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Original Article

Effect of truncating blood sampling in measuring glomerular filtration rate

Kenneth J Nichols[1], Adam Kesner[2], Kuldeep K Bhargava[1], Paige Bennett[1], Christopher J Palestro[1]

[1]Department of Radiology, New Hyde Park, Northwell, NY, USA; [2]Department of Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

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Abstract: Glomerular filtration rates (GFR's) are used to guide patient management. GFR's are based on radioactivity measurements of blood samples sampled at different times. We compared GFR computations from blood collected at 6 times to those collected at 2 timepoints. Thirty-seven GFR studies were performed on 25 patients. After intravenous administration of I-125 sodium iothalamate, 6 plasma samples were obtained at 5 min, 10 min, 15 min, 3 hr, 3.5 hr and 4 hr after injection, then counted in a well counter. Two different GFR calculation tools were applied to each set of 6 plasma counts (Methods 1 and 2), and a 2-sample algorithm (Method 3) computed GFR using only 3 hr and 4 hr data. Linear correlation between Method 1 and 2 GFR's was stronger than for Method 3 versus Methods 1 and 2 ($r = 1.00$ versus $r = .91$, $P < .0001$). Bland-Altman limits of agreement were larger ($P < .0001$) for Method 3 versus Methods 1 and 2 (-39.5 to +22.0 ml/min/1.73 m^2) than for Method 1 versus 2 (-7.6 to +4.5 ml/min/1.73 m^2). Method 3 overestimated lower GFR's and underestimated higher GFR's. Methods 1 and 2 agreed exactly in identifying 3 cases of GFR < 74 ml/min/1.73 m^2 ($\kappa = 1.00$), while Method 3 detected only 1 of the three ($\kappa = .48$). To avoid underdiagnosing low GFR's, larger GFR sample sizes are preferable to smaller sample sizes.

Keywords: Renal function, glomerular filtration rate, I-125 sodium iothalamate, curve fitting, algorithms

Introduction

One of the physiological parameters used to evaluate renal function is glomerular filtration rate (GFR), which measures the rate of flow of filtered liquids through the kidney. In routine practice, clinicians commonly use an estimated GFR (eGFR) based on patient age, gender, weight, race, and serum creatinine levels [1]. The eGFR is a readily available and inexpensive estimation of functioning kidney nephrons, which contain glomeruli that filter waste from the blood. However, in some clinical scenarios, eGFR is not adequate or can be erroneous. For example, in oncology patients, chemotherapeutic drugs can harm the kidneys, and therapeutic dosing depends on more exact measurements of renal function [2], helping clinicians determine the maximum amount of drug for successful treatment with minimal toxicity to the kidneys. Other scenarios where more exact measurements of GFR are required include antibiotic dosing, acute and chronic kidney disease, renal transplant donor evaluations, and unusually thin or obese patients [3].

One of the best tests available to monitor renal function is radionuclide evaluation of GFR, which measures the amount of radiotracer cleared by the kidneys over time. This requires the administration of radiotracer followed by blood samples collected over several hours [4]. From these blood samples, radioactive counts of plasma over time are recorded and the GFR is calculated [5]. For all patients regardless of age, a GFR value of 60-90 ml/min/1.73 m^2 is considered an indicator of early kidney disease [6]; the lower limit of normal GFR for a 20-year-old patient is 74 ml/min/1.73 m^2 [7]. Some calculation methods use one blood sample [8], some use two samples [9], and others use as many as 9 blood samples [10].

At our institution, blood samples are acquired at 6 different time points for GFR calculations. Because there are several algorithms available to compute GFR from plasma samples, we wished to determine whether GFR measurements were the same when computed from two different algorithms applied to the same 6 data samples per patient. Also, as there are several different protocols used to collect blood samples at different times for different time durations, we also wished to determine whether GFR measurements using a 2-sample algorithm were equivalent to the 6-sample GFR measurements.

Materials and methods

Patient population

Our Institutional Review Board approved this study (Study IRB#23-0781), for which a waiver of patient consent was obtained. We performed a retrospective data analysis of 37 GFR studies performed on 25 patients [17 males and 8 females aged 1-73 years (mean age = 16 ± 19 years; median age = 5 years)]. Sixteen patients had 1 study, 6 patients had 2 studies, and 3 patients had 3 studies, with a maximum of 3 months between initial and final studies.

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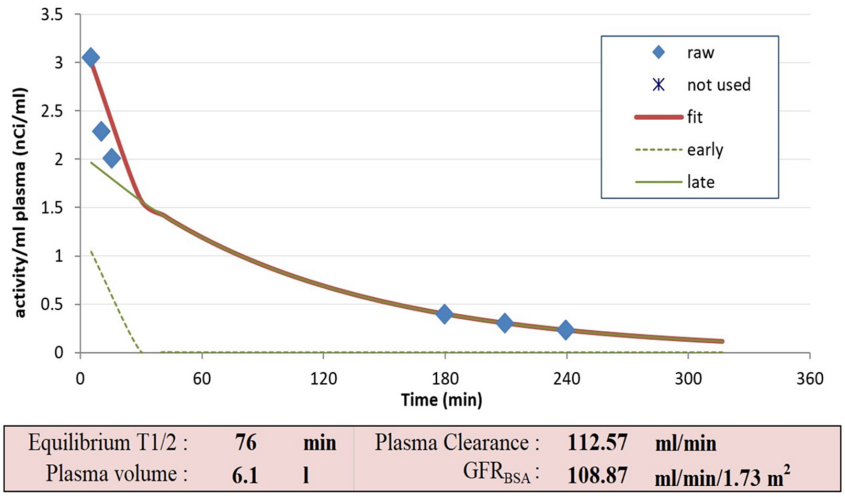

Figure 1. Method 2 curve-fitting to 6 blood plasma count samples.

Thirty-two studies were performed on patients prior to bone marrow transplantation, chemotherapy, or for further evaluation of possible chemotherapy-induced renal toxicity. Four were performed for evaluation prior to renal transplant donation. One study was performed for suspected renal insufficiency.

Data acquisition

Patient height and weight were recorded in order to estimate body surface area (BSA), for calculating GFR. A radioactive standard was prepared using 0.9-1.1 MBq (25-30 μCi) I-125 sodium iothalamate diluted in 500 mL of normal saline. The agent was supplied as "Glofil®" (Iso-Tex Diagnostics, Inc., Pearland, TX) [11], which has been commercially available continuously since it was first approved by the FDA 1962. It is an ionic radiopaque contrast agent that is indicated to measure GFR [11].

The largest bore butterfly needle possible (21 gauge) was placed in the patient's arm vein and an initial 3-mL blood sample was removed for subsequent radioactive background measurement. Then, 0.9-1.1 MBq (25-30 μCi) of I-125 sodium iothalamate was administered intravenously, while a stopwatch was used to mark the time of injection. Subsequently, six 3-mL blood samples were drawn over 4 hours, at time intervals as close as possible to 5 min, 10 min, 15 min, 3 hrs, 3.5 hrs and 4 hrs following injection. Blood samples were centrifuged to isolate 1-mL aliquots of plasma. 0.1-mL samples were counted in a calibrated well counter for 3 minutes per sample, along with 0.1-mL of the radioactive standard, background plasma sample and water background.

Data processing

Three different methods of calculating GFR were applied to each patient's plasma sample well-counter activity measurements. Methods 1 and 2 both perform a bi-exponential fit to a 2-compartment model and assume a non-equilibrium early phase component followed by an equilibrium late phase reflecting renal clearance of tracer. The early phase of tracer clearance is rapid due to high concentration of radioactivity that is not yet in equilibrium with the extracellular fluid volume; the late phase of tracer clearance is assumed to reflect renal clearance alone following equilibrium (**Figure 1**) [4]. Each curve component is assumed to have a fixed flow rate. Both Methods 1 and 2 implement a validated approach to compute plasma clearance rate (PCR) and GFR values from the 6 plasma samples [12].

Since 2007, the "Cleveland Clinic Plasma Disappearance Protocol" (Method 1) has been used at our institution. This method requires an input of 6 independently acquired plasma samples. The "Denver GFR v2.02e", an open source GFR research worksheet (Method 2) [13], was applied to the same plasma sampling data as a cross-check to GFR values obtained by Method 1.

The single compartment GFR characterization model has been recommended for routine use [14, 15], and is the method commonly employed across the field [4]. It is based upon fitting a late exponential function and requires only 2-4 time points to implement. This method systematically overestimates GFR, but this overestimation is negligible for low renal function and can be corrected for higher function [4]. Method 3 was implemented as a single compartment model, deriving GFR estimates from two time points and using a British Nuclear Medicine Society Correction curve correction [16]. This method used a modified version of "DenverGFR" that performed a mono-exponential fit to the equilibrium late phase data [13]. It used only 3 hr and 4 hr plasma counts (**Figure 2**).

For all three methods, patient height and weight were used to estimate BSA according to a standard formula [17], which was used for GFR normalization. All three methods generated values for PCR, GFR and the biological half-life ($T_{1/2}$) of the equilibrium late phase of renal clearance. Method 3 values for GFR's, PCR's and $T_{1/2}$ values were obtained using data only at 3 and 4 hrs.

Statistical analysis

Statistical analysis was performed using MedCalc software (MedCalc® Statistical Software version 23.1.1; MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2025). Values are reported as the mean ± one standard deviation. The normality of continuous variable distributions was determined using the Shapiro-Wilk test. ANOVA with Bonferroni correction was used to test the significance of differences in measurements from the different computation methods analyzed simultaneously.

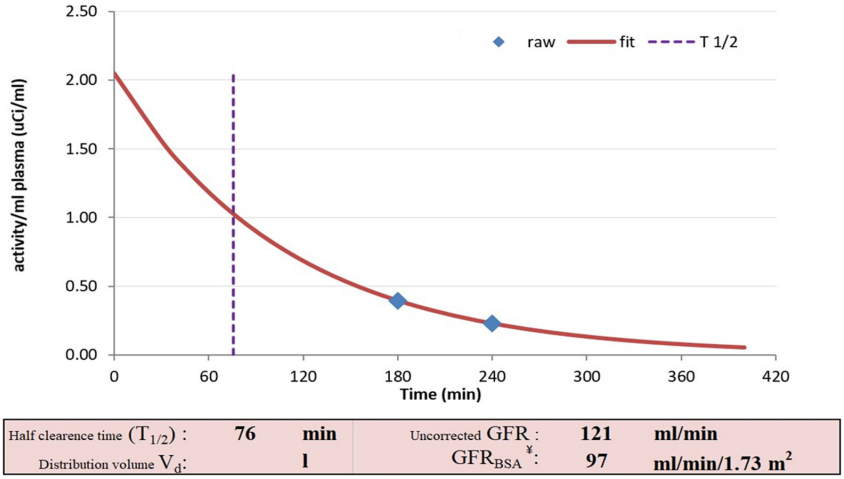

Figure 2. For the same patient as in **Figure 1**, Method 3 curve fitting to 2 plasma count samples, with slightly lower GFR and slightly higher PCR values than Method 2.

Table 1. Comparison of renal parameters obtained by the 3 methods

	GFR (ml/min/1.73 m²)	PCR (ml/min)	T1/2 (min)
Method 1	131 ± 35	84 ± 52	107 ± 61
Method 2	130 ± 35	84 ± 51	109 ± 64
Method 3	122 ± 26	106 ± 67	103 ± 49
ANOVA F-ratio	.94; P = .39	2.11; P = .13	.25; P = .78

GFR, glomerular filtration rate; PCR, plasma clearance rate; $T_{1/2}$, biological half-life of the equilibrium late phase of plasma clearance.

Significance of differences was assessed using the unpaired or paired t-test for normally distributed variables; otherwise, the Mann-Whitney or Wilcoxon test was used. To compare 2-sample Method 3's values to 6-sample methods' values, the means of Method 1 and 2 GFR values were computed.

GFR < 74 ml/min/1.73 m² was defined as abnormal because this value previously had been established as the lower limit of normal [7]. Inter-rater agreement for the different methods to identify patients with a GFR < 74 ml/min/1.73 m² was determined using the kappa (κ) statistic [18], for which the strength of agreement is characterized using standardized terminology as being "slight agreement" for κ < .20, "fair agreement" for κ = .21-.40, "moderate agreement" for κ = .41-.60, "substantial agreement" for κ = .61-.80, and the highest category of "almost perfect agreement" for κ ≥ .81 [18, 19].

Strength of correlation of continuous GFR values among methods was assessed by linear regression, and trends and biases among methods were assessed by Bland-Altman analysis. The concordance correlation coefficient evaluated the degree to which pairs of GFR Methods' measurements fell on the 45° line through the origin [20].

Because of the small number of cases, power analysis was used to determine whether marginal differences were significant. The standard conventions of determining power using α = .05 to avoid errors of type I and β = .20 to avoid errors of type II were used. Test probability (P) < .05 was considered statistically significant, or as adjusted by Bonferroni corrections for ANOVA comparisons among simultaneously evaluated multiple methods.

Results

For Methods 1 and 2, PCR values were not normally distributed (range: 20-216 ml/min, Shapiro-Wilk P = .001), nor were $T_{1/2}$ values (range: 29-365 min, P < .0001), but GFR values were (range: 68-212 ml/min/1.73 m², P = .64). One-way ANOVA demonstrated no significant differences among the 3 methods for GFR, PCR or $T_{1/2}$ values (**Table 1**).

However, for identifying studies with GFR < 74 ml/min/1.73 m², the 2-sample Method 3 had "moderate agreement" with the 6-sample Methods 1 and 2 (κ = .48), whereas Methods 1 and 2 agreed exactly and had the highest level of "almost perfect agreement" in characterizing cases (κ = 1.00) [18, 19]. Three studies were abnormal by Methods 1 and 2, but only 1 was abnormal by Method 3 (**Table 2**). Consequently, Method 3 did not agree as well with Methods 1 and 2 as those methods agreed with each other.

Methods 1 and 2 GFR values correlated strongly with one another (**Figure 3A**), and more strongly than did Method 3 values compared to mean Method 1 and 2 GFR's (r = 1.00 versus r = .91, z-statistic = 13.7, P < .001) (**Table 3**). The concordance correlation coefficient was stronger for Methods 1 and 2 GFR data pairs than for Method 3 versus Method 1 and 2 GFR data pairs (.995 versus .83, z-statistic = 7.4, P < .0001). Bland-Altman analysis demonstrated that there were no biases or trends between Methods 1 and 2 (intercept and slope not significantly different from zero), but there were biases and trends for Method 3 GFR values compared to Methods 1 and 2 (**Table 4**). Method 3 overestimated low GFR's and underestimated high GFR's, compared to Methods 1 and 2 (**Figure 3B**). Bland-Altman limits of agreement were considerably larger in comparing Method 3 GFR's to GFR's of Methods 1 and 2 (-39.5 to +22.0 versus -7.6 to +4.5 ml/min/1.73 m²), with greater standard deviation (11.1 versus 2.4 ml/min/1.73 m², F-statistic = 21.3, P < .0001) (**Figure 4**). Power analysis demonstrated that there were more than the minimum number of cases necessary for statistically significant difference between 2-sample and

Table 2. Comparison of methods to identify cases of GFR < 74 ml/min/1.73 m^2 versus Method 1 characterization of cases

	Method 2		Method 3		%
	GFR ≥ 74	GFR < 74	GFR ≥ 74	GFR < 74	cases
Method 1	34	0	34	0	92%
	0	3	2	1	8%
% cases	92%	8%	97%	3%	
kappa	1.00		.48		
Inter-rater agreement	"almost perfect agreement"		"moderate agreement"		

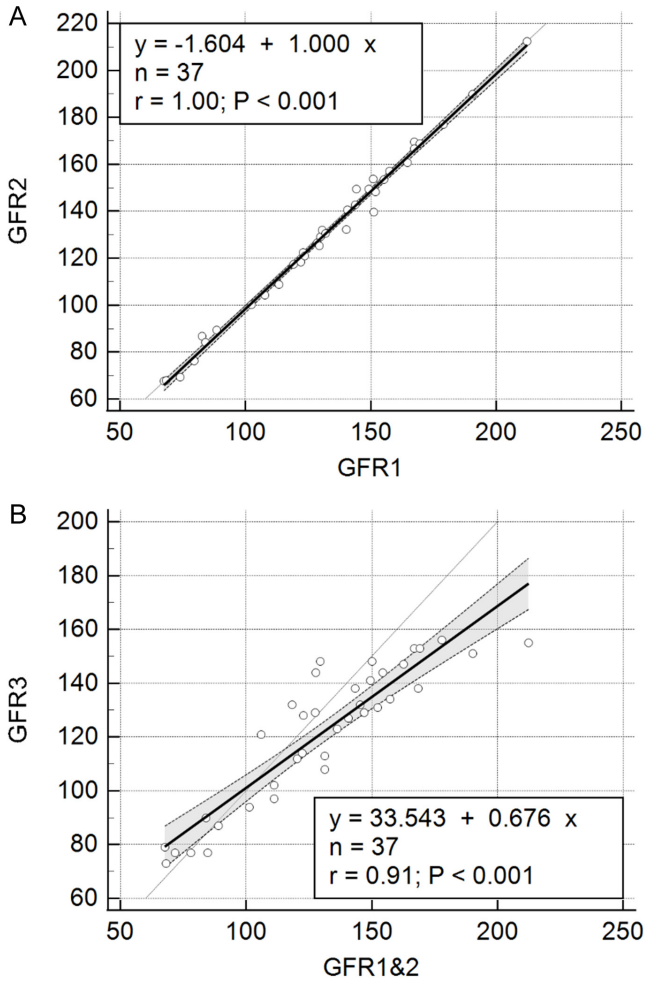

Figure 3. Linear regression plots of (A) Method 2 versus Method 1 GFR and (B) Method 3 GFR versus the mean of Method 1 and 2 GFR values. Correlation was significantly stronger between Methods 1 and 2 than between Method 3 and Methods 1 and 2.

Table 3. Linear regression analysis results comparing GFR values

	Method 2 GFR versus Method 1 GFR	Method 3 GFR versus mean GFR of Methods 1 and 2
r	1.00; P < .0001	.91*; P < .0001
Intercept	-1.6 ± 2.0; P = .43	33.5 ± 7.1*; P <.0001
Slope	1.00 ± .02; P < .0001	.68 ± 0.05*; P < .0001

*P < .05 versus Methods 1 and 2.

6-sample GFR methods for linear correlation (> 5 cases) and concordance correlation coefficients (> 6 cases). There were also sufficient numbers of cases for Bland-Altman differences to be statistically significant between 2-sample and 6-sample GFR methods for intercepts (> 4 cases), slopes (> 4 cases), and limits of agreement (> 16 cases).

Discussion

It is important to have accurate GFR measurements. Low GFR has been reported as a side effect in 31% of patients who have undergone various forms of chemotherapy [21]. Consequently, chemotherapeutic protocols often include assessment of GFR before, during and after initiating treatment.

Estimated GFR (eGFR) is not a direct measurement, but rather is obtained by regression formulas [22, 23], particularly the CKD-EPI creatinine formula, which computes eGFR from serum creatinine, sex, race and age and indexes values to BSA [1]. Nuclear Medicine tracer techniques, in contrast, provide direct physiologic measurement of GFR. For Nuclear Medicine GFR methods that rely on blood sampling, several different methods have been used [16]. Some use one sample [8], some use two samples [9], and others use up to as many as 9 blood samples [10, 24]. While it may be expedient to choose a one-sample method over other techniques, the GFR values obtained are prone to considerable imprecision, with errors as high as 50 ml/min [25]. In handling multi-sampled data to measure renal clearance, dual compartment slope-intercept approaches have fewer computational errors than single compartment slope-only methods [26]. While a 2-sample technique may be easier and more practical compared with techniques utilizing more samples, our data indicate that the simpler method may come at the cost of clinically significant accuracy. We found that acquiring and processing a sufficient number of blood samples to support a bi-exponential time-activity curve characterization, using commonly available software, to be a more reliable approach to GFR estimation, in agreement with the literature [24, 27, 28].

Prior statistical analyses using only 4 blood samples indicated that GFR measurements deviate from correct values by more than 15% in over 8% of patients [29]. Our results agree with that finding, suggesting that imprecision is introduced by decreasing the number of blood samples used to determine GFR. We found considerably more variation for the 2-time-point method than for the 6-sample methods. The Bland-Altman confidence limits of agreement comparing the two 6-sample Methods 1 and 2 were -7.6 to +4.4 ml/min/1.73 m^2 (**Figure 4A**) but were -39.5 to +22.0 ml/min/1.73 m^2 for comparing 6-sample to 2-sample methods (**Figure 4B**). Consequent-

Table 4. Bland-Altman results comparing GFR method differences to mean values

	Method 2 GFR and Method 1 GFR	Method 3 GFR and mean GFR of Methods 1 and 2
r	.05; P = .78	-.58*; P = .0002
Intercept	-2.1 ± 2.0; P = .30	+15.0 ± 4.7*; P = .003
Slope	.004 ± .01; P = .78	-.15 ± .04*; P = .0002
Limits of agreement	-7.6 to +4.5 ml/min/1.73 m^2	-39.5 to +22.0 ml/min/1.73 m^{2*}

*P < .05 versus Methods 1 and 2.

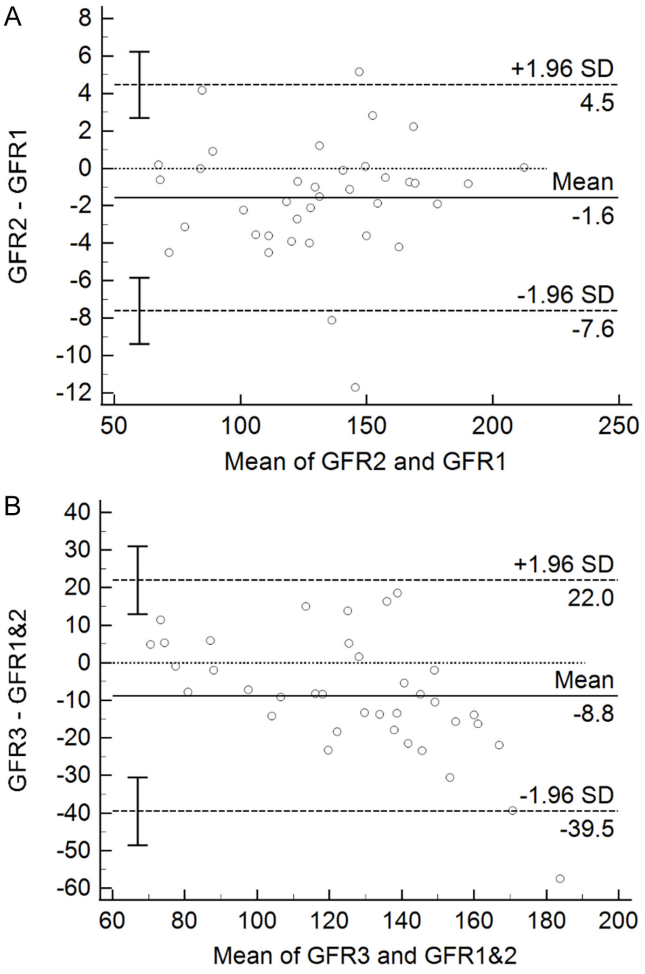

Figure 4. Bland-Altman plots of (A) Methods 1 and 2 GFR values, and (B) of Method 3 and mean of Method 1 and 2 GFR's. Limits of agreement were significantly larger for Method 3 versus Methods 1 and 2 than were the limits of agreement between Methods 1 and 2.

ly, different characterizations can result in whether patients fall into the normal or abnormal GFR range, given specific GFR thresholds of abnormality, different values of which have been suggested [16, 30]. This in turn can affect patients' eligibility for treatment protocols. Some patients have repeated GFR determinations over time, and it is important to have the most accurate values possible for all measurements, to facilitate monitoring renal function over time. In addition to the misidentification of 2 out of 3 abnormally low GFR cases, our findings of overestimation of low GFR's by the 2-point method versus the 6-point methods suggest that using fewer blood samples could lead to undertreatment and failure to detect drug toxicity. Other GFR tracers, such as Cr-51-EDTA and Tc-99m-DTPA, have been used in conjunction with essentially similar bi-exponential fitting approaches [11], and it is reasonable to expect that using fewer blood samples will cause similar misidentification of abnormally low GFR regardless of which tracer is used.

Limitations

In comparing the results of multiple different computation methods, it would have been preferable to have a completely independent reference standard for GFR's, but unfortunately this was not available to us. Furthermore, we had a limited number of cases to evaluate, so ours was a small sample size. In particular, it would have been desirable to have had a larger number of markedly low GFR values. Our finding that ANOVA showed no differences of means was likely masked by the assessment of 3 methods simultaneously (**Table 1**), even though Bland-Altman graphs depicted markedly larger differences between 2-sample Method 3 and the 6-sample Methods 1 and 2, with considerably larger limits of agreement (**Figure 3**).

Conclusion

Detection of abnormally reduced GFR is compromised by use of only 2 plasma samples versus use of all 6 plasma samples.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kenneth J Nichols, Department of Radiology, 270-05 76th Avenue, New Hyde Park, Northwell, NY 11040, USA. Tel: 718-470-7080; Fax: 718-470-9247; E-mail: Knichols@Northwell.edu

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