J of Nuclear Medicine Technology, first published online June 10, 2019 as doi:10.2967/jnmt.119.229153

Validation of glomerular filtration rate measurement with blood sampling from the injection site

Running title: GFR injection site blood sampling

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Word count of the manuscript: 2956

Financial support: There was no external financial support for this research. Costs were covered by departmental budget.

Abstract

Background: Measurement of glomerular filtration rate from the plasma clearance of a radionuclide labeled tracer is reliable and accurate. However, in order to avoid contamination of the blood samples with radioactivity remaining at the injection site, it requires venepuncture in at least two sites: one for the administration of tracer, and the other/s for blood sampling. This is uncomfortable for patients particularly when venous access is difficult. The objective of this study was to validate the use of a single site of venous access in combination with injection site imaging, for glomerular filtration rate measurement.

Methods: Twenty-two adults (≥ 18 years), who were referred for GFR determination were included prospectively. GFR was measured from the plasma clearance of ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) according to international guidelines. After administration of the tracer through an intravenous (IV) cannula, a 60-second static image of the injection site was acquired. A second IV cannula was inserted into the contralateral arm. Venous blood samples were collected at 2, 3 and 4 hours after the administration of the radiotracer from both the injection site (experimental) and contralateral limb (conventional). GFR was calculated using slope-intercept (SI-GFR) and single sample methods (SS-GFR). The median conventional and experimental plasma counts (decay and background-corrected) were compared for the 2, 3 and 4 h venous samples. Conventional GFRs (GFR_{con}) and experimental GFRs (GFR_{exp}) were then compared, with a > 10% difference between GFR_{exp} and GFR_{con} being regarded as significant.

Results: Four individuals had visible residual activity at the injection site. The median 2 h counts at conventional and experimental sampling sites were significantly different (p=0.007), whereas

no significant difference was found at 3 h and 4 h. In cases with a clear injection site image, for SS-GFR the difference between GFR_{exp} and GFR_{con} was > 10% in 1 case, whereas for SI-GFR all differences were < 8%.

Conclusion: In cases with clear injection site images, SI-GFR calculated after injection site blood sampling showed no clinically significant difference to conventional contralateral limb sampling.

Key words: Glomerular Filtration Rate; Blood Specimen Collection; Cannula; Adult; Radioactivity

Introduction

Glomerular filtration rate (GFR) is a standard measure of renal function (*1*). GFR represents the plasma volume presented to the nephrons per unit time during urine formation. It is usually measured in millilitres per minute (*1*). Radionuclide-based techniques allow for the rapid and reliable measurement of GFR from plasma samples taken after intravenous administration of a bolus of radionuclide labeled tracer (*2*). It is frequently measured from the plasma clearance of a radiopharmaceutical such as ^{99m}Tc- diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) or ^{51}Cr-ethylenediaminetetraacetic acid (^{51}Cr-EDTA). While the plasma clearance of ^{51}Cr-EDTA is often considered the standard measure of GFR, particularly in Europe, ^{99m}Tc-DTPA gives similar results with added advantages of being cheaper, more widely available, having higher counting efficiency and allowing for simultaneous imaging (*1,3*).

Radionuclide-based GFR determination in general is relatively time-consuming, labour-intensive, and uncomfortable for the patient. There is thus a need for the technique to be simplified without compromising the accuracy of the result. Current international guidelines for the measurement of GFR state that the tracer should not be injected through the same line as that used for blood sampling (*4*). This is to eliminate the risk of contamination of the blood samples by residual radioactivity in the line (*2*). Consequently, venous access must be obtained from at least two sites, usually in each arm. Frequently, large-bore intravenous catheters, needles or butterflies used for this purpose are uncomfortable for patients and may be distressing for children. In addition, venous access may be difficult in certain individuals (e.g. young children, the elderly, obese patients, or patients receiving chemotherapy), making finding two sites of access more challenging. It also increases the risk of haemolysis of the samples which may invalidate the

measurements (*1*). Some centres give preference to the placing of a Venflon needle with a valve, allowing both tracer injection and repeated blood sampling with only one venepuncture (*5*). However, these may not be routinely available, particularly in resource-limited settings.

Sample contamination may however be insignificant if gamma camera imaging, performed immediately after tracer administration, detects no residual activity in the intravenous catheter/butterfly. If this is indeed the case, a strong argument can be made to support the use of a single intravenous catheter/butterfly for both administration of activity and blood sampling in combination with imaging. This would simplify the procedure and reduce patient discomfort. A rigorous validation of this methodology is likely to contribute to the development of new guidelines, however, there is limited published research that addresses this issue. Therefore, the aim of this study is to validate the use of a single site of venous access in combination with injection site imaging, for GFR measurement.

Materials and methods

The study was approved by the Health Research Ethics Committee of Stellenbosch University (protocol number S17/10/191), and all subjects signed an informed consent form. Adults (≥ 18 years) referred for GFR determination at Tygerberg Hospital, Cape Town, South Africa, over a six-month period (April - September 2018) were invited to participate. Subjects with difficult venous access were excluded.

The GFR studies were performed following departmental protocol which is based on the 2004 British Nuclear Medicine Society guideline (*1*). A median dose of 45 MBq (1.2 mCi) (Range: 36 MBq (0.97 mCi) - 49 MBq (1.3 mCi)) ^{99m}Tc-DTPA (RENATEK, NTP Radioisotopes (Pty) LTD., South

Africa) was administered as a bolus through 20 G or 22 G intravenous (IV) cannulae. In all patients a 60-second static image of the injection site was acquired between 5- and 93-minutes post-injection with the IV cannula in situ using one of three available gamma cameras: Siemens Symbia (low energy all-purpose collimator), a GE Infinia, or a GE Hawkeye (low energy high resolution collimators). If there was visible activity at the injection site on this image, its quantity was calculated, and expressed as a percentage of administered activity, based on previously determined camera sensitivities.

A second 18 G or 20 G IV cannula was inserted into the contralateral arm. Venous blood samples (~10 ml) were collected at 2, 3 and 4 hours after the administration of the radiotracer from the contralateral limb (according to normal practice), and simultaneously from the injection site. For practical reasons, the blood samples from the contralateral limb and the injection site were collected by different people, a technologist and the principal investigator respectively. A small volume (~1 ml) of heparin-saline was injected into both IV cannulae after blood sampling to maintain cannula patency, and prior to taking the following sample, at least 3 ml of blood was first drawn and discarded. Blood samples obtained from the contralateral arm were denoted "conventional (con)" and those obtained from the injection site cannula "experimental (exp)".

The conventional and experimental samples were handled identically. Blood samples were centrifuged at 1000 g for 10 min, duplicate 1 ml plasma and standard samples were pipetted into counting tubes, and all samples were counted simultaneously with a multichannel well counter (VIDEOGAMMA 4880, l'acn scientific laboratories, Italy) following departmental protocol. The same standard samples were used for conventional and experimental GFR calculations. Body surface area (BSA) in m^2 was calculated using the Haycock formula (*6*):

$BSA = w^{0.5378} * h^{0.3964} * 0.024265$, where

w = weight in kg

h = height in cm

GFR was calculated using slope-intercept (SI-GFR) (*7*) and single sample methods (SS-GFR) (*8*). SI-GFR was corrected using the mean Bröchner-Mortensen equation (*1,9,10*). Routine quality control checks were performed for both methods.

In cases with clear injection site images, the average plasma counts (decay- and background-corrected) were calculated for the 2, 3, and 4 h samples from both conventional and experimental sites. A Wilcoxon test was used to compare the median counts. The Shapiro-Wilk test was used to test for normality.

SI-GFR was calculated using all three plasma samples (2, 3, and 4 h), and the 2 h sample was used for SS-GFR calculation. The conventional GFRs were denoted $SI\text{-}GFR_{con}$ or $SS\text{-}GFR_{con}$, and the experimental GFRs $SI\text{-}GFR_{exp}$ or $SS\text{-}GFR_{exp.}$ To determine the agreement between $SI\text{-}GFR_{con}$ and $SI\text{-}GFR_{exp}$, and $SS\text{-}GFR_{con}$ and $SS\text{-}GFR_{exp}$, Bland-Altman analyses were performed. The differences between $SI\text{-}GFR_{exp}$ and $SI\text{-}GFR_{con}$, and $SS\text{-}GFR_{exp}$ and $SS\text{-}GFR_{con}$, were calculated. The proportion of cases with differences of > 10% was calculated. A 10% threshold was selected as it is the coefficient of variation for repeat GFR measurements (*11*). Statistical analysis was performed using MedCalc for Windows version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019).

Results

Twenty-two subjects were included (15 female; median age 53 years; age range 26 – 67 years). Twenty-one participants were known with cancer and were referred for pre-chemotherapy GFR determination. One participant was a healthy volunteer. The absolute GFR ranged between 68 and 122 ml/min (median 79.5 ml/min) and the BSA-corrected GFR ranged between 60 and 118 ml/min/1.73 m^2 (median 89 ml/min/1.73 m^2). Of the 22 participants, 4 had visible activity at the injection site, ranging between 0.09% and 0.3% of the administered dose.

The median 2 h counts at conventional and experimental sampling sites were significantly different (p=0.007), whereas no significant difference was found at 3 h and 4 h. The median difference between experimental and conventional counts, expressed as a percentage of conventional counts, was 4.4% at 2 h, 0.3% at 3 h, and -1.9% at 4 h.

The median differences between GFR_{exp} and GFR_{con} were -1.2% and -2.9% for SI-GFR and SS-GFR, respectively (table 1). For SS-GFR, the difference between experimental and conventional measurements was > 10% in 1 case, whereas for SI-GFR all differences were < 8%. This is illustrated by the Bland-Altman analyses (fig. 1). In 3 out of the 4 cases with visible residual activity SI-GFR_{exp} and SS-GFR_{exp} were higher than GFR_{con} by 20-80 ml/min/1.73m^2, well beyond the 95% limits of agreement derived from cases without visible activity (fig.1).

Discussion

GFR is the best index of kidney function in health and disease, and accurate values are required for optimal decision making (*12*). The evaluation of glomerular filtration rate (GFR) using a bolus injection of a radionuclide tracer and measurement of its plasma clearance has become a widely

used method for the assessment of kidney function (*13*). However, the investigation is time consuming, labour intensive and uncomfortable for the patient.

In this study, we compared the conventional technique of contralateral arm blood sampling to an experimental method with blood samples taken through the injection site cannula, after imaging the injection site to exclude the presence of scintigraphically detectable residual injected activity. The median 2 h counts at experimental sampling sites were significantly higher ($p=0.007$), whereas no significant difference was found at 3 h and 4 h. This is most likely due to higher occult residual activity contaminating the 2 h samples, which is likely to have decreased with later samples at 3 h and 4 h as a consequence of previous sampling.

As expected, experimental GFR was extremely inaccurate in 3 of the 4 patients with visible activity at the injection site. This confirms the need for injection site imaging (or counting over the injection site) to detect residual activity at the injection site, thus precluding the performance of injection site blood sampling. In the 18 cases with clear injection site images, for SI-GFR, the percentage differences between the conventional and experimental sites were all < 8% (range: -6.9% to 7.5%). However, for SS-GFR, in 1 case the difference was > 10 %, with the percentage difference ranging from -3.1% to 12.7%. Using a 10% threshold, there was no clinically significant difference between SI-GFR determined from conventional and experimental sites. For SS-GFR, one case differed by 12.7%. It can therefore be argued from a clinical point of view, that it is acceptable to use injection site blood sampling to determine GFR, at least in patients with challenges to venous access.

Our findings are in line with a previous report by Brändström et al (*14*), in which no difference in GFR was found between contralateral arm and injection site blood sampling. The authors did however stress that the injection site venous catheter was flushed with at least 30 ml of saline following radiotracer injection.

In another study by Gawthorpe et al (*15*), it was concluded that a single-lumen central venous catheter should not be used for tracer injection and blood sampling as it significantly affected GFR result due to contamination. In our study, while we have observed sample contamination to affect the 2 h sample counts, this did not affect GFR measurement clinically significantly, especially if SI-GFR was used. It can be speculated that the addition of a saline flush and/or discarding the first blood sample may further reduce the impact of occult activity at the injection site.

This study has a few limitations. The methodology cannot be applied to ^{51}Cr-EDTA GFR as imaging of the injection site is not possible. A comparable method of measuring residual activity using a hand-held monitor would be required. Detection of residual activity at the injection site on an image is related to the individual camera's sensitivity. Had the department's most sensitive camera been used with an all-purpose collimator in all cases, it is possible that residual activity would have been identified in additional cases. However, it can be argued that this reflects clinical practice where it is likely that injection site imaging would be allocated to whatever machine is available, and that this may not be the most sensitive. The number of participants with visible activity at the injection site was small, however most of these had very inaccurate GFR results. In addition, it is possible that further flushing of the injection site cannula, or obtaining blood

samples through direct IV access, would have improved the results. These are issues for possible further studies.

Conclusion

In cases with clear injection site images, SI-GFR calculated after injection site blood sampling was not clinically significantly different from that obtained using conventional contralateral limb sampling. Therefore, single venous access for both blood sampling and radiopharmaceutical injection, combined with injection site imaging, can be used at least in a clinical setting where patients have difficult venous access.

Disclosure

Jennifer Holness received funding from the South African Medical Research Council under the Division of Research Capacity Development under the Clinician Researcher Development PhD Scholarship Programme.

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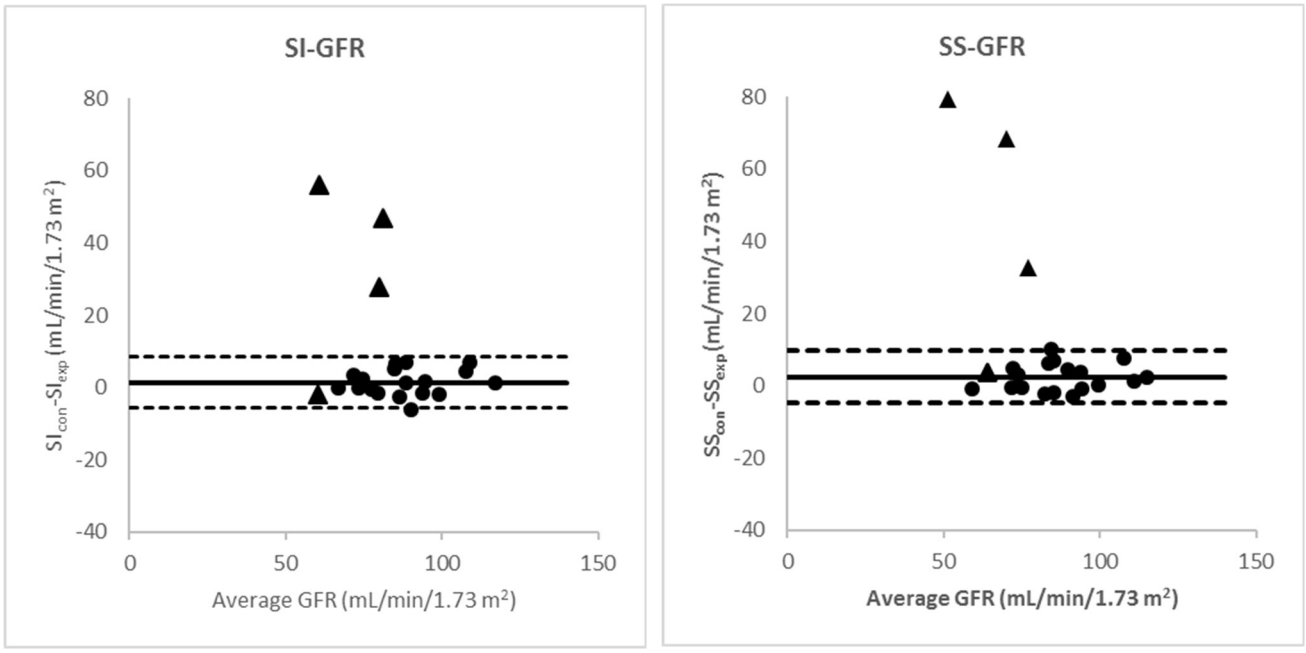

FIGURE 1. Bland-Altman plots of conventional (contralateral limb blood sampling) and experimental (injection site blood sampling) GFR in cases with a clear injection site. (a) SI-GFR_{con} vs. SI-GFR_{exp}. (b) SS-GFR_{con} vs. SS-GFR_{exp}. The solid lines represent the mean differences and the dashed lines the upper and lower 95% limits of agreement. The triangles represent the 4 cases with visible activity at the injection site and are plotted for purposes of comparison. GFR, glomerular filtration rate; SI-GFR, slope intercept GFR; SS-GFR, single sample GFR; LOA, limits of agreement.

TABLE 1. Summary statistics of the differences between experimental and conventional GFR in cases with clear injection sites (n=18)

	Median difference (full range) ml/min/1.73m^2	Median % difference (full range)
SI-GFR	-1.3 (-6.9 to 6.0)	-1.2 (-7.5 to 6.9)
SS-GFR	-2.6 (-10.0 to 2.9)	-2.9 (-10.9 to 3.3)

Median difference is calculated as SI_{exp}-SI_{con} and SS_{exp}-SS_{con}; SI-GFR, slope intercept glomerular filtration rate; SS-GFR, single sample glomerular filtration rate.