Comparison of Methods for Calculating Glomerular Filtration Rate: Technetium-99m-DTPA Scintigraphic Analysis, Protein-Free and Whole-Plasma Clearance of Technetium-99m-DTPA and Iodine-125-Iothalamate Clearance

Jeffrey J. Goates*, Kathryn A. Morton, Wesley W. Whooten, Harry E. Greenberg, Frederick L. Datz, Jerry E. Handy, Anthony J. Scuderi, Alan O. Haakenstad, and Robert E. Lynch

Nuclear Medicine and Research Services, VA Medical Center, Salt Lake City, Utah; Departments of Radiology and Pathology, University of Utah School of Medicine, Salt Lake City, Utah; and University of California at San Francisco School of Medicine, Fresno-Central San Joaquin Valley, California

True glomerular filtration rate (GFR) was measured in normal volunteers and in patients with normal and impaired renal function by the iothalamate clearance (IC) method of Sigman. Within 24 hr, GFR was also determined by two other methods: technetium-99m- (^{99m}Tc) DTPA scintigraphic analysis (SA) utilizing a modification of the Gates computer program, and by measuring disappearance of ^{99m}Tc-DTPA from whole plasma (WPC) and from protein-free ultrafiltered plasma (PFPC). Determinations of GFR by IC and by PFPC methods were virtually identical (mean absolute error 5.36 ml/min, r = 0.99, p > 0.05). GFRs measured in protein-free, ultrafiltered plasma differed significantly from those obtained from whole plasma only in sicker patients and in those taking multiple medications (in whom alterations in protein-binding of DTPA may be seen). The SA method correlated less well with the iodine-125- (^{125}I) IC method than did either the protein-free or whole-plasma clearance methods (mean absolute error 32.36 ml/min, r = 0.74, p < 0.05). However, the SA method provided useful information with respect to differential (split) renal function.

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Glomerular filtration rate (GFR), the volume of plasma ultrafiltrate produced per minute by renal glomeruli, is an important index of renal function. Twenty-four-hour creatinine clearance, one of the most commonly used methods for determining GFR, over-

estimates GFR in patients with impaired renal function, due to a small element of tubular excretion which becomes more significant as glomerular function drops (1,2). The "gold standard" methods for determining GFR include inulin and iothalamate clearance (IC), both of which are expensive, time-consuming, and highly dependent on collection accuracy (3–7). Although several methods for deriving GFR from radioisotopic renography have been described, controversy exists with respect to the consistency and accuracy of GFRs obtained by these methods in humans.

There exist a variety of methods for estimating GFR following injection of technetium-99m- (^{99m}Tc) diethylenetriaminepentaacetic acid (DTPA), an agent commonly used for renal scintigraphy. These methods are well-described in the review by Dubovsky and Russell (8). DTPA is excreted solely by the glomerulus, making it, theoretically, a good agent for the determination of GFR (9,10). Some methods depend on measuring clearance of the agent from blood or urine samples (11–13); others have advocated measuring disappearance of ^{99m}Tc-DTPA by monitoring disappearance of radioactivity from blood by means of a detector placed over the precordium (14). An additional method, a modification of the Schlegel program developed by Gates (15–17), involves computer analysis of scintigraphic images of the kidneys after a single intravenous (i.v.) injection of ^{99m}Tc-DTPA. The Gates program approximates creatinine clearance in adult patients with normal or mildly impaired renal function. Many of the described methods for determining GFR from radioisotopic renography data, particularly those methods which employ analysis of scintigraphic images, are prone to error from a variety of causes. These include technical imaging factors, camera variability, attenuation of activity

by the patient, renal geometry, extracellular localization of DTPA, and variable degrees of protein binding of ^{99m}Tc-DTPA, which can occur because of pharmaceutical preparation and factors that can alter the composition of patient plasma (18,11).

The group at the University of Alabama have developed a method which circumvents the uncertainty introduced into GFR determinations caused by the variable protein binding of ^{99m}Tc-DTPA (19,20). With this method, the clearance of ^{99m}Tc-DTPA is measured in two protein-free, ultrafiltered samples of plasma after a single i.v. injection of the radiopharmaceutical. The goal of the study described herein was to identify the most accurate and convenient method for determining GFR in conjunction with a standard ^{99m}Tc-DTPA renal scan, to validate that method against the "gold standard," in this case iodine-125- (^{125}I) IC. We compared the values for GFR obtained by IC to those obtained by clearance of DTPA from two protein-free plasma samples and by a modification of the Gates program, in which the visual data obtained during a standard ^{99m}Tc renal scan is quantitatively analyzed by a computer program to determine both global and split GFR.

MATERIALS AND METHODS

Subjects

A total of 87 studies were performed on 7 normal volunteers and 24 patients following informed consent in accordance with the Institutional Review Board of the University of Utah and the Salt Lake City VA Medical Center. The patients were referred to the nuclear medicine department for renal scans for a variety of reasons and had renal function that varied from normal to markedly impaired. All subjects were adults, with an average age of 43.9 yr (range 29–78). All but two of the subjects were male. Subjects had GFRs calculated by some or all of several methods. In each case, all GFR determinations in the same subject were made within 36 hr, and 29 of the 31 patients had all studies done during the same day. Subjects with edema or ascites were excluded from this study because of possible alterations in the distribution of radionuclides into the extracellular fluid.

Iothalamate Clearance (IC) Method

The method of Sigman was used for determining GFR by clearance of ^{125}I-iothalamate (Glofil-125, ISO-TEX Diagnostics, Inc., Friendswood, TX) from plasma and urine after an equilibrium has been reached (4). Following an oral water load of 20 cc/kg, a bolus of 50 μCi of ^{125}I-iothalamate was injected intravenously. This was followed by a constant i.v. infusion of 50 μCi of ^{125}I-iothalamate in 70 cc of 5% dextrose in normal saline at a rate of 0.5 cc/min. The infusion was continued for 45 min to allow the iothalamate to attain constant plasma levels before beginning the collections of urine and blood. After 45 min of constant infusion, the subject was asked to empty completely his or her bladder. If the subject had a history of, or was clinically suspected of having urinary retention, if he was known to have an enlarged prostate by physical exam, or if he was elderly, a foley catheter was inserted to insure complete urine collections. If a urinary

catheter was in place, the collection bag was completely drained after the first 45 min of constant infusion and the sample discarded. After an additional 15 min, the urine produced was collected and a sample of blood was obtained in a heparinized syringe. After a second 15-min period, blood and urine were again collected. The constant infusion was continued throughout the collections. All blood samples taken were removed from the arm opposite that used for the iothalamate infusion. Plasma was obtained after sedimentation of cells in a centrifuge at 2,000 rpm for 10 min. Duplicate 0.5-cc aliquots of plasma and urine from each collection were counted in a gamma scintillation counter, averaging the counts from the urine and blood samples for each collection period. GFR was calculated for each collection period based on the formula:

$$GFR = \frac{(\text{urine counts per minute})(\text{urine volume})}{(\text{plasma counts per minute})(\text{time in minutes})}.$$

The average of the two GFR estimates was then calculated. All IC studies were done prior to the Tc-DTPA studies.

Two-Sample Protein-free Plasma Clearance (PFPC) Method

The estimation of GFR using the ultrafiltration of plasma after injection of ^{99m}Tc-DTPA (Sn-complexed, Mediphysics, Paramus, NJ) was performed according to the method described by Rowell and associates (19). In our study, this examination was generally performed in conjunction with the standard ^{99m}Tc-DTPA renal scan. Two equal aliquots containing equal activity and volume of ^{99m}Tc-DTPA were prepared, one used as a standard and the other as the dose to be injected intravenously. Blood was withdrawn into EDTA anticoagulated tubes at 60 and 180 min after the injection from the arm opposite that into which the ^{99m}Tc-DTPA had been injected. The blood samples were then subjected to centrifugation at 2,000 rpm for 10 min and the plasma was removed. To obtain protein-free fluid, the plasma was subjected to centrifugation in Centrifree micropartition tubes (Amicon, Danvers, MA) for 15 min at 2,000 rpm. The resultant ultrafiltrate is 99.9% free of plasma proteins. Duplicate aliquots of the ultrafiltered samples and an equal volume of a standard dilution were counted in a gamma well counter. GFR was estimated by the formula:

GFR
$$= [(D \ln (P_1/P_2)/(T_2 - T_1)) \times e^{((T_1 \ln P_2 - T_2 \ln P_1)/(T_2 - T_1))}]^{0.979},$$

where

D = Dose activity, counts/min.
T_1 = Time of collection of first blood sample in minutes (60).
T_2 = Time of collection of second blood sample in minutes (180).
P_1 = Ultrafiltrate activity (in cpm/ml) at T_1 × 0.94.
P_2 = Ultrafiltrate activity (in cpm/ml) at T_2 × 0.94.

Although radioactivity from the prior injection of ^{125}I-iothalamate contributes to measured radioactivity from injected ^{99m}Tc, we discontinued the practice of determining the ^{125}I present at the start of the study with ^{99m}Tc after the first 14 patients because the error caused by residual ^{125}I was always <1%, even in patients with impaired renal function.

The authors of the two-sample plasma clearance methods have shown that a single sample can also be used to calculate

GFR, with an error of ±8 ml/min. However, we elected to use the more accurate two-sample method, in which Russell et al. have reported an error of only ±4 ml/min (18).

Two-Sample Whole-Plasma Clearance (WPC) Method

The WPC method was performed exactly as the PFPC method described above except that the plasma samples were not ultrafiltered after the blood cells had been removed by centrifugation. Therefore, the plasma proteins remained in these specimens.

Scintigraphic Analysis (SA) Method

The final method we employed for estimating GFR involved the use of a modification of the Gates computer program written for ADAC (Milpitas, CA) for determining global and split GFR from renal scintigraphic images following the administration of ^{99m}Tc-DTPA. The program uses a computer-generated regression formula to allow for direct estimation of GFR, which is proportionate to the fractional renal uptake of ^{99m}Tc-DTPA at the 2–3 min following arrival of tracer in the kidneys. The regression coefficient used is 9.813 and the intercept is −6.825. A formula for a calculation of GFR is derived accordingly: GFR ml/min. = (% total renal DTPA uptake) (regression coefficient) + (intercept). A depth attenuation correction factor used was that of Tonnesen et al: Right kidney depth = 13.3 (weight/height) + 0.7; left kidney depth = 13.2 (weight/height) + 0.7, with weight and height in kilograms and centimeters. Split, or differential GFR, was also determined by the SA method. Since the original Gates program requires that the patient dose be counted under the camera prior to injection, difficulty arose in that our usual 10-mCi adult patient dose of ^{99m}Tc-DTPA for a renal scan resulted in pixel roll-over and/or significant deadtime losses when counted as a single small source (in a syringe). Our modification of this program allowed us to count a small aliquot of known activity (usually 300 μCi or less) under the camera and to introduce subsequently a correction factor to extrapolate to the actual patient dose. Our modification also allowed for a dilution of the residual activity in the dose syringe to be counted after the acquisition was completed to determine the actual patient dose injected. The PFPC, WPC, and SA methods were usually combined so that the subject

required only one injection of ^{99m}Tc-DTPA, with a usual dose of 10 mCi.

In preparation for the DTPA scan, the subjects were hydrated to a normal degree. If they had either ingested a normal meal including fluids within 4 hr, or were receiving maintenance i.v. fluids they were not given additional fluids. If they did not satisfy these conditions, they received either oral or i.v. fluids in the amount equal to 10 ml/kg, prior to the study.

The data for the program were obtained by placing a syringe containing a dilution of the patient dose of ^{99m}Tc-DTPA under a camera at a distance of 30 in. (experimentally determined to be optimal) and obtaining a 1-min image. The patient dose was injected intravenously and a dynamic acquisition was obtained for 6 min and stored in a 128×128×8 matrix at intervals of 15 sec using a low-energy general-purpose collimator. A dilution of the residual activity in the dose syringe was counted for 1 min at the completion of the acquisition, and a correction factor introduced for extrapolation to the true residual activity of the patient dose syringe. The difference between the preinjection adjusted syringe counts and the postinjection adjusted syringe counts was considered the actual patient dose.

Statistical Methods

A comparison of the magnitude of the errors (IC-SA versus IC-PFPC, Table 1) was performed by a t-test (21,22). Linear regression and p values shown in Figures 1 and 2 were performed by the least squares method. Graphs were generated using the software Cricket Graph for Macintosh.
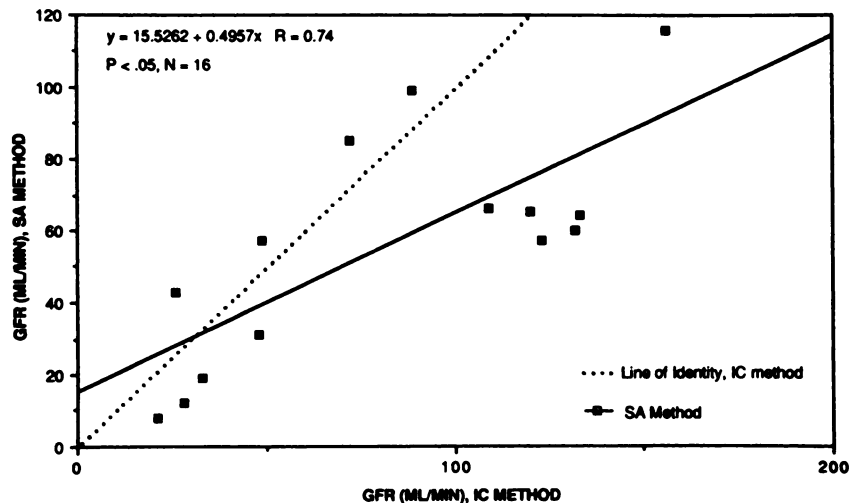
RESULTS

Comparison of GFR by the IC and the PFPC Methods

In 16 subjects, GFR was determined by both the IC and the PFPC methods. The results are shown in Figure 1. The two methods for calculating GFR yield virtually identical results, r = 0.99, p > 0.05, validating the PFPC method as equivalent to the gold standard. The tight correlation between the two methods was maintained both in the patients with normal renal function and in those in whom renal function was markedly impaired.
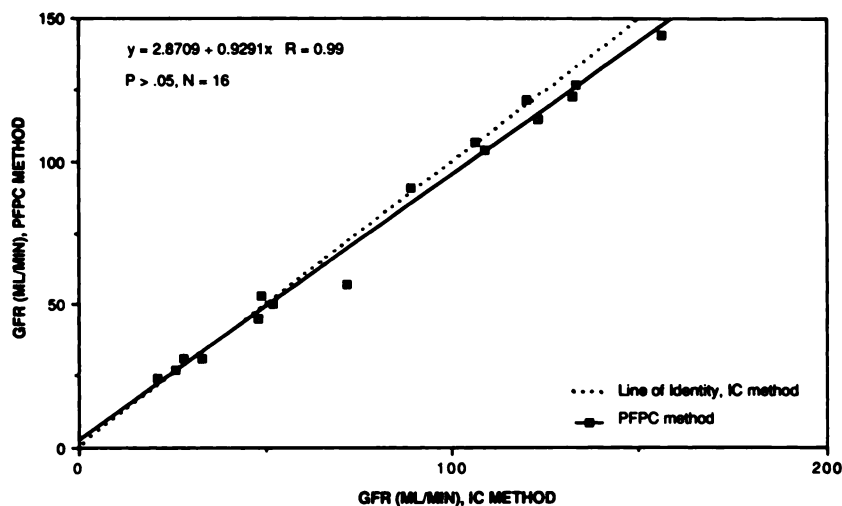
FIGURE 1
Comparison of GFR by clearance of ^{125}I-iothalamate (IC) from urine and plasma and by clearance of ^{99m}Tc-DTPA from two protein-free plasma samples (PFPC). The line of identity of the true GFR (IC) is shown as the dotted line. The solid line represents the regression line of IC versus PFPC.

y = 2.8709 + 0.9291x   R = 0.99
P > .05, N = 16

GFR (ML/MIN), PFPC METHOD

···· Line of identity, IC method
—■— PFPC method

GFR (ML/MIN), IC METHOD

$y = 15.5262 + 0.4957x$ $R = 0.74$
$P < .05, N = 16$

· · · · Line of Identity, IC method

SA Method

GFR (ML/MIN), SA METHOD

GFR (ML/MIN), IC METHOD

FIGURE 2
Comparison of GFR by clearance of ^{125}I-iothalamate (IC) from urine and plasma and by a modification of the Gates program for scintigraphic analysis of renal images following injection of ^{99m}Tc-DTPA (SA). The line of identity of the true GFR (IC) is shown as the dotted line. The solid line represents the regression line of IC versus SA.

Comparison of GFR by the SA and the PFPC Methods

The values for GFR obtained by the SA method from images obtained during the ^{99m}Tc-DTPA renal scan vary in both directions from the "true value" obtained by the IC method (Fig. 2.). However, for subjects with normal renal function, the SA tended to underestimate, rather than overestimate the GFR, often by as much as 50%. The degree of scatter about the line of identity (of the IC method) is much greater for the SA method than for the PFPC method. The error for the SA method was large throughout the range of GFRs.

Analysis of Paired Data for all Three Methods of Determining GFR

The impression that the error was greater for the SA method than for the PFPC method was examined by comparing the errors in patients in whom the GFR was determined by all three methods (IC, SA, and PFPC), (Table 1). When compared to the IC method (the gold standard), the mean absolute error produced by the SA method was significantly greater than that produced by the PFPC method (32.36 ml/min versus 5.36 ml/min, $p < 0.001$).

Comparison of GFR Obtained from the PFPC and the WPC Methods

Having established that the PFPC method was equivalent to the IC (gold standard) method for determining GFR, we compared the values obtained for GFR by the PFPC method to those obtained from two unfiltered whole-plasma samples (WPC method). There was little difference between the values obtained in 71% (12 of 17) of the patients (mean absolute difference 5.3 ml/

TABLE 1
Paired Data for GFR: I-125 Iothalamate Clearance (IC), ^{99m}Tc-DTPA Protein-Free Plasma Clearance (PFPC) and Scintigraphic Analysis (SA) Methods

Subject number	GFR (ml/min) IC method	GFR (ml/min) PFPC method	GFR (ml/min) SA method	Absolute error	Absolute error
1	21	24	8	3	13
2	26	27	43	1	17
3	28	31	12	3	16
4	33	31	19	2	14
5	48	45	31	3	17
6	49	53	57	4	8
7	72	57	85	15	13
8	89	91	99	2	10
9	109	104	66	5	43
10	120	122	65	2	55
11	123	115	57	8	66
12	132	123	60	9	72
13	133	127	64	6	69
14	156	144	116	12	40
Mean absolute error				5.36	32.36
S.E.M. absolute error				1.16	6.73

Mean absolute error (IC-SA) $\gg$ mean absolute error (I-PFPC), $p < 0.001$, (by paired t-test, $t = -4.33$, d.f. = 13).

min, range 0–9.4 ml/min). However, in 5 of the 17 patients (29%), the difference between the PFPC and WPC methods was greater (mean difference 27.8 ml/min, range 12–52 ml/min). In four of these five subjects, the WPC method overestimated GFR when compared to the value obtained by the PFPC method. The five patients all had severe acute or chronic illnesses and were on multiple medications. However, no consistent features were present in the five with the most disparate values with respect to renal function, type of intercurrent or chronic illness, medications, or total serum protein or albumin levels. Additionally, a number of the 12 patients in whom there was good agreement between the PFPC and WPC methods also were very sick and on multiple medications. Therefore, clinical features could not be identified that would enable predication of a population of patients in whom GFR could be accurately measured by the WPC method.

DISCUSSION

Our results clearly indicate that the PFPC method for determining GFR was equivalent to the gold standard, ^{125}I-iothalamate clearance. The PFPC method was very easy to perform. There was no difference in calculated GFR when the blood samples were processed immediately after they were drawn, as opposed to saving the blood samples in EDTA tubes and processing the samples as a "batch" at the end of the day. An ^{131}I-hippuran renal scan could be performed between drawing the 1- and 3-hr blood samples. Down-scatter of ^{131}I-hippuran, with an average patient dose of 300–350 μCi of ^{131}I, resulted in an increase in apparent counts in the ^{99m}Tc window of <5% (for both normal subjects and those with impaired renal function) and was considered insignificant.

In most patients, the WPC method accurately predicted true GFR, and was even easier to perform than the PFPC method. However, in a number of patients there was a large discrepancy in the values obtained by the PFPC and WPC methods. Since we have established that the PFPC method is equivalent to the gold standard, and since it is difficult to predict those patients in whom a discrepancy between the WPC and PFPC method will exist, it is probably prudent to use the PFPC method in determination of true GFR.

Our studies differ somewhat from the results of LaFrance et al. (13), who recently compared GFRs obtained by urinary clearance of ^{125}I-iothalamate after a single subcutaneous injection (5,6) by urinary excretion of ^{99m}Tc-DTPA and by whole-plasma clearance of DTPA. They found that urinary clearances of DTPA and iothalamate were very similar but that whole-plasma clearance of DTPA tended to overestimate GFR, as determined by urinary iothalamate excretion, throughout the range of GFRs. In our experience, urinary clearance of a tracer alone is prone to significant error since many patients are unable to void completely, particularly in the elderly male population such as that at our VA Medical Center. Many of our patient subjects required vesicular catheterization for complete urine collection. In addition, the iothalamate method that we employed, which measures both plasma and urinary clearance following continuous i.v. infusion of tracer, avoids several of the potential pitfalls of the Cohen method. LaFrance et al. noted that plasma clearance of DTPA tended to overestimate the GFR, which would agree with the data on the 29% of our subjects in whom significant discrepancy existed between the WPC and PFPC methods.

Although the SA method was less accurate than the PFPC method, in our hands, it served as a useful method for calculating "split" or differential, renal function. If the error in the SA method results from factors in the patient which are likely to remain constant throughout his illness, such as attenuation, geometry, medication, or binding of DTPA by serum proteins, then the type of error (over- or underestimation of GFR) may remain constant in that particular patient. In this case, the SA method may be extremely useful in documenting the improvement or deterioration of GFR in a given patient.

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FIRST IMPRESSIONS

PURPOSE:
A 29-yr-old male paraplegic was studied for acute bleeding in the right thigh. A ^{99m}Tc-RBC study showed a region of increased activity with a photopenic halo in the center, suggesting an active bleeding site. Angiography showed a 2-cm region of increased opacification fed by a branch of the superficial femoral artery. No extravasation of contrast was seen. The radiological diagnosis was false aneurysm, possibly mycotic. The lesion was treated surgically.

TRACER:
^{99m}Tc-RBC

ROUTE OF ADMINISTRATION:
Intravenous injection

TIME AFTER INJECTION:
15 min

INSTRUMENTATION:
Angiography

CONTRIBUTORS:
J.C. Wallace, MD

INSTITUTION:
Department of Nuclear Medicine, St. John Regional Hospital, Saint John, New Brunswick, Canada.