whether data were skewed or not. Student's t-test, the Chi-square test or ANOVA was used to compare the demographic characteristics and the values of the renal function prediction equations between the BMI categories. The accuracy of the renal function prediction formulae for the different BMI categories was compared using the McNemar test.

The performance of the GFR prediction equations, the GFR prediction equations corrected for BSA and the CG were compared by calculating correlation, Krippendorf's coefficient [16], bias, precision (the SD of the bias) and accuracy for each BMI category.
Spearman's coefficient of correlation was calculated for each BMI category to determine the correlation between the CCR and the results of the MDRD, the MDRD-BSA, the CKD-EPI, the CKD-EPI-BSA and the CG. The Krippendorff coefficient was used as an aggregate measure for method concordance. A Krippendorff coefficient of 1 shows a perfect concordance between two methods and a Krippendorff coefficient of -1 shows a perfect discordance [16].
Bias was defined as the mean difference between the renal function prediction equations and CCR, whereas precision was defined as the SD of this difference. Accuracy (a combination of bias and precision), and the percentage of patients who had an estimated kidney function within 30% and 50% limits of the CCR, were calculated.

Results

Population characteristics and design
The patient characteristics of this study are presented in table 1. The mean (SD) CCR of the overall study population was 112 (45) ml/min. The mean renal function of the overall population estimated with the MDRD, MDRD-BSA, CKD-EPI, CKD-EPI-BSA and CG was 76 (25) ml/min/1.73m^2, 87 (31) ml/min, 78 (24) ml/min/1.73m^2, 90 (30) ml/min and 99 (42) ml/min respectively. The total study population was equally divided between the BMI categories, and included subjects across a wide range of ages (20-92 years of age), with BMI scores ranging between 15-58 kg/m^2. The CCR values ranged between 11-250 ml/min. Although non-significant, there were considerable differences in sex distribution between the BMI categories. No significant differences in age and HbA1c were observed between the BMI categories.
After calculating the results of the GFR prediction equations and the CG equation for each BMI category, only the MDRD result was significantly different between the normal group (BMI <25 kg/m^2) and the overweight group (BMI 25-30 kg/m^2) ($p<0.04$). Between the normal and obese group significant differences were found for the CCR value ($p<0.01$), MDRD-BSA ($p<0.01$) and CG result ($p<0.001$). However, when the GFR prediction equations were not corrected for BSA, no significant differences were found (MDRD equation: $p<0.95$, CKD-EPI equation: $p<0.97$).

	Normal <25 kg/m^2	Overweight 25-30 kg/m^2	Obese >30 kg/m^2
n (%)	243 (29)	295 (35)	306 (36)
Age (in years)*	65 [48-73]	63 [54-71]	63 [56-72]
Sex (men, in %)	57	65	44
Weight (kg)*	70 [63-76]	80 [74-90]	97 [89-109]
BMI (kg/m^2)*	23 [22-24]	27 [26-29]	33 [31-37]
HbA1c (%)*	6.7 [6.0-7.4]	6.6 [5.9-7.4]	6.9 [6.1-7.6]
Serum creatinine (µmol/L)*	78 [66-93]	83 [71-103]	78 [66-98]
BSA (m^2)	1.83 (0.18)	1.95 (0.18)	2.10 (0.21)
Creatinine clearance (ml/min)	104 (41)	109 (42)	121 (51)
CG (ml/min)	85 (34)	91 (34)	116 (49)
CKD-EPI (ml/min/1.73m^2)	82 (24)	77 (24)	77 (23)
CKD-EPI-BSA (ml/min)	87 (28)	87 (29)	94 (31)
MDRD (ml/min/1.73m^2)	82 (28)	77 (29)	79 (30)
MDRD-BSA (ml/min)	85 (29)	84 (29)	92 (34)

Table 1. Patient characteristics. Data are represented as mean (standard deviation). *Data are represented as median [interquartile range]. BMI: body mass index, BSA: body surface area, CG: Cockcroft-Gault equation, CKD-EPI: chronic kidney disease epidemiology collaboration equation, CKD-EPI-BSA: chronic kidney disease epidemiology collaboration equation corrected for body surface area, MDRD: Modification of Diet in Renal Disease (the 4-variable MDRD is used in this study), MDRD-BSA: Modification of Diet in Renal Disease corrected for body surface area.

Correlation and Krippendorff's coefficient

Both GFR estimates, also after a correction for BSA, and the CG result were correlated with the CCR value of each BMI category (see table 2). Overall, the Spearman correlation coefficient was 0.73 for the MDRD, 0.80 for the MDRD-BSA, 0.74 for the CKD-EPI, 0.81 for the CKD-EPI-BSA, and 0.78 for the CG equation. If no correction for BSA takes place, the correlation between the CG and the CCR proves to be superior to the GFR prediction equations in all BMI categories, except for the MDRD equation in the normal weight category (as might be expected). When GFR formulae are corrected for BSA, the correlation between the CKD-EPI-BSA and MDRD-BSA results is stronger than between the CG result and CCR in all BMI categories. Because correlation alone is insufficient to prove the concordance among methods, the Krippendorff coefficient was calculated (see table 2). The best concordance was found between the CG result and CCR within all BMI categories. The CKD-EPI result had the worst concordance with CCR in all BMI categories, even after a correction for BSA.

	CG (ml/min)	MDRD (ml/min/1.73m^2)	MDRD-BSA (ml/min)	CKD-EPI (ml/min/1.73m^2)	CKD-EPI-BSA (ml/min)
BMI <25 kg/m^2	0.82 (0.71)	0.75 (0.58)	0.80 (0.66)	0.76 (0.55)	0.82 (0.68)
BMI 25-30 kg/m^2	0.81 (0.71)	0.78 (0.52)	0.82 (0.62)	0.79 (0.47)	0.82 (0.65)
BMI ≥ 30 kg/ m^2	0.75 (0.75)	0.72 (0.42)	0.77 (0.58)	0.72 (0.33)	0.78 (0.57)

Table 2. Correlation and Krippendorff's coefficient for CG and eGFR values per BMI category. Data are presented as Spearman's correlation coefficient (Krippendorff's coefficient). CG = Cockcroft-Gault equation; MDRD = Modification of Diet in Renal Disease; MDRD-BSA = MDRD corrected for body surface area; CKD-EPI = chronic kidney disease epidemiology collaboration equations; CKD-EPI-BSA = CKD-EPI corrected for body surface area; BMI = body mass index.

Bias and precision

All prediction equations had a negative bias in the various BMI categories. The bias varied widely, as can be seen in figure 1 The higher the BMI, the greater the mean bias for both the MDRD and the CKD-EPI equation (see figure 1). When the MDRD and the CKD-EPI were corrected for BSA, the results were similar. However, the mean bias for these two equations did not increase as much in the higher BMI categories (see figure 1). For the CG equation, a decreasing trend in bias was observed with increasing BMI, from -18.7 ml/min in BMI <25 kg/m^2 to -4.0 ml/min in BMI >30 kg/m^2 ($p<0.001$).

No significant differences in performance were found between the CKD-EPI and the MDRD equations in the normal weight and overweight group ($p=0.88$ and $p=0.50$, respectively). In the obese patient group the MDRD performed significantly better than the CKD-EPI ($p=0.01$).

The precision of all these formulas varied widely in all BMI categories (see table 3).

Accuracy

In table 3, the accuracy of the various renal function estimates is presented for each BMI category. The CG had the best accuracy (>70.4%) in all BMI categories. The CKD-EPI equation had a better accuracy than the MDRD equation in all BMI categories (when a dispersion of 30% was tolerated), although the difference in accuracy was only significant in the overweight group (<0.01); accuracy decreased with increasing BMI. However, when the GFR prediction equations were corrected for BSA, the MDRD-BSA performed significantly better in all BMI categories than the CKD-EPI-BSA ($p<0.01$), and the higher the BMI, the lower the accuracy.

		CG (ml/min)	MDRD (ml/min/1.73m^2)	MDRD-BSA (ml/min)	CKD-EPI (ml/min/1.73m^2)	CKD-EPI-BSA (ml/min)
BMI <25 kg/m^2 (normal weight)	Precision	25.5	28.3	25.9	28.0	24.9
	Accuracy 30%	70.4	63.3	72.0	67.1	67.1
	Accuracy 50%	95.1	94.7	96.3	95.1	95.1
BMI 25-29.9 kg/m^2 (overweight)	Precision	24.9	28.4	25.2	26.9	24.3
	Accuracy 30%	76.9	45.8	67.5	51.9	51.9
	Accuracy 50%	96.9	92.5	97.3	94.2	94.2
BMI ≥ 30 kg/m^2 (obese)	Precision	38.0	36.2	34.8	37.7	33.0
	Accuracy 30%	76.8	34.0	58.5	37.3	37.3
	Accuracy 50%	90.5	82.0	94.4	85.3	85.3

Table 3. Precision and accuracy for the MDRD and the CG per BMI category. Precision defined as one standard deviation of the bias (ml/min(/1.73m^2)). The accuracy is defined as the percentage of the glomerular filtration rate (GFR) estimation within ± 30 and ± 50% range of the respective creatinineclearance measurements. BMI = Body mass index; BSA = Body surface area; CG = Cockcroft-Gault equation; CKD-EPI = chronic kidney disease epidemiology collaboration equations; CKD-EPI-BSA: CKD-EPI corrected for body surface area; MDRD: Modification of Diet in Renal Disease (4-Variable MDRD); MDRD-BSA: MDRD corrected for body surface area.

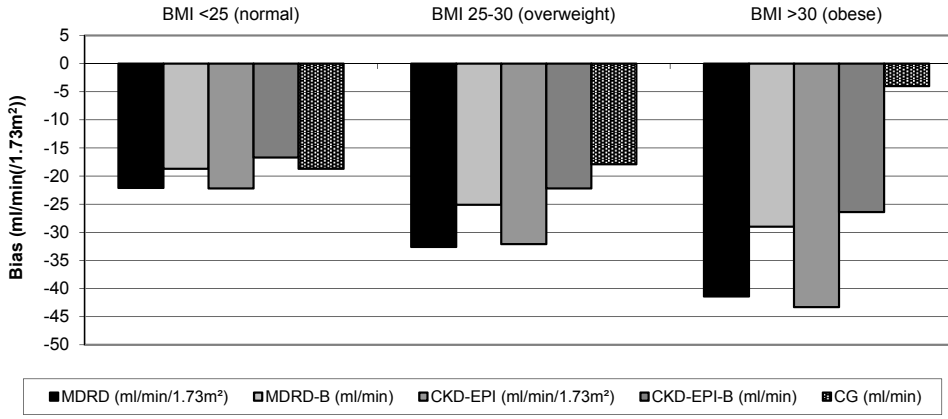

Figure 1 Mean bias per BMI category. Mean bias (ml/min/1.73m^2) of each renal function prediction equation stratified according to BMI category (kg/m^2). Bias is defined as mean difference between the (estimated) renal function using equations and the creatinine clearance. MDRD = Modification of Diet in Renal Disease; MDRD-B = MDRD corrected for body surface area; CKD-EPI = chronic kidney disease epidemiology collaboration equations; CKD-EPI-B = CKD-EPI corrected for body surface area; CG = Cockcroft-Gault equation.

Discussion

This study shows that the CG is a better predictor of renal function than the MDRD and the CKD-EPI in diabetic patients, especially when patients are overweight or obese, at least when CCR is used as a reference value. The MDRD and CKD-EPI equations provided less accurate results for overweight and obese patients. Even though the renal function in the studied population was good (mean CCR 112 ml/min), the recently developed CKD-EPI did not perform significantly better than the MDRD. When the MDRD and CKD-EPI were corrected for BSA, bias and accuracy improved. Even so, the CG outperformed the GFR prediction equations in the overweight and obese patient group.

The limitations of creatinine-based prediction equations in overweight and obese populations have been discussed in literature before [17-22]. A condition frequently encountered in obese patients and patients with diabetes is renal hyperfiltration. The suggested underlying mechanism is that progressive obesity alters renal hemodynamics, leading to an increase in GFR of each single nephron, since the number of nephrons will not increase with increasing body fat [23]. Ultimately, nephrons will function near to or on maximum capacity, i.e. hyperfiltration. Correcting GFR for BSA obscures this problem, as was shown in a cohort of 81 obese patients (BMI 41 ± 9 kg/m^2) with a mean GFR of 101 ± 24 ml/min (measured by Cr-EDTA) and a mean indexed GFR of 76 ± 16 ml/min/1.73m^2 [24]. When the absolute GFR, respectively the indexed GFR, was

used as a reference, the MDRD formula underestimated (mean difference: -11 ± 20 ml/min) respectively overestimated (mean difference: 14 ± 18 ml/min/1.73m^2) the measured GFR. The observed underestimation of the GFR using the MDRD can be expected based on previous literature; however, the overestimation when using an indexed GFR is remarkable and suggests that back-correction for BSA is needed [24].

Although overweight and obesity have almost reached epidemic forms nowadays 'the Caring for Australians with Renal Impairment (CARI) guidelines' are the only guidelines to mention the influence of weight on the GFR prediction equations [25.] These guidelines are also the only ones warning for the unreliable prediction results of the MDRD equation in an overweight and obese population [25]. The influence of weight on renal function equations should however, be considered, especially since many laboratories have started to use automated reporting of the MDRD estimates. More importantly, clinical decisions are based on these renal function estimates.

The observation in our study that the CG equation had the least bias in overweight and obese subjects is supported by previous publications [17-18]. In a population of newly diagnosed T2DM patients with a mean isotopic GFR of 115 ml/min/1.73m^2, the CG equation had the most pronounced bias in lean subjects (mean -20.6 ml/min/1.73m^2, CI -23.9 to -17.3); a bias that diminished with increasing body weight (-5.6 ml/min/1.73m^2 in an obese population). Contrarily, the bias of the MDRD increased (from -21.3 ml/min/1.73m^2 in the normal weight group to -28.9 ml/min/1.73m^2 in the obese group), and the accuracy decreased [19]. The fact that we found more pronounced results compared with the study of Chudleigh et al. might be due to the use of CCR values instead of ^{51}Cr-EDTA values as a reference for renal function.
Verhave et al. studied the performance of the CG and MDRD equations in a diverse cohort of outpatients with serum creatinine levels of less than 1.5 mg/dL (<133 µmol/L) [17]. In their study, a rather similar trend for the CG was found, except that in the obese population an overestimation of + 10.1 ml/min was found. This is in contrast to our study, in which we found a small underestimation. Also in contrast with our findings, the investigators found that the MDRD equation underestimated the GFR to a certain extent (approximately -12.4 ml/min/1.73m^2), irrespective of BMI [17]. It is possible that their results slightly differ from our results because of differences in creatinine measurement. In our study, the creatinine was calibrated to IDMS.
The influence of weight on the CKD-EPI has not yet been evaluated in a cohort of diabetic subjects. In a recently published study performed among potential kidney donors (mean CCR 78.2 ml/min/1.73m^2), the researchers found that the CKD-EPI and the MDRD equations were not influenced by BMI, contrary to the CG [20].

Many of the above mentioned studies, comparing the performance of the CG and the MDRD, use an indexed CG equation (often the standard BSA of 1.73m^2). In our opinion, this is incorrect. Since weight is one of the clinical variables included in the CG equation, a correction for BSA will result in a double correction for weight. This double correction may well have influenced the performance of the equation in these studies. Moreover, a correction to a standard BSA of 1.73m^2 may result in a worse performance of the MDRD and CKD-EPI equations. In patients with a normal BMI, the impact of a correction to a standard BSA of 1.73m^2 is rather small, since 1.73m^2 is approximately the BSA of a non-overweight person. However, such a correction will lead to considerable underestimation of renal function in obese patients, since a lot of obese people have a BSA which grossly exceeds 1.73m^2. When we corrected the values in our study to a standard BSA of 1.73m^2, the MDRD and CKD-EPI equations did indeed perform worse. But when we corrected the data to the actual BSA of participants, the performance of both the MDRD equation and CKD-EPI equation improved considerably.

Another reason why the MDRD equation in our study performed worse than the other equations might be that the average renal function in the study population was good and the MDRD equation has only been validated in a population with impaired renal function (MDRD <60 ml/min/1.73m^2).

Finally, the CCR is not a true reflection of GFR. Still, the CCR is a common way to measure renal function in daily practice; contrarily to various isotopic clearance techniques or other clearance methods that are reserved for research purposes. Unfortunately, details concerning duration of DM, blood glucose lowering treatment and the presence of albuminuria are not available for this population due to the method of data collection. The inclusion of such data would have allowed the analysis to be more complete

Conclusion

In this study, performed among diabetic patients in various weight categories, the CG was the best predictor of renal function compared with the 4-variable MDRD and CKD-EPI when used in an overweight or obese population. The recently developed CKD-EPI equation has no additional value over the existing prediction equations. When the existing prediction equations are used in clinical practice, their disadvantages should be kept in mind when making decisions based on the results of these equations.

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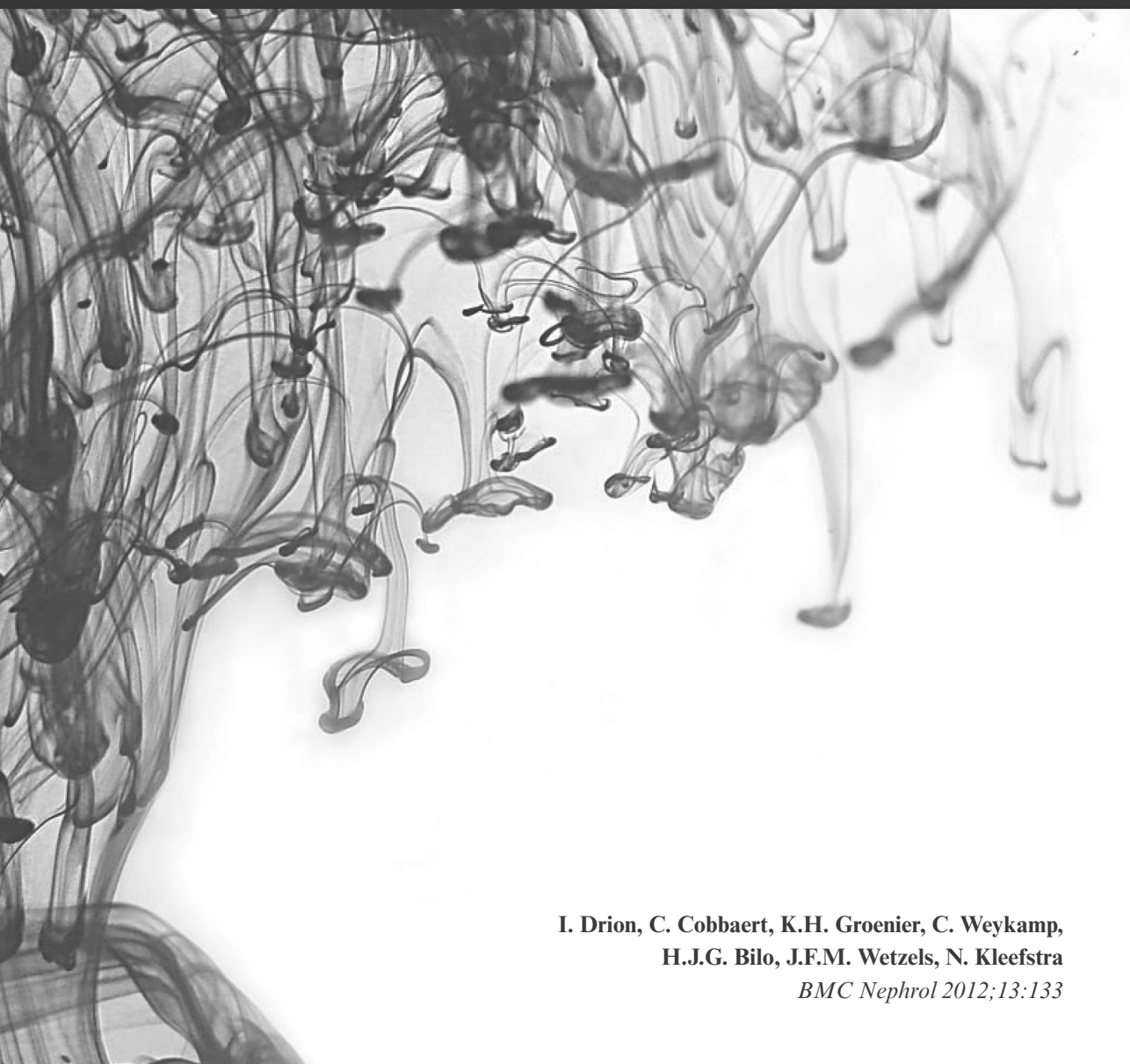
Chapter 4

Clinical evaluation of analytical variations in serum creatinine measurements: why laboratories should abandon Jaffe techniques



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Background and objectives Non-equivalence in serum creatinine (SCr) measurements across Dutch laboratories and the consequences hereof on chronic kidney disease (CKD) staging were examined.

Methods National data from the Dutch annual external quality organization of 2009 were used. 144 participating laboratories examined 11 pairs of commutable, value-assigned SCr specimens in the range 52-262 μmol/L, using Jaffe or enzymatic techniques. Regression equations were created for each participating laboratory (by regressing values as measured by participating laboratories on the target values of the samples sent by the external quality organization); area under the curves were examined and used to rank laboratories. The 10^{th} and 90^{th} percentile regression equation were selected for each technique separately. To evaluate the impact of the variability in SCr measurements and its eventual clinical consequences in a real patient population, we used a cohort of 82424 patients aged 19-106 years. The SCr measurements of these 82424 patients were introduced in the 10^{th} and 90^{th} percentile regression equations. The newly calculated SCr values were used to calculate an estimated glomerular filtration rate (eGFR) using the 4-variable Isotope Dilution Mass Spectrometry traceable Modification of Diet in Renal Disease formula. Differences in CKD staging were examined, comparing the stratification outcomes for Jaffe and enzymatic SCr techniques.

Results Jaffe techniques overestimated SCr: 21%, 12%, 10% for SCr target values 52, 73 and 94 μmol/L, respectively. For enzymatic assay these values were 0%, -1%, -2%, respectively. eGFR using the MDRD formula and SCr measured by Jaffe techniques, staged patients in a lower CKD category. Downgrading to a lower CKD stage occurred in 1-42%, 2-37% and 12-78.9% of patients for the 10^{th} and 90^{th} percentile laboratories respectively in CKD categories 45-60, 60-90 and >90 ml/min/1.73m^2. Using enzymatic techniques, downgrading occurred only in 2-4% of patients.

Discussion Enzymatic techniques lead to less variability in SCr measurements than Jaffe techniques, and therefore result in more accurate staging of CKD. Therefore the specific enzymatic techniques are preferably used in clinical practice in order to generate more reliable GFR estimates.

Introduction

Serum creatinine (SCr) based prediction equations are frequently used in screening and clinical settings in order to estimate the glomerular filtration rate (GFR). The current variability in SCr measurements affects all estimating equations for GFR, including the MDRD equation. Many automated routine methods for SCr measurement exceed the desirable imprecision criterion of ≤ 2.2%; therefore, reduction of analytical bias ≤ 3.4% in creatinine assays by standardization of calibration is needed [1]. It is important to notice that standardization of calibration does not correct for analytical interferences (nonspecificity bias). The bias and nonspecificity problems associated with some of the routine methods must be addressed.

Chronic kidney disease (CKD) staging directly relies on these estimated GFR values. Using accurate SCr measurements is essential, since systematic errors cause unreliable renal function estimates, leading to incorrect drug dose adjustments, misclassifications in CKD staging and incomparability of patient data [2-5].

Since significant interlaboratory variation was observed worldwide, it was internationally confirmed that calibration traceability to higher-order reference methods was needed to realize comparable biochemical measurement results [2,6-7].

Therefore, the European in vitro diagnostics (IVD) directive 98/79/EC, and the laboratory working group of the National Kidney Disease Education Program recommend that in order to improve standardization, clinical laboratory measurements should be traceable to internationally recognized and certified reference materials [8-10]. Since the development of NIST SRM 967 in 2006, a matrix-based IDMS targeted creatinine standard, all essential elements (i.e. methods, laboratories, and materials) needed to complete the creatinine reference system are in place, according to ISO 17511 [11]. Since the complete traceability is in place in vitro diagnostic manufacturers of creatinine assays in Europe are legally obliged to make their products metrologically traceable, regardless of the method applied.

In this study we examine the degree of variability and interchangeability of SCr measurements across all clinical chemistry laboratories in 2009 in the Netherlands, in order to evaluate the situation after global restandardization, using data from the annual national external quality control program of the Dutch external quality assessment (EQA) organization for clinical chemistry laboratories (Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek, SKML). Subsequently, we investigate in a theoretical model, the impact of the variability in SCr measurements between laboratories on estimates of GFR using the 4-variable IDMS-traceable MDRD formula and the consequences hereof on CKD staging of patients, when the data from the SKML are extrapolated to a large cohort of patients.

Methods

In this cross-sectional study, we evaluate the effect of different SCr assays on SCr levels and CKD classification. EQA data are derived from the 2009 EQA program of the SKML. Annually, the SKML creates 11 pairs of frozen commutable, value assigned serum samples spiked with crystalline creatinine, aliquoted in 1 ml vials. A commutable material reflects the characteristics and properties of native patient samples [2,12]. Value-assignment was performed by a joint committee on traceability in laboratory medicine (JCTLM)-endorsed reference laboratory. Each of the 144 laboratories participating in the EQA program in the Netherlands annually receives a set containing 11 pairs of these commutable samples from the SKML and store them intermittently at -80°C. The 11 pairs of EQA-samples cover SCr values in the entire measuring range: 52-73-94-115-136-157-178-199-220-241-262 μmol/L and form a linear sequence; thus each laboratory received and analyzed, the range mentioned before in twofold over the year. The target values for the SCr levels are established by a JCTLM listed reference laboratory (Bonn, Germany) using an Isotope Dilution Gas Chromatography/Mass Spectrometry (ID-GC/MS) method [13-14].

Every other week all routine laboratories thawed one of the EQA samples and measured the SCr concentration applying their routine SCr methods according to the manufacturer's instructions. 91 (63%) versus 48 (33%) of the laboratories used a Jaffe or enzymatic method to measure SCr, respectively. 62 (68%) laboratories using a Jaffe technique applied a modified kinetic Jaffe method; 29 (32%) used a compensated Jaffe method. Few laboratories used dry chemistry to measure SCr; since this group of laboratories was too small to draw conclusions from (n=5), this group was excluded from further analyses. Companies and instruments included Abbott (Abott Park, Il, USA; Aeroset, Architect), Beckman (Brea, Ca, USA; Synchron, Unicel, LX20, Lxi725), Siemens Healthcare diagnostics (The Netherlands; ADVIA 1650, 1800, 2400), Roche Diagnostics (Mannheim, Germany; Integra, Hitachi, Modular, Cobas, Cobas Integra) and Olympus (Tokyo, Japan; AU 400, 600). In total, 39 different instrument method combinations were used to measure SCr.

Data of the SCr measurements as measured by the participating laboratories were reported centrally to the SKML and collected in an completely anonymous database.

Variability SCr extrapolated in cohort

To investigate the impact of the variability in SCr measurements as found in the national EQA database and the eventual clinical consequences hereof in a real patient population, we used an unselected cohort of 82424 patients whose SCr had been measured in 2009 in the Isala Clinics Zwolle, the Netherlands; the details of this population have been

described before [15]. In short, 45.3% of the population was male, age varied from 19-106 years and 38.7% was older than 65 years old. SCr in this cohort was measured using an enzymatic technique (modular P Analyzer, creatinine plus assay; Roche Diagnostics, Mannheim, Germany). In order to obtain SCr reference values that are traceable to the reference data from the EQA program for each patient, we requested the results from the 2009 EQA program from the clinical chemistry department, Isala clinics Zwolle. Based on these results we created a regression equation for the Zwolle laboratory (the exact procedure is extensively described in the statistical analyses section), using inverse regression. Subsequently SCr values as measured in the Zwolle population were introduced in this regression equation in order to calculate the SCr values traceable to the results of the EQA program for each of the 82424 patients. The GFR using these IDMS-traceable SCr values was estimated using the 4-variable IDMS-traceable MDRD formula [16-17].

Statistical analysis

We used SPSS version 16.0 (SAS Institute, Cary, NC, USA) and STATA version 11 (StataCorp, College Station, Texas USA) for statistical analyses. All SCr measurements of the laboratories participating in the EQA program were inspected for outliers (truncated at ± 3 standard deviation (SD)); 3 laboratories were removed from the dataset because more than 50% of the measurements of these 3 laboratories deviated more than 3 SD from the other laboratories. The target reference values from the linearity sequence of the EQA program served as the reference method against which routine methods to measure SCr from participating laboratories were compared, by means of relative and absolute bias. The results were displayed in box and whisker plots for each method group. Relative bias is defined as the mean percentage difference [(measured SCr-target value SCr)/target value SCr] x 100; absolute bias is defined as the mean difference between SCr values measured by individual laboratories and SCr target values; precision is defined as the SD of the absolute bias.

We extrapolated the impact of the non-equivalence in SCr measures (as derived from the laboratories participating in the EQA program), to our patient cohort of 82424 patients. In order to do so, SCr values as measured by laboratories participating in the quality assessment program were regressed on the target values of the samples sent by the SKML, so-called inverse regression, for each of the participating laboratories separately. Regression equations for each of the participating laboratories, (n=47 for Jaffe and n= 39 for enzymatic), who had not changed their technique to measure SCr in 2009, were created. For each of these regression equations (thus for each of the laboratories fulfilling the criteria mentioned above), we calculated an area under the curve (AUC) in the range 73-115 μmol/L. The range of 73-115 μmol/L was chosen since especially these values

of SCr provide eGFR's around the threshold value of 60 ml/min/1.73m^2, sufficient to classify patients as having CKD stage 3. Subsequently, the AUC's of all the participating laboratories were ranked in ascending order for the Jaffe and the enzymatic technique separately, in order to establish a 10th and 90th percentile regression equation for each of the techniques. Then, SCr values from our cohort of 82424 patients were inserted in the 10th and 90th percentile regression equations (for the Jaffe technique and the enzymatic technique as appropriate). These 'newly calculated' SCr values were introduced in the appropriate MDRD equations, thus providing 10th and 90th percentile eGFR values. To get an impression from the clinical implications of the variation in SCr values on CKD staging, we classified the patients according to the K/DOQI guidelines and evaluated the differences in CKD staging when SCr values were measured by Jaffe or the enzymatic methods [18].

Ethical statement

No permission was required from the Medical Ethics Committee as our data only included lab result information, which had been obtained from a laboratory database. No personal patient information was included. Permission to use the national 2009 EQA-data was obtained from the SKML. The laboratories in the dataset were anonymous.

Results

The relative and absolute bias for both Jaffe and enzymatic techniques are shown in figures 1 to 4. The enzymatic method to measure SCr produced the least biased results, which were not significantly different from the target values, whereas the Jaffe technique produced the most biased and imprecise results, which differed significantly from the reference values. The Jaffe technique especially tended to overestimate SCr at low concentrations: 21%, 12%, 10% for the SCr target values 52, 73 and 94 µmol/L, respectively. The enzymatic method had a small bias that was constant over the entire range of SCr values. The precision for Jaffe / enzymatic (per reference value) is: 10 / 3 (52 µmol/L), 10 / 3 (73 µmol/L), 7 / 3 (94 µmol/L), 13 / 4 (115 µmol/L), 7 / 5 (136 µmol/L), 8 / 4 (157 µmol/L), 8 / 5 (178 µmol/L), 9 / 5 (199 µmol/L), 10 / 6 (220 µmol/L), 11 / 5 (241 µmol/L), 5 / 2 (262 µmol/L) for both the Jaffe and the enzymatic method.

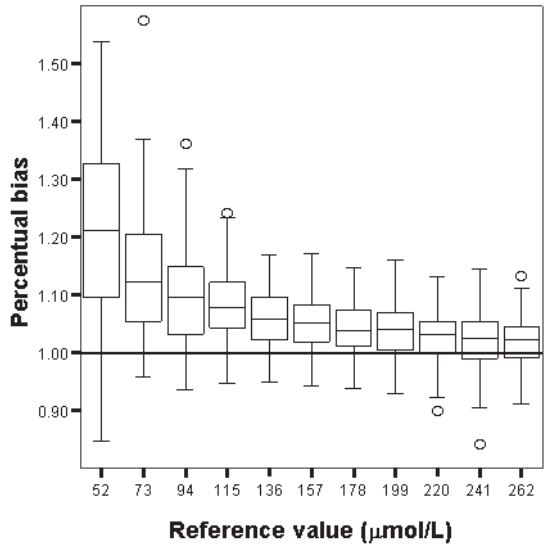

Figure 1. Box and whisker plot showing the percentual bias of the Jaffe technique. Interpretation of the vertical axis e.g. 1.1 means a percentual bias of 10%. The box represents the 25th, 50th and 75th percentile; the whiskers represent the 5th and 95th percentile. The extremes, defined as values more than three times the interquartile range, are the signs above and underneath the whiskers. The grey line represents the 0% bias line.

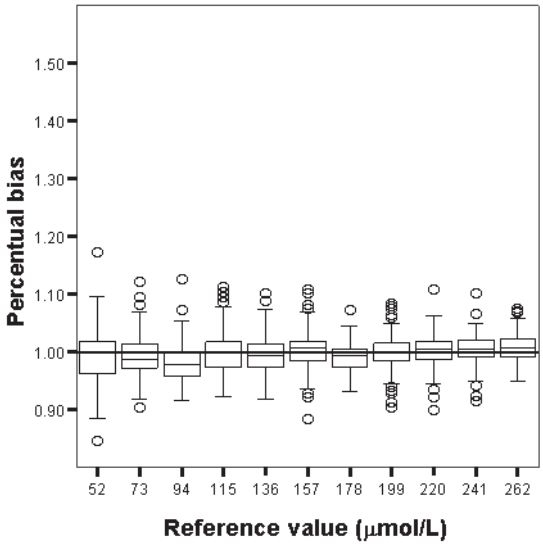

Figure 2. Box and whisker plot showing the percentual bias of the enzymatic technique. Interpretation of the vertical axis e.g. 1.1 means a percentual bias of 10%. The box represents the 25th, 50th and 75th percentile; the whiskers represent the 5th and 95th percentile. The extremes, defined as values more than three times the interquartile range, are the signs above and underneath the whiskers. The grey line represents the 0% bias line.

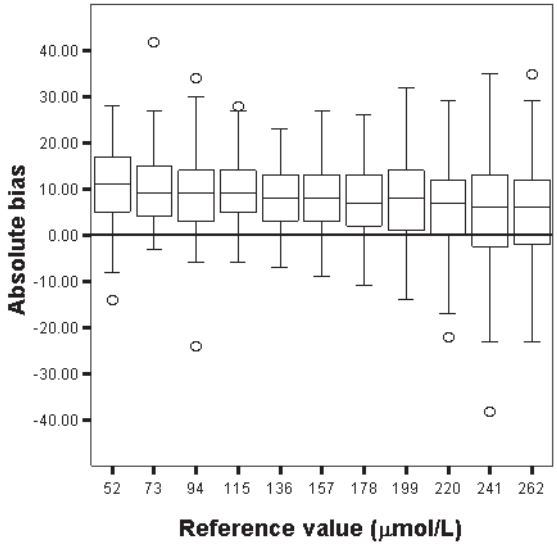

Figure 3. Box and whisker plot showing the absolute bias (μmol/L) for the Jaffe technique. The box represents the 25th, 50th and 75th percentile; the whiskers represent the 5th and 95th percentile. The extremes, defined as values more than three times the interquartile range, are the signs above and underneath the whiskers. The grey line represents the 0 μmol/L bias line.

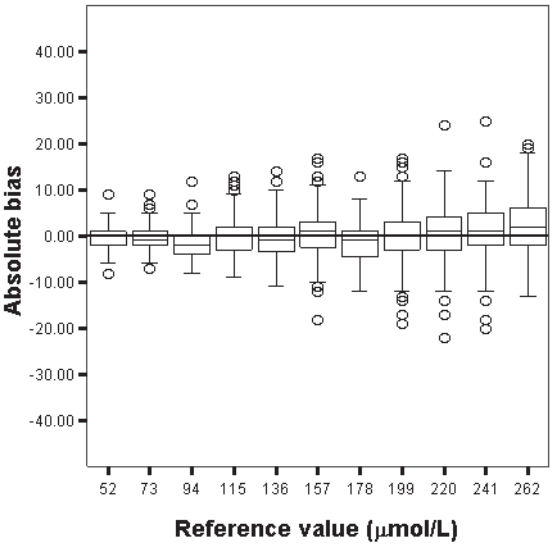

Figure 4. Box and whisker plot showing the absolute bias (μmol/L) for the enzymatic technique. The box represents the 25th, 50th and 75th percentile; the whiskers represent the 5th and 95th percentile. The extremes, defined as values more than three times the interquartile range, are the signs above and underneath the whiskers. The grey line represents the 0 μmol/L bias line.

The impact of the variability in SCr measurements on CKD staging is illustrated in tables 1 and 2. From the tables we can conclude that the differences between the 10th and 90th percentile laboratory are large when a Jaffe technique is used. Downgrading to a lower CKD class was observed using the Jaffe assay for CKD stages: 45-60 ml/min/1.73m^2 (1.1%, 41.9%); 60-90 ml/min/1.73m^2 (1.8, 36.7%) and >90 ml/min/1.73m^2 (12.3%, 78.9%), for the 10th and 90th percentile values respectively. When an enzymatic technique was used, the variability resulted in both upward and downward reclassification of CKD stage. Downward reclassification occurred in 2.1-4.1% of patients, whereas upgrading occurred in 15.6-30.1% of patients.

		MDRD Jaffe p_{10} / p_{90}										
		<30 ml/min/1.73m²		30-45 ml/min/1.73m²		45-60 ml/min/1.73m²		60-90 ml/min/1.73m²		>90 ml/min/1.73m²		**Total**
		p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	
MDRD standardized	<30 ml/min/1.73m²	2523 (99.2%)	2543 (100%)	20 (0.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2543
	30-45 ml/min/1.73m²	0 (0%)	678 (17.3%)	3908 (100%)	3231 (82.7%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3909
	45-60 ml/min/1.73m²	0 (0%)	0 (0%)	90 (1.1%)	3439 (41.9%)	8126 (98.9%)	4777 (58.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8216
	60-90 ml/min/1.73m²	0 (0%)	0 (0%)	0 (0%)	0 (0%)	736 (1.8%)	14598 (36.7%)	39085 (98.2%)	25223 (63.3%)	0 (0%)	(0%)	39821
	>90 ml/min/1.73m²	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3448 (12.3%)	22047 (78.9%)	24487 (87.7%)	5888 (21.1%)	27935
		2523	3221	4018	6670	8863	19375	42533	47270	24487	5888	82424

Table 1. Implications for CKD staging when a Jaffe or a standardized serum creatinine is used. Crosstabulation showing the estimated glomerular filtration rate (GFR) stages when the modification of diet in renal disease equation (MDRD) is calculated with a standardized serum creatinine versus a serum creatinine as measured by a p10 (10th percentile) or a p90 (90th percentile) laboratory using a Jaffe technique.

		MDRD enzymatic p_{10} / p_{90}										
		<30 ml/min/1.73m²		30-45 ml/min/1.73m²		45-60 ml/min/1.73m²		60-90 ml/min/1.73m²		>90 ml/min/1.73m²		**Total**
		p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	p_{10}	p_{90}	
MDRD standardized	<30 ml/min/1.73m²	2483 (97.6%)	2543 (100%)	60 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2543
	30-45 ml/min/1.73m²	0 (0%)	141 (3.6%)	3299 (84.4%)	3768 (96.4%)	610 (15.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3909
	45-60 ml/min/1.73m²	0 (0%)	0 (0%)	0 (0%)	338 (4.1%)	5740 (69.9%)	7878 (95.9%)	2476 (30.1%)	0 (0%)	0 (0%)	0 (0%)	8216
	60-90 ml/min/1.73m²	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	845 (2.1%)	28308 (71.1%)	38976 (97.9%)	11513 (28.9%)	0 (%)	39821
	>90 ml/min/1.73m²	5 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1502 (5.4%)	27930 (100%)	26433 (94.6%)	27935
		2488	2684	3359	4106	6350	8723	30784	40478	39443	26433	82424

Table 2. Implication for CKD staging when an enzymatic or a standardized serum creatinine is used. Crosstabulation showing the estimated glomerular filtration rate (GFR) stages when the modification of diet in renal disease equation (MDRD) is calculated with a standardized serum creatinine versus a serum creatinine as measured by a p10 (10th percentile) or a p90 (90th percentile) laboratory using an enzymatic technique.

Discussion

The present study shows that interlaboratory non-equivalence in SCr assays in the Netherlands was still substantial in 2009, notwithstanding the recent international creatinine restandardization effort. The high variability was largely explained by the ongoing use of Jaffe assays for measuring SCr. Compared with the enzymatic assays, the Jaffe assays had a significantly larger bias, especially for SCr levels in the lower range (reference value range 52-115 μmol/L). Although relative bias decreased when SCr reference values were higher, imprecision remained high. It was of course to be expected that Jaffe methods lead to a positive bias when compared with the ID-/GC-MS method, and that adjustment for this bias would occur when using the appropriate MDRD equation. This has caused the downgrading of patients to a lower CKD category relevantly more often when a Jaffe technique instead of an enzymatic technique was used, especially in categories >45 ml/min/1.73m^2 (up to 79%). In contrast, the use of an enzymatic technique more often resulted in upgrading of the CKD stage, which may be explained by the differences in bias: the Jaffe technique provided higher values of SCr, whereas the enzymatic technique provided slightly lower values.

Ever since SCr is assessed in clinical practice its accuracy has been debated [1,7,19]. Although, SCr measurements are routinely performed, it is one of the most variable laboratory tests [20]. The increasing use of eGFR in clinical practice has renewed the interest on the shortcomings of the SCr methodology [1,21-24]. Since SCr is the most important variable in the renal function estimation equations, calibration of the creatinine assays is necessary to reduce bias in these formulas. This even lead to a modification of the factor used in the MDRD-equation (from 186 to 175 for IDMS traceable creatinine). However, the way this calibration was obtained has been regularly criticized in literature, due to the fact that the formulas were modified after having recalibrated the Jaffe creatinine to an IDMS traceable enzymatic method, having deleted the intercepts since these were not statistically significant.

The substantial bias and between laboratory variance as we found in our study, has been shown in various other studies in which data from proficiency testing (PT) and EQA scheme programs were evaluated [7,25-26]. A European trueness verification study of SCr also showed large interlaboratory variability before the matrix-based SRM 967 standard was available [7]. In our study we would have expected a significantly reduced interlaboratory CV due to global restandardization on SRM 967. However, despite the European IVD directive with stricter regulations, no improvements compared with earlier studies, in which a method group SD of 2.6-11 μmol/L and a median CV of 5% at a SCr concentration of 74 μmol/L, had been reached [27]. The failure to realize amelioration of interlaboratory non-equivalence is explained by the fact that standardization does

not correct for analytical non-specificity problems, as is the case with the Jaffe method [28-29]. These non-specificity problems concern the measurement of many endogenous and exogenous interfering substances such as protein, glucose and ketones when SCr is measured using a Jaffe method [28-31]. Despite many attempts to improve the performance characteristics of the Jaffe reaction, non-specificity remained [7]. This leads to overestimation of the true SCr concentration. Calibration traceability cannot solve this problem nor substitute for improvement of suboptimal routine methods.

Although the enzymatic assay to measure SCr is not free of interference from various substances, it has a better specificity than the Jaffe technique [28]. This was recently confirmed in a multicentre study evaluating IDMS-traceable enzymatic creatinine assays. It showed that the majority of enzymatic methods reached the acceptable total analytical error of 8% for SCr values as low as 36 µmol/L, when adequately calibrated against IDMS, improving the traceability and standardization of creatinine [32].

Moreover, upgrading in CKD stage as we observed when enzymatic assays were used in the MDRD formula may be less relevant in routine clinical practice than the downgrading to a lower CKD stage as occurs when a Jaffe assay was used to measure SCr. E.g. a patient whose eGFR is 57 ml/min/1.73m^2 (CKD stage 3a) or 62 ml/min/1.73m^2 (CKD stage 2) when a Jaffe respectively an enzymatic assay is used to examine SCr, probably have similar risks regarding end-stage renal disease, all-cause and cardiovascular mortality. From studies comparing the prognosis associated with the two most commonly used equations to estimate GFR (the MDRD and the Chronic Kidney Disease Epidemiology collaboration equation, CKD-EPI) during a long follow-up (≥7.5 years) we know that individuals reclassified from CKD stage 3a (eGFR 45-60 ml/min/1.73m^2) to no CKD had lower mortality risk than those not reclassified. Moreover, these participants had an equal risk to those classified as no CKD by both formulas [33-34].

Based on the large batch of evidence in literature showing that alkaline picrate methods are inferior methods to measure creatinine, it is time for laboratories to substitute the alkaline picrate method by enzymatic methods. Moreover, if an increasing number of laboratories apply enzymatic techniques, the number of vendors of commercial enzymatic assays will increase, leading to more competition, which will ultimately reduce the costs of these assays. To bring this in a broader perspective, more accurate and precise measurements of SCr will lead to a reduction of the source of error in GFR estimates and thus errors in the staging of renal failure. Considering the number of patients that are misclassified in this study when using an alkaline picrate technique, clinical laboratories should also consider the implications for overall health costs, since patients are referred based on creatinine based estimates of GFR [35].

Although this study is a theoretical analysis, it is one of the few illustrating the consequences of variations in SCr measurements on GFR estimation and CKD staging for the individual patient. Since the majority of Dutch laboratories is included and we have a large heterogeneous cohort of patients in which we tested our model, we are able to give a good reflection of the consequences it might have for daily clinical practice. Moreover, we have studied creatinine values on 11 different levels against a strong reference method; and the samples used, were all recent instead of remote samples, which are frequently used in other studies. Selection bias may have occurred, since laboratories with too few analyses in the external quality control program were excluded for further analysis in our patient cohort. Moreover, we applied the MDRD formula in a patient cohort with an age range from 19-106 years. This may have introduced bias since, the MDRD has only been validated for patients from 19-70 years, and underestimates the GFR in patients >70 years. However, in clinical practice, laboratories automatically report eGFR's each time a creatinine is measured, also in patients older than 70 years, and clinical decision making is often based on these estimates.

Conclusions

In conclusion, accurate and precise measurements of SCr are required for a more reliable estimation of GFR as support for reliable clinical decision making. Enzymatic techniques measure SCr with substantially less variability than Jaffe techniques as compared with ID-MS reference values. This leads to more reliable estimation of GFR and CKD staging. To allow improvement of reliability of eGFR, specific enzymatic techniques to measure SCr are preferable over unspecific Jaffe techniques.

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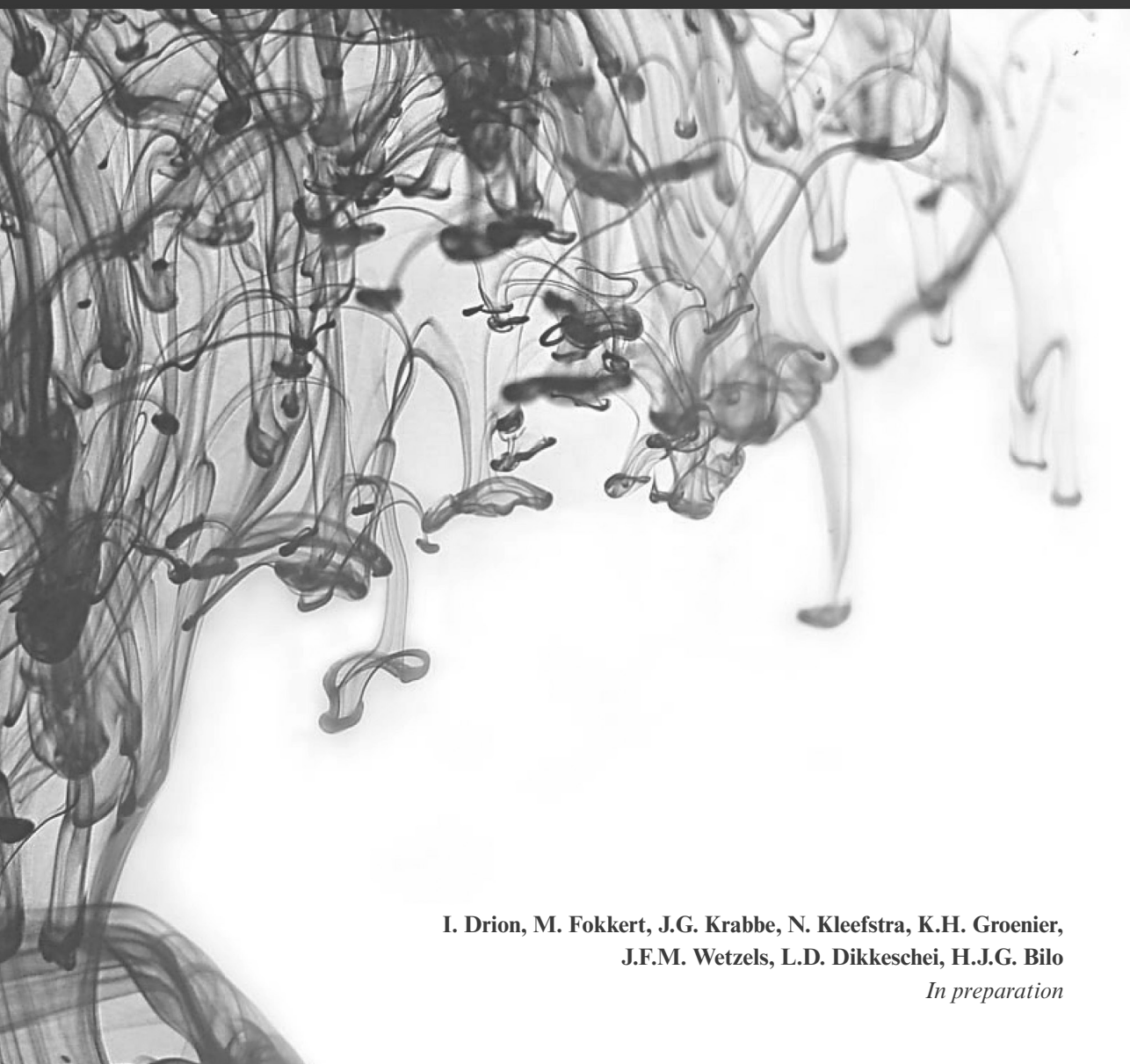
Chapter 5

Measurement of creatinine in urine: an overlooked problem



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In preparation

Background and objectives This study examines the variability between various assays for measuring creatinine in urine, plasma and peritoneal fluid and the effects hereof on the estimated glomerular filtration rate (eGFR) and creatinine clearance (CCR).
Methods This cross-sectional study measured the creatinine concentration in the plasma, urine and peritoneal dialysis fluid of 181 patients, using a compensated Jaffe assay, enzymatic assay and liquid chromatography mass spectrometry (LCMS) technique. The CCR and eGFR were calculated. Scatterplots, Bland-Altman plots and a concordance correlation coefficient (Lin's coefficient) were used to assess (concordance) correlation coefficient, bias and precision.
Results Mean age is 58 (± 15) years, 53% (n=96) is male, median plasma creatinine levels in the non-dialysis group are: 103, 84 and 89 µmol/L for the Jaffe, enzymatic and LCMS assay respectively; median urine creatinine levels are 4.1, 4.6 and 5.7 mmol/L respectively. Lin's coefficient for the CCR is 0.466 and 0.715 when creatinine is measured using a Jaffe and a enzymatic technique respectively. Bias (precision) for creatinine measurements in urine in the non-dialysis group is -1.24 (1.82) mmol/L and -0.75 (1.88) mmol/L for Jaffe and enzymatic respectively.
Discussion Both Jaffe and enzymatic assays provide biased results for creatinine measurements in urine, leading to underestimation of CCR values.

Introduction

Formulas based on plasma creatinine (PCr) are regularly used to estimate the glomerular filtration rate (GFR) [1-3]. These formulas have replaced the old technique of calculating the creatinine clearance (CCR). Still, despite the mainly practical constraints of its use, the CCR is regularly used in daily practice as a (surrogate) marker of the GFR, especially when other renal function estimates are either uncertain or need confirmation. Moreover, the CCR still has an important role in (pre-)dialysis patients, for example to help to decide the right moment for initiating renal function replacement therapy and to assess residual renal function and the adequacy of both hemodialysis and peritoneal dialysis.

Furthermore, since the 24-hour creatinine excretion rate is considered to be relatively constant and independent of 24-hour urinary volume, urinary excretion of biomarkers is often expressed after adjustment for urine creatinine concentration to correct for sampling errors. Since the introduction of creatinine based formulas for estimating GFR in clinical practice, many authors have stipulated the substantial variability in PCr measurements, which lead to unreliable estimation of the glomerular filtration rate when using formulas [4-7]. In order to make techniques for PCr measurement more reliable and results comparable, the European in vitro diagnostics directive has stressed the importance of standardization of PCr measurements to internationally recognized and certified reference materials [8-9]. Clinical laboratories are now expected to perform calibration tests at regular intervals, traceable to an Isotope Dilution Mass Spectrometry (IDMS) reference measurement [8,10-11].

Thus far, the methodological issues involved in measuring creatinine in other fluids than plasma, have received little attention, although the creatinine concentration in urine has an indispensable role in calculating the CCR. In this study we wanted to examine the variability between different techniques (Jaffe, enzymatic, liquid chromatography mass spectrometry) to measure creatinine in plasma, urine and peritoneal fluid (in patients on peritoneal dialysis) and the effects of various measurement techniques on outcome of eGFR equations and CCR in a real-life population with a wide range of renal functions.

Methods

This cross-sectional study was conducted in the Isala Clinics Zwolle, the Netherlands, from June 2010 to January 2011. The laboratory facilities of the Isala clinics provide both primary and secondary health care services for a region with a population of approximately 375.000 inhabitants.

All subjects older than 18 years who came to the clinical chemistry department to deliver a 24-hour urine combined with a PCr assessment, irrespective the reason for 24-hour urinary collection, were informed about the study and were asked to participate. Moreover, patients from the peritoneal and hemodialysis departments were invited by letter before they came to the hospital for the periodic assessment of dialysis adequacy. This procedure includes the collection of a 24-hour urine (provided patients are not anuric), the drawing of blood samples to measure PCr and for PD patients the collection of 24-hour peritoneal dialysate. Moreover, 27 healthy volunteers, who were considered to have a normal renal function, working at the clinical chemistry department, the department of internal medicine or the diabetes centre participated in this study. All patients who decided to participate were asked to give an extra 12-16 ml blood for this study and to give permission to draw 4 samples from their 24-hour urine and 24-hour dialysate (if on peritoneal dialysis). 181 persons gave their informed consent to participate in this study.
Details concerning medication, medical history, comorbidities, BMI, and ethnicity were not available due to the nature of our data collection methods. Based on the known population data in the Zwolle region, it can be safely assumed that the vast majority of this cohort has a Caucasian background.
All plasma and urine creatinine measurement results, volumes from the 24-hour urines, together with screening data regarding demographic characteristics (sex, age) were imported in a database. No personal information was added to protect the anonymity of the patients.

Ethical statement
The study protocol (clinical trials identifier number NCT01575392) has been approved by the Medical Ethics Committee of the Isala Clinics Zwolle. A signed informed consent form was obtained from all participants.

Creatinine measurements and renal function measurements
Creatinine was measured in plasma, urine and peritoneal dialysis fluid using three different techniques: a Roche Modular P Creatinine Jaffe method, rate-blanked and compensated (1936131001V10), an enzymatic technique CREA Plus, (1193597600V12; Roche Mannheim, Germany), and as a reference technique the LCMS technique, verified with NIST SRM 967a (high performance liquid chromatographer 1100, 1200 Agilent, Santa Clara, U.S.), Tandem Mass Spectrometer (Quattro Micro, Micromass (Manchester, England)) was used. The Jaffe and enzymatic assessments in blood were performed in lithium-heparin plasma (BD Vacutainer LH PST™ II 367374. No additives were used in urine and peritoneal samples. All urine, plasma and peritoneal dialysate

samples were stored frozen at -80º Celsius until the moment of examination and were measured in one batch.
CCR was assessed by calculating $U_{creat} \times V/P_{creat}$, where U_{creat} = creatinine concentration in urine, µmol/ml; V = urine volume, ml/min; and P_{creat} = creatinine concentration in plasma in µmol/ml. Moreover, the GFR was estimated using the modification of diet in renal disease study equation (MDRD) using either Jaffe creatinine ($MDRD_{Jaffe}$) or enzymatic creatinine ($MDRD_{enzymatic}$) respectively [1-3]. For the present analysis, all patients were assumed to be Caucasian.

Statistical analysis
Statistical analysis was conducted using SPSS 16.0 (Chicago IL, USA), Stata 12 (StataCorp, Texas, USA). Q-Q plots and histograms were used to assess normality. Continuous variables are presented as mean (± standard deviation) for the normally distributed values and as median (interquartile range) for the non-normally distributed variables.
Scatterplots were created in order to assess the correlation between different laboratory techniques compared to the reference method (LCMS) for both creatinine measurements in plasma and urine. Scatterplots for PCr were only made for samples with a LCMS < 200 µmol/L, since this is the range in which large differences may have important clinical consequences.
Moreover, bias (mean difference between the CCR calculated when a Jaffe/enzymatic technique was used to measure creatinine in plasma and urine and when the LCMS was used to measure creatinine) and precision (standard deviation of the bias) were calculated for CCR as well as the MDRD. Bland-Altman plots were used to visualize the bias and precision of creatinine measurements in plasma and urine.
A concordance correlation coefficient (Lin's coefficient) was calculated in order to evaluate the reproducibility index of the different assays (Jaffe, enzymatic and LCMS) in plasma, urine and dialysate, as well as for the CCR and MDRD, when different assays to measure creatinine were used [12]. The concordance correlation coefficient evaluates the agreement between two readings (from the same sample) by measuring the variation from the 45º line through the origin [12].

Results

181 subjects participated in this study, 27 (15%) of these participants were healthy volunteers, 37 (20%) participants were on hemodialysis, 23 (13%) participants were on peritoneal dialysis, and 94 (52%) participants were patients, who for reasons unknown to the investigators, had to collect a 24-hour urine. Mean age was 58 years (SD 15; 23-88 years) and 53% (n=96) was male.

The creatinine concentration in plasma samples using a Jaffe or an enzymatic technique was performed in 97% (n=175/181) of the participants; the LCMS technique was performed in 96% (n=174/181). The concentration of creatinine in the urine was measured in 79% (n=137/174) of the participants using a Jaffe and an enzymatic technique and in 80% (n=140/174) using the LCMS technique. In 20% of the patients urine data were absent because patients were anuric, or urine samples were lost.

Table 1 shows the median values and interquartile ranges of the measured creatinine values in plasma, urine and dialysate for the non-dialysis and the dialysis population, respectively. As expected, the Jaffe technique gives higher creatinine measurements in plasma than when the enzymatic or LCMS technique are used. Moreover, enzymatic assays have a better concordance correlation coefficient, meaning that it had a better agreement with the LCMS technique compared to the Jaffe. Figure 1a shows the scatter plot of the Jaffe and enzymatic technique versus the LCMS. As expected, the creatinine values measured with the Jaffe technique were higher compared to the LCMS than when measured using an enzymatic technique. To illustrate the aforementioned more clearly, figure 1b and 1c were created showing the bias of both techniques compared to the LCMS.

		Jaffe	**Enzymatic**	**LCMS**	Lin's coefficient *Jaffe-LCMS*	Lin's coefficient *Enzymatic-LCMS*
Non-dialysis	Plasma (µmol/L) n=116	103 (91; 134)	84 (73; 113)	89 (72; 113)	0.958 [0.943; 0.969]	0.988 [0.984; 0.992]
	Urine (mmol/L) n=110	4.1 (2.7; 6.0)	4.6 (3.0; 6.7)	5.7 (3.7; 7.8)	0.757 [0.673; 0.822]	0.804 [0.729; 0.859]
CAPD	Plasma (µmol/L) n=22	780 (655; 923)	758 (538; 913)	782 (587; 873)	0.963 [0.916; 0.984]	0.973 [0.936; 0.988]
	Urine (mmol/L) n=15	3.6 (3-4.4)	3.9 (3.2-4.7)	3.8 (3.0-5.1)	0.947 [0.862; 0.980]	0.977 [0.934; 0.992]
	Dialysate (µmol/L) N=18	454 (316; 605)	462 (321; 630)	415 (270; 602)	0.972 [0.929; 0.989]	0.965 [0.913; 0.986]
HD	Plasma (µmol/L) n=37	738 (619;887)	714 (600;868)	719 (569; 923)	0.957 [0.919; 0.978]	0.959 [0.922; 0.979]
	Urine (mmol/L) n=11	2.9 (2.1; 4.1)	3.2 (2.3; 4.4)	4.9 (3.3; 6.5)	0.370 [0.019; 0.662]	0.430 [0.004; 0.723]

Table 1. Creatinine measurements in patients not on dialysis and on dialysis. Data are depicted as median and interquartile range (IQR). Creatinine concentration is in µmol/L (plasma and dialysate) or mmol/L (urine). CAPD: continuous ambulant peritoneal dialysis; HD: hemodialysis

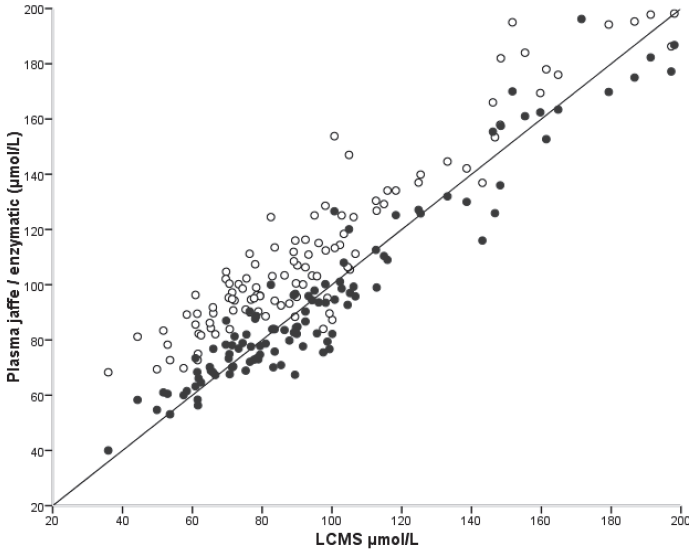

Figure 1a. Creatinine measurements in plasma. The black bullets represent creatinine values measured by the enzymatic technique. The open bullets represent creatinine values when a Jaffe technique was used. Only data of patients with plasma creatinine values <200 µmol/L are represented in this figure. LCMS: liquid chromatography mass spectrometry.

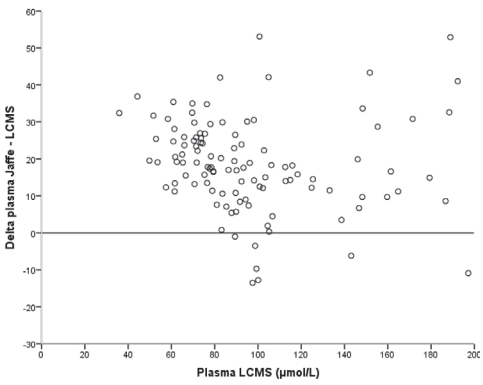

Figure 1b

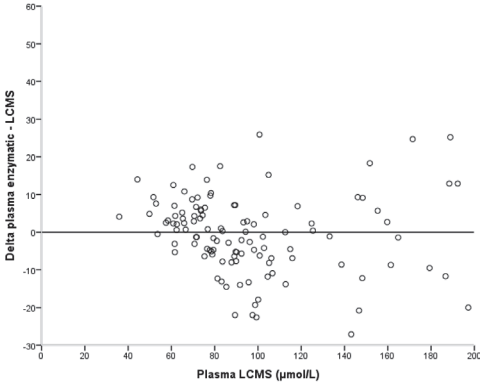

Figure 1c

The figures above represent the absolute bias of both the Jaffe (figure 1b) and the enzymatic (figure 1c) technique for participants with plasma creatinine values <200 µmol/L.

In urine, the differences in creatinine measurements between Jaffe and enzymatic were less evident compared to plasma measurements, both the Jaffe and the enzymatic assay provided biased results for urine creatinine (table 2). The scatterplots (figure 2a, 2b and 2c) show a very wide scatter for both Jaffe and enzymatic measurements when compared to LCMS, with a stronger tendency to under- than to overestimate. Concordance correlation coefficients were lower in urine measurements. Although concordance correlation coefficients were low, the enzymatic technique had a slightly better concordance correlation coefficient compared to the Jaffe assay in urine (table 1). In table 2, bias and precision are displayed in numbers for both the non-dialysis and the dialysis population. Measurements are less biased when the enzymatic technique is used, both for creatinine measurements in plasma and urine. The precision is more or less similar for plasma and urine measurements. This leads to substantial bias en imprecision in the CCR and the MDRD for especially the CCR and the MDRD when calculated using the Jaffe technique, and to a lesser extend for enzymatically measured creatinine values.

		Jaffe		**Enzymatic**	
		Bias	Precision	Bias	Precision
Non-dialysis	Plasma	15.8 (µmol/L)	18.2	-1.96 (µmol/L)	12.6
	Urine	-1.24 (mmol/L)	1.82	-0.75 (mmol/L)	1.88
	CCR	-31.9 (ml/min)	30.1	-12.9 (ml/min)	29.0
	MDRD	-11.1 (ml/min/1.73m^2)	14.7	0.0 (ml/min/1.73m^2)	9.5
Dialysis	Plasma	24.6 (µmol/L)	58.5	4.8 (µmol/L)	59.1
	Urine	-1.02 (mmol/L)	1.30	-0.75 (mmol/L)	1.32
	CCR	-0.95 (ml/min)	1.59	-0.55 (ml/min)	1.08
	MDRD	0.16 (ml/min/1.73m^2)	1.16	0.04 (ml/min/1.73m^2)	0.95

Table 2. Bias and precision of creatinine measurements for the non dialysis population. LCMS = Liquid Chromatography Mass Spectrometry, MDRD = Modification of Diet in Renal Disease study. Creatinine concentrations are in µmol/L (plasma and dialysate) or mmol/L (urine), CCR (creatinine clearance) is in ml/min; estimated glomerular filtration rate is ml/min/1.73m^2 (MDRD). Bias is the mean difference between plasma/urine/CCR/MDRD$_{Jaffe}$ or plasma/urine/CCR/MDRD$_{enzymatic}$ and plasma/urine/CCR/MDRD$_{LCMS}$, whereas precision was defined as the SD of these differences.

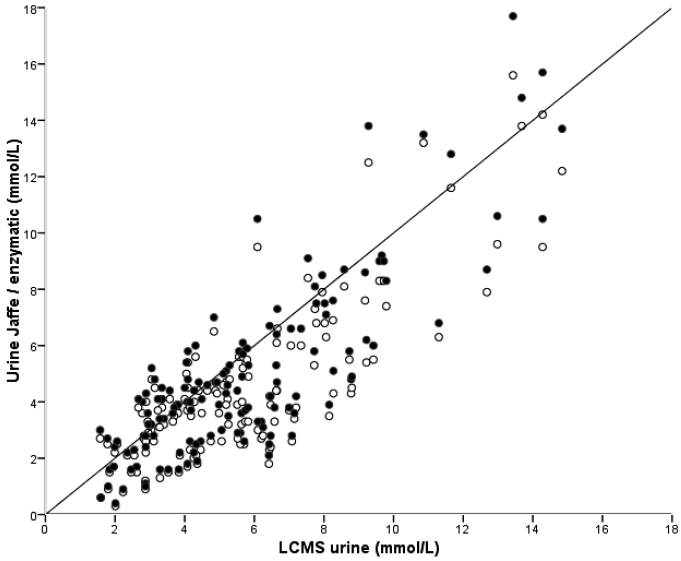

Figure 2a. Creatinine measurements in urine. The black bullets represent creatinine values measured by the enzymatic technique. The open bullets represent creatinine values when a Jaffe technique was used.

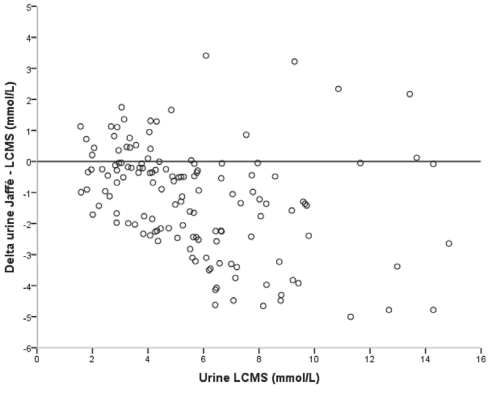

Figure 2b

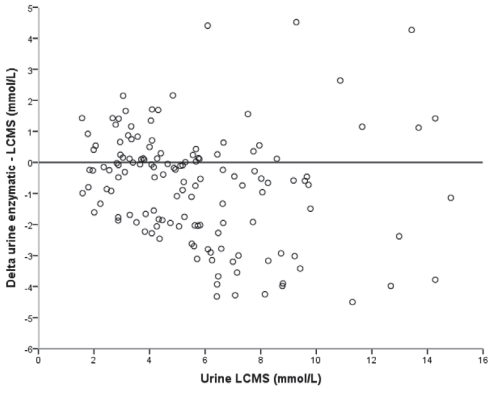

Figure 2c

The figures above represent the absolute bias of both the Jaffe (figure 1b) and the enzymatic (figure 1c) technique in urine.

The consequences of the different techniques to measure creatinine on CCR and the MDRD are shown in table 3. An underestimation of the CCR is observed both when Jaffe and enzymatic assays are used to measure creatinine in urine and plasma compared to the LCMS in the non-dialysis population. These differences are less obvious when the MDRD is used. Moreover, the reproducibility of the enzymatic assay was much better than the reproducibility of the Jaffe assay and had more narrow confidence intervals.

		Jaffe	Enzymatic	LCMS	Lin's coefficient *Jaffe-LCMS*	Lin's coefficient *Enzymatic-LCMS*
Non-dialysis	CCR (ml/min)	51 (34; 72)	72 (44; 102)	89 (53; 113)	0.466 [0.362; 0.559]	0.715 [0.613; 0.793]
	MDRD (ml/min/1.73m^2)	60 (44; 73)	70 (51; 89)	69 (50; 86)	0.746 [0.676; 0.803]	0.940 [0.916; 0.958]
Dialysis	CCR (ml/min)	3 (1; 5)	3 (1; 5)	3 (1; 7)	0.948 [0.912; 0.969]	0.982 [0.964; 0.991]
	MDRD (ml/min/1.73m^2)	6 (5; 8)	6 (5; 7)	6 (5; 7)	0.948 [0.916; 0.968]	0.968 [0.947; 0.981]

Table 3. Creatinineclearance and eGFR in patients not on dialysis and on dialysis. Data are depicted as median and interquartile range (IQR). CCR: creatinineclearance rate, MDRD: the modification of diet in renal disease equation.

Discussion

This study shows that both the enzymatic and the Jaffe assay provide biased and imprecise results for urine creatinine. Although both methods provided lower creatinine values compared with the LCMS technique, the Jaffe assay was most biased. Our study confirmed the well known bias of the techniques for the assessment of PCr, with the Jaffe technique being more biased than the enzymatic technique. As a result, CCR values were underestimated. Moreover, the Jaffe technique had lower reproducibility than the enzymatic technique.

Due to interfering substances, a higher reading was expected for Jaffe PCr measurements [6,13-14]. Due to the high creatinine content in urine (mmol/L versus μmol/L in plasma), the urinary Jaffe measurement should have less interferences by other substances and therefore would show results more comparable to the enzymatic and LCMS measurements [15-17]. Taking this into account, the observation of lower results in urinary measurements when using a Jaffe technique was unexpected and inexplicable.

Such differences do have consequences. Firstly, normal ranges of CCR are mostly based on studies, in which Jaffe techniques have been used to measure creatinine. Based on the presented results, normal ranges might have to be reestablished when using an enzymatic technique as prime technique in a laboratory. MDRD differences are bothersome in some aspects, and the differences found emphasize once again the nature of the MDRD formula: it is an estimate, and when abnormal, should lead to further analysis, not be taken to represent the true value.

Clinically, such differences in the MDRD has consequences, since variability in SCr measurements affects SCr based prediction equations such as the MDRD [5]. CKD staging directly relies on these estimated GFR values, and inaccurate SCr measurements

may result in misclassification of patients [4,18]. The variations in CCR due to the huge differences in urinary measurement results are more bothersome from a clinical point of view. However, the differences found with the different techniques do force a rethinking of this assumption. Furthermore, further research should be initiated both to explain these differences and to redefine normal and abnormal ranges for CCR. Although in subjects with end-stage renal disease differences are definitely less bothersome, also in this group the lack of precision remains a focus of attention.

Conclusions

Jaffe and enzymatic assays measuring creatinine in urine are biased and imprecise, leading to the underestimation of the CCR. In plasma samples the unreliable nature of Jaffe measurements compared to a reference method (LCMS) was shown again, at least in the (near)normal ranges. Using an enzymatic technique, results show a more consistent pattern, and are grossly comparable to the results using LCMS.

Based on the urinary results and its consequences for calculating CCR, those ranges need to be redefined as well. However, in our opinion, first more research should be focused trying to explain these differences in urinary measurements before advocating changes in ranges. Based on our study results, accepting such a switch would have as a consequence redefining normal ranges.

As it is, normal ranges for PCr have been redefined already. Still, the differences in PCr outcomes also have consequences for the MDRD formula outcomes. Calculating an eGFR using an enzymatic technique will show slightly higher results in (near)normal ranges. This has consequences for the classification of subjects with a slightly impaired renal function.

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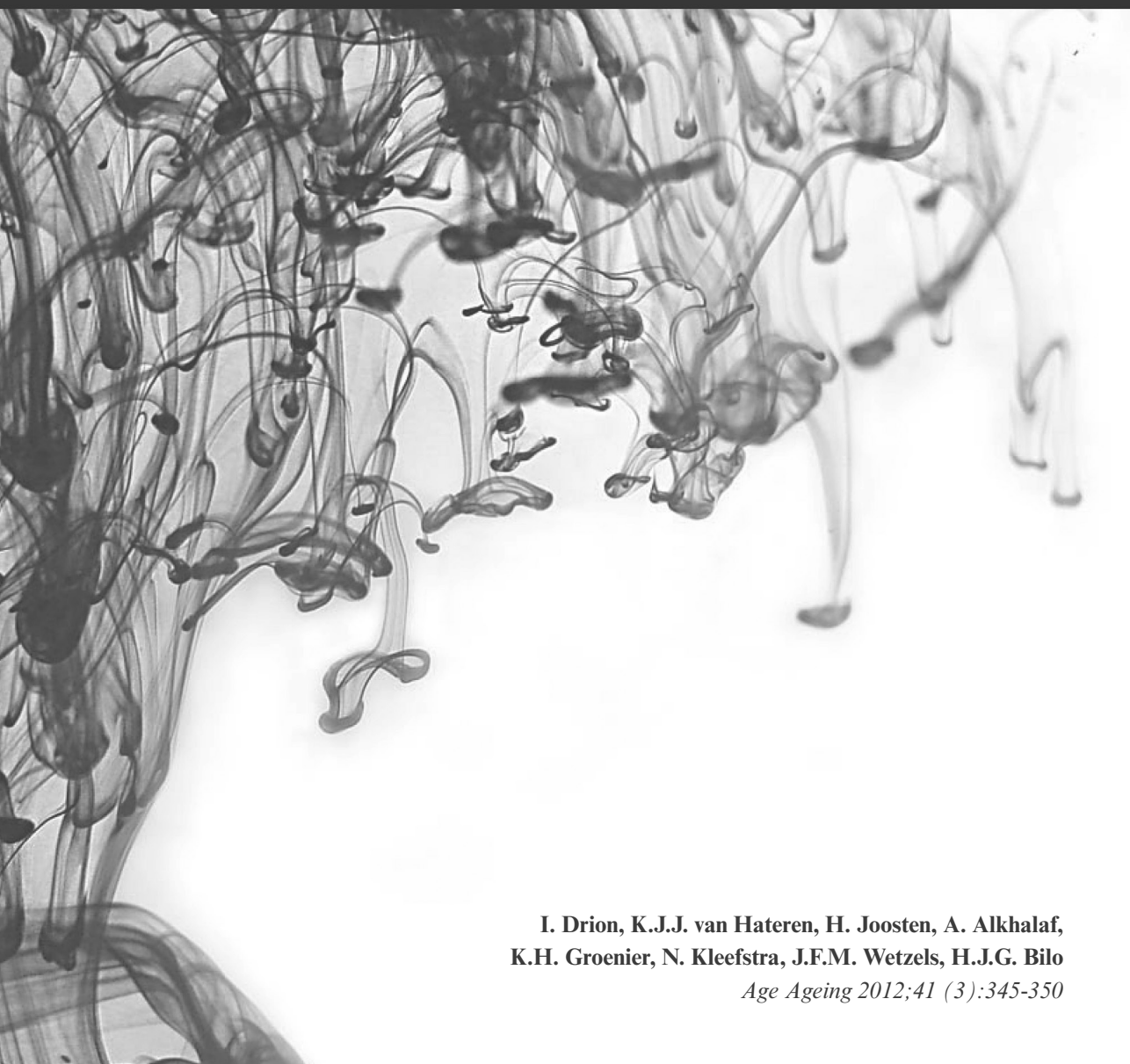
Chapter 6

Chronic kidney disease and mortality risk among older patients with type 2 diabetes mellitus



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Age Ageing 2012;41 (3):345-350

Background and objectives An impaired glomerular filtration rate (GFR) and albuminuria are associated with increased mortality in patients with type 2 diabetes. In spite of increasing numbers of elderly patients with type 2 diabetes, this association is sparsely investigated in this patient group. Therefore, we wanted to analyze the association between a decreased estimated GFR (eGFR), albuminuria and mortality in elderly patients with type 2 diabetes mellitus.

Methods From 1998-1999, 810 patients with type 2 diabetes ≥ 65 years participated in this prospective observational study. Mortality data were collected in 2009. With Cox proportional hazard modelling the association between eGFR, albuminuria and mortality was investigated. Analyses were performed in age strata: 65-75 (n=471), >75 (n=339) years.

Results An eGFR <45 and 45-60 ml/min/1.73m^2 is associated with increased cardiovascular mortality in patients of 65-75 years, hazard ratio (HR): 3.29 (1.58-6.86) and 1.78 (1.09-2.90), respectively; in those >75 years increased cardiovascular mortality was observed when eGFR was <45 ml/min/1.73m^2: 2.42 (1.47-3.69). Compared with patients of 65-75 years, an eGFR >60 ml/min/1.73m^2 and normo-albuminuria, fully adjusted HRs for cardiovascular mortality were 2.26 (1.04-4.92) and 4.86 (2.33-10.15) for those aged 65-75 years, an eGFR of 45-60 ml/min/1.73m^2 and normo-albuminuria or albuminuria, respectively; HRs were 1.33 (0.67-2.66) and 2.01 (1.02-3.94), respectively for those >75 years.

Conclusions An eGFR of 45-60 ml/min/1.73m^2 in type 2 diabetes patients was associated with increased mortality in subjects aged 65-75 years but not in those >75 years. Albuminuria is associated with increased mortality in elderly >65 years.

Introduction

Chronic kidney disease (CKD) is an independent risk factor for cardiovascular morbidity and mortality as well as all-cause mortality [1-3]. As a consequence, there has been increasing focus on the prevention and early detection of CKD.
Some of the present CKD guidelines recommend follow-up and treatment when the estimated glomerular filtration rate (eGFR) falls below 60 ml/min/1.73m^2 [4]. However, a substantial part of the elderly population has an eGFR <60 ml/min/1.73m^2. The clinical significance of moderate reductions of eGFR in elderly people is still debated [5-7]. Some argue that, in the absence of other abnormalities, an age-related decrease in eGFR is physiological; others state that a reduction in eGFR in individuals >65 years) may reflect the high prevalence of kidney disease risk factors at older age[8]. In spite of the uncertainties regarding clinical significance, follow-up of renal function is indicated, since older patients can also have an underlying renal disease or factors adding to the progression of kidney disease.
The number of older people with type 2 diabetes mellitus (T2DM) is increasing thanks to earlier diagnosis and better survival. Therefore complications, such as diabetic nephropathy occur more frequently [9] and screening for kidney disease has become a cornerstone of diabetes care [10]. However, the association between eGFR, albuminuria and mortality has been sparsely investigated in older diabetic patients [11]. Moreover, classic cardiovascular risk factors seem to have a diminished effect when assessed in patients >75 years [12-13]. Therefore, we aimed to investigate the association between eGFR, albuminuria and mortality in older patients with T2DM, stratified according to age (65-75 years and >75 years).

Methods

In 1998, the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study was initiated in the Zwolle region, the Netherlands. The design and details of this study have been presented elsewhere [14]. Briefly, the ZODIAC study is part of a shared care project, in which general practitioners are assisted by hospital-based nurses specialised in their care of patients with T2DM. At baseline, patients being treated by a specialist of internal medicine (20%) or patients with a very short life expectancy (including patients with active cancer) or insufficient cognitive abilities as judged by the general practitioner, were excluded. Ultimately, general practitioners excluded 5% of the patients treated in primary care for T2DM.
Approximately 90% (n=1357) agreed to participate; four patients were excluded because of insufficient baseline data. For the present study we selected all patients ≥65 years with

complete information on all confounders (n=810). The ZODIAC study was approved by the medical ethics committee, and all patients provided informed consent.

Data collection

Baseline data were collected from 1998-1999 and consisted of a full medical history including assessment of macrovascular complications, medication use, diabetes duration and tobacco consumption. Macrovascular complications were defined as a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke or transient ischaemic attack. Laboratory and physical assessment data were collected annually and included glycated haemoglobin (HbA_{1c}), non-fasting lipid profile, plasma creatinine (a kinetic colorimetric Jaffé method was used (Modular P Analyzer, Roche Almere, the Netherlands), albumin-to-creatinine ratio (ACR, assessed in a spot morning urine sample using immunonephelometry (Behring Nephelometer; Mannheim, Germany)), blood pressure (measured twice with a Welch Allyn sphygmomanometer), bodyweight and height.

Renal function was estimated by the Modification of Diet in Renal Disease equation (MDRD) [15]. MDRD was categorized into 3 classes: <45, 45-60 and ≥60 ml/min/1.73m^2. Albuminuria was defined as an ACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women. The overall cohort (n=810) was divided into a low (65-75 years, n=471) and a high age group (>75 years, n=339).

Clinical endpoints

Two clinical endpoints were examined: all-cause and cardiovascular mortality. In 2009, the vital status and cause of death were retrieved from records maintained by the hospital and general practitioners.

Statistical analyses

SPSS version 16.0 (SAS Insitute, Cary, NC, USA) and STATA version 11.0 (Stata Corp., College Station, TX, USA) were used for statistical analyses.

A Cox proportional hazard model was used to investigate the association between eGFR, ACR and mortality with adjustment for selected confounders. The associations were investigated for the eGFR as a categorical variable as well as a continuous variable (using baseline MDRD values) and for albuminuria as a categorical variable. Hazard ratios (HRs) for covariates were calculated for changes in eGFR of 10 ml/min/1.73m^2.

The following possible confounders were selected: age, gender, smoking (dichotomous), body mass index, systolic blood pressure, history of macrovascular complications (dichotomous), diabetes duration, HbA_{1c}, use of carbasalate calcium, use of lipid lowering medications, the total cholesterol-HDL ratio and albuminuria (dichotomous),

the eGFR was added and albuminuria was removed as a confounder when the association between (normo)albuminuria and mortality was tested.
Three different models were analyzed: model 1 (crude), model 2 (including all selected confounders) and model 3 in which all selected confounders were used, except variables that were already used in the MDRD (sex and age). The latter model was performed to reduce the phenomenon of multicollinearity and to evaluate the influence of omitting these variables on the association between renal function predicted by the MDRD, and mortality.
Cox regression analyses were performed to investigate the association of albuminuria with all-cause and cardiovascular mortality; these analyses were repeated in eGFR categories ≤ and >60 ml/min/1.73m^2 with and without albuminuria. Since stratification by the level of ACR may be warranted in terms of association with mortality, we tested the interaction between the MDRD (as continuous and as categorical variable) and the ACR.
For Kaplan-Meier curves eGFR baseline values were categorised into three different groups: <45, 45-60 and >60 ml/min/1.73m^2.

Results

Baseline characteristics of the population are shown in table 1. Patients >75 years had an eGFR <60 ml/min/1.73m^2 (n=213; 63%) and albuminuria (n=176; 52%) more frequently than patients aged 65-75 years (n=195, 41% and n=209, 44%, respectively).
274 patients (81%) in the high age group and 170 patients (36%) in the low age group died after a follow-up time of 10 years. In 27 patients (3%) the cause of death was unknown, 19 patients were lost to follow-up. The proportion of deaths attributable to cardiovascular causes was 43% in the high age group and 42% in the low age group.

Renal function estimates and plasma creatinine
Tables 2 and 3 (for table 3, please see the table appendix 1 in the supplementary data on the journal website http://ageing.oxfordjournals.org) present the hazard ratios (HRs) for cardiovascular and all-cause mortality for eGFR categories and (normo)albuminuria. The MDRD as a continuous variable was associated with both increased all-cause as well as cardiovascular mortality in all models of both age groups. After adjusting for confounders, the cardiovascular mortality risk increased by 64% [95% confidence interval (95% CI): 33-96%] and 47% [95% CI: 25-75%] for every 10 ml/min/1.73m^2 decrease in eGFR in the low and high age group, respectively (model 3).

	65-75 years	>75 years
Characteristic	*n*=471	*n*=339
Age (years)	71 [68, 73]	79 [77, 83]
Men	192 (41%)	122 (36%)
Body mass index (kg/m^2)	29 (5)	27 (4)
Systolic blood pressure (mmHg)	159 (24)	156 (25)
Diastolic blood pressure (mmHg)	84 (11)	81 (11)
Current smoking	66 (14%)	32 (9%)
Cholesterol-HDL ratio	5.2 (1.5)	4.9 (1.6)
HbA_{1c} (mmol/mol)	58	57
Macrovascular complications present	175 (37%)	150 (44%)
Receiving antihypertensive treatment	263 (53%)	234 (62%)
Receiving carbasalate calcium	65 (14%)	61 (18%)
Receiving lipid lowering treatment	61 (13%)	15 (4%)
Duration of diabetes mellitus type 2 (years)	6 [3, 12]	8 [4, 13]
Plasmacreatinine (µmol/L)	92 [82, 105]	98 [85, 115]
MDRD (ml/min/1.73m^2)	63 [55, 71]	56 [48, 66]
<45	6%	21%
45-60	35%	42%
>60	59%	37%
Albuminuria present	209 (44%)	176 (52%)

Table 1. Baseline characteristics

Patients with an eGFR <45 ml/min/1.73m^2 in the high age group, had an increased risk for all-cause and cardiovascular mortality compared with the reference category (>60 ml/min/1.73m^2). Such a relationship was not observed for eGFR values between 45 en 60 ml/min/1.73m^2. In contrast in patients aged 65-75 years, cardiovascular mortality risk was increased in patients with eGFR values between 45-60 ml/min/1.73m^2. The HRs of model 1 and 2 for all-cause mortality in this age group were not significant for patients with an eGFR <60 ml/min/1.73m^2. However, the results of model 3 show that the risk of all-cause mortality was increased for patients with an eGFR value below 60 ml/min/1.73m^2 compared with higher levels. Figure 1 and 2 show the association between eGFR and cardiovascular mortality and all-cause mortality, respectively, in both the low (A) and the high (B) age group.

Albuminuria

In both age groups, albuminuria was associated with all-cause and cardiovascular mortality (tables 2 and 3). Compared with participants aged 65-75 years with an eGFR >60 ml/min/1.73m^2 and normo-albuminuria, the fully adjusted HRs for cardiovascular mortality were 2.26 (95% CI 1.04-4.92) for impaired eGFR (45-60 ml/min/1.73m^2) and normo-albuminuria, and 4.86 (95% CI 2.33-10.15) for those with an eGFR of 45-60 ml/min/1.73m^2 and albuminuria. For participants aged >75 years, HRs were 1.33 (0.67-2.66) and 2.01 (1.02-3.94), respectively.

No interaction between the MDRD and ACR was present for both age groups (MDRD categorical: p-value 0.085 and 0.575; MDRD continuous p-value 0.767 and 0.386 for patients aged 65-75 respectively >75 years).

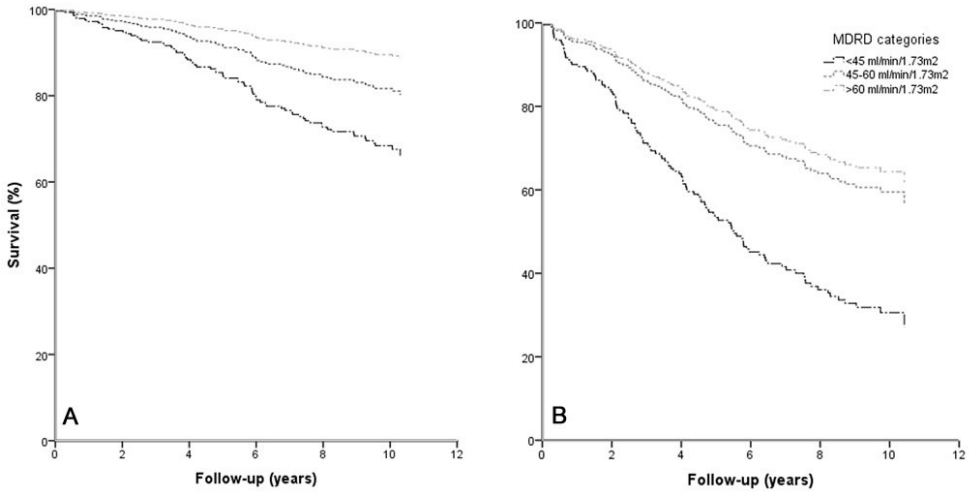

Figure 1. Kaplan Meier curves showing the association between estimated glomerular filtration rate and cardiovascular mortality for patients aged 65-75 years (A) and patients aged >75 years (B).

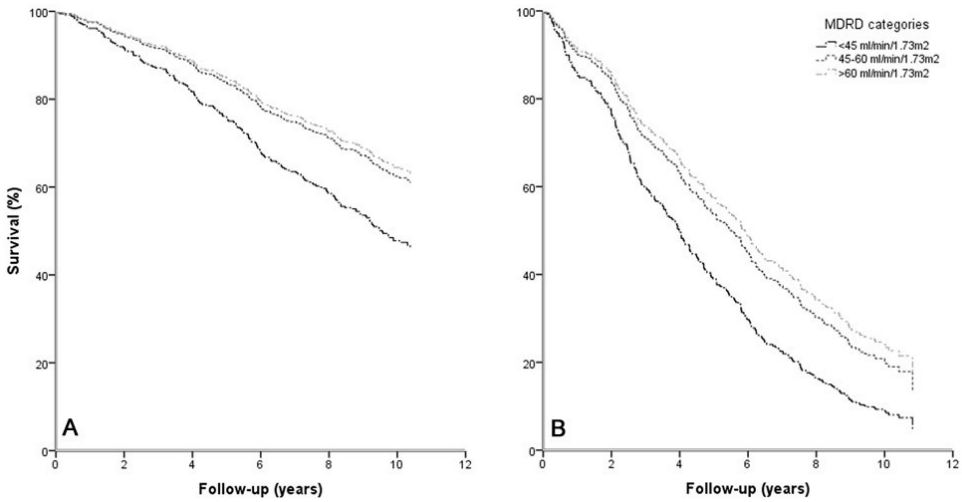

Figure 2. Kaplan Meier curves showing the association between estimated glomerular filtration rate and all-cause mortality for patients aged 65-75 years (A) and patients aged >75 years (B).

		ALL-CAUSE MORTALITY					
		65-75 years			• 75 years		
		*Model 1	#Model 2	$Model 3	*Model 1	#Model 2	$Model 3
Estimated glomerular filtration rate	Continuous Per 10 ml/min/1.73m^2 decrease	1.16 (1.03-1.32)	1.22 (1.06-1.41)	1.19 (1.05-1.35)	1.12 (1.03-1.25)	1.12 (1.02-1.27)	1.16 (1.05-1.28)
	>60 ml/min/1.73m^2	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
	45-60 ml/min/1.73m^2	1.10 (0.81-1.50)	1.11 (0.79-1.57)	1.82 (1.10-2.99)	0.99 (0.75-1.31)	1.03 (0.76-1.38)	1.18 (0.74-1.87)
	<45 ml/min/1.73m^2	1.48 (0.85-2.59)	1.72 (0.94-3.14)	3.57 (1.69-7.54)	1.63 (1.18-2.25)	1.63 (1.15-2.30)	2.69 (1.63-4.43)
Albumin-to-creatinine ratio	normoalbuminuria	1.00 (ref)	1.00 (ref)	NA	1.00 (ref)	1.00 (ref)	NA
	albuminuria	2.04 (1.52-2.73)	1.52 (1.11-2.07)	NA	1.45 (1.14-1.85)	1.60 (1.23-2.08)	NA

Table 2. Hazard ratios for eGFR and all-cause mortality. * Model 1 is unadjusted. # Model 2 is adjusted for: age, gender, smoking (dichotomous), body mass index (BMI), systolic blood pressure, a history of macrovascular complications (dichotomous), diabetes duration, HbA_{1c}, albuminuria (dichotomous), the total cholesterol-HDL ratio, the use of carbasalate calcium and lipid lowering treatment; $ Model 3 is adjusted for the same variables as in model 2 except for those who are already used for calculation of the MDRD (age, sex). In model 2 ACR no correction takes place for albuminuria (dichotomous); in model 2 ACR, correction for eGFR does occur. NA: not applicable; ACR: albumin creatinine ratio.

		CARDIOVASCULAR MORTALITY					
		65-75 years			>75 years		
		*Model 1	#Model 2	$Model 3	*Model 1	#Model 2	$Model 3
Estimated glomerular filtration rate	Continuous Per 10 ml/min/1.73m^2 decrease	1.61 (1.35-1.96)	1.69 (1.37-2.08)	1.64 (1.33-1.96)	1.43 (1.22-1.67)	1.45 (1.20-1.69)	1.47 (1.25-1.75)
	>60 ml/min/1.73m^2	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
	45-60 ml/min/1.73m^2	1.95 (1.20-3.16)	1.87 (1.08-3.23)	1.78 (1.09-2.90)	1.15 (0.73-1.79)	1.08 (0.67-1.73)	1.15 (0.73-1.81)
	<45 ml/min/1.73m^2	3.57 (1.75-7.29)	3.65 (1.65-8.07)	3.29 (1.58-6.86)	2.56 (1.59-4.12)	2.51 (1.51-4.19)	2.42 (1.47-3.96)
Albumin-to-creatinine ratio	normoalbuminuria	1.00 (ref)	1.00 (ref)	NA	1.00 (ref)	1.00 (ref)	NA
	albuminuria	2.60 (1.63-4.15)	1.94 (1.18-3.21)	NA	1.59 (1.09-2.32)	1.49 (0.99-2.24)	NA

Table 3. Hazard ratios for eGFR and cardiovascular mortality. * Model 1 is unadjusted. # Model 2 is adjusted for: age, gender, smoking (dichotomous), body mass index (BMI), systolic blood pressure, a history of macrovascular complications (dichotomous), diabetes duration, HbA_{1c}, albuminuria (dichotomous), the total cholesterol-HDL ratio, the use of carbasalate calcium and lipid lowering treatment; $ Model 3 is adjusted for the same variables as in model 2 except for those who are already used for calculation of the MDRD (age, sex). In model 2 ACR no correction takes place for albuminuria (dichotomous); in model 2 ACR, correction for eGFR does occur. NA: not applicable; ACR: albumin creatinine ratio.

Discussion

In this study renal function loss was related to both increased all-cause and cardiovascular mortality. In patients >75 years, increased all-cause and cardiovascular mortality was only observed when the eGFR was <45 ml/min/1.73m^2, this in contrast to elderly patients aged 65-75 years in which an increased risk for cardiovascular mortality was observed when renal function estimates dropped below 60 ml/min/1.73m^2. Albuminuria was independently associated with all-cause and cardiovascular mortality irrespective of eGFR in both age groups. The fact that in model 3 the risk of all-cause mortality was increased for patients with an eGFR value <60 ml/min/1.73m^2 compared with higher levels, shows that multi-collinearity occurs when age and sex are, next to its presence in the MDRD formula, also used as a confounder (such as in model 2).

Thus, age seems to be an important effect modifier in CKD. A meta-analysis in general population cohorts showed independent and joint associations of albuminuria and eGFR <60 ml/min/1.73m^2 on cardiovascular and all-cause mortality [16]. However, the number of patients >70 years was relatively small and associations were not evaluated in separate age cohorts. Other studies investigating the consequences of a reduced eGFR in patients >75 years in the general population have shown that if normoalbuminuric, mortality risk is only increased when eGFR is <45 ml/min/1.73m^2 [17-19]. Moreover, older patients had higher rates of death and lower rates of end-stage renal disease than younger patients at comparable levels of eGFR [20]. From a cross-sectional study in older people, it appeared that an eGFR <45 ml/min/1.73m^2 mainly identifies a smaller sub-group of people >75 years with significant comorbidity, impaired functional state and a high risk of potentially reversible consequences (e.g. anaemia) [21].
Most of the above mentioned studies contained only few diabetes patients or patients >75 years. A study among diabetic patients >65 years showed that albuminuria and an eGFR <60 ml/min/1.73m^2 were independent risk factors for mortality [2]. However, the observed relationship might have been largely attributable to the proportion of patients with an eGFR <45 ml/min/1.73m^2. Our results show that even in normo-albuminuric patients with T2DM, >75 years, an eGFR of 45-60 ml/min/1.73m^2 is not associated with an increased risk for cardiovascular and all-cause mortality, in contrast to those aged 65-75 years. Our results are confirmed by a recent meta-analysis in normo-albuminuric patients at high risk for CKD (n=106690, 40% had diabetes), whose risk for all-cause mortality was increased at eGFR levels <60 ml/min/1.73m^2 [3]. However, in subjects ≥65 years, significance was reached at a lower level (<45 ml/min/1.73m^2), as opposed to subjects <65 years. No specific analyses were made for patients >75 years.

The attenuation of the association of mortality with certain eGFR stages in older patients as we observed; was not found for albuminuria. This is confirmatory with previous studies. An independent association between proteinuria and mortality has been shown in patients with and without diabetes. In the HUNT II study, the presence of micro-albuminuria or high-normal ACR ratios was associated with increased cardiovascular mortality below the threshold of 75 ml/min/1.73m^2 compared with those with normo-albuminuria [18]. A more recent study found in a largely male cohort that the ACR in diabetes patients >65 years was independently associated with mortality at all levels of eGFR [11]; an observation that is in agreement with our study. In contrast to our study, a large study in primary care, investigating the association between dipstick proteinuria, eGFR and mortality in patients aged >75 years, the presence of dipstick proteinuria did not add to cardiovascular mortality risk. This is remarkable since one would expect that especially when a dipstick is used, the risk of cardiovascular mortality would have been higher [19]. Also another study in older individuals referring patients with CKD stage 4 did not find a statistically significant association between level of proteinuria and risk of death; 33% was >75 years) [22]. An explanation for the discrepancy in the two last mentioned studies and our study results has not been found.

The absence of an association of moderate reduction in eGFR with mortality at older age may have been caused by the fact that the MDRD was not developed for use in older patients. Moreover, creatinine is a poor marker of renal function in these patients leading to inaccuracy [23-24]. Secondly, older patients have higher background mortality and a higher prevalence of comorbidity [25]. Finally moderate reductions in eGFR may reflect a physiological decline in renal function with advancing age [26-27]. Since albuminuria reflects another pathway of kidney damage than eGFR, this may explain we found no attenuation of the association between albuminuria and increased cardiovascular mortality [28-29].

Strengths and limitations

Our study has some methodological aspects that need discussion. Firstly, our study cohort is rather small, especially the group with an eGFR <45 ml/min/1.73m^2. Therefore the results should be interpreted with caution. Due to the small numbers no differentiation in micro- and macroalbuminuria was made since the number of patients in the separate groups would become too small. Secondly, we have used uncalibrated plasma creatinine measurements. This might have induced systematic errors in eGFR values. Fortunately, all creatinine measurements were performed in the same laboratory, so interlaboratory variation was excluded. Selection bias may have occurred, since patients with a short life expectancy and patients treated in hospital for their diabetes were excluded. Finally,

the MDRD has not been validated in patients >70 years. Strengths are the prospective nature, the possibility to take into account many possible confounders with few missing data, and the long follow-up.

In conclusion, patients >75 years with T2DM and an eGFR of 45-60 ml/min/1.73m^2 are not at increased risk for all-cause and cardiovascular mortality compared with their counterparts with an eGFR >60 ml/min/1.73m^2. In contrast albuminuria at all levels of eGFR is strongly associated with increased all-cause and cardiovascular mortality, and therefore may have potential as a more discriminative risk stratification tool in the large group of older patients with moderate reductions in eGFR (45-60 ml/min/1.73m^2). In this study, as in most studies of CKD in older individuals, patients with moderate decrements of renal function account for a large proportion of the older population with CKD, which suggests that the current staging system, taking into account eGFR only, may not be a reliable tool for older patients, at least when used to assess increased cardiovascular risk.

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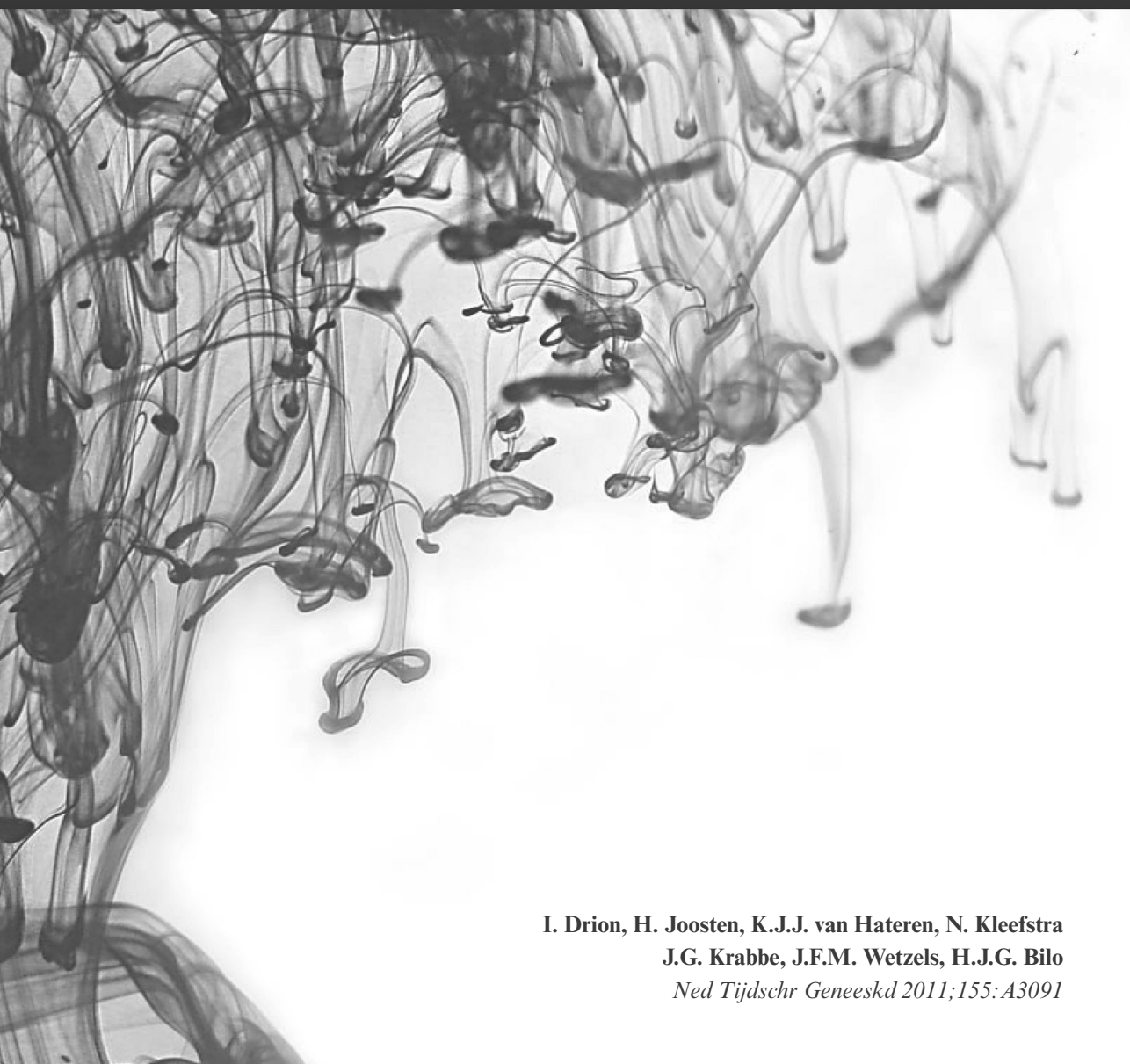
Chapter 7

Employing age-related cut-off values results in more targeted referral to secondary care



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Background and objectives To describe the consequences of using age-related cut-off values for renal function on the burden for primary and secondary care in the Netherlands, when following the Dutch national transmural agreement (LTA) for 'Chronic renal impairment', rather than the 'Kidney disease outcome quality initiative' (K/DOQI) guidelines.

Methods In this observational cross-sectional study, patients whose serum creatinine was examined in 2009 were identified from the laboratory registry of the Isala Clinics in Zwolle, The Netherlands. The glomerular filtration rate (GFR) was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation. Burden of care was defined as the necessity for referral or consultation in secondary care. The number of people that would have been referred using the K/DOQI guideline that refers all those with an eGFR <60 ml/min/1.73m^2, was compared with a situation using age-related cut-off values in the referral policy.

Results The study population contained 82,424 people; 45.3% were men; age range was 19-106 years; 38.7% were >65 years. 19% of the population (n = 15,637) had an eGFR <60 ml/min/1.73m^2 and would have been referred had the K/DOQI guidelines been applied; 11,935 of those 15,637 were >65 years. The use of the LTA for 'Chronic renal impairment', that includes age as one of the criteria, would have resulted in the referral of 3,303/15,637 patients (2,011 of those 3,303 were >65 years), and resulted in consultation with a nephrologist for 5,748/15,637 patients (3,338/5,748 were >65 years). The majority of patients aged >65 years and with an eGFR <60 ml/min/1.73m^2 (55%) could be treated in primary care without consultation of secondary care or referral.

Conclusion The categorization applied by the current LTA for 'Chronic renal impairment', whereby age-related cut-off values are used in the referral policy, will result in more targeted referral to secondary care, especially in the elderly patient group, when compared with application of the K/DOQI guidelines.

Introduction

In 2002 the Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines were introduced to uniformly define and classify chronic kidney disease (CKD), to recognize patients with CKD in an early stage, and to improve the prognosis of this patient group [1]. In these guidelines CKD is devised in 5 stages (table 1). The classification in stage 1 and 2 is, apart from the estimated glomerular filtration rate (eGFR), especially based on the presence of persisting (micro-) albuminuria or erythrocyturia of glomerular origin [1]. From stage 3 onwards, the classification is solely based on the GFR, as estimated by the Modification of Diet in Renal Disease formula (MDRD) [2-3].

Stage	eGFR
1	>90 ml/min/1.73m^2 and persisting (micro-) albuminuria or persisting and specific sediment abnormalities (dysmorphous erythrocytes and / or cell cylinders).
2	60-90 ml/min/1.73m^2 and persisting (micro-) albuminuria or persisting and specific sediment abnormalities (dysmorphous erythrocytes and / or cell cylinders).
3	30-60 ml/min/1.73m^2
4	15-30 ml/min/1.73m^2
5	<15 ml/min/1.73m^2

Tabel 1 Stages of chronic kidney disease according to ´the Kidney Disease Outcomes Quality Initiative (K/DOQI-guidelines). The glomerular filtration rate is estimated by the Modification of Diet in Renal Disease (MDRD) formula.

According to the K/DOQI guidelines approximately 10% of the Dutch population has CKD, of whom approximately 60% has an eGFR <60 ml/min/1.73m^2 [4]. If the eGFR is <60 ml/min/1.73m^2 these guidelines recommend additional research to an eventual underlying renal disease. Moreover, the cardiovascular risk profile has to be examined as well as the risk for further renal function deterioration [1]. Therefore, in some countries this threshold is an indication for referral to a nephrologist.

In 2009 a national guideline for CKD ('de Landelijke Transmurale Afspraak (LTA) chronische nierschade') was introduced in the Netherlands [5]. The LTA gives advices for the management and referral of patients diagnosed with renal function loss. For patients with proteinuria or erythrocyturia of glomerular origin, referral is advised irrespective of the eGFR. When proteinuria or erythrocyturia are absent and an eGFR <60 ml/min/1.73m^2 is found, age becomes an important factor in the decision process for further diagnostics and / or referral (table 2). For patients younger than 65 years, consultation or referral to a nephrologist is advised when the eGFR is <60 ml/min/1.73m^2, for those older than 65 years consultation or referral is only advised when the eGFR is <45 ml/min/1.73m^2. This classification is based on the fact that renal function loss in elderly is often based on a (physiologic) deterioration of renal function; healthy elderly often have

an eGFR <60 ml/min/1.73m^2. The median eGFR for a healthy 70-79 year old man or woman is 79 and 72 ml/min/1.73m^2; the 5th percentile is 54 respectively 52 ml/min/1.73m^2 [6-9]. Not using an age criterion, would mistakenly result in a larger percentage of elderly being referred to a nephrologic centre only on the basis of an eGFR, while the eGFR is appropriate for their age. However, one should keep in mind that also in elderly with a reduced eGFR an underlying renal disease or factors adding to the progression of kidney disease might be present.

In this study the consequences of using age-related cut-off values for renal function on the burden for primary and secondary care in the Netherlands, following the Dutch national transmural agreement (LTA) for 'chronic renal impairment', rather than the 'Kidney Disease Outcome Quality Initiative' (K/DOQI) guidelines will be evaluated.

	Age categories according to the 'LTA chronische nierschade'	
eGFR	≤65 jaar (n=50550)	>65 jaar (n=31874)
<30 ml/min/1.73m^2	549 (1.1%)	2011 (6.2%)
30-45 ml/min/1.73m^2	743 (1.4%)	3338 (10.5%)
45-60 ml/min/1.73m^2	2410 (4.8%)	6586 (20.7%)
>60 ml/min/1.73m^2	46848 (92.7%)	19939 (62.6%)

Referral to nephrologist Consultation nephrologist Follow-up primary care

Tabel 2. Implications of the 'Landelijke Transmurale Afspraak (LTA) chronische nierschade' on referral patterns from primary care to a nephrologic centre. For each of the age cateogories (≤ 65 and >65 years) the number of people per eGFR category is presented. Different colours indicate which patients can be followed in primary care (■), which patient group needs referral to a nephrologist (□), and in which patient group consultation of a nephrologist will be sufficient (▒).

Methods

Study design and study population

In this retrospective observational cross-sectional study all serum creatinine data, that had been analyzed in the Isala clinics in 2009, were identified. Ultimately, serum creatinine data of 224455 patients (from primary and secondary care) were collected in a database. The adherence region of the Isala Clinics encompasses 130 general practices, taking care for a population of approximately 375000 patients. No personal information was added to the database for patient anonymity reasons. Therefore, we did not have any information on specific medical indications for measuring serum creatinine, the presence or absence of albuminuria and erythrocyturia, ethnicity, medical history and medication use.

Data regarding the demography of Zwolle and its adherence region were withdrawn from a regional database (*http://www.overijssel.nl/overijssel/cijfers-kaarten/inwonersaantal*),

to provide inside in the frequency of renal function measurements in the different age categories of the study population.
All subjects <18 years (n=5142) were excluded. When serum creatinine had been assessed more than once in the indicated period, the two most recent values were added to the database (n=34756). The definitive database contained data from 82424 patients. No permission was required from the Medical Ethics Committee as our data only included laboratory result information, as obtained from a laboratory database.

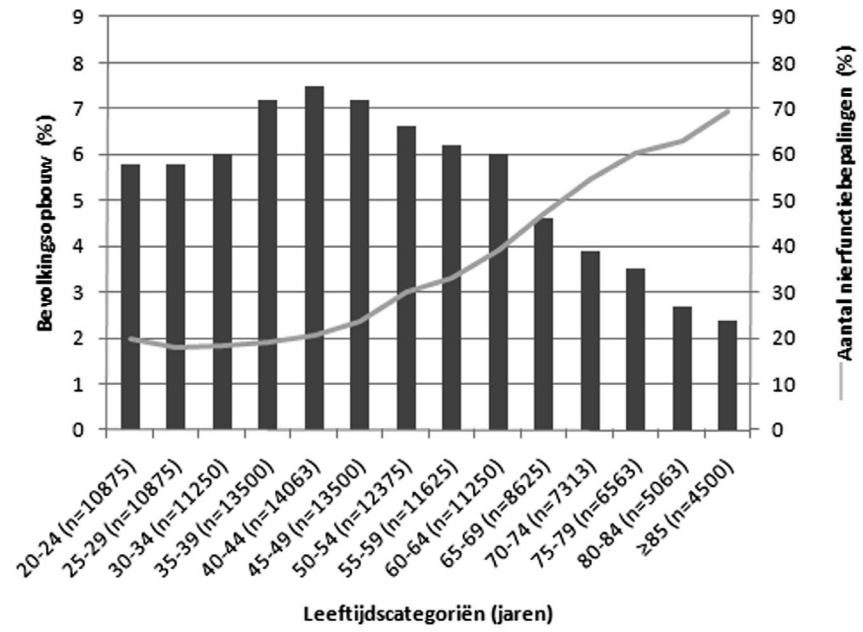
Serum creatinine measurements and the MDRD
All serum creatinine measurements were measured enzymatically using a modular P Analzer (creatinine plus assay, Roche Diagnostics, Mannheim, Germany). The GFR was estimated using the MDRD: 175* (serum creatinine (µmol/L)/88.4)$^{-1.154}$ * age (years)$^{-0.203}$) * factor (factor=1 for men; factor = 0.742 for women) [2-3].

Statistical analysis
Analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Data regarding age distribution in the adherence region of the Isala clinics were presented per age category of five year, for men and women separately. Consequently, the number of renal function measurements per age category was calculated. Since we did not have any information on albuminuria and haematuria, the number of patients with K/DOQI stage 3-5 was assessed. Subsequently, the study population was classified according to the categories as applied in the LTA: eGFR <30, 30-45, 45-60 and ≥60 ml/min/1.73m^2. Moreover, the prevalence of patients with an eGFR <60 ml/min/1.73m^2 was assessed. Ultimately, the number of patients categorized in each eGFR category and the number of patients that would be referred to a nephrologist, when the K/DOQI guidelines or the LTA guidelines were applied, was evaluated. Finally we studied the effect of repeated serum creatinine measurements on the need for referral to a nephrology ward. In patiens having an extra serum creatinine assessment after 2-12 weeks or after more than 3 months we assessed in how many patients still an indication for referral existed after this period.

Results

Serum creatinine data of 82424 patients were used; 45.3% were men, 38.7% was >65 years, age ranged from 19-106 years.
Figure 1a and 1b show the frequency of renal function measurements per age category in Zwolle and its adherence region. These figures demonstrate that renal function measurements are more frequently performed in elderly people.

A



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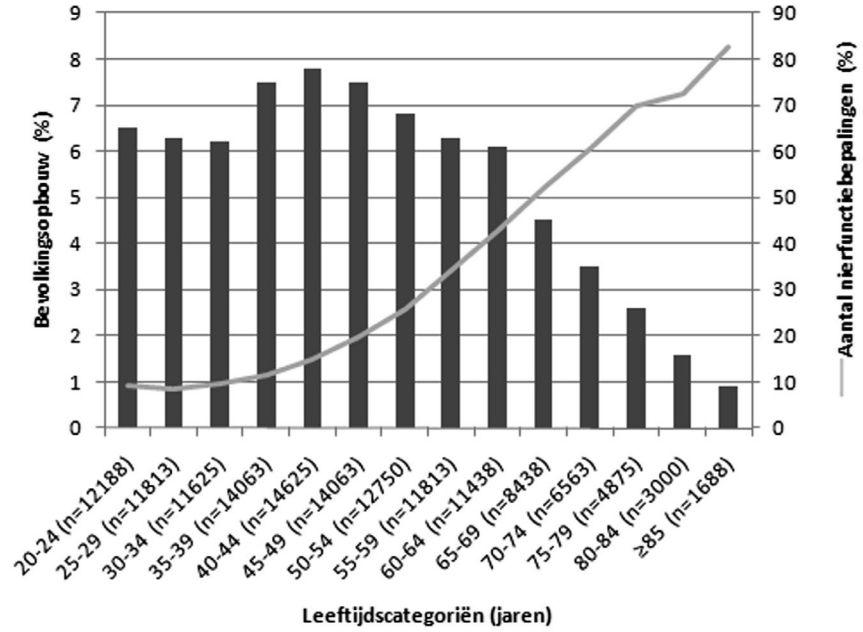

Figure 1 A en B. Population structure (bars with scale on left vertical axis) depicter per age category (horizontal axis) in Zwolle and the adherence area of the Isala Clinics in 2009; the number of renal function measurements (line chart with scale on right vertical axis) per age category. Figure A: men; figure B women. (Reference: http://www.overijssel.nl/overijssel/cijfers-kaarten/inwonersaantal).

In figure 2 the prevalence of people with a renal function <30, 30-45, 45-59 and ≥60 ml/min/1.73m^2 per age cohort is shown. In the group of patients aged >65 years, the number of subjects with an eGFR <60 ml/min/1.73m^2 is almost linearly increasing, from 22.2% in the group aged 65-70 years to 58% in the group older than 85 years.

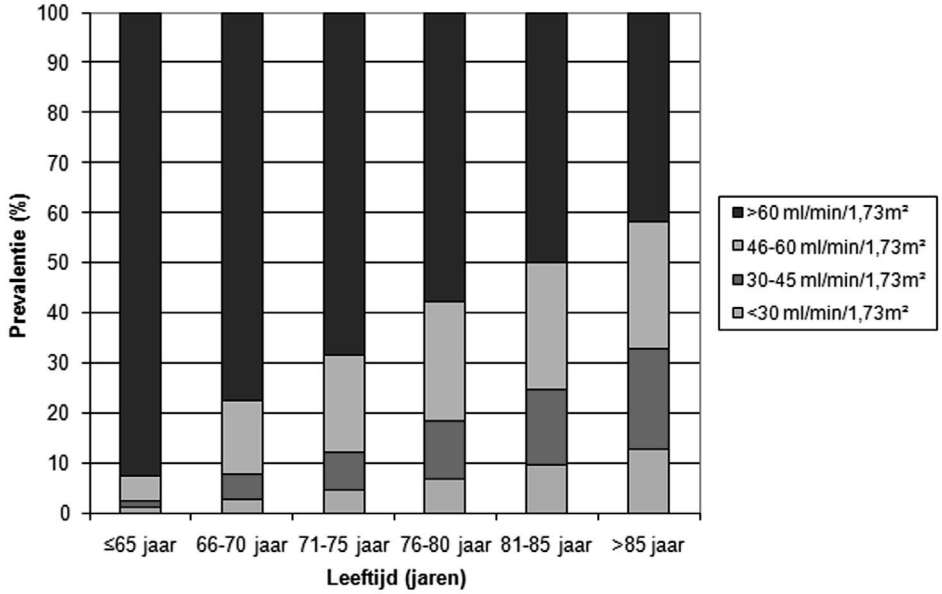

Figure 2. Bars ranging from dark-grey to light-grey, represent the prevalence of patients with an estimated glomerular filtration rate of <30, 30-45, 46-60 and >60 ml/min/1.73m^2, respectively, depicted per age category.

The implications of using age-related cut-off values for renal function (as proposed in the LTA guidelines) on referral patterns, is shown in table 2. When every patient having an eGFR <60 ml/min/1.73m^2, would be referred to secondary care, 19% (n=15637) of the total study population would have been referred. When the LTA guidelines using age-related criteria would be applied, only 4% (n=3303) of the total study population would have been referred. This is a difference of 12334 patients. For 3% (n=2410) of the patients aged ≤65 years, consultation of a nephrologists could take place instead of referral. In the population >65 years with an eGFR <60 ml/min/1.73m^2 83% (9924 of the 11935) is not referred any longer; for 6586 of these subjects follow-up can take place in primary after a screening (blood pressure, weight, specific laboratory measurements (see LTA guidelines) [4]) and in the remaining 3338 people consultation or referral to secondary care would have been advised.

In the group of patients in whom renal function was reassessed after 2-12 months compared with those with a repeated renal function measurement after more than 3

months, 15% respectively 18% did not need consultation or referral any longer after the second serum creatinine measurement.

Discussion

We assessed the consequences of the application of age-related cut-off values following the LTA guidelines rather than the K/DOQI guidelines on the burden for primary and secondary care in a patient population with potential renal impairment. Using age-related thresholds, as is stated in the LTA guidelines, leads to more directed referrals from primary to secondary care. Moreover, repeated measurements will result in a reduction of referrals to secondary care of approximately 16%. Especially in the patient group older than 65 years the number of referrals to secondary care will increase but to a lesser extent. 55% of the patients with an eGFR <60 ml/min/1.73m^2 could be followed-up in primary care, in 28% of the patients consultation of a nephrologist may occur and in 17% of the patients referral to a nephrologist is indicated. Because of this the workload in primary care will increase. Irrespective of age, an eGFR <60 ml/min/1.73m^2 implies that certain advices and measures have to be evaluated in these patients [4].

When the K/DOQI guidelines were published, automatic reporting of the MDRD became routine practice, and an eGFR <60 ml/min/1.73m^2 was considered to be pathological, the number of patients needing referral was expected to increase [10-11]. This hypothesis was confirmed in a Canadian population, in which the number of referrals to a nephrology ward increased with 70% [12]. It mainly concerned middle-aged people, women, patients with diabetes mellitus and patients with hypertension.

Renal function loss and age

Implicitly related to whether or not we should apply age-related thresholds in the referral policy of patients with CKD is the question, whether renal function in elderly should be considered physiological or pathological. This remains a point at issue worldwide. Approximately 56% of the population older than 75 years has a MDRD of 30-60 ml/min/1.73m^2 [13-14]. The clinical relevance hereof is unclear. Population studies have shown that the relative risk for mortality associated with CKD is lower for elderly people [15-16]. One study demonstrated how mortality risk attributable to CKD varied with age [17]: an eGFR of 50-59 ml/min/1.73m^2 in the patient group aged 18-54 years was associated with a higher mortality. However, in the elderly patient group (>65 years) an eGFR >50 ml/min/1.73m^2 was not associated with increased mortality (HR 1.02 [95% CI 0.99-1.05]). The absence of an association with mortality in the elderly patient group with a moderate reduction of renal function could result from inaccuracies in the estimation of the GFR

using the MDRD in elderly patients, since the MDRD has not been validated in the age category >70 years. Moreover, serum creatinine is not a good indicator of renal function in elderly [18-19]. However, it could also be true that differences in relative risk for mortality and eGFR indeed exist between different age categories [15].
In a representative primary care population of patients older than 75 years it has been demonstrated that when renal function decreases there is a moderate independent increase in total and cardiovascular mortality, especially in men and subjects with an eGFR <45 ml/min/1.73m^2. Therefore, the identification and treatment of CKD in elderly should focus on the select group of patients with a severely decreased renal function (<45 ml/min/1.73m^2) [20].

Risk appraisal in elderly
It will be complicated to determine an exact threshold for increased cardiovascular risk exclusively associated with a reduced eGFR [21]. It is plausible that this threshold is lower for the elderly population. Perhaps, an eGFR is insufficient as measure of outcome to determine cardiovascular morbidity and mortality in elderly. Possibly albuminuria is needed as marker of damage. In several studies a strong association has been shown between the degree of albuminuria and mortality risk [22-23]. Recently it has become clear that in elderly patients with diabetes the albumin/creatinine ratio is independently associated with mortality in all eGFR categories and can be a good handle for risk stratification in a large group of patients with moderate reductions of eGFR. To be able to make definitive conclusions, research in well defined cohorts is essential.

Strengths and weaknesses
This study is a reflection of daily clinical practice, in which renal function is often assessed in elderly patients. Moreover, by using a calibrated serum creatinine the chance of under- or overestimation of the number of subjects with renal function loss is reduced [24-25].
Since patients included in this study were at risk for CKD, this study is not a reflection of the general population; the prevalence of CKD in the study population will be higher than in the general population [9,26]. To give an illustration: 42-44% of the patients aged over 85 years in the NBS had an eGFR <60 ml/min/1.73m^2, compared with 58% in our cohort.
The GFR was estimated using the MDRD. The MDRD is developed in a population with renal disease. Moreover, the MDRD has a reduced individual precision, resulting in a systematic underestimation in GFR values >60 ml/min/1.73m^2. Therefore, a part of the study population might have been categorized in the wrong category. However, in clinical practice decisions are also based on the eGFR, which makes this study a

representation of daily clinical practice. Finally, the prevalence of CKD in this population is underestimated, since data on albuminuria and erythrocyturia are lacking.

Conclusion

The use of age-related thresholds in the referral policy of patients with CKD, may result in more targeted referrals to secondary care. At the moment, a good definition of pathologic or physiologic moderate renal impairment in the elderly patient group is lacking. Perhaps that albuminuria should be adapted as an extra marker in the definition of significant renal function loss at older age. Until then, nomograms should be used in the elderly individual to evaluate whether there is stable renal impairment or progressive deterioration of renal function loss compared with subjects of the same age and sex. Repeated MDRD measurements in accordance with the LTA guidelines are important to prevent needless referrals.

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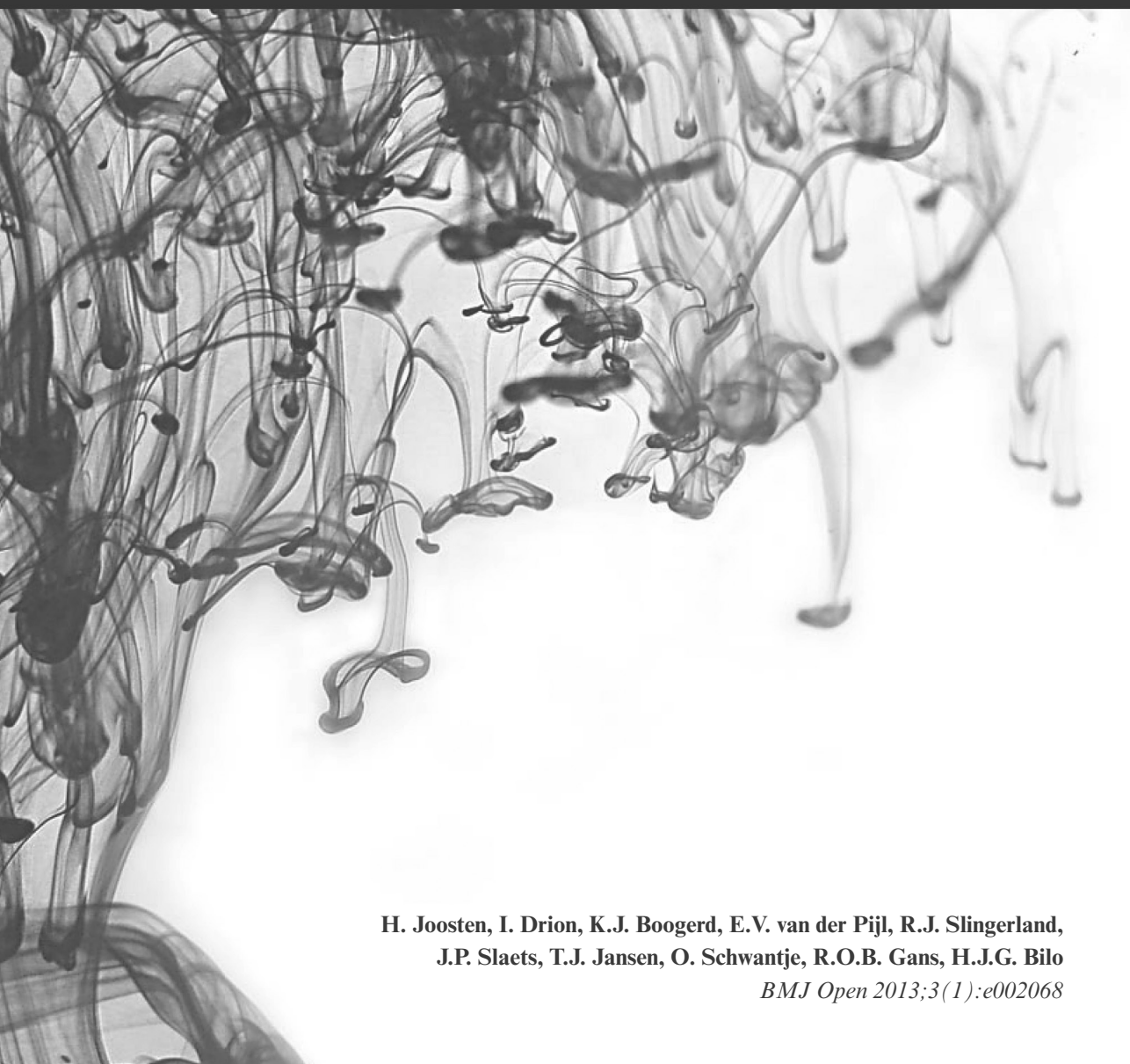
Chapter 8

Optimising drug prescribing and dispensing in subjects at risk for drug errors due to renal impairment: improving drug safety in primary health care by low eGFR alerts



H. Joosten, I. Drion, K.J. Boogerd, E.V. van der Pijl, R.J. Slingerland, J.P. Slaets, T.J. Jansen, O. Schwantje, R.O.B. Gans, H.J.G. Bilo

BMJ Open 2013;3(1):e002068

Background and objectives Iatrogenic injury due to medication errors is known as adverse drug events (ADEs). Renal impairment is a risk factor for ADEs. The aim of this study was to assess the risk of medication errors in subjects with renal impairment, defined as an estimated glomerular filtration rate (eGFR) ≤40 ml/min/1.73m^2, in a primary care setting. Moreover, the effectiveness of generating automatic eGFR ≤40-alerts and medications reviews involving community pharmacists was evaluated.

Methods 22 community pharmacists and 65 general practitioners participated in this clinical survey. The total number of ambulatory subjects with an eGFR ≤ 40-alert during the study period of one year, the number of medication errors related to renal impairment, the type and number of proposed drug adjustments recommended by the community pharmacist and acceptance rate by the prescribing physicians. Classification of all medication errors on their potential to cause an adverse drug event (ADE) and the actual occurrence of ADEs (limited to those identified through hospital record review) one year after the introduction of the alerts were measured.

Results Creatinine measurements were performed in 25929 adults. An eGFR ≤40-alert was indicated for 5.3% (n=1369). This group had a median [IQR] age of 78 [69,84] years, and in 73% polypharmacy (≥5 drugs) was present. In 15% (n=211) of these subjects, a medication error was detected. The proportion of errors increased with age. Pharmacists recommended 342 medication adjustments; mainly concerning diuretics (22%) and antibiotics (21%). The physicians' acceptance rate was 66%. Of all the medication errors, 88% were regarded as potential ADEs, with most classified as significant or serious. At follow-up, the ADE risk (n=40) appeared highest when the proposed medication adjustments were not implemented (38% versus 6%).

Conclusions The introduction of automatic eGFR-alerts identified a considerable number of subjects who are at risk for ADEs due to renal impairment in an ambulatory setting. The nationwide implementation of this simple protocol could identify many potential ADEs thereby substantially reducing iatrogenic complications in subjects with impaired renal function.

Introduction

Safe medication management is an important health care topic, as medication errors are a significant source of iatrogenic injury to patients [1-7]. Injuries resulting from such errors are known as adverse drug events (ADEs). Various factors are associated with ADEs, including patient characteristics, lack of medication monitoring, and prescription errors [4-6,8]. Studies on medication related hospital admissions estimate that 21-91% of admissions were potentially preventable [1,6,9,10]. Important patient determinants for ADEs are increasing age, female gender, polypharmacy, noncompliance and co-morbidities such as cognitive dysfunction or renal impairment [1-4,7,8,10].

Renal impairment is a well-known risk factor for ADEs, but often remains unrecognized by physicians and pharmacists [11-14]. Even in high-risk patients such as elderly and those with diabetes, health care workers are not always sufficiently alert [15-17]. Various studies reported considerable dosing difficulties and subsequent medication errors in patients with renal impairment [10,12,17-19]. Therefore, intensified collaboration between health care workers (such as general practitioners (GPs), pharmacists, and nephrologists) is recommended with exchange of relevant patient information (medical history and co-morbidities) and more effective use of routinely collected data from electronic patient records such as laboratory results relating to renal function) [2,6,20-23].

In this 1-year observational study, we aimed to evaluate the number of subjects at risk for medication errors due to renal impairment (defined as an estimated glomerular filtration rate (eGFR) ≤40 ml/min/1.73m^2) and the effectiveness of providing automatically generated eGFR ≤40-alerts towards community pharmacists in a shared pharmaceutical care model. In addition, we classified all medication errors for their potential to cause ADEs and evaluated the actual number of ADEs in those with a medication error after a period of one year.

Materials and methods

Setting

This study was conducted in Zwolle, which is a city in the north of the Netherlands with a population of more than 89,000 adults [24]. All of the primary care pharmacies (n=11) and the general practices (n=24) participated in this study. Their characteristics are shown in table 1. Dutch patients are generally registered at one single pharmacy and GP practice, which promotes continuity of care and reliable information regarding each individuals' medication use. Secondary care in this region is delivered by the Isala

Clinics, a 1000+ bed teaching-hospital in Zwolle. All standard laboratory investigations requested in both primary and secondary care are performed in one laboratory, which uses a single electronic system for data handling.

	Characteristics	Pharmacists	GPs
Participants			
	Number (%)	22 (100)	65 (100)
	Sex, n (%)		
	Male	9 (40)	42 (65)
	Female	13 (60)	23 (35)
	Years in practice, n (%)		
	0-10	10 (45)	25 (39)
	11-20	9 (41)	15 (23)
	21-30	0 (0)	21 (32)
	>30	3 (14)	4 (6)
	Position in practice, n (%)		
	(Joint) owner	6 (27)	45 (70)
	Employee	16 (73)	20 (30)
Practice			
	Number (%)	11 (100)	24 (100)
	Practice type, n (%)		
	Independent	9 (80)	-
	Chain	2 (20)	-
	Overall number of patients, n	114033	117147
	Practice size, median [IQR]	10.000 [7000, 14000]	3426 [2691, 6586]
	Prescription system, n (%)		
	Computer-based	11 (100)	24 (100)

Table 1. Characteristics of participating pharmacists and general practitioners (GPs), and their practices. IQR=Interquartile Range; GP=general practitioner.

Design and case-finding

This prospective observational study was conducted between February 1st 2009 and January 31st 2010. During this period, all consecutive adults in whom a serum creatinine was measured in the ambulatory setting who had an eGFR at or below the cut-off point of 40 ml/min/1.73m^2 were identified, irrespective of the reason for laboratory testing. This threshold was based on guidelines advising dosage adjustment in renal impairment [25,26] and also chosen from a practical point of view. A higher cut-off-point of 50-60 ml/min/1.73m^2 was expected to exceed an acceptable workload, and the generation of many alarms induces the risk of ignoring and overriding alerts. Each week the laboratory automatically generated a report for any ambulatory patients with an eGFR ≤40 ml/min/1.73m^2 for the pharmacists.

Study protocol

A predefined protocol was followed after the pharmacist received a report on an eGFR ≤40 ml/min/1.73m^2 (figure 1). First, the patients' pharmacist checked the actual medication regimen for current errors related to renal impairment. Numbers and types of errors were registered. Medication errors were based on Dutch Pharmacists guidelines including 'the National Formulary on drug prescribing in renal impairment' and the 'National Shared Care Guidelines on Chronic Kidney Disease (CKD)' [25,26]. Second, the pharmacist alerted the prescribing physician (GP or clinician) on the low eGFR and, if appropriate, an adjusted medication regimen was recommended. Pharmacists contacted prescribing physicians by telephone or (if unreachable) by email. Finally, an alert warning for a low eGFR (eGFR ≤40-alert) was activated in the patient's pharmacy record. This eGFR ≤40-alert then appeared with every future new prescription. After this first laboratory notification, follow-up eGFR results were also reported to the pharmacists. When an eGFR recovered well beyond the cut-off value during follow-up (specified as an eGFR >50 ml/min/1.73m^2), the eGFR ≤40-alert was removed from the pharmacy record.

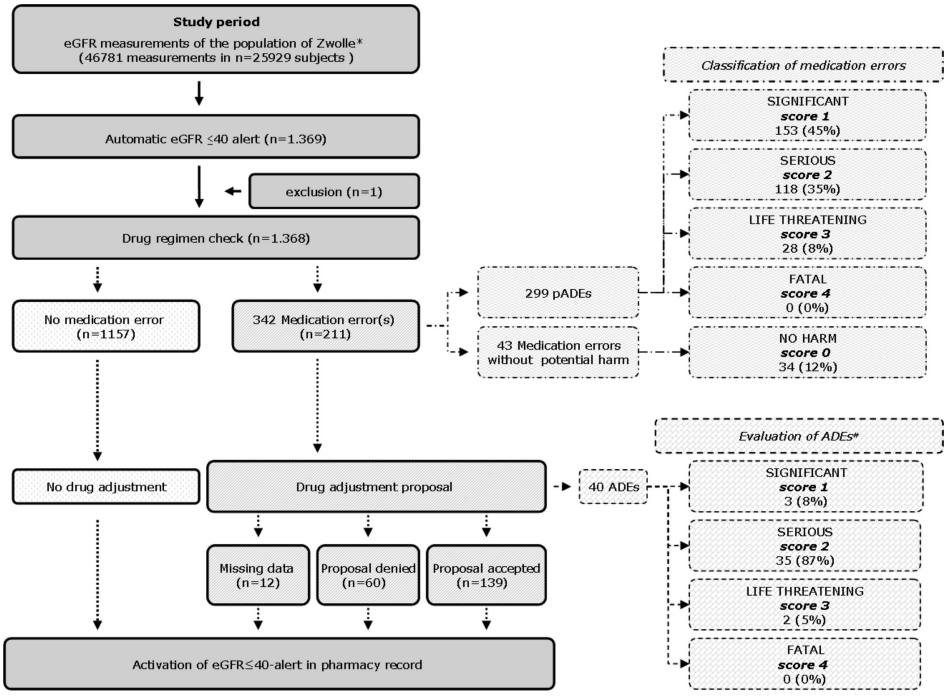


Figure 1 Flow chart summarizing study method and selection of study population. * from both primary and secondary care; # from hospital records *(January 2011)*.
eGFR=estimated glomerular filtration rate (ml/min/1.73m^2); ADE=adverse drug event; pADE=potential adverse drug event.

The study was approved by the local medical ethics committee and conducted in accordance with the guidelines of the Declaration of Helsinki. All pharmacists and GPs informed their patients about the study through flyers, issued both at the pharmacies and at the GP practices. The patient folder and the Isala Clinics website also contained information about the stepwise eGFR ≤40-alert protocol, the sharing of laboratory data, and medication monitoring. The study had an opt-out policy, therefore, subjects who did not wish to participate in this pharmacovigilance study were excluded from the weekly reporting. It should be emphasized that the final decision about making any medication changes after an alert (and informing the patient) was considered to be the responsibility of the prescribing physician.

Definitions and calculations

Serum creatinine was measured with an enzymatic essay (Modular, Roche, Mannheim, Germany) and eGFR was calculated with the enzymatic MDRD formula [27]. The only medications included were those prescribed by health care professionals, and topical or over the counter (OTC) products were excluded. Actual medication use was assessed by documenting all current prescriptions according to the Anatomical Therapeutic Chemical (ATC) classification system [28] at the moment of the first eGFR ≤40-alert. Polypharmacy was defined as the chronic (>1 year) use of 5 or more drugs.

Data collection

For all identified subjects with an eGFR ≤ 40-alert, demographics and medication information were collected. Any medication adjustment recommendations were recorded, which included the patient's medical record number, the pharmacist, the type and daily dose of the medication, and the prescribing physician (GP or clinician). The physician's response to the pharmacist's recommendation was also recorded. Finally, the amount of time the pharmacists spent on every eGFR ≤40-alert was documented.

Classification and tracking of (potential) adverse drug events

To evaluate the impact of eGFR ≤40-alerts two pharmacists (EP and KB) independently evaluated all medication errors on the potential to cause an ADE (defined as a potential ADE (pADE)). They received a database that was anonymized by an investigator not involved in the eGFR-alert processing (HJ). A methodology developed for classification of medication errors and (p)ADEs.[29] They judged and classified the theoretical severity of the medication error, yielding a score of 0-4 (0=drug error without significant harm, 1=potentially significant, 2=potentially serious, 3=potentially life threatening, 4=potentially fatal) (table 2). To reach a consensus, all discrepant ratings were discussed with both pharmacists and two nephrologists (HB and HJ). Examples of pADE

classifications are listed in table 2. The best assessment of the number of ADEs proved to be from the documentation on ADEs in the hospital records.[30] Therefore, one year after the end of the study, the hospital records of all subjects in whom a medication error was detected, were reviewed. This review was performed by two nephrologists (HJ and HB) who independently checked the occurrence of ADEs. ADEs were based on admission and discharge diagnosis in the patients' medical records. The relationship of the ADE with the 'suspected' agent was double checked by evaluating whether the medication regimen at admission in the hospital record matched with the pharmacy record at the date of admission. After review of the hospital records HJ and HB discussed their findings for reaching consensus.

Score	Potential severity	Examples
0	Drug error without potential harm	Not applicable
1	Significant	-Gastro-intestinal complaints -Therapeutically ineffective dose according to eGFR -Mild neurological effects (e.g. motoric dysfunction) -Hepatic dysfunction -Any significant event identified by patient which does not require change in therapy
2	Serious	-Hypoglycemia -Nephrotoxicity or increased risk nephrolithiasis -Electrolyte disturbances (e.g. hyperpotassiemia) -Altered mental status due to sedation -Myopathy or rhabdomyolysis -Gastrointestinal bleed
3	Life threatening	-Lactic acidosis -Cardiac arrhythmia -Decline in mental status with risk of falling -Respiratory failure requiring intubation (e.g. bronchospasms)
4	Fatal	Death

Table 2. Categories of potential adverse drug events according to severity

Data analysis

The main outcome measures were the incidence of eGFR ≤40-alerts, the number and types of medication errors, and the number and types of medication adjustment proposals. Secondary outcome measures were the time required for pharmacists to process the eGFR ≤40-alerts, the adherence of physicians to the proposed adjustments, risk factors for medication errors, and the severity of medication errors. In addition, after one year of follow-up, we checked the incidence of ADEs in subjects in whom a medication error was detected. Statistical analysis was performed with SPSS version

16.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean and standard deviation (SD) when normally distributed. Otherwise, median and interquartile range [IQR] were used. For normally distributed data, the differences in baseline characteristics were evaluated with the independent samples *t*-test. For nonparametric data Mann-Whitney *U* test was used. Differences in distribution were calculated using the chi-square tests.

Results

Incidence of eGFR ≤40-alert and characteristics of the study population

During the study period 46781 creatinine measurements were performed in 25,929 subjects. In 5.3% (n=1369) of cases, an eGFR ≤40-alert was indicated. One patient indicated no willingness to participate for privacy reasons, leaving 1368 subjects for analysis (figure 1). Their characteristics are summarized in table 3. Overall, 56% was female, the median age was 78 [69,84] years (distribution is shown in figure 2) and the median eGFR was 34 [27,38] ml/min/1.73m^2. Overall, polypharmacy was present in 73% (n=993) with a mean number of medications per patient of 7 (range 0-21). An overview of the actual medication use in the study population (which reflects comorbidities) according to the ATC classification is given in *Appendix A*.

Variable	
Number of subjects, n (%)	1368 (100)
Demographics	
Age (years), median [IQR]	78 [69,84]
Male, n (%)	601 (44)
Diabetes, n (%)	346 (25)
Renal variables	
eGFR (ml/min/1.73m^2), median [IQR]	34 [27,38]
Serum creatinine (μmol/ml), median [IQR]	152 [128,186]
Actual drug regimen	
Number of drugs, median [IQR]	7 [4,9]
Polypharmacy, n (%)	993 (73)

Table 3. Characteristics of the study population. IQR=interquartile range; eGFR=estimated glomerular filtration rate.

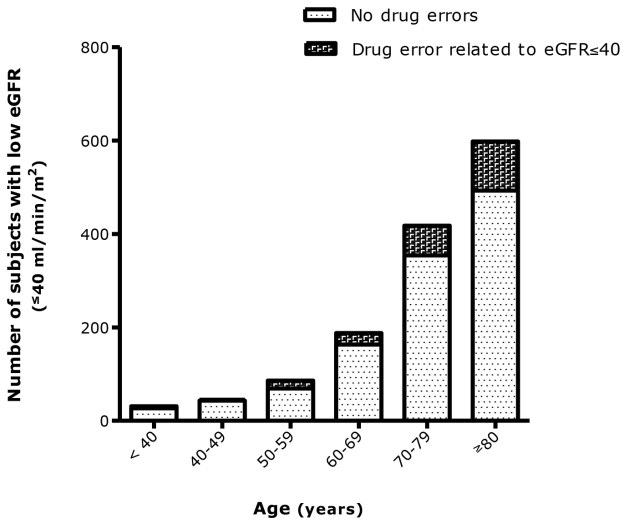


Figure 2 Age distribution of study population and risk of medication error per age category.

Number and type of medication errors

Overall, 342 medication errors were detected in 211 patients with an eGFR ≤40-alert (15% of the study population) (figure 1). The proportion of errors increased with increasing age (figure 2). The types of medication most commonly associated with errors were diuretics (22%), antibiotics (21%), and anti-gout medications (15%) (figure 3).The majority of these medications (77%) were prescribed by GPs. An overview of the type of medication errors that were identified by the pharmacists is given in figure 4.

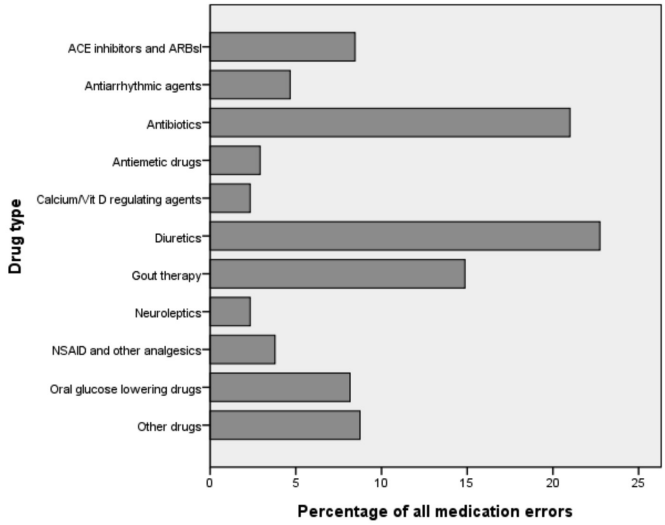


Figure 3 Drug groups associated with medication errors related to renal impairment.

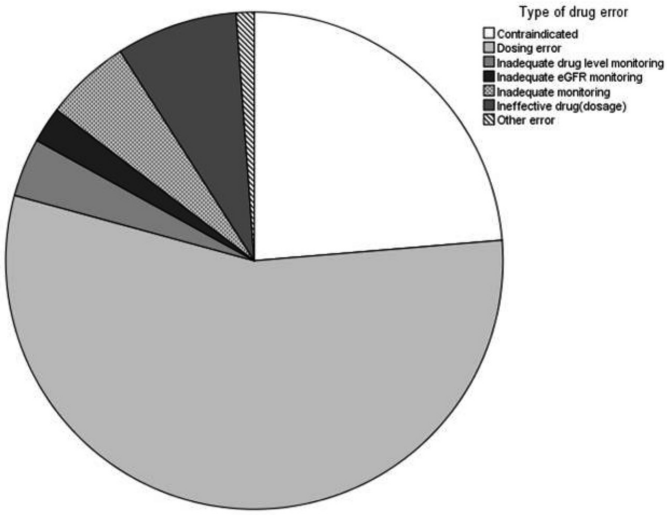


Figure 4 Type of medication errors identified by the pharmacists.

Physicians' compliance with medication adjustment recommendations

Figure 5 gives an overview of the frequency and types of medication adjustment recommendations. The most common recommendations were 'change dosage' (55%), followed by 'stop medication (24%). In 31% (n=105) the proposal concerned a new prescription. Physicians complied with the recommendation in 66% (n=226) of cases. In 28% (n=96) of cases, the pharmacists' advice was rejected and the medication regimen remained unchanged. The main reasons for rejection included already increased alertness with intensive monitoring by the prescribing physician (often being an internist or nephrologist) and an inadequate response to lower dosages in the past. The majority of rejected recommendations included diuretics and renin-angiotensin-aldosterone system (RAAS) blockers like ACE inhibitors and ARB drugs. In some cases, the recovery of renal function was expected or underestimation of renal function was presumed, both of which were generally checked with a 24-hour creatinine clearance. Overall, acutely reduced eGFR did not account for an important subset of the eGFR ≤40 alerts towards the community pharmacists (n=3). Notably, in 22 of the 96 cases, the medication was soon changed anyway, due to a further decrease in the eGFR or the occurrence of an ADE. Therefore, from the latter it seems plausible that with the eGFR ≤40-alert the physician's awareness of the risk for an ADE was triggered. Data on rejection or agreement lacked in 6% (n=20) of cases.

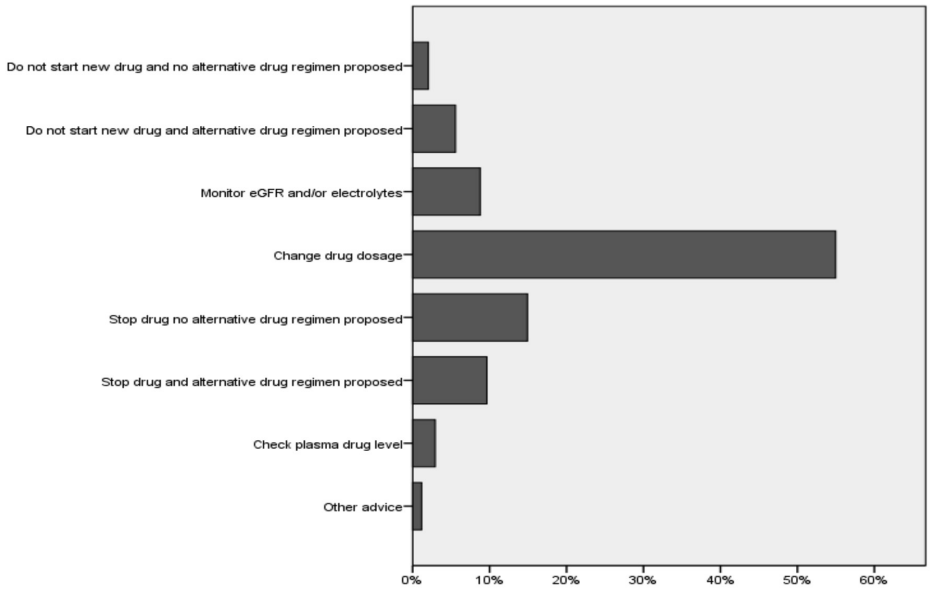


Figure 5 Type and frequency of recommended medication adjustments by community pharmacists.

Potential risk factors for medication errors in patients with eGFR≤40 alerts

Compared with the subjects without medication errors (n=1157), subjects for whom medication adjustments were recommended (n=211) were more often female (59% versus 41%, p=0.04) and had a lower eGFR (median 34 [28,38] versus 29 [2,34] ml/min/1.73m^2, p<0.001, respectively). Notably, the latter had higher rates of polypharmacy (70 versus 89%, p<0.001, mean number of medications 6.6 (3.8) versus 8.2 (3.5), p<0.001).

Effectiveness: potential ADEs and occurrence of ADEs after follow up

Overall, 88% (n=299) of the medication errors were regarded as relevant pADEs (score>0). These were mainly judged to be either significant or serious. An overview of the number and potential severity of pADEs in the study population is given in figure 1. Overall, 40 ADEs were identified in hospital records within one year after the study period in the group of subjects with medication errors, including two life-threatening ADEs (bradycardia due to digoxin intoxication and acute kidney injury with lactic acidosis associated with persistent metformin use). The number and severity of ADEs are shown in figure 1. Importantly, the ADE risk was higher in subjects whose medication regimen remained unchanged (n=60) as compared with subjects whose medication regimen was adjusted as recommended by the pharmacist (n=139); 38% versus 6%, respectively.

Effectiveness: workload and time investment of the pharmacists

After receiving an eGFR ≤40-alert, the pharmacist needed an average of 11 minutes (range 5-13 minutes) to check an individual's medication regimen for errors. When taking into account the time needed for consultation with the prescribing physician, pharmacists required an average of 20 minutes to process one eGFR ≤40-alert triggering a medication adjustment. All pharmacists judged the time investment as feasible, particularly considering the fact that each pharmacy received an average of only one alert per week. Retrospectively we evaluated the feasibility of different thresholds for kidney function alerts by calculating the number of low eGFR-alerts that would have been generated during the study period using different cut-offs for renal impairment (<30, <50 and <60 ml/min/1.73m^2, respectively, see Appendix B).

Overall, 904 eGFR ≤40-alerts were activated in the records of the participating pharmacies at the end of the study period, as 16% (n=214) of the population died and in 250 subjects, the most recent eGFR was at least twice >50 ml/min/1.73m^2. Therefore, on average, every primary care pharmacy had 82 patients with an activated eGFR ≤40-alert. If we translate this to a standard Dutch GP practice (±2300 patients), simple laboratory data sharing identified approximately 23 patients per practice who need drug adjustment(s) or extra alertness in medication management.

Discussion

The main findings of this study were that an eGFR ≤40-alert was indicated in 5.3% of the adult population of a Dutch city in whom a creatinine measurement was performed in an ambulatory setting and that in these subjects 342 medication errors (mainly involving antibiotics and diuretics) were detected during the year following the introduction of an automatic eGFR ≤40-alert system. The majority of the medication errors was regarded as relevant pADEs, necessitating medication adjustments as recommended by pharmacists. Physicians complied in 66% of cases. ADE risk increases with age, polypharmacy, and in instances when the proposed medication adjustments were initially rejected. Overall, automatically generated low eGFR-alerts in primary care seemed effective, easy to implement, and, importantly, improve both the pharmacists' and the physicians' awareness of medication safety.

Comparison with other studies

Despite the fact that medications are usually both prescribed and dispensed in the primary care setting, most studies on (p)ADEs have been hospital-based.[3,9,10] We aimed to study the incidence of (p)ADEs in a shared pharmaceutical care model with a central role for community pharmacists. Three primary health care studies on this topic reported lower

pharmacist drug proposal rates (0.7-1.9%) than the 15% we found.[31-33] These studies were performed in a general population, while we selected a high-risk population of subjects with renal impairment. In line with our results, primary and ambulatory care studies evaluating pharmacists' drug proposals in vulnerable subgroups like the elderly or subjects with cardiovascular risk factors also reported higher rates.[12,17,34,35] Two recent studies, also concerning subjects with renal impairment, identified problems related to inappropriate prescribing in over 20% of patients.[18,36]

Patients with renal impairment are especially vulnerable to medication errors.[12,13,18] Various strategies to improve drug safety in these patients have been studied, such as educational wards rounds, immediate clinician-pharmacist feedback, or dose adjustment according to renal function at hospital discharge.[12,18,37-42] However, despite the fact that most prescribing takes place in the primary health care setting, the majority of the strategies implemented so far have been tailored to hospital settings and are therefore not suitable for primary care. Others have demonstrated the effectiveness of 'computerized physician order entry' and 'clinical decision support' in reducing medication errors in case of renal impairment.[39-41] However, computerized drug prescribing alerts do not always guarantee a reduction of prescribing errors,[43] partly because such alerts are often overridden or ignored by prescribing physicians.[41,44-46] This phenomenon is also reflected in our data, as in 28% of cases pharmacist recommendations were rejected by the prescribing physician.

A central role for community pharmacists in improving medication safety in primary care has been recognized. Many pharmacists are gradually extending their role as integral members of the medical team around the patient, thereby taking an important position in a shared care environment.[21,47,48] This has not only been induced by legislative issues,[21,25] but also recommended in various guidelines and studies to counteract problems associated with multiple medication prescribers.[20,26,32,48] This is important in view of our ageing population in which complex drug therapy will only increase, polypharmacy is common and renal impairment widespread.[49,50] A recent review showed notable differences in ADE prevalence rates by age groups increasing from 5% for adults up to 16% for the elderly.[7] Therefore, in complex cases (as with renal impairment) the close collaboration between community pharmacists and physicians is essential to prevent ADEs.

The alert method we have investigated here could be a simple solution to address this.

Our strategy included three steps to reduce medication errors in patients with renal impairment. First, automatic laboratory alerts were generated, second these alerts were linked to pharmacy data to judge the need for drug adjustments, and third, pharmacists

discussed recommended changes with physicians. Several studies investigated the impact of the above steps. The introduction of automatically generated laboratory alerts had varied effects on the prescribing physician.[41,51,52] Authors suggested that such passive alerts did not have enough of an impact. There is limited data on the effect of extending the alerts so that the community pharmacist was also involved.. Other studies showed that when the pharmacy data were linked with the laboratory renal data, the medication dosage could be beneficially adjusted..[12,42,53] We aimed to optimize medication safety in cases of renal impairment by combining the aforementioned steps and tailored our strategy for application in the primary care setting.

Implications for clinical practice

The estimated prevalence of both moderate (30-59 ml/min/1.73m^2) and severe (15-29 ml/min/1.73m^2) renal insufficiency in the adult American and Dutch population is 4.5% and 5.3% respectively.[26,54] Therefore, the number of subjects potentially susceptible to related medication errors is substantial. If we compile our pADE-rate towards nationwide figures (based on 12,500,000 adults in the Netherlands), our type of data sharing could intercept more than 40,000 potential ADEs related to renal impairment each year. This would undoubtedly increase health care safety with already available data and (hopefully) decrease the costs of ADE related morbidities. Drug safety management might be further improved by extending patient data exchange towards other important parameters, such as medication allergies, platelet counts, electrolyte concentrations, INR, liver enzymes, and plasma drug levels.

Strengths and weaknesses of the study

Some limitations of this study have to be noted. First, our study design does not allow determining individual health care effects, nor overall cost-benefits. This would necessitate a more complex study design as was for example used in the population-based randomized controlled renal drug alert effectiveness trial of Bhardwaja et al., or a 'before and after' design [36]. However, participating GPs and pharmacists indicated that the protocol improved their awareness of medication errors related to renal function impairment. Second, data on the incidence of ADEs before start of the study project were not available in our region; therefore a possible change in ADE incidence as a result of our interventions cannot be determined. Besides, the incidence of ADEs is likely underestimated due to underreporting, missed recognition, and lack of recording in daily clinical practice. Our study also has several strengths. First, our intervention can be easily implemented in various health care settings. We simply extended the availability of laboratory renal data which were not shared formerly. Second, physicians valued the pharmacists' involvement in improving health care delivery. The acceptance percentage

of the pharmacists' was fairly good (67%), as compared with previous studies (24-82%)[17,32,34,37,51] and our prescription ratio between GPs and hospital-based physicians (77:23%) reflects the normal distribution of prescriptions in the Netherlands (82:18%)[55.] However, to improve the overall efficiency of the eGFR-alerts, also variables influencing physicians' (non-) adherence towards pharmacists' recommendations (like type and duration of medication use) should be further studied. Third, the time investment was acceptable and costs were low. Finally, we chose for a safe, but also feasible threshold for renal function alerts. However, as thresholds for dosage adjustment vary between different guidelines, a higher cut-off of ≤50 or 60 ml/min/1.73m^2, or drug specific thresholds could be discussed.[25,26,36,56] Besides, as the Cockcroft-Gault (CG) formula is often used in pharmacokinetic studies and for drug dosing recommendations, the implications of the use of renal function estimates like the MDRD equations for drug dosing, is under debate. Several studies have compared drug dosing recommendations based on the CG with those based on the MDRD[57-59]. In summary, the accuracy of the MDRD seems comparable to the CG [57-59]. Based on these studies, in our opinion, the MDRD is a reasonable alternative to the CG for drug dosing. This is of importance especially since there is an increasing trend of clinical laboratories reporting the MDRD along with serum creatinine, which is also recommended by national and international organizations [26,60].

Some guidelines advise a higher cut-off point for dose adjustments (creatinine clearance 50-60 ml/min),[11,61] but this was expected to result in an amount of alerts exceeding an acceptable workload. Moreover, as the MDRD tends to underestimate true GFR, we presumably already included subjects with true GFR >40 ml/min.[62]

Conclusions and policy implications

The introduction of automatic renal function alerts in the ambulatory care setting, with the involvement of both GPs and community pharmacists, revealed that a considerable part of the population is at risk for ADEs due to impaired renal function. Extending the availability of renal laboratory data to community pharmacists resulted in their presenting the prescribing physicians with a considerable number of medication adjustment recommendations. We feel that nationwide implementation of this simple protocol could potentially identify many pADEs and substantially reduce the risks of iatrogenic damage in persons with decreased renal function.

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SUPPLEMENTAL DATA

ATC CLASSIFICATION		Number (%)
A	**ALIMENTARY TRACT AND METABOLISM**	**1764 (19.1)**
A01	STOMATOLOGICAL PREPARATIONS	4
A02	DRUGS FOR ACID RELATED DISORDERS	556
A03	DRUGS FOR FUNCTIONAL GASTROINTESTINAL DISORDERS	27
A04	ANTIEMETICS AND ANTINAUSEANTS	15
A05	BILE AND LIVER THERAPY	7
A06	LAXATIVES	194
A07	ANTIDIARRHEALS, ANTIINFLAMMATORY/ANTIINFECTIVE	30
A09	DIGESTIVES, INCL. ENZYMES	6
A10	DRUGS USED IN DIABETES	558
A11	VITAMINS	179
A12	MINERAL SUPPLEMENTS	185
A15	APPETITE STIMULANTS	1
A16	OTHER ALIMENTARY TRACT AND METABOLISM PRODUCTS	2
B	**BLOOD AND BLOOD FORMING ORGANS**	**1107 (11.9)**
B01	ANTITHROMBOTIC AGENTS	902
B02	ANTIHEMORRHAGICS	2
B03	ANTIANEMIC PREPARATIONS	203
C	**CARDIOVASCULAR SYSTEM**	**4064 (43.8)**
C01	CARDIAC THERAPY	400
C02	ANTIHYPERTENSIVES	28
C03	DIURETICS	1145
C04	PERIPHERAL VASODILATORS	1
C07	BETA BLOCKING AGENTS	767
C08	CALCIUM CHANNEL BLOCKERS	316
C09	AGENTS ACTING ON THE RENIN-ANGIOTENSIN SYSTEM	830
C10	LIPID MODIFYING AGENTS	577
D	**DERMATOLOGICALS**	**3 (0.03)**
G	**GENITO URINARY SYSTEM AND SEX HORMONES**	**147 (1.6)**
H	**SYSTEMIC HORMONAL PREPARATIONS**	**254 (2.8)**
J	**ANTIINFECTIVES FOR SYSTEMIC USE**	**165 (1.9)**
L	**ANTINEOPLASTIC AND IMMUNOMODULATING AGENTS**	**100 (1.0)**
M	**MUSCULO-SKELETAL SYSTEM**	**312 (3.4)**
N	**NERVOUS SYSTEM**	**846 (9.2)**
P	**ANTIPARASITIC PRODUCTS, INSECTICIDES,REPELLENTS**	**6 (0.06)**
R	**RESPIRATORY SYSTEM**	**417 (4.6)**
S	**SENSORY ORGANS**	**6 (0.06)**
V	**VARIOUS**	**36 (0.5)**
OVERALL		**9227 (100)**

Appendix A. Overview of drug use according to the Anatomic Therapeutical Chemical (ATC) classification in the study

eGFR threshold (ml/min/1.73m^2	Number of patients	Change in workload (%)
<30	647	-47%
<40 (current study design)	1369	*reference*
<50	2696	*+196%*
<60	5041	*+368%*

Appendix B The calculated number of patients in the study setting and the change in amount of patients (workload) when applying different thresholds for eGFR-alerts.

Appendix C. Technical details of automatic laboratory alerts.

In the management database system of our laboratory the relationship and indexes of different types of data are embedded. We defined a query in this database to select our study population. This query included: test code (eGFR), ambulatory laboratory requests (excluding clinical eGFR data), and data were filtered on age ≥18, eGFR ≤45 and zip codes of the city of Zwolle. The query was run periodically (weekly). A module matching the patients' unique Citizens Service Number (CSN) with the patient's pharmacy code was developed for this project, which enabled us to address the eGFR-alerts to the right community pharmacy. The fact that in The Netherlands patients are generally registered at one single community pharmacy (en thus have a one personal pharmacy code) facilitates this method.

Chapter 9

Discussion, recommendations and future perspectives

The studies in this thesis were aimed at assessing the degree of (un)reliability of the formulas that are most frequently used for estimating the glomerular filtration rate (GFR). Differences between formulas were evaluated, and the influence of variations in creatinine assays on the outcomes of these formulas as well as the creatinine clearance (CCR) were studied. Furthermore, the aim was to study the effect of using different formulas on clinical decision making and referral policy. Finally a new medication alert system warning to prevent medication errors in patients with CKD was evaluated.

The introduction and consequences of the use of formulas to estimate renal function

At first formulas estimating renal function were developed to reflect CCR, the best used of which was and is the Cockcroft Gault formula. When methods were developed to specifically and reliably measure the glomerular filtration rates (GFR), formulas were developed to estimate GFR as well. The role of both types of formula (estimating CCR and estimating GFR) in clinical decision making is still under discussion.

One also has to consider the fact that the definition of renal function varies. When basing the conclusion of renal function only on serum creatinine, pitfalls are manifold. A 24-year old male bodybuilder and a frail old lady will have completely different renal functions, even when their serum creatinine will be the same, e.g. 100 µmol/L.

When assessing renal function by measuring creatinine both in serum and in urine, one has to realize that creatinine is excreted both in the glomeruli and in the tubuli (and a small amount is lost in the gut), with a rather wide variation in tubular excretion (estimated at 10 to 40% of the amount excreted through the glomeruli).

When measuring GFR with tracer elements, results are translated towards a standardized body surface area of 1.73m^2, thus not representing the true GFR of the tested individual. This routine recalculation quite often results in a lower GFR outcome, since adults tend to have a higher BSA than 1.73m^2.

These differences have consequences, which add to the unreliability of the estimate formulas. When looking at renally cleared medication and indications and dosages adjusted according to renal function, there are quite some definitions of decreased renal function. In former times, cut-off points were formulated in relation to serum creatinine, irrespective of the technique of creatinine measurements. In more recent years, eGFR has predominantly been used. since formal studies assessing circulating medication levels with varying dosages at different renal functions are relatively scarce, these estimates are more or less the best we have at the moment.

Attention points

Despite the ambition to see estimates as being reliable enough to reflect renal function, there still remain doubts [1]. A major issue is that these formulas were often derived from information gathered in highly selected patient cohorts and nonetheless are applied in heterogeneous patient populations, quite often as a screening tool to gain an impression of the actual renal function, since serum creatinine as a separate entity knows its pitfalls as sole assessment of renal function.

Over time, different formulas to estimate GFR have been developed in different populations, the most important being: the modification of diet in renal disease formula (MDRD) and the chronic kidney disease epidemiology collaboration equation (CKD-EPI) [2-3]. The remodeling of especially the MDRD formula by its creators indicates, that these formulas apparently do perform reasonably well in a grid scale and for the total group, but are much less precise in estimating the GFR in an individual [4-5].

Facts and fallacies in relation with a decreased renal function

The need to know a more exact renal function also in screening circumstances is driven by various reasons, explanations, and assumptions. In general, a decreased renal function, and especially renal function loss combined with proven renal damage, is associated by many with an increased risk of cardiovascular (CV) disease and end stage renal disease [6-8]. Furthermore, many medications are cleared through the kidney, and being unaware of the degree of renal function decrease may result in potentially major or even lethal side effects through overdosing [9-10].

As for the first point regarding the association with adverse CV and renal outcome, literature shows a definitely mixed picture. In younger persons with overt renal disease and renal function loss, this seems to hold true. Whether applying the same rules to elderly people with a decreased renal function but no signs of renal disease, is very doubtful [11-13]. Other means to assess, assume or suspect renal disease are needed besides the information on renal function per se, whether measured by proven techniques or by estimation formulas.

As for the second point (medication use), renal function assessment definitely is of major importance; especially in the elderly, as for drug dosing. It does not matter whether the impaired renal function is associated with decreased function with age or with renal damage by disease: countering medication dosing mistakes remains the predominant issue.

When putting into a model the separate variables of the MDRD-4 formula, the combination of the separate variables actually leads to a better estimation of (cardiovascular) mortality, than the formula itself [14]. Those factors are part of the clinical assessment, which is performed by every well thinking health care professional

with an interest in nephrology: knowing age and sex, measuring serum creatinine, so one might question the need to use an estimation formula for other purposes than estimating renal function.
Therefore, with all the second thoughts regarding the use of estimation formulas, the eGFR should always be seen as an alert signal, and an abnormal outcome should be seen in the light of the clinical observations with possible implications for the person, for whom the formula is used. One always needs to know whether there are (other) signs of renal damage, whether renal function is stable or not, and what the implications are, when a certain GFR estimate is used for clinical decision making. As an example: when in subjects with diabetes metformin is used as an oral glucose lowering drug and the eGFR falls below 30 ml/min/1.73m^2, metformin will have to be discontinued as primary treatment according to current guidelines; this in turn might result to the need to start insulin, a major change in treatment with many practical consequences. Under such circumstances it would be worthwhile to actually measure a CCR in the old-fashioned way, and to use this information not only as leading in decision making regarding medication doses, but also as a reference when assessing renal function thereafter. When at a certain time point eGFR shows an outcome of 34 ml/min/1.73m^2, but a CCR performed at the same time results in an outcome of 55 ml/min, this allows for the mental calculation of a correction factor, at least as long as renal function does not change too dramatically in time.

Renal function prediction equations

In **chapter 2 and 3** of this thesis, the implications of using different eGFR equations on CKD staging were evaluated in a population of patients with diabetes mellitus. **Chapter 2** focused on the differences in performance of the MDRD and the CKD-EPI formula, as well as the consequences of their use on chronic kidney disease staging. Both the MDRD formula and the CKD-EPI formula were found to have a substantial bias and imprecision compared with the measured 24 hr CCR. The two scatterplots (see figures 1a and 1b) illustrate the substantial variability between an eGFR calculated with a formula and CCR, both in patients with renal impairment as well as in patients with a 'normal' renal function. Unfortunately, due to the absence of mortality data in the cohort studied in chapter 2, it is unclear whether the patients who were down-/upstaged when using the CKD-EPI also had a higher or lower mortality risk, respectively. A large meta-analysis of data from general population cohorts showed that the CKD-EPI equation categorized the risk for mortality more accurate than the MDRD equation [15]. However, we should remain reticent to embrace the CKD-EPI only because it produces better risk profiles, since some inherent limitations of the MDRD remain essentially unchanged in the CKD-EPI [16].

The fact that the CCR was used in chapter 2 makes the interpretation of these data complex. As already mentioned, the CCR is not a true reflection of GFR and measures both the glomerular function as well as the tubular secretion of creatinine, leading to an overestimate of 10-40% compared with the inulin clearance. Of course, the fact that the absolute difference between the measured GFR and the CCR in the population studied was unknown, complicated the interpretation of bias and accuracy. Although no firm conclusion can be drawn based on the results of chapter 2, it does raise questions about the role of the eGFR as a screening tool in clinical practice. The bias and imprecision of the MDRD has also been observed in studies that did have a measured GFR (mGFR). The accuracy of the MDRD in this study deteriorated with declining mGFR and thus led to misclassifications of chronic kidney disease. This makes the eGFR a tool with multiple limitations for diagnosing CKD in individuals [4].

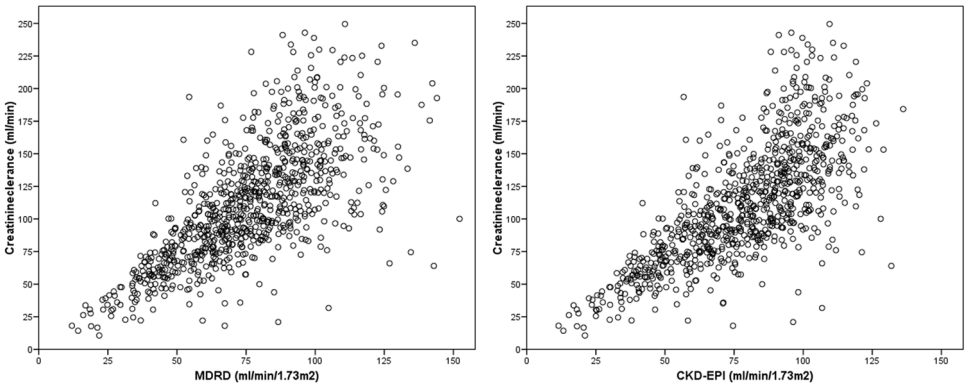

Figure 1a. shows the correlation between the CCR (ml/min) and the CKD-EPI (ml/min/1.73m^2).
Figure 1b. shows the correlation between the CCR (ml/min) and the MDRD (ml/min/1.73m^2).

Chapter 3 more specifically evaluated the impact of differences in body weight. Worldwide, the prevalence of obesity as well as the complications associated with obesity, such as diabetes, hypertension and renal function loss, are increasing [17-18]. Also in patients with overweight and obesity, the use of renal function prediction equations led to interpretation difficulties due to bias [19]. When compared with the CCR as a reference value, the CG turned out to be the best predictor of renal function compared with the MDRD and the CKD-EPI formulas. Our findings are in contrast with the conclusions of a large French study that evaluated the influence of body size on the predictive ability of eGFR equations. Froissart et al showed that the MDRD formula was less biased, more precise and more accurate than the CG formula in all patients [20]. Our and Froissart's study differed in some important aspects: we have used CCR as reference method whereas Froissart used a golden standard technique for the assessment

of GFR. Moreover, in our study the MDRD and CKD-EPI formulas calculated eGFR in ml/min/1.73m^2, whereas CG and CCR were not BSA-corrected. In contrast, the French investigators have used BSA corrected values for all equations and the actual GFR measurement. We must acknowledge that our analysis was biased in favor of the CG formula. Indeed, when we adjusted the data of our patient cohort to the actual BSA of the participants, the performance of both the MDRD equation and the CKD-EPI equation improved. However, we question the use of an indexed CG, since weight is one of the clinical variables included in the CG equation. A correction for BSA will thus result in a double adjustment for weight. Such a double adjustment may influence the performance of the equation in this study.

An important point to be stressed when using formulas to estimate renal function in a population with overweight is the correction for a standard BSA of 1.73m^2, whereas the average Dutch adult has a body surface of approximately 2.1 m^2. By definition, this means that in general an eGFR outcome will be lower than the actual renal function in the average adult. The correction for a standard BSA of 1.73m^2 occurs both in the MDRD and the CKD-EPI, in spite of the fact that body mass indices (and thus the BSA) are increasing in most populations during the last decades [21]. This also implies for example, that with a comparatively large BSA and calculating medication dosages based on GFR estimate formulas corrected for 1.73m^2, medication dose might be too low to be sufficiently effective in an individual. Therefore, from a clinical point of view, correcting eGFR for actual BSA could help to achieve more adequate medication dosages in quite a few patients.

It is still unclear which estimate is best to approach the real glomerular function in overweight and obese patients. All current formulas have substantial bias in this population. Future research should be performed to evaluate which technique to estimate renal function gives the best prediction regarding mortality, morbidity or progressive renal function loss.

So far, all formulas to estimate renal function have their specific limitations when estimating renal function. The fact that the reliability of renal function estimates in populations with different characteristics from the original study population poses restrictions with regard to their general applicability, was already acknowledged in the Kidney Disease Outcomes Quality Initiative guidelines [22]. This has also been stressed in a cohort of patients with DM. This cross-sectional study of patients with normo- or microalbuminuria who had their renal function measured annually during 8 years by means of a iohexol plasma clearance showed that both the MDRD and the CKD-EPI did neither provide reliable estimates of GFR at baseline nor of the changes over time (e.g. short-term reductions when implementing blood glucose lowering therapy as well

as antihypertensive therapy, and the deterioration of GFR over time associated with renal disease progression) [5].
Although the CKD-EPI was developed to overcome the problem of imprecision and systematic underestimation of the MDRD in patients with normal to high normal serum creatinine (SCr) levels, our study indicated that the MDRD and CKD-EPI formulas proved to be equally imprecise in estimating the GFR in patients with diabetes or obesity, a fact which has also been stressed in literature previously [5,23-25]. Therefore, changing from one equation to the other will not result in a clinically relevant improvement of the reliability of the estimate for an individual subject.

Evaluating the results of the performance of renal function prediction equations in this thesis as well the plethora of literature discussing this subject, one may conclude that it is and will be very hard to create a renal function prediction equation that functions accurately, and – thus – reliably, in any patient population with all differences in race, sex, age, posture, and renal function.

Kidney function and ageing

Although there are still many uncertainties regarding the use of renal function prediction equations in clinical practice, most clinical laboratories report an eGFR every time SCr is measured. Often these reported eGFR values are seen as reliable outcome measures to be accepted at face value, although they should rather be interpreted as a signal for further thought and - when indicated - action. The obligation of clinical chemistry laboratories to report an eGFR every time SCr is assessed, may be interpreted as a disguised screening tool for CKD [1,26]. Although the ambition to identify renal function loss in an early stage is valuable, a substantial number of patients will be erroneously classified as having a disease, needing additional diagnostics. In the end, an early identification of a disease is only meaningful when adequate treatment or early intervention elicited by this early identification results in lower morbidity and mortality rates or a reduced risk for the development of ESRD or other complications related to renal function loss [1].
In this respect, an important group of patients to be mentioned is the large group of elderly individuals, since a substantial part of individuals with stage 3 CKD (eGFR 30-60 ml/min/1.73m^2) is older than 65 years [27]. It is subject to debate whether this population is at increased risk to progress to a higher CKD stage, in the absence of other features of kidney damage (especially the presence of albuminuria). As is shown in this thesis in **chapter 6**, the oldest elderly population (>75 years) with type 2 diabetes mellitus seems to be one of the target populations that will be erroneously classified as having a kidney disease when eGFR is used as the only indicator for this classification, because

the moderate reductions in eGFR observed in that population were not associated with an increased (cardiovascular) mortality risk, this in contrast to the population aged 65-75 years. In contrast, albuminuria irrespective of eGFR proved to be an independent marker of mortality in both the group aged 65-75 years as well as those older than 75 years.

Studies analyzing mortality risk of CKD according to a measured GFR level do not exist. Using an eGFR may be considered as a limitation since creatinine based equations lack precision around the value of 60 ml/min/1.73m^2. However, the aforementioned attenuation of the association between mortality and eGFR in elderly that was found in this thesis confirms the findings of previous studies [11-12,28-30]. Also, data as presented by the Chronic Kidney Disease Consortium, including more than 2 million participants from 46 different cohorts (both general and high-risk cohorts) with a follow-up time of 5.8 years, show the same attenuation of the association. In this cohort 7.3% was older than 75 years. There was a significant age interaction; mortality risk always started to increase in the eGFR range 60-75 ml/min/1.73m^2. However, in those aged 75 years or older it reached statistical significance at an eGFR of 56 ml/min/1.73m^2 or lower (HR 1.35 [95% CI 1.23-1.48]) [11]. Although significance is reached, the relevance is questionable; isn't it biological age we are looking at and isn't it time to start using age related cut-off values (such as in the LTA) in international guidelines or even better gender as well as age-related thresholds?

Although causality cannot be proven from the observational study such as performed in this thesis, the results of this study do raise questions about the need for referral to specialist nephrological care of the (oldest) elderly patient population with a modest decrease in renal function. Prospective studies are needed in order to be able to make valid recommendations both for valid cut-off points, for additional discriminating diagnostics, and – in case of true and clinically significant renal function loss - appropriate treatment in elderly patients.

The ageing kidney and referral mechanisms in elderly

Another argument supporting other referral criteria for the elderly population is the biological variability in GFR [21,31-33]. As was observed in the Nijmegen Biomedical Study, eGFR decreases with age [31,33]. Also in a study of Poggio et al, evaluating GFR reference values, it was shown in a population of 1057 living kidney donors whose renal function was measured using ^{125}I-Iothalamate, that the 5th percentile at 60 years was 64 and 60 ml/min/1.73m^2 for men and women respectively. GFR decreased 8 ml/min/1.73m^2 per 10 years. From this results one may expect the value of the 5th percentile in healthy subjects older than 70 years to be lower than 60 ml/min/1.73m^2 [21,34].

Ignoring this age-related decrease, without taking into account other markers of kidney damage (such as albuminuria) will lead to an astronomically high number of patients that would need to be referred to a nephrologist based on an eGFR value <60 ml/min/1.73m^2 (as suggested in the K/DOQI guidelines), as is demonstrated in this thesis in **chapter 7**. In an observational cross-sectional study of 82424 patients, the impact of the introduction of age-related cut-off values for estimated glomerular filtration rate (eGFR) on consultation and referral patterns from primary care to nephrologists were analyzed. 19% (n=15637) of this population had an eGFR <60 ml/min/1.73m^2. If age related cut-off values would have been used, 3303 patients would have been referred, and for 5748 patients the nephrologist would have been consulted. For the majority of patients (55%) follow-up could take place in primary care. Using age-related thresholds, as is advocated in the LTA guidelines, will lead to more relevant and focused referrals from primary to secondary care, resulting in the referral of only the selection of patients with complex renal problems or those that need nephrological care in a predialysis phase. Of course, using the MDRD formula in chapter 7, which has not been validated in an elderly population, this may have introduced some misclassifications. However, also in clinical practice decisions are frequently made based on this eGFR in the elderly population. The fact that the Dutch national transmural agreement (LTA) for 'Chronic renal impairment' applies age-related cut-off values, is a first step in a more differentiated approach of the elderly patient with eGFR loss [35]. An additional step in the approach of the elderly patient could be the addition of albuminuria as a risk stratification marker in classification schemes, in order to identify those elderly patients with a reduced eGFR having clinically relevant CKD (see also the addendum). After all, the impact of proteinuria on cardiovascular morbidity and mortality risk has been largely confirmed by data from previous trials and could therefore be a good differentiator for risk stratification in the rather large group of patients with moderate reductions of eGFR [6,8,29,36].

Creatinine

Creatinine is by far the most frequently used measure to assess renal function. However, there are many limitations related to using creatinine as a marker of renal function: variations in the production, variations in the (tubular) secretion, the variability between the techniques to measure creatinine, as well as the presence of exogenous creatinine sources (see table introduction) [37-38]. All these variables may have an effect on serum creatinine and this should be accounted for when using formulas to estimate renal function.

The method of creatinine measurement is rather important, since its accuracy to reflect true creatinine concentrations will have impact on the accuracy of all renal function estimating equations. Rather small differences in creatinine measurements have substantial influence on the estimated glomerular filtration rate (eGFR) [39-42]. Since significant interlaboratory variations in creatinine measurements were observed worldwide, it was internationally confirmed that calibration traceability to higher-order reference methods was needed to realize comparable biochemical measurement results. Since the development of NIST SRM 967 in 2006, in vitro diagnostic manufacturers of creatinine assays in Europe are legally obliged to make their products traceable, regardless of the method applied [43-47].

Improving the accuracy of SCr measurements (both Jaffe and enzymatic) as included in the various equations estimating the GFR should be seen as imperative in order to diminish at least the impact of the variability of this factor, when striving to improve the reliability of the formulas to estimate GFR. However, a study from 2008 showed that despite the European IVD directive, an unacceptable inter-laboratory variation was observed, which was mainly due to calibration differences. Especially the between laboratory variation of Jaffe-based methods did not decrease, notwithstanding the interventions made [44].

In **chapter 4** of this thesis the implications of the variability in SCr measurements (using a Jaffe or an enzymatic technique) between laboratories after this global restandardization was evaluated in a theoretical model as well as the impact thereof on the MDRD and CKD staging in the Netherlands. It was reconfirmed that especially the Jaffe method is inaccurate with a high non-specificity bias, which most probably occurs as a result of analytical interferences of field methods by plasma proteins. Although the enzymatic technique to measure serum creatinine has interference with various substances as well (see table introduction), the specificity remains better than the specificity of the Jaffe method [48]. This finding is supported by data from a multicentre study evaluating IDMS-traceable enzymatic creatinine assays [49]. The high non-specificity of especially the Jaffe method results in inaccurate estimates of the GFR when using equations, with the most important clinically relevant impact when patients are inaccurately downgraded to a more severe CKD category. Applying an enzymatic technique on the other hand, more often resulted in change to a less severe CKD stage, which is less relevant in routine clinical practice than downgrading to a more severe CKD stage, although undeserved staging to a less severe CKD stage also may have consequences.

It has also been shown in other studies that due to plasma pseudo-creatinine chromogens true SCr concentrations are overestimated, even when an eventual calibration error is corrected by alignment to IDMS [50]. Especially in the low ranges of SCr, Jaffe assays are

inaccurate and less precise than enzymatic assays. Enzymatic techniques measure SCr with substantially less variability than Jaffe techniques as compared with IDMS reference values. This leads to more reliable estimation of GFR and CKD staging. Therefore, it can only be concluded that to improve reliability of renal function estimates, using the Jaffe assays to measure creatinine in clinical practice should be discarded and replaced by the enzymatic technique.
In **chapter 5** the variability in creatinine measurements using a Jaffe, an enzymatic and an LCMS assay in urine are evaluated, as well as the implications thereof on the CCR. Quite remarkably, an unexpected and inexplicable tendency of the Jaffe assay towards lower results in urinary measurement was found. Although the enzymatic assay showed a more consistent pattern compared with the Jaffe method, the reliability of the urinary measurements appeared not optimal with the use of an enzymatic technique as well. Since the CCR has an important role when other renal function estimates either show a questionable result or need confirmation, in pre-dialysis, to assess the adequacy of hemo- and peritoneal dialysis, and to examine the urinary excretion of biomarkers to correct for sampling errors. These differences in urinary measurements are bothersome. Further research should take place to explain these differences; furthermore, redefining the normal and abnormal ranges for CCR could be the consequence of the presented findings.

Cystatin C
Since creatinine based equations suffer from the limitations associated with using serum creatinine as a marker of renal function, an alternative biomarker cystatin C has been introduced. Cystatin C is less sensitive for differences in muscle mass or diet. Formulas estimating GFR based on cystatin C have shown variable results regarding their accuracy in GFR estimation in different populations [51-54]. Since a standardized calibration has become available for cystatin C, a new GFR prediction equation has been introduced, combining serum creatinine and cystatin C: the CKD-EPI mix [54-57]. Whether measurement of cystatin C levels and incorporating this information in the eGFR formula will improve patient care is still unknown, and not studied for this thesis. Further studies in more specific patient groups have to follow in order to determine the position of this new biomarker.

Medication safety

A theme inextricably related to renal function is medication safety. Renal function loss often alters the pharmacokinetics of medication and therefore makes patients prone for renal damage due to too high concentrations of some drugs when dose adjustments are

inadequate, and more vulnerable to adverse drug events [9-10,58-59]. The results presented in **chapter 8**, showed that the use of GFR estimates in clinical practice may be used as a tool to select high-risk populations in order to optimize patient safety. Moreover, it illustrates that the number of subjects at increased risk for adverse drug events (ADEs) due to renal impairment is substantial [60]. The elderly population, whose renal function is often impaired and whose constituents often are on 5 or more different medications (polypharmacy), especially benefited from this intervention. By means of a relatively simple intervention, the issuance of eGFR alerts, community pharmacists were able to provide valuable medication adjustment recommendations to prescribing physicians. The implementation of this simple protocol nationwide in the Netherlands might possibly lead to the early recognition of more than 40,000 potential ADEs because of renal function loss annually. This probably will lead to a (cost) reduction of morbidities caused by ADE as well as increase health care safety.
In our ageing population in which polypharmacy and renal impairment are common, there are strong arguments to promote more effective use of patients records such as renal function data between health care workers (general practitioners, nephrologists and pharmacists) in order to allow monitoring of safe drug therapy [22,61-64]. This is also recommended in different guidelines and studies to counteract problems created by multiple drug prescribers. As GP's are key players in drug therapy (accounting for over 80% of drug prescriptions in the Netherlands), links on information with regard to renal function between community pharmacists and GPs supported by proper and accorded protocols are essential to reduce or even prevent drug errors.

One must conclude that despite the efforts over the last 15 year, the accuracy of the eGFR formulas for the individual remains an issue. It is doubtful that a more precise formula will be developed soon, at least when based on easily available personalized information.
The outcome of the formula calculation should therefore be considered an indicator, not as a true value. An abnormal value (maybe sometimes even a normal value) should only be seen as the starting point for further clinical thinking and assessment, and not as an infallible guide. Especially in elderly people, moderate renal function decrease without signs of renal damage and within the range of their age, should be seen as part and parcel of their life and age, and conveys to us a completely different message than in younger people. Insufficiently treating possible risk factors for CV disease and progression of renal damage is one thing, but treating a substantial part of the general population undeservedly just because of an estimate not interpreted within a well defined context is another. "Prevent or slow down harm and disease" has always to be weighed against "do no harm and do not promote unnecessary anxiety".

Conclusions

- To date, all measures that are available to estimate renal function in daily practice are extremely inaccurate.
- The CKD-EPI formula that was developed to overcome the problem of imprecision and underestimation of GFR in patients with normal to high SCr levels by the MDRD, was shown to be as imprecise as the MDRD in estimating renal function in a cohort of patients with diabetes mellitus and a preserved renal function.
- In an overweight and obese population, the CG formula surprisingly seemed to be the best predictor of renal function when compared with the MDRD and the CKD-EPI. When making clinical decisions, limitations of renal function prediction equations should always be kept in mind.
- An eGFR of 45-60 ml/min/1.73m^2 in patients with diabetes mellitus type 2 is associated with increased mortality in patients aged 65-75 years, but not in those >75 years. Albuminuria is associated with increased mortality in all patients >65 years. Mortality risk related to renal function thus varies with age. Therefore, the age related cut-off values as applied in the Dutch 'Landelijke Transmurale afspraak chronische nierschade', will aid to prevent unnecessary referral to secondary care, especially of elderly patients with moderate renal function loss. Moreover, we suggest albuminuria to be added as a marker of renal damage in order to identify elderly people with relevant renal function loss and – thus - CKD.
- Accurate and precise measurements of SCr are required to improve the reliability of the eGFR formulas as support for reliable clinical decision making. Enzymatic techniques measure SCr with substantially less variability than Jaffe techniques as compared with IDMS reference values. This leads to less unreliable estimation of GFR and CKD staging. To allow improvement of reliability of eGFR, specific enzymatic techniques to measure SCr are preferable over unspecific Jaffe techniques.
- Both the Jaffe and the enzymatic assay provide biased results for urine creatinine, leading to an underestimation of the CCR. More research should be focused trying to explain these differences in urinary measurements before advocating changes in ranges.
- Extending the availability of renal function data towards community pharmacists can be an effective intervention to improve drug safety. An intensified collaboration between community pharmacists and prescribing physicians resulted in a considerable amount of drug adjustments aiming to maximize therapeutic efficacy and minimize the risk of adverse events in ambulant patients with renal function impairment.

Recommendations and future perspectives

In this thesis the pitfalls of using creatinine based renal function estimating equations are sketched. Based on the conclusions of this thesis the following recommendations can be made:

1. It should be severely questioned whether serum creatinine-based equations estimating glomerular function should be used as the only tool to identify and monitor progression of renal function (loss), especially in patients at risk for renal function loss, such as patients with diabetes mellitus. A better performing and more reliable formula is needed in this population. Whether cystatin-based equations could be used, should be further investigated in future research. Until that time one should be very well aware that the GFR is *estimated* by these formulas, and – thus – an abnormal result should be the trigger for further analysis, not be considered a reliable outcome to be accepted at face value.

   In the meantime we should consider whether we should not stop estimating the GFR by means of formulas and start measuring GFR by appropriate techniques such as iohexol or look for other exogenous biomarkers of glomerular filtration that are purely filtered by the glomerulus, at least in those subjects suspected of a decrease in renal function or progressive renal function loss.

2. Once a decreased renal function is found, the outcomes should be interpreted in the light of the personal characteristics and diet of the patient and one should keep in mind the many flaws of these formulas. A 24-hour CCR should be considered when an abnormal eGFR is found.

3. Especially in patients with over- or underweight, but also in non-obese populations, indexation of GFR for BSA may induce relevant differences. When the (estimated) GFR is used for clinical decision making (drug dosing etc.) as well as in the follow-up of renal function, a measured GFR would be preferable in these patient categories. Future research should occur in a large cohort of healthy adults with a wide range of body sizes in order to determine whether BSA indexation should be abandoned or can be maintained.

4. Accurate and precise measurements of creatinine are required for a reliable estimation of GFR as support for clinical decision making. Enzymatic techniques measure serum creatinine with substantially less variability than Jaffe techniques as compared with ID-MS reference values. Therefore, laboratories in

the Netherlands (and preferably worldwide) should abandon the Jaffe assay to measure creatinine. Creatinine measurements in urine remain a point of concern. Before proposing a redefinition of the normal ranges regarding the CCR, more research should be done to explain the differences in urinary measurements.

5. As long as it is unclear when renal function loss in elderly patients is significant, every time a moderately decreased renal function is found in patients >65 years, we should evaluate whether there is a stable moderately decreased renal function or progressive loss of renal function, by comparing them to eGFR values of peers of the same age and sex. Furthermore, other indices pointing towards possible damage should be considered, such as the presence of persistent (micro) albuminuria.

6. The differentiation of the LTA in age related cut-off values has been a first step to prevent excessive referral rates of elderly people with moderate renal function loss to a nephrologist. Even better would be a situation in which laboratories present every time a serum creatinine is measured and an eGFR is calculated in patients >65 year, the median eGFR value (with 5^{th} and 95^{th} percentile) based on the nomograms like those of the Nijmegen biomedical study. Further research should focus on a method to better identify patients who are at greatest risk of CKD that without intervention would progress to end stage / symptomatic renal disease.

7. Increased collaboration with community pharmacists improves health care safety and awareness of medication errors related to renal function impairment in primary care, thereby substantially reducing iatrogenic complications in subjects with impaired renal function. Extending the availability of laboratory renal data which were not formerly shared is relatively straightforward with minimal expense. Therefore, the nationwide implementation of this relative simple protocol can be recommended.

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Chapter 10

Summary

A clear definition and classification system, to raise awareness for chronic kidney disease (CKD) and to recognize CKD adequately worldwide, was developed in 2002; the so-called Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines. The (estimated) glomerular filtration rate ((e)GFR) and the presence of albuminuria / proteinuria became the two most important components of this classification scheme. Care pathways for patients with various CKD stages have been developed. Since these pathways also involve assessment and (sometimes major) treatment decisions, a proper estimation of GFR is pivotal for good patient care.

Currently, various equations estimating the GFR are used. Before the introduction of these equations, the serum creatinine concentration (SCr) was used as an estimate of renal function in clinical practice. However, a major drawback of using SCr as an estimate of the GFR, is the hyperbolic relation between SCr and GFR. In research settings the inulin clearance (which is considered to be the gold standard) and other isotopic methods are frequently used to measure the GFR. However, these methods are cumbersome and costly, and therefore not applicable in daily practice. Less costly and complex is the 24-hour creatinine clearance (CCR), which for its accuracy is dependent on the accuracy of the 24-hour urine collection. In principle, CCR will be higher than GFR, since, in general, creatinine will also be secreted at a tubular level besides the glomerular loss.

As already mentioned before, several renal function prediction equations have been developed over time: the Cockcroft-Gault equation (CG; 1976), and the more recently developed Modification of Diet in Renal Disease equation (MDRD; 1999), the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI; 2009). All these GFR prediction equations contain SCr as a variable. Therefore, using accurate SCr measurements is essential, since systematic errors cause unreliable renal function estimates leading to incorrect drug dose adjustments, misclassifications in CKD staging and incomparability of patient data.

Apart from the variability in creatinine measurements, there are still many open ends when using eGFR estimating equations in daily (clinical) practice; the use of the equations may give biased results when used in populations that differ from the population these equations originally were developed in. Moreover, we need more insight in the differences between the various equations, the use of threshold values of eGFR in relation to age, the implications of variations in creatinine assays on the predictions equations, and the role of eGFR on pharmacovigilance.

This thesis focused on the different aspects of the various equations estimating renal function. First: the performance of the two currently most frequently used equations estimating the glomerular filtration rate (GFR) and the impact thereof on chronic kidney disease (CKD) staging, second: the performance of these equations in an overweight and

obese population, third: the influence of using different techniques to measure creatinine on renal function estimating equations, fourth: the variability between creatinine assays in urine and the influence hereof when calculating the CCR, the association between renal function and mortality at different ages, and fifth: staging chronic kidney disease (CKD) while applying age-related cut-off levels and the consequences thereof on referrals to the nephrology department, as well as how a medication alert system warning for a decreased renal function may improve medication safety.

In **chapter 2** the performance of the Modification of Diet in Renal Disease formula (MDRD) was compared with the more recently developed Chronic Kidney Disease Epidemiology Collaboration Equation (CKD-EPI) in a group of 916 diabetic patients whose SCr and 24-hour CCR had been measured in the Maxima Medical Centre in Eindhoven, the Netherlands. Age ranged from 18-92 years and the CCR ranged from 11 to 250 ml/min/1.73m^2. The MDRD and CKD-EPI were found to be equally imprecise when compared to the CCR: 26.3 [25.1-27.6] and 25.6 [24.5-26.9] ml/min/1.73m^2, respectively. With regard to consequences in staging of CKD, 17.1% of the women was staged in a lower CKD category when compared to the MDRD. A larger percentage of diabetes patients <65 years was diagnosed as having CKD stage 3-5 when the MDRD was used. The classification of renal impairment in patients with diabetes can be simplified by using equations estimating the GFR, as long as one realizes oneself that the precision of both the MDRD and the CKD-EPI is low. Compared to the MDRD, the CKD-EPI gives higher estimates of GFR in young patients with diabetes, leading to a lower prevalence estimate of CKD on population level. Moreover, the performance of the CKD-EPI equation in diabetic patients has to be determined in a study in which a gold standard to measure renal function is used as a comparator.

Both the MDRD, the CKD-EPI (both estimating the GFR) and the CG (estimating the CCR) have been developed in populations with specific patient characteristics. No clear advices exist regarding which formula is best used for optimal estimation of kidney function in overweight and obese patients, since it is uncertain whether these equations give accurate estimates of renal function in these populations. All these equations correct for body weight, each on its own way. The Cockcroft-Gault formula (CG) uses weight as a variable because it is a crude estimate of muscle mass and therefore also of creatinine production. The MDRD and the CKD-EPI have been indexed for a body surface of 1.73m^2 (the mean body surface of a non-overweight person). In **chapter 3** data of the same cohort as evaluated in *chapter 2* were used. The influence of overweight and obesity on the performance of the CG, the MDRD, and the CKD-EPI was assessed (and compared to the CCR), as well as the influence hereof on the number

of patients that is misclassified as having chronic kidney disease. The renal function estimates were classified in weight categories: body mass index (BMI) <25 kg/m^2; BMI 25-30 kg/m^2 (overweight); BMI >30kg/m^2 (obese).The CG had the greatest accuracy in the overweight and obese patient group (76.9 en 76.8% were correctly classified; versus 45.8% (MDRD) and 34% (CKD-EPI) in the overweight group and 1.9% (MDRD) and 34% (CKD-EPI) in the obesity group. All renal function prediction equations are biased when used in overweight or obese diabetic populations, at least in those with a preserved renal function. Therefore, when using renal function prediction equations in clinical practice, their disadvantages should be kept in mind when making decisions based on the outcomes of these equations.

Many of the currently used equations to estimate GFR use SCr. In the Netherlands two techniques are used to measure creatinine: the Jaffe and the enzymatic technique. However, both techniques do show varying results when measuring the creatinine concentration, which in turn will affect the equation outcomes that are used to estimate the GFR. In **chapter 4** the problem of non-equivalence in SCr measurements across Dutch laboratories and the consequences thereof on CKD staging were explored. Data collected in 2009 by the annual national external quality assessment (EQA) organization for clinical chemistry laboratories (Stichting Kwaliteitsbewaking Medische Laboratorium diagnostiek, SKML) were used. The Jaffe technique overestimated the SCr when compared to a reference method (21%, 12% and 10% for SCr target values of 52, 73 and 94 μmol/L); the enzymatic technique gave more accurate measurements of creatinine (0%, -1% and -2% for SCr target values of 52, 73 and 94 μmol/L). When these Jaffe SCr values were used to estimate GFR, they gave an underestimation of renal function in 1-79% of the patients. When the enzymatic technique was used, only 2-4% of the patients were reclassified according to the CKD classification system. Thus, SCr as measured by a Jaffe technique leads to a substantial overestimation and stages patients in a lower CKD category than when an enzymatic technique is used compared to a reference method. Therefore, it is strongly advisable to use specific enzymatic techniques in clinical practice in order to generate more reliable GFR estimates.

Thus far, methodological issues involved in measuring creatinine in other fluids than plasma, have received little attention, although the creatinine concentration in urine has an indispensable role in calculating the CCR. **Chapter 5** examines the variability between the Jaffe, enzymatic and liquid chromatography mass spectrometry in plasma, urine and peritoneal fluid and the effects thereof on the eGFR and CCR in a population of 181 patients, including a spectrum from healthy subjects to dialysis patients, from the Isala Clinics, Zwolle. Bias (precision) for creatinine measurements in urine in the non-dialysis

group were -1.24 (1.82) and -0.75 (1.88) mmol/L for the Jaffe and enzymatic technique compared to LCMS, respectively. Bias (precision) for the CCR in the non-dialysis group were -31.9 (30) and -12.9 (29) ml/min for the Jaffe and enzymatic technique compared to LCMS, respectively. The enzymatic assay showed a more consistent pattern for creatinine measurements compared to the Jaffe method. However, the reliability of the urinary measurements appeared not optimal with the use of an enzymatic technique. These differences in urinary measurements are bothersome, since the CCR is important in clinical practice in pre-dialysis, to assess the adequacy of hemo- and peritoneal dialysis, and to examine the urinary excretion of biomarkers to correct for sampling errors. Further research should take place to explain the differences in urinary measurements before advocating changing the ranges of the CCR.

CKD is a risk factor for (cardiovascular) morbidity and mortality. Therefore, this is an additional reason to detect CKD in an early stage. In elderly patients it is unclear whether moderate renal impairment is a physiological process (without major clinical consequences) or whether it is associated with increased (cardiovascular) morbidity and mortality. In **chapter 6** we evaluated whether an association exists between a decreased eGFR (eGFR <45 and 45-60 ml/min/1.73m^2), albuminuria and mortality in patients with type 2 diabetes stratified according to age (65-75; >75 years). Information on 810 patients with diabetes mellitus type 2 was derived from the database of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study, a study which was initiated in the Zwolle region, the Netherlands in 1997. Patients had been followed for 9.8 years and were divided in three groups based on their eGFR: <45, 45-60, >60 ml/min/1.73m^2. A moderately decreased renal function (eGFR 45-60ml/min/1.73m^2) was not associated with an increased all-cause and cardiovascular mortality risk in those older than 75 years, provided there was no albuminuria. Albuminuria, irrespective of eGFR proved to be an independent marker of mortality in this elderly population and therefore is potentially useful as a more discriminative risk stratification tool in the large group of older patients with moderate reduction in eGFR. Although an eGFR of 45-60 ml/min/1.73m^2 is not associated with increased mortality in those >75 years, patients still may have an underlying cause and risk factors adding to progression of renal function. Moreover, irrespective the age, an eGFR <60 ml/min/1.73m^2 implies that certain lifestyle advices may have to be given and measures to be taken (see LTA chronic renal impairment).

In 2002 the Kidney Disease Outcome Quality Initiative (KDOQI) guidelines were developed to define and classify CKD uniformly and to recognize patients with CKD in the earliest possible stage to improve the prognosis of this patient group. Both the eGFR

as well as the presence of albuminuria and renal haematuria became components of the classification scheme of CKD. The KDOQI guidelines state that an eGFR <60 ml/min/1.73m^2 requires referral to a nephrologist for additional diagnostics, irrespective the age. However, a substantial part of the elderly have moderate (probably physiological) renal function loss with increasing age, without having an underlying (renal) disease or increased risk of mortality. Therefore, in the Dutch national guidelines (LTA) age-related cut-off values have been adapted. **Chapter 7** describes a cross-sectional analysis, examining the impact of the introduction of age-related cut-off values on consultation and referral patterns from general practitioners to nephrologists in a population of 82,424 patients. Median eGFR was 79 (65, 93), age ranged from 1-106 years; 31898 (39%) was >65 years All these patients had their SCr examined in 2009 in the Isala Clinics. When the KDIGO guidelines were used 19% (15,637 patients) would have been referred to secondary care. When applying age-related cut-off values, 3303 patients would have been referred, in 5748 patients peer consultation by a nephrologist would have occurred and 6586 patients would not have been referred or consulted about at all. The use of these age-related cut-off values in referral policy will result in fewer as well as at the same time more targeted referrals to nephrology departments, especially in the elderly patient group. However, fewer referrals to secondary care will also result in an increased number of patients that need to be monitored and treated in primary care. We suggest that the addition of albuminuria as a marker of renal damage may be of value for optimal risk stratification and targeted referral of elderly.

Iatrogenic injury due to medication errors is known as an adverse drug event (ADEs). Renal impairment is a risk factor for ADEs. When renal impairment is diagnosed, often the dosing of medications has to be adapted. Adaptations can only be done when the doctor and pharmacist are aware of the renal function of the patients. In **Chapter 8** the problem of medication errors in a community based population was assessed from a preventive perspective. It was investigated how many people were at risk for medication errors due to renal impairment and whether intensification of the cooperation between pharmacist and primary (community pharmacists and general practitioners) and secondary health care workers (clinical chemists and nephrologists) resulted in a decrease of the number of medication errors. This prospective observational study was conducted in the Zwolle region. Every time a decreased renal function (as assessed by MDRD and defined as an eGFR ≤40 ml/min/1.73m^2) was found, the laboratory sent a signal to the community pharmacist. The renal function of 25,929 persons was tested during this shared-care project. 1368 (5.3%) subjects were identified with an increased risk for an eventual adverse drug event (ADE), since they had an eGFR ≤40 ml/min/1.73m^2. In 15% (n=211), a medication error was detected. 88% hereof was regarded as potential

adverse drug event, most classified as significant or serious. The proportion of errors increased with age. Pharmacists recommended 342 medication adjustments, and in 66%, the dosage advice of the pharmacists was adopted by the doctor. Higher age, female sex and polypharmacy (defined as use of 5 or more medications) were identified as potential risk factors for renal function-associated medication errors. This study showed that an intensified collaboration between community pharmacists and prescribing physicians results in a considerable amount of drug dose adjustments and lead to improved medication safety in patients with renal impairment. Moreover, awareness for this problem was raised in health care workers. The results underline the potentially valuable role of community pharmacists in medication monitoring and provide evidence that exchange of medical patient information is relevant to safer drug therapy.

Based on the results of this thesis the following conclusions are warranted:

- The accuracy of the eGFR formulas for the individual remains doubtful, and outcomes of these equations should be used as an indicator, not as a true value.
- The Jaffe method for measuring creatinine should be abandoned.
- In elderly people, moderate renal function decrease without signs of renal damage, should be seen as part and parcel of their life and age.
- Extending the availability of renal function data towards community pharmacists can be an effective intervention to improve drug safety.

Chapter 11

Nederlandse samenvatting

De nieren hebben een belangrijke functie in het menselijk lichaam. De nieren zuiveren het bloed en hebben een belangrijke rol in de vochthuishouding. Als de nieren beschadigd raken, leidt dit tot nierfunctieverlies, ziekte en een verhoogde kans op overlijden. Nieren kunnen beschadigd raken door verschillende ziekten (zoals suikerziekte), maar ook door factoren als een verhoogde bloeddruk, roken en overgewicht. Door gezond te leven kan de kans op nierziekten worden verkleind, omdat je dan minder kans hebt deze risicofactoren te ontwikkelen. Vaak wordt nierschade pas in een laat stadium opgemerkt omdat een verminderde nierwerking vaak pas klachten geeft als er nog maar plusminus 30-45% van de oorspronkelijke nierfunctie over is.
Nierschade kan worden opgespoord door middel van bloedonderzoek (het meten van de creatinine concentratie, een afbraakproduct van creatininefosfaat afkomstig uit spierweefsel dat met een vrij constante snelheid wordt geproduceerd) en urineonderzoek (het meten van de hoeveelheid eiwit; als er eiwit in de urine zit betekent dit dat er sprake is van schade aan de nierfilters). Er is sprake van nierschade als de creatinine concentratie in het bloed verhoogd is of als er eiwit in de urine zit (albuminurie). Een andere manier om een indruk van de nierfunctie te krijgen is het meten van de creatinineklaring. Bij deze klaring wordt niet zozeer gekeken hoe hoog de concentratie creatinine in het bloed is (deze is naast de nierfunctie ook afhankelijk van onder andere de hoeveelheid spierweefsel dat iemand heeft), maar naar hoe goed de nieren in staat zijn om het creatinine vanuit het bloed in de urine te krijgen. Daarnaast kan de klaring ook worden gemeten met stoffen als inuline en radioactief gelabelde stoffen als ^{125}I-iothalamaat en ^{51}Chroom-EDTA, maar deze worden in de dagelijkse praktijk eigenlijk niet gebruikt, maar wel bij wetenschappelijk onderzoek.

Huisartsen hebben een belangrijke rol in het opsporen van nierschade en risicofactoren voor hart- en vaatziekten. In 2002 werd er een classificatiesysteem ontwikkeld met als doel chronische nierschade in een vroeger stadium te herkennen. In plaats van de tot dan toe gebruikte creatinine bepaling in het bloed (die onvoldoende betrouwbaar was gebleken om een goede schatting van de nierfunctie te maken), werden er nu formules gebruikt om de nierfunctie te schatten. In deze schattingsformules worden naast het creatinine ook leeftijd, geslacht en ras (alle bepalende factoren voor de hoeveelheid spierweefsel die iemand gemiddeld heeft) meegenomen. De meest frequent gebruikte formules zijn de Cockcroft-Gault (CG) formule (uit 1976), de MDRD formule (uit 1999), en de CKD-EPI formule (uit 2009). De beperking van het gebruik van deze formules is echter dat ze in specifieke patiëntengroepen zijn ontwikkeld, waardoor het maar de vraag is of ze toegepast kunnen worden in patiëntengroepen die hiervan afwijken.
In 2002 werd het classificatiesysteem van chronische nierschade gepresenteerd in een belangrijke richtlijn voor chronische nierschade (zie tabel 1). Naast het al dan niet

aanwezig zijn van albuminurie, berust dit classificatie systeem op een schatting van de nierfunctie met een formule. Het gebruik en de keuze van de formule voor het schatten van de nierfunctie heeft belangrijke gevolgen voor het classificeren van de chronische nierschade en daarmee ook voor het verwijs-/ en behandelbeleid van patiënten.
Nierfunctiebepalingen worden veel gebruikt in de dagelijkse praktijk. Ondanks de bovengenoemde beperkingen van schattingsformules worden ze voor uiteenlopende patiëntengroepen gebruikt en worden er op basis van deze schattingen veel beslissingen genomen.

Stadium	Beschrijving
1	eGFR ≥ 90 ml/min/1,73m^2 én persisterende (micro)albuminurie of persisterende en specifieke sedimentsafwijkingen ((afwijkende) rode bloedcellen in de urine)
2	eGFR 60-89 ml/min/1,73m^2 én persisterende (micro)albuminurie of persisterende en specifieke sedimentsafwijkingen ((afwijkende) rode bloedcellen in de urine)
3	eGFR 30-59 ml/min/1,73m^2
4	15-29 ml/min/1,73m^2
5	<15 ml/min/1,73m^2 of dialyse

Tabel 1. Classificatie systeem van chronische nierschade

Doelstellingen en samenvatting van dit proefschrift
In dit proefschrift kijken we kritisch naar het gebruik van deze nierfunctie schattingsformules: 1) hoe nauwkeurig en precies zijn deze formules, 2) welke invloed hebben deze formules op het (juist) vaststellen van nierschade, 3) maakt het voor de betrouwbaarheid van de uitkomst van formules uit welke techniek wordt gebruikt om de creatinine concentratie in het bloed te meten, 4) is een verminderde GFR even bedreigend voor zowel jonge als oude mensen, 5) wat is het effect van het hanteren van leeftijdsgebonden afkapwaardes voor de eGFR op het aantal diagnoses 'nierschade' en dus op het doorverwijzen van het aantal patiënten naar nefrologen (medisch specialisten in nierziekten), 6) kunnen deze formules, wanneer de samenwerking tussen apothekers en artsen wordt geïntensiveerd, helpen om de medicatieveiligheid voor mensen met nierschade te verbeteren?

In **hoofdstuk 2** worden de MDRD en de CKD-EPI formules met elkaar vergeleken in een groep patiënten met suikerziekte. Beide schattingsformules hadden een brede foutmarge en waren niet erg precies. Deze foutmarge heeft belangrijke gevolgen voor het classificeren van nierschade; 17,1% van de vrouwen werd door de CKD-EPI in een lagere nierschade stadium ingedeeld in vergelijking met de MDRD en dus als 'chronisch (nier) ziek' beschouwd. Het voordeel van schattingsformules is dat het berekenen en indelen van nierschade bij deze mensen eenvoudiger is. Uit onze studie blijkt dat zorgverleners zich bewust moeten zijn van de foutmarges van beide formules.

Zoals eerder genoemd zijn de verschillende schattingsformules in geselecteerde groepen mensen ontwikkeld. Daarom zijn deze formules soms moeilijk te vertalen naar specifieke patiëntgroepen (zoals mensen met suikerziekte, overgewicht of ouderen).
Overgewicht is een toenemend probleem in Nederland. De MDRD en de CKD-EPI zijn echter ontwikkeld in een groep mensen met een normale lengte en een normaal gewicht. In de CG formule wordt gewicht (als grove schatting van de spiermassa, en dus creatinine productie) als variabele meegenomen in de formule, wat spaak loopt als iemand veel overgewicht (vetmassa) heeft. In **hoofdstuk 3** hebben wij daarom de invloed van overgewicht op 3 nierfunctie schattingsformules (de CG, MDRD en CKD-EPI) vergeleken. In deze studie hebben wij gebruik gemaakt van onderzoeksgegevens van 916 patiënten met suikerziekte. Uitkomsten van de schattingsformules werden vergeleken met de creatinineklaring in verschillende gewichtsgroepen: 1) normaal (body mass index (BMI) <25 kg/m^2; een index die de verhouding tussen lengte en gewicht bij personen weergeeft en wordt gebruikt om een indicatie te krijgen of er sprake is van over- dan wel ondergewicht), 2) overgewicht (BMI 25-30 kg/m$^{2)}$ en 3) ernstig overgewicht ofwel obesitas (BMI>30 kg/m^2). In het algemeen was er voor alle schattingsformules een aanzienlijke foutmarge bij zowel de mensen met overgewicht als obesitas. In verhouding was de CG het nauwkeurigste in de overgewicht en de obese patiëntengroep (76,9% en 76,8% werden correct geclassificeerd respectievelijk; versus 45,8% (MDRD) and 34% (CKD-EPI) in de overgewicht groep and 51,9% (MDRD) en 34% (CKD-EPI) in de obesitas groep. In de dagelijkse praktijk moet dus steeds weer rekening worden gehouden met de beperkingen van nierfunctieschattende formules in de groep patiënten met overgewicht.

Veel van de gebruikte formules om de nierfunctie te schatten doen dit met behulp van de creatinine concentratie in het bloed. Deze creatinine concentratie kan je op verschillende manieren in het laboratorium bepalen. In Nederland zijn er twee technieken die veel worden gebruikt, de zogenaamde 'Jaffé techniek' en de 'enzymatische techniek'. Echter, er is veel variabiliteit tussen deze twee technieken om serum creatinine te bepalen. Dit heeft gevolgen voor de betrouwbaarheid en de uitkomsten van nierfunctieschattende formules, als ook voor de classificatie van chronische nierschade. In **hoofdstuk 4** is nagegaan wat de consequenties zijn van de variabiliteit in serum creatinine bepalingen in de Nederlandse laboratoria voor het classificeren van chronische nierschade in een grote patiëntengroep. Zoals verwacht overschatte de Jaffé techniek de serum creatinine waarde ten opzichte van de gouden standaard (de meest nauwkeurige maat om creatinine te meten), de liquid chromatography mass spectrometry (LCMS). De enzymatische techniek daarentegen evenaarde nagenoeg de gouden standaard. Wanneer met de Jaffé creatinine waardes een schatting werd gemaakt van de GFR, leidde dit bij 1-79% van de patiënten tot een onderschatting van de nierfunctie, waardoor ze in een lagere (dus

ernstiger) categorie in de chronische nierschade classificatie terecht kwamen. Bij de enzymatische techniek gebeurde dit slechts bij 2-4% van de patiënten. Daarom is het ten zeerste aan te bevelen om de Jaffé techniek niet meer te gebruiken voor het bepalen / schatten van de nierfunctie, en heeft het de sterke voorkeur dat laboratoria overgaan op het gebruik van een enzymatische techniek voor het bepalen van het serum creatinine.

Naast de formules die worden gebruikt om de nierfunctie te schatten, wordt ook nog steeds de creatinineklaring gebruikt om een indruk van de nierfunctie te krijgen. De creatinineklaring wordt o.a. gebruikt om het juiste moment voor het starten van nierfunctievervangende behandeling (dialyse) te bepalen of wanneer de uitkomsten van nierfunctie schattingsformules onzeker zijn. In h**oofdstuk 5** is gekeken naar de variabiliteit van de creatininebepaling in urine wanneer een Jaffé dan wel een enzymatische techniek wordt gebruikt, ten opzichte van de gouden standaard. De creatinine concentratie is bepaald in bloed (plasma), urine en dialyse vloeistof in een groep van 181 patiënten. Lagere creatinine concentraties in urine ten opzichte van de gouden standaard werden gemeten wanneer de Jaffé werd gebruikt en in iets mindere mate als de enzymatische techniek werd gebruikt. Omdat de Jaffé bepalingen in het plasma ook de verwachte onnauwkeurigheid lieten zien, leidde dit tot onderschatting van de creatinineklaring. Alvorens over te gaan tot het aanpassen van de normaalwaarden van de 24-uur creatinineklaring, zal er eerst meer onderzoek moeten plaatsvinden om de gevonden verschillen te verklaren.

Chronische nierschade is een risicofactor voor het ontwikkelen van hart- en vaatziekten, het overlijden aan hart- en vaatziekte, en verhoogd ook de totale sterftekans van een patiënt. Daarom probeert men de afgelopen jaren om chronische nierschade in een zo vroeg mogelijk stadium op te sporen. Bij ouderen is onduidelijk of achteruitgang van de nierfunctie een proces passend bij het ouder worden is, of dat het als ziekte moet worden beschouwd. In **hoofdstuk 6** werd daarom onderzocht of er een verband is tussen de geschatte nierfunctie, albuminurie en sterfte bij mensen met ouderdomssuiker(ziekte) in verschillende leeftijdsgroepen (65-75; >75 jaar). Dit werd onderzocht in een groep patiënten die voor hun suikerziekte onder behandeling waren bij de huisarts. Een groep van 810 patiënten met suikerziekte werden 9,8 jaar gevolgd. Mensen werden op basis van hun nierfunctie ingedeeld in drie groepen: <45 ml/min/1,73m^2 (ernstige nierschade), 45-60 ml/min/1,73m^2 (matige nierschade), >60 ml/min/1,73m^2 (geen nierschade). Een nierfunctie <60 ml/min/1,73m^2 was in de groep 65-75 jaar geassocieerd met meer sterfte aan hart- en vaatziekten. Echter, bij mensen ouder dan 75 jaar werd pas meer sterfte aan hart- en vaatziekten gezien bij een nierfunctie <45 ml/min/1,73m^2, mits er geen sprake was van albuminurie. Albuminurie bleek ook bij patiënten >65 jaar geassocieerd met een

toegenomen sterfte. Bij ouderen moet er dus steeds worden nagegaan of er sprake is van een matige maar stabiele nierschade, of dat er sprake is van een gestage achteruitgang van de nierfunctie met aanwezigheid van albuminurie.

In 2002 werd een richtlijn ('the Kidney disease outcomes quality initiative (K-DOQI)-richtlijn) opgesteld waarin een classificatiesysteem wordt voorgesteld met als doel om patiënten met nierschade in een vroeg stadium te herkennen en zo de prognose van deze groep te verbeteren (tabel 1).

Patiënten < 65 jaar

	Normo/micro-albuminurie	Macro-albuminurie
eGFR > 60 ml/min/1,73m^2		
eGFR 45-60 ml/min/1,73m^2		
eGFR 30-45 ml/min/1,73m^2		
eGFR < 30 ml/min/1,73m^2		

Patiënten > 65 jaar

	Normo/micro-albuminurie	Macro-albuminurie
eGFR > 60 ml/min/1,73m^2		
eGFR 45-60 ml/min/1,73m^2		
eGFR 30-45 ml/min/1,73m^2		
eGFR < 30 ml/min/1,73m^2		

Begeleiding in de eerste lijn
Consultatie nefroloog en eventueel gezamenlijke behandeling
Verwijzing naar de tweede lijn

Tabel 2. Indicaties voor beleid in de eerste lijn, tweede lijn en gezamenlijke behandeling.

Afhankelijk van in welk stadium van nierschade een patiënt valt, worden er adviezen gegeven ten aanzien van verdere diagnostiek of verwijzing. Bij ouderen is er regelmatig sprake van een iets verminderde, maar stabiele nierfunctie. In de Nederlandse richtlijnen (de landelijke transmurale afspraak chronische nierschade) is er daarom voor gekozen om naast de schatting van de nierfunctie en de albuminurie, ook de leeftijd (≤ of >65 jaar) in ogenschouw te nemen in de besluitvorming tot verdere diagnostiek en verwijzing.

In **hoofdstuk 7** hebben wij de impact van deze leeftijdsgebonden afkapwaardes op het verwijsbeleid naar het ziekenhuis van de LTA (tabel 2) vergeleken met de afkapwaardes uit de K-DOQI richtlijnen (tabel 1).
Deze studie werd gedaan in een onderzoeksgroep van 82424 patiënten uit de regio Zwolle. Wanneer de KDOQI richtlijnen zouden worden gehanteerd zou 19% (15637 mensen)

van de populatie worden verwezen voor verder (poliklinisch) onderzoek in ziekenhuis. Bij het gebruik van leeftijdsgebonden afkappunten zouden 3303 patiënten zijn verwezen en 5748 mensen een ziekenhuisspecialist nierziekten hebben geconsulteerd. Het gebruik van leeftijdsgebonden afkappunten zal dus leiden tot meer gerichte verwijzingen naar de afdeling nierziekten in met name de groep ouderen en waarschijnlijk leiden tot minder zorgkosten.

De nieren spelen een belangrijke rol in het verwerken van medicatie en het verwijderen van hun afvalproducten. Als de nierfunctie gestoord is, kan medicatiegebruik sneller leiden tot bijwerkingen, en daarnaast worden de nieren extra gevoelig voor bepaalde medicijnen als ze slechter werken. Daarom moet bij veel medicijnen de dosering worden aangepast aan de nierfunctie en moet een apotheker de medicatielijst van mensen met nierschade extra in de gaten houden. Aanpassingen kunnen alleen worden gedaan als arts en apotheker op de hoogte zijn van de nierfunctie van de patiënt en dit is lang niet altijd het geval. Daarom is in **hoofdstuk 8** geëvalueerd hoeveel mensen risico liepen op medicatiefouten door nierfunctiestoornissen, en of het intensiever samenwerken tussen apotheker, (huis)arts en klinisch chemici resulteerde in een afname van het aantal medicatiefouten.
Dit onderzoek vond plaats in Zwolle van februari 2009 tot maart 2010. Elke keer dat er een verminderde nierfunctie werd vastgesteld in het laboratorium, is er vanuit het laboratorium een signaal naar de apotheek gestuurd. In totaal is van 25929 personen de nierfunctie getest. Hiervan had 1368 (5,3%) een verminderde nierfunctie. Bij deze mensen werd door de apothekers gecontroleerd of de dosering van de gebruikte medicatie de juiste was. Bij 15% van de studiepopulatie waren aanpassingen nodig in het medicatiegebruik vanwege de nierfunctie. Dit werd telefonisch besproken met de voorschrijvend arts. In 66% zijn de door de apotheek voorgestelde wijzingen in de dosering van de medicijnen overgenomen door de arts. Hoge leeftijd, het vrouw zijn en polypharmacie (gedefinieerd als het gebruik van 5 of meer medicijnen) werden aangewezen als mogelijke risicofactoren voor nierfunctie gerelateerde medicatie fouten. Deze studie laat zien dat een intensievere samenwerking tussen apothekers, klinisch chemici en (huis)artsen resulteert in een aanzienlijke hoeveelheid aanpassingen in de dosering van medicijnen en leidt tot een verbeterde medicatie veiligheid bij patiënten met nierfunctiestoornissen. Bovendien, is er meer bewustzijn gekweekt bij gezondheidszorg medewerkers ten aanzien van nierfunctie en medicatiegebruik. Apothekers vonden het systeem nuttig en de tijdsinvestering van medicatiecontroles de moeite waard. De resultaten onderstrepen de mogelijk waardevolle rol van apothekers bij het monitoren van de medicatie en bieden bewijs dat uitwisseling van medische patiënten informatie relevant is voor veiligere medicatietherapie.

Conclusies

Alle formules die worden gebruikt om nierfunctie te schatten zijn onnauwkeurig in populaties die afwijken van de populatie waarin deze formules zijn ontwikkeld. Om te komen tot een zo nauwkeurig mogelijke schatting van de nierfunctie, moeten enzymatische technieken worden gebruikt om de creatinine concentratie in het bloed te meten. Het blijft onduidelijk welke techniek het meest betrouwbaar is voor het bepalen van de creatinine concentratie in urine. Bij patiënten met suikerziekte >75 jaar is een matig gestoorde nierfunctie (eGFR 45-60 ml/min/1,73m^2) niet geassocieerd met een verhoogde kans op sterfte, mits er geen sprake is van albuminurie. Het hanteren van leeftijdsgebonden afkappunten in de richtlijn chronische nierschade zoals die in Nederland wordt gebruikt lijkt dan ook gerechtvaardigd en leidt tot minder onnodig verwijzen van oudere patiënten naar het ziekenhuis voor aanvullend onderzoek. Een intensievere samenwerking tussen apothekers en artsen bestaande uit een alarmeringssysteem waarbij een verminderde nierfunctie door apothekers kan worden gesignaleerd leidt tot een betere medicatieveiligheid bij patiënten met nierschade.

Dankwoord

List of publications

Previous dissertations

Dankwoord

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