GFR meets mTOR: value of different methods to measure and estimate GFR & (side) effects of mTOR inhibition in renal transplantation
Baas, M.C.

Citation for published version (APA):
General Introduction
Part I
GENERAL INTRODUCTION

The glomerular filtration rate (GFR), the sum of the filtration rate of all functioning nephrons, is used to express kidney function. For many reasons, adequate measurement of renal function is important in patients at risk for or suffering from overt renal failure. Early detection of renal impairment is relevant since it can have major implications for decisions about treatment. Moreover, exact calculation of GFR necessary in the evaluation and follow-up of possible beneficial or harmful interventions. Furthermore, the dose of many drugs has to be adjusted for renal function; overdosing can cause an increase in adverse side effects or can further worsen kidney function. Finally, GFR is an important predictor for all-cause mortality due to especially cardiovascular disease and is thereby a prognostic factor in itself.

The Kidney Disease Outcomes Quality Initiative (K/DOQI) has provided guidelines to optimize the care of patients with chronic kidney disease (CKD), according to a five-stage classification that is based on kidney function. Stage I: GFR ≥ 90 ml/min/1.73m² with signs of kidney damage (defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies), stage II: 60 - 89 ml/min/1.73m² with signs of kidney damage, stage III: 30 - 59 ml/min/1.73m², stage IV: 15 - 29 ml/min/1.73m² and stage V: < 15 ml/min/1.73m². Each stage of kidney disease asks for a different clinical action plan and is accompanied by its own hazard ratio for mortality and morbidity. Therefore, accurate classification of patients with CKD is important for appropriate treatment and evaluation.

Many attempts have been made to find an ideal method to determine GFR. GFR can be measured or can be estimated. So far, inulin clearance or the use of radioactive markers are considered as the gold standard, but these methods are expensive and cumbersome. Plasma creatinine is the best known and most often used endogenous marker for GFR.

In the next sections, the currently used methods for GFR determination will be reviewed and new methods for GFR measurement and GFR estimation will be discussed.

Methods for GFR measurement

Two approaches to measure GFR can be used. The first one uses a continuous infusion of a tracer. Before the actual measurements are done, a steady plasma level of the tracer is required, enabling the calculation of renal clearance from urine collections. Confounders such as extrarenal clearance and variation in distribution are minimised in this way. The other approach is the single injection method, which measures whole body clearance from the plasma disappearance rate. The main advantage of the latter method is its relative simplicity.
Inulin clearance

Inulin is a starch (fructose polymer) found in the tubers and roots of many plants. Inulin clearance is considered the gold standard, since it has all the characteristics of an ideal marker. Inulin is a low molecular weight solute (5200 D), not bound to plasma proteins and is therefore freely filtered by the glomerulus. No reabsorption or secretion takes place in the tubular system. There is only minimal secretion into the bile\(^2\). The classic method of measuring inulin clearance includes intravenous administration of a priming dose followed by a constant infusion\(^3\). GFR is calculated as \(U \times V/P\), where \(U\) = inulin concentration in the urine, \(V\) = the urine flow rate per unit of time, determined by bladder catheterization and \(P\) = the inulin concentration in plasma.

However the use of this method has important drawbacks: measurement in laboratories is complex and cumbersome, inulin is not widely available\(^4\) and the use of a bladder catheter for investigation should be avoided.

\(^{125}\text{I}-\text{labeled iothalamate}/^{131}\text{I}-\text{labeled hippuran continuous infusion method}\(^5\)

This method was first introduced in 1977, enabling simultaneous determination of GFR with \(^{125}\text{I}-\text{iothalamate}\) and effective renal plasma flow (ERPF) with \(^{131}\text{I}-\text{hippuran}\). This makes it possible to calculate the filtration fraction (FF). The administration of \(^{131}\text{I}-\text{labeled hippuran}\) makes it possible to correct for incorrect urine collection, making bladder catheterization unnecessary and improving accuracy\(^6\).

\(^{51}\text{Cr}-\text{EDTA}\) continuous injection method\(^7\)

\(^{51}\text{Cr}-\text{EDTA}\) is a widely accepted and commonly used method using a single bolus injection with measurement of the plasma disappearance rate of \(^{51}\text{Cr}-\text{EDTA}\). Similar to each injected substance, the plasma concentration curve after a single intravenous injection consists of two phases: an initial rapid decrease representing the distribution of the injected marker in the extra-cellular volume and a second slower decrease representing its clearance. \(^{51}\text{Cr}-\text{EDTA}\) is mostly filtered by the glomeruli but extra renal clearance can be as high as 10\(^%\). The radiation load is minimal. Other filtration markers used to determine GFR are \(^{99}\text{Tc}-\text{diethylenetriaminepenta-acetic acid}\) (\(^{99}\text{Tc-DTPA}\)) or iohexol (a non-ionic, low osmolar radiocontrast medium).

Methods for GFR estimation

GFR estimation by plasma creatinine

Since methods to measure GFR are labour intensive as well as expensive, endogenous markers are used as an alternative to estimate GFR. As mentioned earlier, plasma creatinine is the best known and most commonly used endogenous marker for estimation of GFR, since Popper et al. first described the use of creatinine as a marker for GFR in 1937\(^9\). Creatinine is a breakdown product of creatinephosphate in muscle tissue. The word ‘creatinine’ (or ‘kreatinine’) is derived from the Greek word ‘kreas’, which means flesh. It is produced at a relatively constant rate, depending on the muscle mass, and filtered in the glomeruli but also actively secreted in the proximal
tubule. Tubular secretion contributes normally to 10 - 40% of renal creatinine removal, but increases when GFR decreases, causing plasma creatinine to remain in the normal range until GFR drops below 60-70 ml/min ('creatinine blind range'). Significant nephron loss can therefore have occurred before a change in creatinine occurs. If GFR decreases below 10 ml/min, extrarenal clearance increases, probably mediated by intestinal microflora\textsuperscript{10-12}. Although plasma creatinine is the most commonly used marker, it has several other limitations. It is influenced by muscle mass and therefore by age, gender and race. Medication, for example prednisolone, can also affect plasma creatinine\textsuperscript{13, 14}. Furthermore, the method used for its determination contributes to variation in the measured value of plasma creatinine. The alkaline picrate (Jaffé) method is influenced by non-creatinine chromogens like glucose, acetoacetate and antibiotics\textsuperscript{15, 16}. Therefore, this method is nowadays more often replaced by an enzymatic determination. But also the enzymatic method can be influenced by exogenous compounds, like dopamine, 5-fluorocytosine and bilirubin\textsuperscript{17}.

Another factor which is important to interpret plasma creatinine values, is whether the creatinine assay is calibrated to the reference isotope dilution mass spectroscopy (IDMS) creatinine standard. Errors in calibration make little difference in estimating severely decreased GFR (<30 mL/min/1.73 m\textsuperscript{2}), but result in progressively larger differences at higher GFRs\textsuperscript{18}. This factor is especially important when creatinine values of different laboratories are compared.

To estimate GFR, creatinine clearance can be calculated using the following formula: GFR = U x V / P (i.e filtered load), where ‘P’ is the plasma concentration of creatinine, ‘U’ the urine concentration of creatinine and ‘V’ the volume of the urine. This calculation does not correct for tubular secretion. Measurement of creatinine clearance using this method becomes more reliable after the administration of cimetidine, which inhibits tubular secretion\textsuperscript{19-21}.

To overcome some other limitations of creatinine as a marker for GFR, several formulas have been constructed to correct for the influences of weight, age, gender and/or race. The most commonly used formulas (and their advantages and limitations) are listed in the next section.
### Creatinine-based formulas

**Table 1.** Plasma creatinine (Pcr) is expressed in μmol/l, urea in mmol/l, albumin in g/l, weight in kg and height in m.

<table>
<thead>
<tr>
<th>formula</th>
<th>Population in which the formula is developed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cockcroft and Gault</strong>&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Creatinine clearance = ((140 - \text{age}) \times \text{weight})/\text{Pcr} &lt;br&gt; (male, multiply result by 1.23, female: multiply result by 1.05) &lt;br&gt; 236 Canadian patients (209 male)</td>
</tr>
<tr>
<td><strong>6-variable MDRD</strong>&lt;sup&gt;23&lt;/sup&gt;</td>
<td>GFR = 170 x (Pcr ÷ 88.4)&lt;sup&gt;-0.999&lt;/sup&gt; x age&lt;sup&gt;-0.176&lt;/sup&gt; x (P&lt;sub&gt;urea&lt;/sub&gt; x 2.8)&lt;sup&gt;-0.170&lt;/sup&gt; x (P&lt;sub&gt;albumin&lt;/sub&gt;/10)&lt;sup&gt;-0.318&lt;/sup&gt; &lt;br&gt; (female: multiply result by 0.762, black multiply result by 1.180) &lt;br&gt; 1628 patients with CKD</td>
</tr>
<tr>
<td><strong>4-variable MDRD</strong>&lt;sup&gt;24, 25&lt;/sup&gt;</td>
<td>GFR = 175 x (Pcr ÷ 88.4)&lt;sup&gt;-1.154&lt;/sup&gt; x age&lt;sup&gt;-0.203&lt;/sup&gt; &lt;br&gt; 1628 patients with CKD</td>
</tr>
<tr>
<td><strong>CKD-EPI</strong>&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Female and Pcr ≤ 62 umol/l: &lt;br&gt; GFR = 144* x ((Pcr / 88.4) / 0.7)&lt;sup&gt;-0.529&lt;/sup&gt; x (0.993)&lt;sup&gt;Δ&lt;/sup&gt; &lt;br&gt; Female and Pcr &gt; 62 umol/l: &lt;br&gt; GFR = 144* x ((Pcr / 88.4) / 0.7)&lt;sup&gt;-1.209&lt;/sup&gt; x (0.993)&lt;sup&gt;Δ&lt;/sup&gt; &lt;br&gt; Male and Pcr ≤ 80 umol/l: &lt;br&gt; GFR = 141** x ((Pcr / 88.4) / 0.9)&lt;sup&gt;-0.411&lt;/sup&gt; x (0.993)&lt;sup&gt;Δ&lt;/sup&gt; &lt;br&gt; Male and Pcr &gt; 80 umol/l: &lt;br&gt; GFR = 141** x ((Pcr / 88.4) / 0.9)&lt;sup&gt;-1.209&lt;/sup&gt; x (0.993)&lt;sup&gt;Δ&lt;/sup&gt; &lt;br&gt; * use 166 if black &lt;br&gt; ** use 163 if black &lt;br&gt; 8254 patients with various causes of CKD and healthy kidney donors</td>
</tr>
<tr>
<td><strong>Nankivell</strong>&lt;sup&gt;27&lt;/sup&gt;</td>
<td>GFR = 6.7/Pcr + weight/4 – urea/2 – 100/height&lt;sup&gt;2&lt;/sup&gt; &lt;br&gt; (male: add 35, female: add 25) &lt;br&gt; 146 renal transplant patients</td>
</tr>
</tbody>
</table>

All formulas have their specific limitations. The Cockcroft and Gault formula generally overestimates GFR because of tubular secretion of creatinine. Especially in patients with a bodymass index above 30 kg/m<sup>2</sup>, whereas it systematically underestimates GFR in elderly people<sup>28</sup>. The 6-variable MDRD formula is derived from patients in the United States with non-diabetic renal disease, with a mean
GFR of 40 ml/min/1.73m². The included patients were largely Caucasian, but the formula seems also accurate across a wide range of subgroups. To simplify the 6-variable MDRD formula, the 4-variable (abbreviated) MDRD was constructed in which measurement of urea and albumin are not necessary. There are two formulas of the abbreviated MDRD, depending on the method used for the determination of creatinine (i.e. whether creatinine values are standardised to creatinine reference materials using gold standard techniques or not). The MDRD and the abbreviated MDRD underestimate GFR in young women and also overestimate GFR in patients with a body mass index below 18.5 kg/m², since these patients have a very low creatinine, reflecting low muscle mass.

Furthermore, the MDRD formulas are less accurate at GFR > 60 ml/min/1.73m² and systematically underestimate true GFR. Attempts have been made to construct new formulas for healthy persons. Recently, the CKD-EPI equation has been developed to be more accurate at higher GFRs. The CKD-EPI indeed performs better in the higher ranges of GFR, leading to higher estimates of kidney function and resulting in less CKD (defined as estimated or measured GFR < 60 ml/min/1.73m²) on a general population based level by reclassification of low-risk individuals. However, it may lead to an increased incidence of CKD in the elderly (> 70 years) since the formula results in lower GFR estimates in this group compared to the MDRD formula.

The Nankivell formula was specially developed for renal transplant recipients (RTR), using 711 GFR measurements in 146 patients. Thus many data points were repeated measures in the same patients and not independent samples. Although derived in kidney transplant recipients, the Nankivell equation did not perform well in that same group of patients, and was even less accurate than the MDRD equation.

But when GFR is above 60 ml/min/1.73m², the accuracy and precision of the formula improved and exceeded that of the MDRD and Cockcroft-Gault formula. Recently, it was demonstrated that the CKD-EPI performed better in RTR than the MDRD, although all equations to estimate GFR overestimate measured GFR.

So far, none of these formulas seem to be good enough to substitute measured GFR, but they seem acceptable to discriminate patients with chronic kidney disease.

**New methods and markers**

Since the above mentioned methods and markers for GFR determination have their specific limitations, the search for new ones continues.

A new method to measure GFR is the single injection method of ‘Gd-DTPA’, using gadolinium DTPA (Magnevist®). This method is a non nuclear method and can therefore be used in facilities without a nuclear department. Plasma concentrations of gadolinium are measured using an ELISA technique. The whole body clearance is calculated from the plasma disappearance rate. Although recently a number of articles has been published describing the occurrence of nephrogenic systemic fibrosis after exposure to gadolinium in patients with end-stage renal failure, this is a rare event occurring in patients receiving gadolinium in the context of an MRI scan.
The incidence of nephrogenic systemic fibrosis appears to depend on the total dose of administered gadolinium. Since GFR measurement with Gd-DTPA uses an amount of gadolinium that is only 5% of the dose used with MRI, the risk of developing nephrogenic systemic fibrosis is seems negligible. So far, GFR measurements using Gd-DTPA have only been performed in dogs and in a small group of patients, including renal transplant patients.

Relatively new endogenous markers are cystatin C and beta-trace protein (βTP). Cystatin C is a low molecular weight protein (13.3 kD) that functions as a cysteine protease inhibitor. It is produced at a constant rate by all nucleated cells and removed from the body by glomerular filtration, thereafter reabsorbed and broken down in the proximal tubule. Cystatin C production is thought not to be influenced by sex, age, bodyweight or muscle mass although this is contradicted by some reports. However, cystatin C is influenced by thyroid function; in overt hypothyroidism, but also in subclinical states. In hyperthyroidism cystatin C concentrations increase and normalize after euthyroidism is achieved. In hypothyroidism, the opposite occurs. Whether this reflects a change in GFR is not completely clear; plasma creatinine values rise when hyperthyroidism is corrected and decrease when hypothyroidism is corrected. Treatment with corticosteroids also increases cystatin C levels by increasing the production of cystatin C. Several studies showed that cystatin C is a more sensitive indicator of mild reductions of renal function than creatinine. Moreover cystatin C is not removed during intermittent hemodialysis or continuous venovenous hemodiafiltration and it has been advocated as a marker for residual renal function in dialysis patients.

Table 2. Cystatin C (cysC) is expressed in mg/l

<table>
<thead>
<tr>
<th>Formula</th>
<th>Population in which the formula is developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoek62</td>
<td>GFR = -4.23 + 80.35/cysC 93 patients with various causes of renal disease</td>
</tr>
<tr>
<td>Larsson71</td>
<td>GFR = 77.24 x cysC^{1.2623} 100 patients with various causes of renal disease</td>
</tr>
<tr>
<td>Rule69</td>
<td>Native CKD GFR = 66.8 x cysC^{1.30} 460 healthy subjects, patients with various causes of renal disease and renal transplant patients</td>
</tr>
<tr>
<td></td>
<td>Transplant recipient GFR = 76.6 x cysC^{1.16}</td>
</tr>
</tbody>
</table>

Several formulas have been constructed for cystatin C to express GFR. The formulas are each extracted from and validated in different patient populations and the ones used in the articles in this thesis are summarized in Table 2.

Creatinine- and cystatin C based formula

From pooled data from the MDRD study, the African American Study of Kidney Disease (AASK), the Collaborative Study Group (CSG) and a clinical population in...
Paris\textsuperscript{76} a creatinine and cystatin C combined formula was constructed\textsuperscript{76}. This formula was more accurate than the formulas based on creatinine or cystatin C alone, but this formula requires further testing in various patients groups.

Beta-trace protein (BTP), also known as lipocalin prostaglandin D2-synthase, is another low molecular weight protein (22-26 kDa, depending on the degree of posttranslational glycosylation\textsuperscript{77}). It is mainly produced in the central nervous system (leptomeninges, choroid plexus epithelium and oligodendrocytes) and forms one of the principal proteins of the cerebrospinal fluid (CSF), which contains the highest BTP concentration of all body fluids. Male gonads, the human heart, spleen, bone marrow, thymus also produce BTP and it has been found in serum, urine, ascites and seminal plasma. The concentration of BTP in serum and urine is very low compared to that in CSF\textsuperscript{78-81}. Glycosylation patterns of BTP differ in cerebrospinal fluid, serum and urine. BTP in the brain (bBTP) is smaller, due to truncated oligosaccharide side-chains and absent sialyzation. bBTP is metabolized in the liver and therefore cleared from the circulation; the sialyzed glycoforms are eliminated by glomerular filtration\textsuperscript{77}. Both isoforms of BTP can be distinguished by immunonephelometry. BTP is advocated as a early marker for deterioration of renal function in the ‘creatinine blind range’\textsuperscript{77}, \textsuperscript{82-84}. BTP might also be of value to estimate residual renal function in patients treated with hemodialysis (HD) or hemodiafiltration (HDF), since HD and HDF do not induce clinical relevant alterations on BTP serum levels. However, HDF reduced BTP levels by more than 20% in some patients. BTP serum concentrations appeared strongly associated with residual diuresis (i.e. probably GFR) in these patients\textsuperscript{85}.

Like cystatin C, BTP also appears to be influenced by the use of corticosteroids. In contrast to cystatin C, BTP decreases during the use of prednisolone\textsuperscript{86, 87}. The mechanism underlying the decrease in BTP is not elucidated yet. Down-regulation of BTP synthesis, permeability changes of the blood-brain barrier or increased extrarenal elimination are proposed mechanisms\textsuperscript{77, 87}. A comparison between eGFRs using cystatin C and BTP, showed no benefit of BTP over cystatin C\textsuperscript{87}.

The following GFR-equations have been constructed in adults for estimation of GFR, using BTP plasma-levels (Table 4):
Many renal transplant patients develop renal failure. This can be multi-factorial. In the first months after transplantation acute rejection is the main cause of kidney deterioration. Later on, calcineurin-inhibitor toxicity, chronic rejection, chronic allograft nephropathy and BK-nephropathy become important. Each of these conditions demands a different treatment. Early detection is critical, so that further diagnostic procedures such as a kidney biopsy can be performed without delay.

In the past years renal transplantation with a kidney from a living donor has gained more and more grounds due to organ shortage and excellent results. Nowadays half of the kidney transplantations in the Netherlands are performed with living donors. This has increased the urge to accurately assess renal function in potential donors, ensuring that pre-donation renal function is above a certain minimal standard and minimizing the risk of future renal failure in these donors. Reliable assessment of pre-donation kidney function is therefore necessary to exclude those patients with diminished kidney function.

Renal function in HIV-infected patients can be affected by the HIV-infection itself, manifesting as interstitial nephritis, collapsing FSGS or thrombotic microangiopathy, or by other HIV-related infections, like post-infectious glomerulonephritis. Furthermore, nephrotoxicity is the dose-limiting toxicity associated with certain components of highly active retroviral therapy (HAART), such as cidofovir, adefovir and tenofovir.

Fabry's disease is an X-chromosomal-linked lysosomal storage disorder. Due to deficient activity of the lysosomal enzyme α-galactosidase A, glycosphingolipids, mainly globotriaosylceramide (Gb-3) or ceramide trihexoside, accumulate in lysosomes of various cell types. The kidney is also affected because of lysosomal storage in endothelial, mesangial, interstitial cells and podocytes. Proteinuria is an early sign of renal involvement. Microvascular endothelial deposits of Gb-3 cause ischaemic injury, global glomerulosclerosis, tubular atrophy and interstitial fibrosis. Renal replacement therapy may already be necessary in the third and fourth decade of life. Renal function is an important predictor of mortality. Since enzyme replacement therapy (ERT) became available for patients with Fabry disease, the course of the disease has altered. Studies have shown that the treatment benefit of ERT is more pronounced in patients with a better renal function at baseline.

### Table 4. Beta-trace protein (βTP) is expressed in g/l, urea in mmol/l

<table>
<thead>
<tr>
<th>Formula</th>
<th>Population in which the formula is developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR = 112.108 x βTP^0.662 x urea^0.280</td>
<td>163 Renal transplant patients</td>
</tr>
<tr>
<td>(female: multiply result by 0.88)</td>
<td></td>
</tr>
<tr>
<td>GFR = 89.85 x βTP^0.5541 x urea^0.3018</td>
<td>187 Caucasian renal transplant patients</td>
</tr>
</tbody>
</table>
Breunig et al. showed that a decline of GFR to 71 ml/min already was a risk factor for the development of a clinical end point (death, cardiac and cerebrovascular event, renal failure)\textsuperscript{92} stressing the importance of, adequate monitoring of renal function to assess the effect of ERT.

\textbf{AIM AND OUTLINE OF PART I OF THIS THESIS}

GFR is measured and estimated in renal transplant recipients, healthy kidney donors, HIV-infected patients and patients with Fabry disease. As mentioned above, these patients are at risk of developing renal failure and normal formulas to estimate GFR may not apply to them because of low muscle mass, interference of medication with the markers for GFR or because their GFR is above 60 ml/min and creatinine will not be able to detect renal deterioration. \textit{Chapter 2} discusses the value of formulas using other markers than plasma creatinine, i.e. cystatin C and $\beta$-trace protein, to estimate GFR in Fabry patients treated with enzyme replacement therapy. The new non nuclear method to measure GFR with gadolinium (Gd-DTPA) as an alternative to $^{51}$Cr-EDTA is examined in \textit{chapter 3}, in which simultaneous GFR measurements with Gd-DTPA and $^{51}$Cr-EDTA are performed in renal transplant recipients, healthy kidney donors, HIV- and Fabry patients.

Since creatinine is cleared from the circulation by dialysis and continuous venovenous hemofiltration (CVVH), it cannot be used as a parameter of renal function in patients who receive this form of renal replacement therapy. However, in some situations, it would be very useful to be able to estimate residual renal function to determine if renal replacement therapy is still necessary. In \textit{chapter 4}, cystatin C is examined as an alternative marker for residual GFR in critically ill patients treated with CVVH because of acute renal failure.
REFERENCES


20. Kemperman FA, Silberbusch J, Slaats EH, Prins AM, Krediet RT, Arisz L. Follow-up of GFR estimated from plasma creatinine after cimetidine administration in


34. Stevens LA, Schmid CH, Greene T et al. Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m2. Am J Kidney Dis 2010; 56(3):486-495.


58. Bjarnadottir M, Grubb A, Olafsson I. Promoter-mediated, dexamethasone-


