Glomerular Filtration Rate (GFR) determination via individual kinetics of the inulin-like polyfructosan sinistrin versus creatinine-based population-derived regression formulae

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Abstract

Background
In renal patients estimation of GFR is routinely done by means of population-based formulae using serum creatinine levels. For GFR determination in the creatinine-blind regions or in cases of reno-hepatic syndrome as well as in critical cases of live kidney donors individualized measurements of GFR (mGFR) employing the kinetics of exogenous filtration markers such as the inulin-like polyfructosan sinistrin are necessary. The goal of this study is to compare mGFR values with the eGFR values gained by the Modification of Diet in Renal Disease (MDRD4) and Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) formulae.

Methods
In 170 subjects comprising persons with normal renal function or with various stages of kidney diseases (CKD 1-4) GFR was measured by application of intravenous bolus of sinistrin and assessment of temporal plasma concentration profiles by means of pharmacokinetic methods (mGFR). Comparison of mGFR with MDRD4 – and CKD-EPI-derived eGFR values was performed by means of regression analysis.

Results
Good agreement of mGFR and eGFR values was observed in patients with poor renal function (GFR below 60 ml/min/1.73m²). In cases of normal or mildly impaired renal function, GFR determination by MDRD4 or CKD-EPI tends to underestimate GFR. Furthermore, in such individuals a large spread of eGFR values presents a particular difficulty.

Conclusions
For routine purposes or for epidemiological studies in cases of poor renal function eGFR methods are generally reliable. But in creatinine-blind ranges (GFR above 60 ml/min/1.73m²) eGFR values are unreliable and should be replaced by clinically and physiologically suitable methods for mGFR determination.
**Background**

For routine applications or for epidemiological studies, GFR values are estimated on the basis of individual serum creatinine measurements by means of regression formulae, e.g., the MDRD4 and CKD-EPI formulae [1-4]. These so-called eGFR formulae are derived from population-data describing the relations between GFR values obtained by a kinetic methods (mGFR) and the corresponding concentrations of serum creatinine [5].

Unfortunately, these formula-derived GFR-values are problematic in individuals with normal or mildly impaired renal function. Several reasons are responsible for this problem, such as, e.g., the natural variation of the original population data in combination with the confidence intervals increasing with decreasing creatinine concentrations, and the increasing impact of analytical uncertainties of creatinine measurements particularly in low concentration ranges. In addition, disturbed creatinine production, e.g. in patients with liver diseases, may also cause severe limitations for the application of creatinine-based eGFR formulae. Rather, formulae-derived GFR estimations tend to be of use in the presence of moderately or severely impaired renal function, i.e. GFR below 60 (ml/min)/1,73 m² only [6, 7].

For accurate assessment of renal function above this threshold pharmacokinetics of exogenous markers such as $^{125}$-iothalamate or $^{51}$-EDTA or inulin should be used [8]. Sinistrin, an inulin-like polyfructosan, is a physiologically and clinically advantageous GFR marker, which is only filtered by the glomeruli and is metabolically inert [9-12]. In contrast to the original ‘gold standard’ method of constant intravenous infusion of inulin for determination of glomerular filtration rate [13], modern techniques use intravenous bolus injections of test substances and algorithms for fitting of bi-exponential functions to the temporal concentration profiles observed. GFR is determined by dividing the dose applied through the area under the curve estimated with the fitted function parameters [14]. Attainment of a steady state is not required, and only raw guesses of the kinetic constants are required before starting the fitting algorithm.

The aim of the present study is to compare the ranges of validity and the information content which can be gained from the commonly used formulae-based estimations of GFR (MDRD4 and CKD-EPI) with a kinetic technique using
sinistrin in a single injection application as standard for comparison, particularly in CKD 1-2. Thereby, the paper emphasizes the use of a kinetic method employing a physiological marker especially suitable for the renal evaluation of live kidney donors or kidney transplant patients. To our knowledge this paper represents the first report on the application of this kinetic procedure in subjects with normal renal function as well as in patients with chronic kidney disease (CKD 1-4).

**Methods**

All of the included measurements were performed during routine visits within the standard care program of the Division of Nephrology and Hemodialysis at the Medical University of Graz. An ethical approval was obtained from the ethical committee of the Medical University of Graz to use the results of this examinations for the presented study.

**Subjects.**

Clearance measurements were performed in 170 subjects (100 females, 70 males), mean age 45 years (range 18.7 – 89.5), mean serum creatinine 1.22 mg/dl (range 0.5 – 6.7), with different underlying diseases: 12 subjects were screened prior to living kidney donation, and 36 patients after kidney transplantation. 35 patients suffering from breast cancer were tested prior to chemotherapy, 9 patients with kidney stones prior to lithotripsy, 32 patients had mild hypertension, 14 diabetes, 21 glomerulonephritis, 5 patients had a history of unilateral nephrectomy, 5 patients were tested after recompensation of an acute renal failure, and 1 subject had a unilateral duplex kidney. Each subject gave written informed consent.

**Clinical procedures and techniques of clinical chemistry.**

Serum creatinine was determined by the Jaffe method (Hitachi, Roche Diagnostic GmbH, Mannheim, Germany) using a kinetic colorimetric assay. The temporal concentration profiles of sinistrin (Inutest®; Fresenius-Kabi, Linz, Austria) after intravenous injection of a bolus of 2500 mg were determined by drawing samples from venous blood and centrifuging the samples. In these
samples the initial concentration of blood glucose was determined. Measuring
the marker concentrations was done as follows: sinistrin was hydrolyzed to
yield fructose monomers. Fructose was enzymatically converted to glucose.
The latter was enzymatically oxidized using NAD, and the resulting NADH was
assessed photometrically by the extinction of UV [15]. The difference between
the total glucose concentration and the initial one is calibrated to constitute a
measure of the sinistrin concentration. The results for sinistrin clearance are
given standardized in (ml/min)/1,73 m².

Model description and biometric methods

Experiments with sinistrin have shown that its elimination kinetics after a bolus
injection can be adequately described by a two-compartment model as
depicted in Fig. 1 [16]. The well-perfused part of the extracellular fluids is
considered as the central volume into which the exogenous marker sinistrin is
injected, and from which it is on the one hand exchanged with the so-called
peripheral compartment comprising the less perfused part of the interstitium,
and on the other hand eliminated via the kidneys [17]. Mathematically such a
system can be represented by the well-known two-compartment model of
pharmacokinetics containing characteristic system constants, namely, the
relative transfer rates for the substance exchanges between the two
compartments, the eliminating flow from the central compartment to the
outside and the volume of the central compartment. From these system
constants, or parameters in a mathematical sense, can be derived other model
parameters such as the clearance (GFR), the peripheral volume, and a
characteristic retention time in the peripheral volume. GFR especially is
determined as the product of the relative rate of elimination and the central
volume.

The adaptation of the two-compartment model to the experimentally
determined kinetic data profile, yielding the system constants as well as their
respective standard estimation errors due to the noise in the experimental
data, was done by embedding the analytical solution of the model [18] into a
nonlinear regression procedure [19]. However, it could also be done by
employing a commercially available pharmacokinetic software package (SAAM
II, Software Application for Kinetic Analyses, Version 2.0, ©University of
Washington) [20].
GFR estimations based on serum creatinine were performed by means of the MDRD4 – and CKD-EPI formulae [21]. The abbreviated four-variable MDRD eGFR was calculated as follows:

eGFR (ml/min/1.73m²) = 186 x SCr^{-1.154} x age^{-0.203} (x 0.742 if female) (x 1.21 if black).

The CKD-EPI GFR was calculated gender specifically and stratified by creatinine levels according to the following equations:

Female with SCr ≤ 0.7 mg/dl
eGFR (ml/min/1.73m²) = 144 x 0.993^{Alter} x (SCr / 0.7)^{-0.329} (x 1.15 if black)

Female with SCr > 0.7 mg/dl
eGFR (ml/min/1.73m²) = 144 x 0.993^{Alter} x (SCr / 0.7)^{-1.209} (x 1.15 if black)

Male with SCr ≤ 0.9 mg/dl
eGFR (ml/min/1.73m²) = 141 x 0.993^{Alter} x (SCr / 0.9)^{-0.411} (x 1.16 if black)

Male with SCr > 0.9 mg/dl
eGFR (ml/min/1.73m²) = 141 x 0.993^{Alter} x (SCr / 0.9)^{-1.209} (x 1.16 if black)

Comparison between kinetically measured mGFR and creatinine-based eGFR values was performed by linear regression and correlation techniques. Regression lines and their 95%-confidence limits were determined using MATHCAD [22, 23].

Results
In order to illustrate the method chosen for the measurement of GFR, the result of fitting a pharmacokinetic two-compartment model to the observed temporal concentration profile of sinistrin is given for an individual with normal renal function (Fig. 2).

The correlation between kinetically determined GFR and creatinine-based eGFR globally reveals a poor correspondence between the two kinds of values, since the regression line does not indicate equivalence of the two kinds of values (Fig. 3, Table 1, total GFR range).

However, by sorting the data into a range of poor renal function and another one of mildly reduced or normal renal function (on the basis of the kinetically
determined GFR values), it can be seen from the regression analyses that despite considerably strong individual deviations, there is, on average, a much better correspondence between the two kinds of GFR values in the range of poor renal function (Fig. 4, Table 1, GFR range < 60 ml/min). In the range of poor renal function the kinetic method of GFR determination and the MDRD4-as well as the CKD-EPI-formulae GFR estimation show a high correlation of the two kinds of GFR values which confirms the correctness of both kinds of estimates in the range of low renal function. In the range of mildly restricted or normal renal function, the formula-based estimates of GFR values are to be expected as insecure because of the large spread of the data points and because of the almost invariant level for the low serum creatinine values due to the inverse character of the relation between serum creatinine levels and measured renal function. There is almost no equivalence in the range of good renal function (Fig. 5, Table 1, GFR range > 60 ml/min).

There is no real difference between the MDRD4- and the CKD-EPI-methods over the GFR ranges studied. As can be seen from Table 1, the results of the regression analyses using either MDRD or CKD-EPI-eGFR values as dependent variable are nearly identical, both over the total GFR range and in the subranges below and above 60 ml/min/1.73m². As expected, therefore, a linear regression analysis between MDRD and CKD-EPI results yields a nearly perfect agreement with an intercept of 0.24 (95% c.i., -2.0 – 2.5), a slope of 0.96 (0.93 – 0.98) and a correlation coefficient of 0.98 (0.976 – 0.987). There are also no significant differences in the overall diagnostic powers of the two eGFR formulae, taking 60 ml/min/1.73 m² as GFR cut-off value gained by the kinetic procedure. Thus, for the MDRD-formula the diagnostic specificity is 0.875, whereas for the CKD-EPI-formula it is 0.891. The diagnostic sensitivity for the MDRD-formula is 0.902, whereas for the CKD-EPI-formula it is 0.878.

Discussion

The purpose of the present study is to compare the application of compartment analysis techniques using kinetic data of the GFR marker sinistrin with formula-derived GFR estimates based on creatinine levels. The kinetic procedure used here as a standard for comparison, has been validated previously by successfully predicting in individual patients the concentration-profile of a constant infusion experiment using results obtained by a bolus experiment beforehand. In the present paper the model-derived GFR values
were used to assess the reliability of eGFR estimates obtained by the MDRD4- and CKD-EPI-formulae in individuals with poor renal function as well as in subjects with normal or only mildly disturbed kidney function. Similar problems have been studied in the literature [24, 25]. A weak point of the present study is that a retrospective data base arising from everyday clinical routine had to be used. However, similar results as in Fig. 3 were presented previously using GFR values gained with constant infusion of inulin versus creatinine based eGFR values [26].

In patients with high serum creatinine values eGFR methods may suffice despite many objections against it [27-30]. As Table 1 shows, there are no relevant differences between the GFR estimates gained by either the MDRD4 or CKD-EPI formulae. The necessity for kinetic methods, however, arises in the ranges of low serum creatinine because of the flatness of the formula-based curves describing the relation between serum creatinine values and adherent measured GFR values [31, 32]. For long-term studies kinetic techniques allow one to determine measures of the error bounds of the characteristic system constants [33]. For a critical judgment of the measured values in individual patients reliable error measures of the various pharmacokinetic parameters as they can be gained only by a model-fitting procedure are indispensable.

Besides the inherent weakness of eGFR values in the creatinine-blind ranges of low creatinine levels associated with laboratory errors, physiological aspects are also to be considered in judging renal function. Especially individual variations in distribution volumes are not taken into account by eGFR formulae. The application of an exogenous marker such as sinistrin and the appropriate model-identification of the kinetics involved enables one to determine the individual mGFR and measures of the distribution process such as the extracellular volume in the individual subject [34]. Additionally, estimation of the precision of the fitted system constants is easily possible in kinetic methods by means of sensitivity analysis. No such feature is offered by GFR determinations based on single marker concentrations, such as that of creatinine.

Sinistrin is a clinically and physiologically suitable marker and the bolus method assessed by compartmental kinetic analysis is a both reliable and clinically practicable procedure for the measurement of GFR. This is of utmost
importance in subjects requiring higher than routine accuracy and precision as, e.g., in situations of live kidney donor evaluations [35]. As stated in the Amsterdam consensus guidelines [36] kidneys from live donors with a GFR < 80 ml/min are associated with relative risk of graft loss of 2.28 compared to those with higher GFR prior to nephrectomy. In this context GFR measurement applying sinistrin kinetics appears to be of particular use for exact determination of GFR in subjects with normal or mildly impaired renal function. In subjects with normal or close to normal renal function commonly used GFR equations based on serum creatinine are unreliable and thus should be avoided for the evaluation of live kidney donors.

Conclusion

For correct determination of GFR, particularly in subjects with normal or slightly impaired renal function (CKD 1-2), kinetic procedures using an exogenous physiologically and clinically suitable marker such as sinistrin present themselves as individualized GFR measurements in contrast to population-derived estimations by means of formulae employing endogenous markers. Additionally, a model-based technique enables one to assess consecutive clearance tests with a pharmacological or dietetic load in between. Such tests can be used for obtaining information on the properties of the renal functional reserve and the renal micro-vasculature, e.g., on changes in its permeability caused by renal injuries [37]. Dynamic renal function testing of this sort is not possible by means of endogenous markers [38].

Summarizing it can be stated that both the accuracy and precision of mGFR values achievable by the kinetic procedure using sinistrin as exogenous marker as well as the possibility for evaluation of the renal functional reserve appear to be of decisive importance in cases of live kidney donors. Finally although so far eGFR methods using creatinine levels only have been considered as sufficiently satisfactory, recent developments suggest combinations of creatinine and cystatin C in regression formulae as necessary improvements in GFR estimations [39]. However, any such progress still suffers from the drawback of employing statistical formulae derived from population data based on endogenous markers and not from individual kinetic measurements based on exogenous markers.

The authors declare that they have no competing interests.
Authors' contributions

SZ made substantial contributions to conception and design and performed GFR measurements. WS, GR and WE performed statistical analysis, contributed to interpretation of data, and revising critically for important intellectual content. AM performed analytical procedures, DW performed GFR measurements and ARR made substantial contributions in drafting the manuscript and revising it critically for important intellectual content and has given final approval of the version to be published. The final version of this paper was approved by all authors.

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References


**Figures**

**Figure 1 Two-compartment model**

*Fig. 1:* Two-compartment model of sinistrin distribution and elimination.

**Figure 2 Temporal concentration profile of sinistrin**

*Fig. 2:* Temporal profiles of observed (small circles) and model-adapted concentration of sinistrin(smooth line) after an intravenous bolus of 2500 mg sinistrin in a normal subject. The measured concentration profile is represented by circles (o), whereas the model-adapted one is represented by the smooth line (-).

**Figure 3 Regression lines for eGFR and mGFR for 0 < GFR < 150 ml/min/1.73m²**

*Fig. 3:* Comparison of kinetically determined GFR and creatinine-based eGFR values (MDRD, left; CDK-EPI, right). Shown are the individual measurements as well as the regression lines and their 95% confidence intervals.

**Figure 4 Regression lines for eGFR and mGFR for 0 < GFR < 60 ml/min/1.73m²**

*Fig. 4:* Regression line together with 95%-confidence interval curves of kinetic and GFR value gained with the MDRD formula (left) and with the CKD-EPI formula (right) in patients with impaired renal function.

**Figure 5 Regression lines for eGFR and mGFR for 60 < GFR < 150 ml/min/1.73m²**

*Fig. 5:* Regression line together with 95%-confidence interval curves of kinetic and estimated GFR value gained with the MDRD formula (left) and the CKD-EPI formula (right) in patients with mildly impaired CKD 1-2) or normal renal function.
### Table 1 - MDRD and CKD-EPI Results

Table 1: Results of the linear regression analyses of the creatinine-based eGFR values (dependent variable) versus the kinetically determined GFR values (values in parentheses are the 95% confidence limits).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>Slope</th>
<th>R*</th>
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<tbody>
<tr>
<td><strong>Total GFR range</strong></td>
<td></td>
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<tr>
<td>MDRD (Fig. 3, left)</td>
<td>14.9 (7.0 – 22.9)</td>
<td>0.64 (0.55 – 0.72)</td>
<td>0.76 (0.69 – 0.82)</td>
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<tr>
<td>CKD-EPI (Fig. 3, right)</td>
<td>14.7 (6.9 – 22.5)</td>
<td>0.67 (0.59 – 0.75)</td>
<td>0.78 (0.72 – 0.84)</td>
</tr>
<tr>
<td><strong>GFR range &lt; 60 ml/min</strong></td>
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<tr>
<td>MDRD (Fig. 4, left)</td>
<td>-6.7 (-24.4 – 11.0)</td>
<td>1.03 (0.66 – 1.41)</td>
<td>0.67 (0.45 – 0.81)</td>
</tr>
<tr>
<td>CKD-EPI (Fig. 4, right)</td>
<td>-9.8 (-29.5 – 9.9)</td>
<td>1.13 (0.71 – 1.55)</td>
<td>0.66 (0.44 – 0.80)</td>
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<tr>
<td><strong>GFR range &gt; 60 ml/min</strong></td>
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<tr>
<td>MDRD (Fig. 5, left)</td>
<td>29.2 (13.3 – 45.2)</td>
<td>0.51 (0.38 – 0.64)</td>
<td>0.52 (0.38 – 0.64)</td>
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<tr>
<td>CKD-EPI (Fig. 5, right)</td>
<td>29.6 (14.5 – 44.7)</td>
<td>0.55 (0.40 – 0.69)</td>
<td>0.56 (0.43 – 0.67)</td>
</tr>
</tbody>
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*R, linear correlation coefficient.
Figure 1

Dose of sinistrin application

Central volume

Relative rate of sinistrin transfer from central to peripheral volume

Relative rate of sinistrin transfer from peripheral to central volume

Relative rate of sinistrin elimination via kidneys

Peripheral volume
Figure 3