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

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Gadolinium for the Assessment of Glomerular Filtration Rate?


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Submitted
ABSTRACT

Background Accurate measurement of renal function using gold standard techniques remains expensive, time consuming and cumbersome. Therefore, the search for cheaper and easier applicable endogenous and exogenous markers continues. Here we studied the performance of a recently developed method using functional immunoassay technology to determine the blood clearance of the nonradioactive marker gadolinium-DTPA (GFR_{Gd-DTPA}) to assess glomerular filtration rate (GFR).

Methods GFR_{Gd-DTPA} was measured in 33 patients at risk of or with overt renal failure. The GFR_{Gd-DTPA} results were compared to both the GFR determined with ^{51}Cr-EDTA (GFR_{^{51}Cr-EDTA}) and the estimated GFR using creatinine- and/or cystatin C-based formulas. The various estimated GFR and measured GFR methods were compared using Bland and Altman analysis. Bias was defined as the mean difference between reference GFR and investigated GFR. Limits of agreement were defined as mean difference -2SD and mean difference +2SD and precision as ± 1 SD. Accuracy was defined as the percentage of measurements that fell within 30% of the reference GFR.

Results Compared to GFR_{^{51}Cr-EDTA}, GFR_{Gd-DTPA} showed a bias of 9.3 ml/min/1.73m² and precision 16.7 ml/min/1.73m². Accuracy of GFR_{Gd-DTPA} was low with only 72.7% within 30% of GFR_{^{51}Cr-EDTA}. GFR estimated by formulas using creatinine and/or cystatin C correlated better with GFR_{^{51}Cr-EDTA} than with GFR_{Gd-DTPA}.

Conclusion In our study, assessment of GFR_{Gd-DTPA} with functional immunoassay technology did not prove to be accurate and precise enough. GFR_{Gd-DTPA} therefore seems to be unsuitable to replace GFR measurement with radioisotope based techniques.
INTRODUCTION

In patients at risk or with overt renal failure, adequate measurement of glomerular filtration rate (GFR) is warranted and often of utmost importance. In case of deterioration of renal function, treatment of the underlying cause and/or adjustment of medication must be initiated without delay.

Many methods are available to determine GFR. Radioisotopes have been studied as filtration markers because they can be used without continuous intravenous (IV) infusion. Moreover, analysis is relatively simple, compared to assessment of GFR with inulin, which is considered gold standard but cumbersome and time-consuming. Recently, GFR measurement using the MRI contrast agent Magnevist® has been proposed as an alternative nonradioactive method.

Apart from measuring GFR (mGFR), many formulas have been developed to estimate the GFR based on plasma creatinine values in combination with other clinical parameters such as weight, gender and age. One of the first and most frequently used formula was that of Cockcroft and Gault, introduced in 1976. Since then, many more formulas have been constructed to estimate GFR (eGFR) from surrogate markers of kidney function using plasma creatinine and more recently cystatin C levels.

In our hospital, the single bolus injection method with 51-chromium-ethylenediaminetetra-acetic acid (51Cr EDTA) is used as gold standard method to determine GFR. In search of a method to measure GFR without the use of radioisotopes, we tested the above mentioned method using a single injection with Gadolinium-Diethylene triamine penta-acetic acid (Gd-DTPA, Magnevist®) and compared this with mGFR using 51Cr EDTA and with eGFR using formulas containing plasma values of creatinine and/or cystatin C.

METHODS

Patients

Patients seen at the outpatient clinic of the Academic Medical Center were eligible to participate in the study in case of: (1) Otherwise healthy kidney donors, in need of a GFR measurement as part of preoperative evaluation, (2) Renal transplant recipients with stable transplant function, (3) HIV infected patients seen in the outpatient clinic who participated in an earlier GFR trial (Vrouenraets et al, manuscript submitted) and (4) Patients with Fabry disease, an X-linked lysosomal storage disease characterized by gradual deterioration in kidney function, in need of a yearly GFR measurement during treatment with enzyme replacement therapy. The patients had to be at least 18 years old, had to show stable renal function as estimated by a stable plasma creatinine during the previous year and had to give written informed consent to participate. Patients were excluded if their eGFR was < 30 ml/min/1.73m² because of the risk of nephrogenic systemic sclerosis, after administration of gadolinium based...
contrast agents\textsuperscript{16,17}. Other exclusion criteria were pregnancy or a known allergy to either \textsuperscript{51}Cr-EDTA or Gd-DTPA.

Institutional review board approval for the study was obtained. The study was conducted in accordance with the declaration of Helsinki.

### Measurement of GFR

Patients were instructed not to drink coffee or tea from the evening before and not to eat or drink protein rich products at the day of measurement, as these substances could interfere with GFR measurements. Diuretics were withheld on the day of GFR measurement. GFR was simultaneously measured using a single bolus injection with \textsuperscript{51}Cr-EDTA and a single bolus injection with Gd-DTPA.

GFR measurement using \textsuperscript{51}Cr-EDTA was performed according to the guidelines of the British Society of Nuclear Medicine\textsuperscript{25}. After a blood blank (P1) is drawn, a single bolus of 3.7 MBq \textsuperscript{51}Cr-EDTA was injected in an ante-cubital vein. The syringe was weighed and radioactivity was measured before and after injection to exactly determine the injected amount of \textsuperscript{51}Cr EDTA. Blood samples (P2-4, 10 ml each heparinized) were drawn at 2, 3, and 4 hours after the injection with \textsuperscript{51}Cr-EDTA. Samples P2-4 were taken from the contra-lateral ante-cubital vein. The samples were centrifuged and 4 ml of plasma was transferred into counting tubes. These were counted together with two 4 ml standards taken as aliquots from 1 MBq \textsuperscript{51}Cr-EDTA diluted to 500 ml. Activities of \textsuperscript{51}Cr-EDTA in the different samples were determined in a well-type scintillation counter. \textsuperscript{51}Cr-EDTA clearance was calculated using the slope intercept method, assuming a one-compartment model. After correction for overestimation\textsuperscript{18}, GFR was corrected for body surface area using the Haycock formula\textsuperscript{19} (mGFR\textsubscript{\textsuperscript{51}Cr-EDTA}).

Functional immunoassay technology (FIT) was used to determine GFR after a single bolus injection with Gd-DTPA (469.01 mg dimegluminegadopentetate/ml, Magnevist®) as described by Reinhardt et al\textsuperscript{6}. The blood clearance method was used to determine GFR. After the blood blank (P1) was drawn, a single bolus of 10 ml/kg 469.01 mg Gd-DTPA/ml was injected in the same ante-cubital vein as \textsuperscript{51}Cr-EDTA. The syringe containing Gd-DTPA was weighed before and after injection on an Exacta P5 Bovenweger scale (Optech). The exact injected amount (in ml) of Gd-DTPA was determined as follows: \((\text{weight syringe}_{\text{before}} - \text{weight syringe}_{\text{after}}) \times \text{density}_{\text{Gd-DTPA}}\) (g/ml), where the density of Gd-DTPA is 1.195 g/ml at room temperature. Blood samples (P2-5, 4.5 ml serum) were drawn 1, 2, 3, and 4 hours after injection. Samples P2-5 were taken from the contra-lateral ante-cubital vein. Samples were centrifuged and the serum was stored in the refrigerator at 4 °C until analysis (samples could be stored in the refrigerator for at least 6 months; personal communication, Reinhardt, January 2008).

Blood Gd-DTPA concentrations were determined by ELISA using a FIT-GFR kit of BioPAL, Inc., Worcester, MA. For calculation of GFR, a one-compartment blood clearance method was assumed. The data were fit to a one-exponential decay
function: Y=Be-bX. The function was integrated over the limits zero to infinity to obtain the area-under-the-curve (AUC): AUC=B/b (mg\*min/ml). Next, the GFR value was obtained by dividing the administrated dose by the AUC. GFR was corrected for body surface area using the Haycock formula\textsuperscript{19} (mGFR\textsubscript{Gd-DTPA}).

Estimation of GFR (eGFR)
GFR was estimated using the formulas listed in table 1. Plasma creatinine values were measured by an enzymatic PAP+ (phenol/4-aminoantipyrine) assay on a Roche Modular analyzer (Roche, Almere, the Netherlands). The plasma creatinine determinations were calibrated according to the IDMS traceable creatinine standard. Cystatin C levels were measured in heparinized plasma samples by the N Latex Cystatin C test kit, a particle-enhanced immunonephelometric method, on a BN ProSpec analyzer (Siemens, Breda, the Netherlands).

Table 1. Formulas used to estimate GFR.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Equation</th>
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<tbody>
<tr>
<td>re-expressed 4-variable (abbreviated) MDRD\textsuperscript{4}</td>
<td>GFR = 175 \times \left( \frac{\text{Pcr}}{88.4} \right)^{1.154} \times \text{age}^{-0.203} (Female: multiply result by 0.742, if African-American multiply result by 1.210)</td>
</tr>
<tr>
<td>CKD-EPI\textsuperscript{9}</td>
<td>Female and \text{Pcr} \leq 62 \text{ umol/l}: \text{GFR} = 144^{*} \times \left( \frac{\text{Pcr}}{88.4} \right) \times 0.7^{0.329} \times (0.993)^{0.993} \times \text{age} \times (Female: multiply result by 0.742, if African-American multiply result by 1.210) \times \text{age}</td>
</tr>
<tr>
<td>Hoek formula\textsuperscript{11}</td>
<td>\text{GFR} = -4.23 + 80.35/cysC</td>
</tr>
<tr>
<td>Stevens formula\textsuperscript{14}</td>
<td>\text{GFR} = 177.6 \times \left( \frac{\text{Pcr}}{88.4} \right)^{0.65} \times \text{cysC}^{0.57} \times \text{age}^{0.20} (female: multiply result by 0.82, black: multiply result by 1.11)</td>
</tr>
</tbody>
</table>

Statistical analysis
Statistical analyses were performed using SPSS16. Demographic data were expressed as median (range). For comparison of the various eGFR methods with mGFR, the Bland and Altman analysis was used\textsuperscript{20}. Bias was defined as the mean difference between reference GFR and investigated GFR. Limits of agreement were defined as mean difference -2SD and mean difference +2SD and precision as ± 1 SD. Accuracy was defined as the percentage of measurements that fell within 30% of the reference GFR. Differences in bias and accuracy of the various methods for GFR determination, were tested with the Wilcoxon signed ranks test and McNemar’s test, respectively. A p < 0.05 was considered to indicate a statistically significant difference.
### RESULTS

Forty-seven patients were included in this study: 8 candidate kidney donors, 22 renal transplant recipients, 7 HIV-1 infected patients and 10 patients with Fabry disease. The demographic data and the results of the measured GFR with the two methods are displayed in Table 2.

**Table 2.** Demographic data of 47 patients who underwent GFR measurement with $^{51}$Cr EDTA. Data are expressed as median (range).

<table>
<thead>
<tr>
<th></th>
<th>Kidney donors (n = 8)</th>
<th>Renal transplant recipients (n = 22)</th>
<th>HIV patients (n = 7)</th>
<th>Fabry patients (n = 10)</th>
<th>Total (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.1 (28.4–76.1)</td>
<td>58.3 (22.4–78.8)</td>
<td>48.8 (33.4–68.6)</td>
<td>48.8 (21.0–55.6)</td>
<td>52.8 (21.0–78.8)</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>2/6</td>
<td>12/10</td>
<td>7/0</td>
<td>6/4</td>
<td>27/20</td>
</tr>
<tr>
<td>$\text{GFR}_{51\text{Cr}-\text{EDTA}}$ (ml/min/1.73m$^2$)</td>
<td>86.7 (77.6–108.7)</td>
<td>48.3 (22.6–86.0)</td>
<td>80.3 (69.8–99.2)</td>
<td>88.5 (42.9–119.0)</td>
<td>69.8 (22.6–119.0)</td>
</tr>
<tr>
<td>$\text{GFR}_{\text{Gd-DTPA}}$ (ml/min/1.73m$^2$) (n)</td>
<td>76.6 (5.2–105.3)</td>
<td>44.8 (13.7–69.1)</td>
<td>79.7 (75.9–93.5)</td>
<td>77.0 (28.7–118.1)</td>
<td>51.2 (5.2–118.1)</td>
</tr>
</tbody>
</table>

In all patients GFR was measured with $^{51}$Cr-EDTA. However, only 33 patients could be included in the comparative analysis with Gd-DTPA: in 3 patients GFR Gd-DTPA could not be determined due to omission of blood withdrawal and in 11 patients, due to a slow inclusion rate, blood samples containing Gd-DTPA had been stored longer than 180 days (maximum number of days during which blood samples are allowed to be stored after collection until analysis of this parameter).

Figure 1 shows the Bland and Altman analysis of $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$ versus $m\text{GFR}_{\text{Gd-DTPA}}$. Figure 2 shows the Bland and Altman analysis of $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$ and $m\text{GFR}_{\text{Gd-DTPA}}$ versus eGFR.

Table 3 lists the accuracies of $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$ versus $m\text{GFR}_{\text{Gd-DTPA}}$ and of $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$ and $m\text{GFR}_{\text{Gd-DTPA}}$ versus eGFR. Compared to $^{51}\text{Cr}$-EDTA, Gd-DTPA showed a bias of 9.3 ml/min/1.73m$^2$. However, the limits of agreement were wide (-24.2 to 42.8 ml/min/1.73m$^2$). When the most extreme outlier was excluded from the analysis, bias (limits of agreement) improved to 7.0 (-13.8 to 27.8) ml/min/1.73m$^2$. Accuracy was low, 72.7% was within 30% of $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$. GFR estimated by either creatinine and/or cystatin C corresponded better with $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$ than with $m\text{GFR}_{\text{Gd-DTPA}}$. Of the equations used to estimate GFR, GFR estimated with the CKD-EPI formula demonstrated the overall best accuracy and precision in comparison to $m\text{GFR}_{51\text{Cr}-\text{EDTA}}$. 


Figure 1. Bland and Altman analysis of measured GFR with 51Cr-EDTA versus measured GFR with Gd-DTPA (n = 33).

Table 3. Comparison between the accuracy (percentage of measurements within 30% of reference GFR) of GFR measured with $^{51}$Cr-EDTA and Gd-DTPA respectively (n = 33). * McNemar’s test.

<table>
<thead>
<tr>
<th>$^{51}$Cr-EDTA versus</th>
<th>% within 30%</th>
<th>Gd-DTPA versus</th>
<th>% within 30%</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-DTPA</td>
<td>72.7</td>
<td>MDRD</td>
<td>39.4</td>
<td>0.001</td>
</tr>
<tr>
<td>MDRD</td>
<td>72.7</td>
<td>CKD-EPI</td>
<td>39.4</td>
<td>0.003</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>72.7</td>
<td>Hoek</td>
<td>28.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Hoek</td>
<td>56.3</td>
<td>Stevens</td>
<td>21.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Stevens</td>
<td>62.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIT GFR

No adverse events were observed during and after the administration of Gd-DTPA (Magnevist®). After a median (range) follow-up of 523 (118 – 810) days, there were no reports of nephrogenic systemic sclerosis.

After exclusion of the samples containing Gd-DTPA stored beyond 180 days, the mean time to analysis of was 47 (5 - 175) days (i.e. storage time). When all samples were combined (included those stored longer than 180 days) there was a significant correlation between storage time and bias (mGFR$_{^{51}$Cr-EDTA} - mGFR$_{Gd-DTPA}$, figure 3) This correlation disappeared when the storage time was reduced to a maximum of 120 days.

DISCUSSION

We measured GFR using a recently proposed nonradioactive method with the MRI contrast agent Gd-DTPA (Magnevist®) and used the $^{51}$Cr-EDTA based GFR as a reference. Bias and precision of the Gd-DTPA based method deviated significantly
from the $^{51}$Cr-EDTA based GFR. Accuracy of mGFR$_{Gd-DTPA}$ was low with only 72.7% within 30% of mGFR$_{^{51}Cr-EDTA}$. GFR measurement with Gd-DTPA versus mGFR$_{^{51}Cr-EDTA}$ performed similar or worse in comparison to estimated GFR with formulas using creatinine and/or cystatin C versus mGFR$_{^{51}Cr-EDTA}$. Furthermore, eGFR correlated better with mGFR$_{^{51}Cr-EDTA}$ than with mGFR$_{Gd-DTPA}$.

Measurement of GFR using Gd-DTPA was recently proposed as an alternative, nonradioactive, method to assess renal function$^6$. In a population of 20 patients with a GFR ranging from 10.9 to 102.1 ml/min/1.73m$^2$, among whom 12 renal transplant recipients, concentrations of Gd-DTPA were determined using a simple ELISA of blood and urine samples taken 1, 2, 3 and 4 hours following a single bolus injection of Gd-DTPA. Thereafter, GFR was calculated using the blood clearance method,
assuming a one-compartment model or from the classic UV/P method. An excellent performance of this Gfr method was found in comparison to gold standard Gfr measurement using $^{125}$Iothalamate. Although our study population was comparable, we could not reproduce these results.

Reinhardt et al demonstrated that, in comparison to the blood clearance Gfr calculation, the UV/P method showed a (non significant) better accuracy (90 versus 95%, in their study defined as percentage within 20% of reference Gfr, and better precision (3.0 versus 6.2 ml/min/1.73m$^2$) in their study defined as percentage within 20% of reference Gfr, and better precision (3.0 versus 6.2 ml/min/1.73m$^2$)6. Although there was a trend in better accuracy in favor of the UV/P method we chose the blood clearance method, since this method is less cumbersome. An explanation for the difference in accuracy (72.7% within 30% and only 63.6 within 20% of mGfr$_{^{51}Cr}$-edta) and precision (16.7 ml/min/1.73m$^2$) between our study and the study of Reinhardt et al. (90% and 6.2 ml/min/1.73m$^2$, respectively) could be the storage time of the samples. Even when we excluded the samples analyzed beyond the maximum storage time of 180 days, there was an inverse correlation between bias and storage time of the samples (figure 3), resulting in a significant underestimation of Gfr when storage time increased. This correlation only disappeared when storage time was limited to 120 days or less. This effect most probably points to degradation of Gd-DTPA in the samples over time.

Another possibility that could contribute to the differences between our study and the study of Reinhardt, is the fact that we used another radioisotope based method as reference Gfr, namely $^{51}$Cr-EDTA instead of $^{125}$Iothalamate. Although not often compared ‘head to head’, measured Gfr can vary when different markers are applied $^{21, 22}$. Next to inulin, radioactive markers are considered gold standard. However, tubular secretion is suggested for $^{125}$Iothalamate $^{22}$ and extrarenal clearance for DTPA and $^{51}$Cr-EDTA$^{23, 24}$. There are indications that DTPA clearance is systemically higher

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**Figure 3.** Correlation between bias (GFR$_{^{51}Cr}$-EDTA – GFR$_{Gd}$-DTPA) and delay (in days) between the date the Gd-DTPA samples were obtained and the date the Gd-DTPA samples were analyzed (storage time). Correlation disappeared when the storage time was reduced to 120 days or less
than that of EDTA\textsuperscript{25, 26}, although we found underestimation of Gd-DTPA clearance in comparison to that of \textsuperscript{51}Cr-EDTA. Nevertheless, the various formulas to estimate GFR corresponded better with mGFR using \textsuperscript{51}Cr-EDTA than mGFR using Gd-DTPA. This let us to conclude to prefer mGFR\textsubscript{\textsuperscript{51}Cr-EDTA} over mGFR\textsubscript{Gd-DTPA}.

Nephrogenic systemic fibrosis (NSF) is a much feared complication of gadolinium administration in the context of MRI in patients with a reduced GFR (< 30 ml/min/1.73m\textsuperscript{2})\textsuperscript{27, 28}. The incidence of NSF depends on the total dose of administered gadolinium; GFR measurement using Gd-DTPA needs a gadolinium dosage that is only 5% of the dose used with MRI. Despite this low dose of gadolinium administration, we excluded patients from the study with an eGFR of less than 30 ml/min/1.73m\textsuperscript{2}. However, two patients turned out to have a mGFR of 22.6 and 28.1 ml/min/1.73m\textsuperscript{2}, both renal transplant recipients. None of the patients developed NSF during a median (range) follow-up of 530 (145 -810) days.

Exact measurement of GFR remains difficult and even gold standard methods can result in different values of renal function. One may even wonder if exact determination is possible and whether an observed decline in measured GFR actually reflects a pathologic decrease in kidney function. For, many physiologic factors can influence GFR for example fluid intake, diet and the circadian rhythm\textsuperscript{29}. Nevertheless, functional immunoassay technology using the nonradioactive MRI contrast agent gadolinium-DTPA, recently proposed as a simple and easy applicable method for the assessment of renal function, appeared in our hands, safe but unsuitable to replace GFR measurement with radioisotope based methods in clinical practice.

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