Technical Aspects of a New Technique for Estimating Glomerular Filtration Rate Using Technetium-99m-DTPA

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Technetium-99m-DTPA has previously been proposed as an appropriate radiopharmaceutical for the determination of glomerular filtration rate (GFR). Some ^{99m}Tc-DTPA preparations have been found to be inadequate for estimating the GFR because of partial binding of the ^{99m}Tc-DTPA to plasma proteins. This protein bound entity which cannot be filtered by the glomerulus results in an error in the calculated GFR from plasma clearance. Our methods use a single injection and either one or two blood samples to estimate the GFR utilizing a protein-free ultrafiltrate of the plasma. Both the one-sample and two-sample methods are easy to perform. The one-sample method is sufficiently accurate for clinical use. The two-sample method is suggested for investigational purposes where special accuracy is required. The technique employed in both of these methods to correct for protein binding results in an accurate estimate of the GFR using ^{99m}Tc-DTPA.

Glomerular filtration rate (GFR) can be defined as the volume of the plasma ultrafiltrate produced in 1 min in renal glomeruli (*1*). The GFR cannot be measured directly but can be calculated from the rate of clearance of tracer activity from plasma following a single i.v. injection of a suitable radiopharmaceutical. Technetium-99m-diethylenetriaminepenta-acetic acid (^{99m}Tc-DTPA) has previously been proposed as an appropriate radiopharmaceutical for the determination of GFR. Some ^{99m}Tc-DTPA preparations have been found to be inadequate for estimating the GFR because of partial binding of the ^{99m}Tc-DTPA by plasma proteins (*2*).

This protein bound entity which cannot be filtered by the glomerulus results in an error in the calculated GFR from plasma clearance. An accurate GFR measurement is possible even with significant protein binding if the binding is measured for each individual patient and the appropriate correction made. We have previously proposed two methods for measuring GFR that require separate measurement of plasma protein binding. These methods involve a single injection and either one or two blood samples (*2*).

The concise methods and technical aspects of estimating GFR using ^{99m}Tc-DTPA by a new technique, in which plasma protein binding is not measured separately, are presented. Instead of separately counting plasma and a protein free ultrafiltrate of the plasma in order to correct the plasma counts for protein binding, the new simplified procedure requires only counting the ultrafiltrate to estimate GFR.

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MATERIALS AND METHODS

The procedures for both methods are similar, and are described in detail in Appendices A and B. The procedure basically entails: preparing two or more 5-mCi aliquots of ^{99m}Tc-DTPA; injecting one aliquot and setting one aliquot aside for the standard; and withdrawing a single blood sample at 180 min after injection for the single-sample method. In addition to the 180-min sample, a 60-min sample is withdrawn if the two-sample method is used. The procedure continues with centrifuging the blood sample, ultrafiltering the plasma, aliquoting the ultrafiltrate, diluting the standard, aliquoting the diluted standard, counting the samples, and calculating the results.

RESULTS AND DISCUSSION

The two methods presented (Appendices A and B) have proven to be quite accurate and easy to perform. Their scientific validation has been presented elsewhere (*2*). The single-sample method can approximate the actual GFR with only an 8-ml/min error that provides enough accuracy for clinical use. The two-sample method can approximate the GFR value with only a 4-ml/min error that is recommended for special accuracy [i.e., investigational purposes (*2*)]. This procedure has the advantage of not requiring that the patient be present in the department, as the procedure can be performed at bedside. In conclusion, measurement of the glomerular filtration rate can be accurately estimated with ^{99m}Tc-DTPA without separate protein binding measurements. Instead of separate protein binding measurements, a protein free ultrafiltrate of the plasma can be utilized to estimate the GFR.

APPENDIX A

Single Blood-Sample Method

1. Prepare ^{99m}Tc-DTPA by following the package insert directions.
2. Prepare patient dose and standard. Aseptically withdraw (air-free) two or more 5-mCi aliquots using a 3-cc syringe with a 22-gauge needle. (For accurate measurement in a 3-cc syringe, the volume should be at least 1 ml.) One

of the 5-mCi aliquots will be set aside as the standard, and the remainder will be used for patient doses.

3. Calibrate the doses and the standard in a dose calibrator. The percent difference between the dose and the standard should not under any circumstances exceed 5%. One should strive for 2%–3% difference.

Example: Calculation of Percent Difference
Standard 5.112 mCi Dose 5.009 mCi

$$\frac{(5.112 - 5.009)\ \text{mCi}}{5.112\ \text{mCi}} \times 100\% = 2.0\%$$

4. Inject the patient. Flush the syringe at least three times with blood and record the time of injection. Extravasation of even a minute amount of the dose will invalidate the study. This can be checked with a scintillation camera or with a survey meter that has been partially shielded with lead foil.
5. Assay and record the residue in the syringe. If it is >3% of the dose, make an appropriate correction in the calculation.
6. Instruct the patient about the importance of the sample being drawn 3 hr after injection.
7. Standard aliquot preparation.
The ^{99m}Tc-DTPA standard used to calculate the GFR is a 1:10,000 dilution of the standard dose. Dilute the standard any time during the 3-hr period as follows:
A. Add ~ 50 ml of deionized water into each of two 100-ml volumetric flasks.
B. Carefully inject the standard aliquot into one volumetric flask labeled 1:100 dilution. Rinse the syringe into the 1:100 flask by filling it from a container of deionized water, ~ 3–5 times. Assay the residue in the syringe. If the residue is > 2% of the dose, rinse again, including the needle cap. Fill to the mark and mix well. With a volumetric pipette, pipette 1 ml of this solution into the other 100-ml volumetric flask labeled 1:10,000 dilution. Fill to the mark and mix well. Label two test tubes for the standard and carefully pipette 100 μl of the 1:10,000 dilution into each tube. Cap the tubes immediately and set aside.
8. Procedure for the 3-hr blood sample.
A. Use a vein other than the injection vein. Withdraw 10 ml of blood into a lavender stoppered (EDTA) collection tube. Record the exact time of withdrawal.
B. Mix the blood well with anticoagulant. Centrifuge for 10 min.
C. Remove the sample as soon as the centrifuge stops. Insert a serum separator and fill the ultrafiltration apparatus.
9. Procedure for ultrafiltration.
A. Ultrafiltration is carried out in duplicate by the Centrifree apparatus.* Fill the assembly three-fourths full with plasma. Be sure both assemblies have equal amounts of plasma (hold side by side). Place the filled device in a fixed-angle head centrifuge. Be sure the centrifuge is balanced. Centrifuge for 15 min at a speed not > 2,000 g. The Centrifree apparatus must be centrifuged in an angle-head centrifuge because a swinging bucket head will result in inadequate ultrafiltration.
B. As soon as the centrifuge stops, carefully retrieve the Centrifree apparatus. Dropping the apparatus can cause splashing of the ultrafiltrate on the sides of the container, which results in inadequate ultrafiltrate volume. Remove the filtrate cup containing the clear, colorless ultrafiltrate and pipette immediately. Pipette 100 μl from each assembly into test tubes labeled ultrafiltrate. Cap each tube immediately.
10. Procedure for counting.
Count the two standard tubes and the two ultrafiltrate tubes in a gamma scintillation counter set for the 140 keV ^{99m}Tc photopeak with a 20% window. Background correction should be made and the samples should be counted for a sufficient length of time to ensure good counting statistics.
11. Calculation of GFR.

$$GFR = A \ln (D/P) + B,$$

where D = Dose activity, counts/min
P = Ultrafiltrate activity × 0.94, counts/min-ml
T = Time between injection and withdrawal of blood sample (min)
A = −0.278T + 119.1 + 2,405/T
B = 2.866T − 1,222.9 − 16,820/T

(when T = 180 min, A = 82.42, and B = −800.5). Because normal human plasma is 94% water and 6% protein, when the protein is filtered out of 1 ml of plasma only 0.94 ml of ultrafiltrate remain. This gives rise to the factor of 0.94 in the above equation when ultrafiltrate is used in place of plasma.

Example:

T = 180 min A = 82.42 B = −800.5
Standard (1:10,000) activity = 76,070 cts/min-ml
Ultrafiltrate activity = 23,633 cts/min-ml
D = Standard (1:10,000) activity × 10,000 ml = 76,070 × 10,000 = 7.6070 × 10^8 cts/min
P = 23,633 × 0.94 = 22,215.0 cts/min-ml
GFR = 82.42 [ln (7.607 × 10^8/22,215.0)] + (−800.5)
GFR = 82.42 (10.44) + (−800.5)
GFR = 860.5 + (−800.5)
GFR = 60.0 ml/min

APPENDIX B

Two Blood-Sample Method

This method is only required when special accuracy is needed (i.e., for investigational purposes). This method requires the withdrawal of a 60-min blood sample in addition to the 180-min sample. Each sample should be processed immediately after withdrawal as previously described. The data are calculated as follows:

$$GFR = \left[\frac{D \ln (P_1 / P_2)}{T_2 - T_1} \exp \left(\frac{(T_1 \ln P_2) - (T_2 \ln P_1)}{T_2 - T_1} \right) \right]^{0.979},$$

where D = Dose activity, counts/min
P_1 = Ultrafiltrate activity at $T_1 \times 0.94$
P_2 = Ultrafiltrate activity at $T_2 \times 0.94$
P_1 and P_2 are in counts/min-ml.

Example:

T_1 = 60 min P_1 = 29,528 cts/min-ml × 0.94
T_2 = 180 min P_2 = 9,194 cts/min-ml × 0.94
Standard activity (1:10,000) = 56,884 cts/min-ml
D = 56,884 × 10,000 ml = 5.6884×10^8 cts/min
P_1 = 27,756.3 cts/min-ml
P_2 = 8,642.4 cts/min-ml

$$GFR = \left[\frac{5.6884 \times 10^8 \ln (27{,}756.3 / 8{,}642.4)}{180 - 60} \times \exp \left(\frac{(60 \ln 8{,}642.2) - (180 \ln 27{,}756.3)}{180 - 60} \right) \right]^{0.979}$$

$$GFR = \{5.5309 \times 10^6 \exp [(543.9 - 1{,}841.6) / 120]\}^{0.979}$$
$$GFR = [5.5309 \times 10^6 \exp (-10.82)]^{0.979}$$
$$GFR = 110.6^{0.979}$$
GFR = 100.2 ml/min.

FOOTNOTE

*Amicon Centrifree Micropartition System (MPS-1), Amicon Corporation, Danvers, MA.

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