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Estimating Analytical Errors of Glomerular Filtration Rate Measurement

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BACKGROUND: Few studies are available on how to optimize time points for sampling and how to estimate effects of analytical uncertainty when glomerular filtration rate (GFR) is calculated.

METHODS: We explored the underlying regression mathematics of how analytical variation of a kidney filtration marker affects 1-compartment, slope-and-intercept GFR calculations, using 2 or 3 time points following a bolus injection, and used this to examine the results from 731 routine 3-point iohexol plasma clearance measurements.

RESULTS: GFR calculations inflated analytical uncertainty if the time points were taken too late after the bolus injection and too close after each other. The uncertainty in GFR calculation was, however, the same as the analytical uncertainty if optimal time points were used. The middle of the 3 samples was of little value. The first sample should be taken as early as possible after the distribution phase. Sampling before the patient specific half-life of the kidney filtration marker resulted in an exponential error inflation whereas no error inflation was seen when sampling occurred later than 2 half-lives. Theoretical GFR uncertainty could be lowered 3.2-fold if individually optimized time points for sampling had been used in our 731 clearance measurements. Using Taylor expansions to approximate the moments of transformed random variables, the uncertainty of an individual GFR measurement could be calculated in a simple enough way to be applicable by laboratory software.

CONCLUSIONS: We provide a theoretical foundation to select patient-optimal time points that may both limit errors and allow calculation of GFR uncertainty.

Introduction

The glomerular filtration rate (GFR) is often used for dosing of chemotherapeutics (1), to follow treatment effects, confirm the status of chronic kidney disease and follow its progress, evaluate renal function in kidney donors, and to determine future risk of disease (2). It is therefore important that GFR is accurately measured.

GFR is often measured by the rate of elimination of a kidney filtration marker that only resides in the extracellular space, has very limited protein binding, is chiefly eliminated by excretion, and is not reabsorbed. The most frequently used kidney filtration markers are iohexol (2–5), ^{51}Cr-EDTA, Diethylenetriamine pentaacetate, iothalamate (6, 7), and inulin. Renal inulin clearance measured under continuous inulin infusion and urine collection is regarded as the "gold standard" but the rate of elimination of kidney filtration markers from plasma without urine collection is often a sufficiently good measure of the GFR.

One way to determine GFR is to measure the area under the plasma concentration elimination time function (also known as area under the curve, AUC) after a bolus injection of a given dose of a kidney filtration marker. Mean GFR during the elimination is then simply the injected amount of the kidney filtration marker divided with AUC. The situation is similar to that of the traveled distance divided by the area under the velocity function giving the mean speed.

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If the AUC is determined from multiple time points (dotted line, Fig. 1A), the mean GFR during the elimination of the kidney filtration marker will be correctly determined with little error. This multi-point GFR is considered as a reference method when errors in other GFR protocols are assessed (7, 8).

Because it is impractical to collect a large number of samples, simplified versions like the slope-and-intercept GFR have been developed (9, 10) where 2 to 4 samples are collected when the kidney filtration marker has had time to mix with the extracellular water and its decreasing concentration with time is only due to its filtration by the kidneys, which is called the elimination phase (Fig. 1A). The AUC under the triangle formed by the regression line and its intercepts is calculated, and an adjustment is made for the concentration peak that occurs directly after injection before the kidney filtration marker has had time to distribute and mix with extracellular body water, which is called the distribution phase (Fig. 1A) (9).

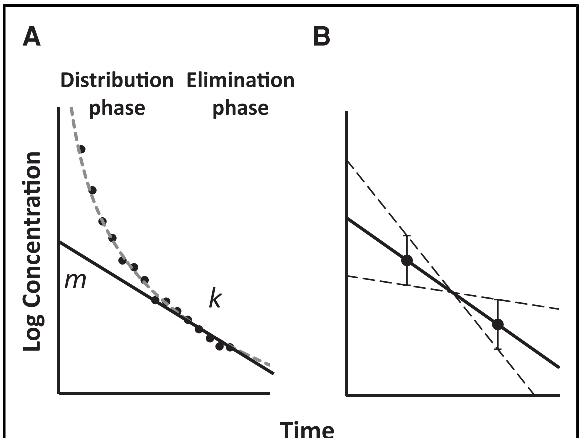

Fig. 1. Visual representation of a time–concentration plot following a bolus injection of a kidney filtration marker. (A), Actual time–iohexol concentration plot with sufficient number of samplings and optimal regression (gray dotted line). The GFR is the injected amount of the kidney filtration marker divided by the area under the dotted time–concentration plot (AUC). When this number of samples is collected, the AUC can be correctly determined and the error in the GFR calculation will be low. The solid line shows 1-compartment regression from the same data. The AUC from this regression is used to calculate the patients GFR. The missing area during the distribution phase is compensated using factorization (9). (B), Demonstration of a 2-point, 1-compartment regression and its variation due to analytical uncertainty. Given an analytical uncertainty, the AUC and the intercept (*m*) will vary in the way shown by the dashed lines. It is this uncertainty that is examined in this study.

The slope-and-intercept GFR method, however, generates an uncertainty in the AUC determination since it does not measure the AUC directly, but infers it from the slope (*k*) and the regression line intercept (*m*) (Fig. 1A). Analytical errors then tend to inflate the errors by the regression mathematics (Fig. 1B and online Supplemental Fig. 1) (10, 11).

Studies of the uncertainty of slope-and-intercept GFR show that the main cause of deviation from multi-point GFR is poor modeling of the true AUC, which results in a mean difference of around ±10%. Plotted data, however, show that GFR may deviate up to 70% in individual cases (8). Unfortunately, all quality control methods to find these outliers have failed (8).

Analytical errors, variation in the injected amount of the kidney filtration marker, and variations in measured time since injection adds errors to the GFR calculations, which amount to 8% to 10% in repeated measurements on individuals with normal GFR (12, 13). GFR uncertainty inflates due to the regression mathematics if the time points are taken too close to each other during the elimination phase (14, 15). Most routine laboratories that measure slope-and-intercept GFR use 2, 3, or 4 venous blood samples collected at between 2 and 5 h post-injection unless GFR adjusted for body surface area, relative GFR, is expected to be <30 mL/min/1.73 m^2 (7, 16). Simulation studies show that if GFR is <60 mL/min/1.73 m^2, the error inflation becomes problematic (10) and that later sampling times, for instance a 24 h sample, can mitigate this problem (11).

Despite a rich literature describing slope-and-intercept GFR methods, we failed to find studies that describe in a useful way how analytical uncertainty affects the slope-and-intercept GFR method and how to choose optimal time points for sampling, although thorough work on this has been done for the 1-point method (17). Here we explored the mathematics of how analytical uncertainty of a kidney filtration marker affected GFR calculations.

Materials and Methods

MATHEMATICAL FRAMEWORK

The mathematical framework examined how 1-compartment, slope-and-intercept GFR calculation (9) expanded a given measurement error. The expanded coefficient of variation (CV) of the GFR (CV_{GFR}) was derived from the measurement imprecision (CV_{method})

of the plasma concentration of a kidney filtration marker. A simplified description of the 1-compartment, slope-and-intercept GFR calculation and how the regression mathematics inflates errors is shown in Fig. 1A, and Supplemental Fig. 1 and Appendix A in the online Supplemental Material. The errors from GFR calculations given a measurement CV are denoted CV_{GFR}. The actual CV_{GFR} was derived from simulations described in the online Supplemental Material, where 1-compartment, slope-and-intercept GFR calculations from 100 000 trials with fixed time points, a fixed terminal slope (k), and a randomly distributed measurement error similar to previous studies of the errors of GFR calculations were employed (14, 15). The standard deviation (SD) from these 100 000 GFR calculations was then divided by the mean from the same data to calculate CV_{GFR} for this particular set of time points and terminal slope. The online Supplemental Material also includes a description of one way to calculate an approximate CV_{GFR} using mathematical formulae derived from Taylor expansions for the moments of transformed random variables that, in contrast to simulations, potentially can be used in conventional laboratory software. All equations and mathematics are provided in the online Supplemental Material.

STUDY GROUP

All 731 patients with a GFR $\geq$10 mL/min/1.73 m^2 undergoing iohexol clearance measurements with the 3-point method between February 1, 2020 and October 20, 2021, at the Sahlgrenska University Hospital, Gothenburg, Sweden and that had complete data sets, were included. During this time, the administration of iohexol and blood sampling were carried out by the same facility. All patients received an intravenous bolus dose of 3235 mg iohexol (5 mL Omnipaque at 300 mg I/mL) and venous blood samples were drawn using serum gel vacuum tubes, most often after approximately 3, 4, and 5 h (online Supplemental Table 1). Information on the iohexol clearance data was retrieved from the hospital's laboratory database. Sex, age, weight, length, sampling times, iohexol concentrations, and estimated GFR (eGFR) from either creatinine or cystatin C concentrations were available for all patients (online Supplemental Table 1). All patient data were anonymized before analysis in MATLAB and CV_{GFR} were calculated as described in the online Supplemental Material.

CHEMICALS AND INSTRUMENTATION

Unless otherwise indicated, the chemicals used were of pro-analysis grade or above and obtained from Merck KGaA. Iohexol (Omnipaque 300 mg I/L) and ioversol (Optiray, 300 mg I/L) were obtained from GE Healthcare AB and from Gothia Medical AB, respectively. Solvents and additives used for the mobile phases were of LC-MS grade. Ultra-pure water (>18 MΩ/cm) was prepared inhouse using a Milli-Q water purification system from Merck.

IOHEXOL MEASUREMENTS USING LC-MS/MS

Iohexol was quantified in serum samples by Ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) using a modified version of a previously described method (18). The details of the modified method and its analytical performance (19) are described in the online Supplemental Material.

CREATININE AND CYSTATIN C MEASUREMENTS

Cystatin C and creatinine were analyzed on the serum sample obtained before the iohexol administration. Cystatin C was analyzed on a Roche Cobas 6000 (Roche Diagnostics) using the Tina-quant Cystatin C Gen.2 reagent (Roche). Creatinine was analyzed on an Alinity c (Abbot) using the Alinity c Creatinine (Enzymatic) Reagent Kit. The CVs for both assays were <5% within the range measured in the patients.

Results

EFFECT OF ANALYTICAL UNCERTAINTY ON GFR CALCULATIONS

Based on the theoretical framework described in the online Supplemental Material, we used simulations to examine how the uncertainty of 2-point or 3-point, 1-compartment, slope-and-intercept GFR calculations (Fig. 1A and B) was affected by a given analytical uncertainty (CV_{method}) (Fig. 1B). CV_{GFR} was independent of the intercept (m) and hence the patient's body size. As the CV was a ratio, the value of m was cancelled out in the calculations (online Appendix B). The CV_{GFR} could be the same as the CV_{method} if optimal time points were used (Table 1 and Supplemental Fig. 2). The middle point did not add much to the precision at any GFR value (online Supplemental Fig. 3). If the time points were too early in relation to the kidney function marker's half-life, the CV_{GFR} increased exponentially (Fig. 2). The first time point should be as early as possible after the distribution phase to limit uncertainty of the intercept (m) and hence the patient's estimated body water. The third point should be as late as possible, but the positive effect of late sampling decreased sharply between 1 and 2 half-lives of the kidney filtration marker (Fig. 3, Table 1).

ESTIMATING THE UNCERTAINTY OF PATIENT IOHEXOL PLASMA CLEARANCE MEASUREMENTS

We examined the CV_{GFR} of 731 three-iohexol plasma clearance measurements (online Supplemental Table 1) using simulation protocols (online Appendix C). The

Table 1. Optimized time points for the 3-point method to the nearest 30 min period for different slopes (*k*).[a]

rGFR, mL/min/1.73 m2[b]	Optimal t_1	Optimal t_2	Optimal t_3	CV_{GFR}, %	*k*
5	120	1410	1440	2.8	−0.0005
15	120	1410	1440	1.4	−0.0010
25	120	750	1140	1.4	−0.0015
35	120	510	870	1.4	−0.0020
45	120	360	720	1.4	−0.0025
55	120	240	630	1.4	−0.0030
60	120	150	570	1.4	−0.0035
70	120	150	450	1.4	−0.0040
80	120	150	390	1.4	−0.0045
90	120	150	330	1.4	−0.0050
100	120	150	270	1.4	−0.0055
110	120	150	240	1.5	−0.0060
120	120	150	210	1.5	−0.0065
130	120	150	180	1.6	−0.0070

[a]As an example, the time points for a patient with an estimated *k* of −0.0020 (GFR of 35 mL/min/1.73 m2) can be $t_1 = 120$ min, $t_2 = 510$ min, and $t_3 = 870$ min. If 870 min is inconvenient, a later time point, for example the next morning, can be used as long as the kidney filtration marker concentration will remain above the limit of quantification. If 2 time points are used, the first (t_1) and the third (t_3) time point in the table can be used.

[b]The approximate relative GFR (rGFR) comes from a regression made on the relative GFR for the 731 patients and rounded to the nearest multiple of 5.

time points were adequately chosen among patients with a normal relative GFR, whereas time points that were too early were often used among patients with a relative GFR <60 mL/min/1.73 m^2 (Fig. 4A). If the theoretically optimal time points from Table 1 had been used, the CV_{GFR} could on average have been lowered 3.2-fold in all patients, 3.9-fold in patients with a GFR ≤60 mL/min/1.73 m^2, and 5.0-fold in patients with a GFR <40 mL/min/1.73 m^2. In simulations, where patient eGFR was used to optimize sampling times from Table 1, as suggested in online Supplemental Fig. 4, the median CV_{GFR} would have decreased from 3.7% to 1.5% (Fig. 4B). Tables with examples of CV_{GFR}-optimized time points that could be used in clinical routine are provided in online Supplemental Tables 2 and 3. The CV_{GFR} did not correlate with the difference between the relative GFR and the estimated GFR (eGFR) using creatinine or cystatin C concentrations (online Supplemental Fig. 5). Finally, we found that in addition to simulations, a set of formulae derived from estimating the moments of the transformed random variables with Taylor expansions could approximate the CV_{GFR} given a terminal slope (*k*), 2 time points, and CV_{method} (Fig. 5 and online Supplemental Material).

Discussion

In contrast to the substantial literature concerning the uncertainty of eGFR from creatinine or cystatin C concentrations (20, 21), the uncertainty of routine GFR measurements has been given less attention. In theory, if the time plot of continuous kidney filtration marker concentrations after a bolus injection is known, it would be possible to get the true mean GFR during the kidney filtration marker elimination from the AUC (8). In reality, the plot using a few blood samples collected after the distribution phase is inferred.

This approach has several potential sources of error, including the distribution phase never being measured, the final slope being used to extrapolate the patient's distribution volume, and the fact that the methods were developed on less than 100 individuals, in whom ^{51}Cr-EDTA was used as the kidney filtration marker (9). Finally, the relative GFR is calculated using the patient's estimated body surface area, a value that is known to correlate poorly with the extracellular volume (22). It is likely that errors caused by simplifications are independent of each other. This means that errors sometimes converge and result in both gross underestimations or overestimations of the true GFR in individual patients

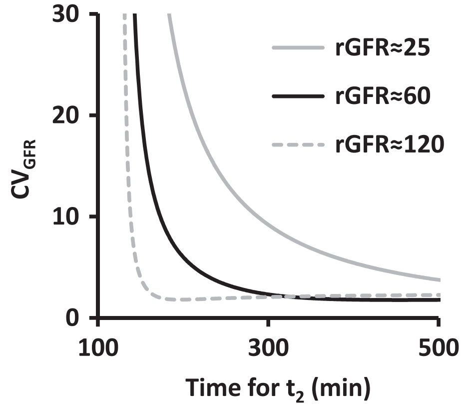

Fig. 2. The CV_{GFR} of the 2-point GFR method given a first sampling time of 120 min and a variable second sampling time (time for t_2) at different relative GFR (rGFR). For each rGFR, there is a time point where the decrease in the CV_{GFR} with time is close enough to zero, and extending the second sampling time further does not result in lower CV_{GFR}. For some slopes there is a theoretical local minimum before the convergence, but the difference between that value and the value at convergence is negligible. The approximate rGFR values are taken from Supplemental Table 1 and the algorithm used to calculate the CV_{GFR} is given in Appendix C in the Supplemental Material.

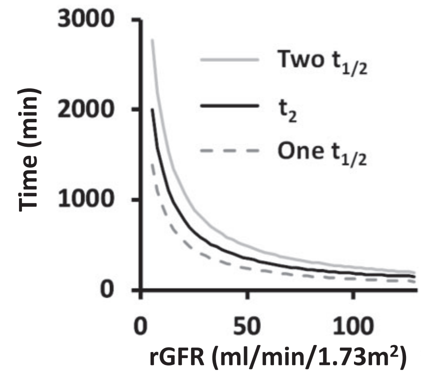

Fig. 3. Relation between the kidney filtration marker elimination half-life ($t_{1/2}$) and the time point when the CV_{GFR} is essentially the same as the CV for the kidney filtration marker ($CV_{GFR} \approx CV_{method}$). Data is for a 2-point method with a first measurement at 120 min. The time point when $CV_{GFR} \approx CV_{method}$ is between 1 and 2 elimination half-lives. For some patients the optimal latest time point is later than 24 h. The approximate relative GFR (rGFR) values are taken from Supplemental Table 2 and the algorithm used to calculate the CV_{GFR} is given in Appendix C in the Supplemental Material.

(8). It is therefore crucial to control and reduce the errors from the simplifications.

By examining the theoretical mathematical framework for the 3-point, slope-and-intercept GFR method described by Bröchner-Mortensen (9), we found that the optimal time points were between 1 and 2 half-lives of the kidney filtration marker and that the middle point could be omitted.

During our study, we found that a general theoretical formula to calculate the CV_{GFR} exactly, given an arbitrary number of measurements, was not feasible. The main reason was that the measurement error could not be tracked in a precise way after the applied mathematical transformations and the regression (Supplemental Fig. 1). However, we found that using Taylor expansions to approximate the moments of the transformed random variables in the 2 time-point case resolved these issues and resulted in a mathematical solution that can be applied to most laboratory information systems.

Using simulations to calculate the CV_{GFR}, we found that our own GFR measurements often used time points that were too early, which amplified CV_{GFR}. It is known that measured GFR and eGFR sometimes diverge substantially from each other. The discrepancy between our iohexol clearance measurements and eGFR did not correlate with CV_{GFR} (Supplemental Fig. 5). The main reason behind differences between measured GFR and eGFR in individual patients must be due to other reasons, for instance an inter-individual variation in production rate of creatinine and cystatin C per unit body volume.

The optimal individual time points for sampling (Table 1 and Supplemental Tables 2 and 3) can be preferentially chosen from the individual patient's eGFR or previous GFR measurements (Supplemental Fig. 4). After the GFR measurement, the final slope (k), the actual first and last time points, and the local CV_{method} for the kidney filtration marker can be put into the equations from the Taylor expansion described in the online Supplemental Material to calculate a CV_{GFR} for this particular patient. Individual CV_{GFR} can then be used as a basis for determining whether the GFR results are accurate enough for clinical routine, e.g., for adjusting doses of cytostatic drugs, or whether the injection and sampling should be reperformed with more appropriate time points. In this way, our theoretical work can be used both to limit CV_{GFR} and provide an estimate of CV_{GFR} for clinical service. It is important to stress,

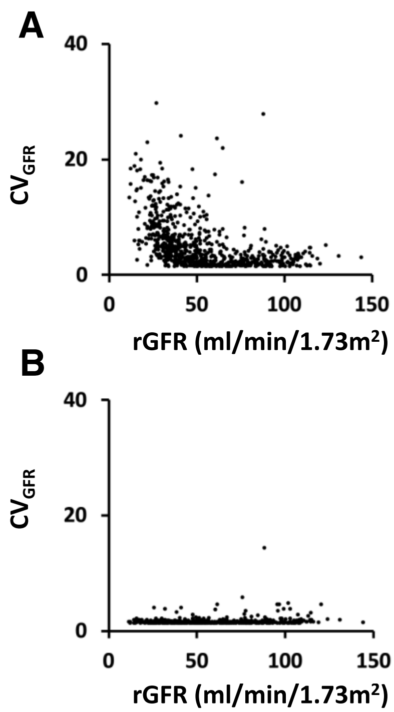

Fig. 4. The CV_{GFR} of 731 three-point iohexol plasma clearance measurements using the 3 actual time points (A) or eGFR-optimized time points from Table 1 (B). The simulations that generated actual CV_{GFR} used patient-specific time points and iohexol concentrations with an added CV_{method} of 2.5% as described in Appendix C in the Supplemental Material. The eGFR-optimized CV_{GFR} used the patients' eGFR to choose the 3 time points and the iohexol concentrations projected from the patients' final slope with an added CV_{method} of 2.5%.

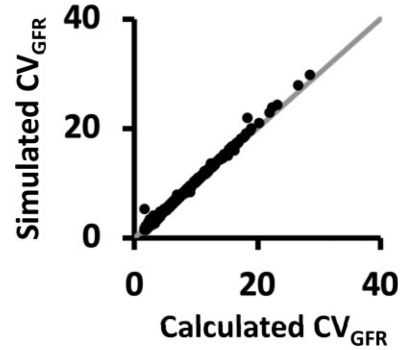

Fig. 5. Comparison of CV_{GFR} from simulation and calculation on the 731 three-point iohexol plasma clearance measurements. CV_{GFR} from simulations plotted against CV_{GFR} calculated by the set of formulae described in the Supplemental Material (simulated CV_{GFR} and calculated CV_{GFR}).

however, that the uncertainty of GFR measurements also rests on many other factors that must be accounted for and further studies need to be done to validate our concept.

This study has several limitations. It is a theoretical study of the error inflation produced by the regression mathematics in GFR calculations based on a given measurement uncertainty of a kidney filtration marker. All patient data are retrospective similar to previous studies on the same subject (10). We have not validated the patient-optimal times suggested in Table 1 and Supplemental Tables 2 to 5.

Second, we only examined errors in 1-compartment GFR calculations. Errors and the resulting overestimation of the patient's GFR due to sampling during the distribution phase when the injected kidney filtration marker is still mixing with extracellular water has not been examined (23). The mixing time has been shown to be around 120 min (24) but may be delayed in patients above 70 years of age (25), in patients with large extracellular volume (26), with low kidney function, and in patients with edema or ascites (27). In these instances, a delayed first time point up to 5 h is sometimes recommended (27) but will as a consequence increase uncertainty of the intercept (m) and therefore increase CV_{GFR}. The balance between the risk of overestimating GFR by sampling in the distribution phase and the risk of amplifying CV_{GFR} by choosing a late first time point must be carefully considered in each patient case.

In addition, any unknown errors due to improper sampling and sample mishandling are not considered. In the 3-point GFR method, the coefficient of determination (r^2) of the 3 points is often used to check the validity of the measurements. If r^2 is >0.95, it is likely that no major error occurred during the sampling or analysis. This check is not possible if only 2 time points are used and it might be counterproductive to remove the middle timepoint.

Finally, it may not be possible to use some of the optimal time points in Table 1 in a facility that operates with normal working hours. However, if needed, the last sampling time point may be delayed without affecting the CV_{GFR} too much as long as the kidney filtration marker concentration in the last sample remains above the limit of quantification of the analytical method.

In summary, when using this new approach to calculate the CV_{GFR}, our own GFR measurements are suboptimal due to sampling too early in patients with low

GFR and that this can possibly be overcome by using more optimal time points.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: GFR, glomerular filtration rate; AUC, area under the curve; CV_{GFR}, coefficient of variation of the GFR; CV_{method}, coefficient of variation of the kidney filtration marker measurement method; eGFR, estimated glomerular filtration rate.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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