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ORIGINAL ARTICLE

Validation of plasma clearance of ^{51}Cr-EDTA in adult renal transplant recipients: comparison with inulin renal clearance

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Summary

Plasma clearance of ^{51}Cr-EDTA (^{51}Cr-EDTA-Cl) is an alternative method to evaluate glomerular filtration rate (GFR). This study aimed to investigate the concordance between ^{51}Cr-EDTA-Cl and renal inulin clearance (In-Cl) in renal transplant recipients as well to determine the repeatability of ^{51}Cr-EDTA-Cl in kidney donors. Forty four kidney recipients and 22 kidney donors were enrolled. Simultaneous measurements of ^{51}Cr-EDTA-Cl and In-Cl were performed. A single dose of 3.7MBq of ^{51}Cr-EDTA was injected and the plasma disappearance curve was created by taking blood samples at 2, 4, 6 and 8 h after injection. Bland and Altman statistical approach was used to quantify the agreement between In-Cl and ^{51}Cr-EDTA-Cl and to determine the better concordance between all possibilities of measure for the ^{51}Cr-EDTA-Cl. The mean of In-Cl was 44.5 ± 17.9 ml/min/1.73 m^2. There was a positive correlation between In-Cl and all possible measurements of ^{51}Cr-EDTA-Cl. ^{51}Cr-EDTA-Cl with two samples taken at 4 and 8 h or at 4 and 6 h presenting the narrow limits of agreement and a difference (bias) of 2.8 and 2.7 ml/min, respectively. Two plasma sampling for ^{51}Cr-EDTA-Cl was a reliable method to measure GFR compared with In-Cl and comprises a suitable method to be used in kidney transplanted patients.

Introduction

Glomerular filtration rate (GFR) is accepted as the best index of renal function [1–3] and has been reported as an independent surrogate marker of long-term kidney allograft survival [4,5].

Serum creatinine and equations derived from it as well as the creatinine clearance have been used to evaluate GFR [6–8]. However, renal function measurements based on serum creatinine overestimate GFR and have a low sensitivity to detect renal dysfunction [9,10]. On the other hand, the renal inulin clearance (In-Cl), the gold-standard to measure GFR, is very cumbersome to perform in clinical practice. Moreover, methods that need urine collection are difficult to execute in clinical practice and can lead to errors in GRF measurement because of urinary losses and incomplete urinary bladder emptying.

Plasma clearance of ^{51}Cr-EDTA (^{51}Cr-EDTA-Cl) was described in 1967 by Garnett *et al.* [11] as an alternative method to measure GFR and has been used in clinical nephrology since then. In spite of its use for almost four decades, studies comparing In-Cl and ^{51}Cr-EDTA-Cl have either not included renal transplant recipients [12–22] in their population study or included only a few [23]. Besides, none of the studies has evaluated the ^{51}Cr-EDTA-Cl bias and its accuracy.

Kidney transplant recipients are a special group because they usually have a lower GFR in a range where most of the methods proposed for estimating GFR show a poor accuracy. Besides, they are frequently under the effects of drugs that influence GFR such as calcineurin inhibitors (CNIs) and angiotensin-converting-enzyme inhibitors (ACEIs) [24,25]. Therefore, the validation of plasma ^{51}Cr-EDTA-Cl versus In-Cl in such population seems to be mandatory and it has never been done before.

The purpose of this study was to establish: (i) the performance of ^{51}Cr-EDTA-Cl in comparison with In-Cl in renal transplanted patients presenting a wide range of renal function; (ii) a strategy for abbreviated blood sampling for ^{51}Cr-EDTA-Cl in this population; and (iii) the within-subject repeatability of ^{51}Cr-EDTA-Cl, using single-kidney, adult, stable, live kidney-donors.

Materials and methods

Study design

To asses the performance of plasma ^{51}Cr-EDTA-Cl, the design of the study planned to carry out measurements of both plasma ^{51}Cr-EDTA-Cl and In-Cl in stable renal transplanted patients with a wide range of GFR estimated by Cockcroft–Gault equation.

To determine the interday coefficient of variation (CV) of ^{51}Cr-EDTA-Cl, in the same individual in similar conditions, two ^{51}Cr-EDTA-Cl with time interval of 2 weeks, were planned in kidney donors with at least 12 months after donation.

The protocol was approved by the local ethical committee (reference number 1042/03) and all participants gave written informed consent.

Study protocols

Simultaneous measurements of plasma ^{51}Cr-EDTA-Cl according to the single injection technique and In-Cl by the continuous infusion method were performed.

Patients were admitted to our clinical research clinic. After a protein-restricted diet and a 12-h overnight fast, at around 7:30 AM, they were requested to drink 400 ml of water before the initiation of the tests and 200 ml every half-an-hour thereafter, to maintain a high rate of urine during the study.

They rested supine with an indwelling polyethylene catheter inserted into a cubital vein in both arms. Inulin and the radiotracer were administered by intravenous infusion and blood samples were obtained from the opposite catheter.

Drugs which affect renal function (as ACEIs and CNIs) were given at the sixth hour after starting the study.

Recruitment and sample size

The population planned for the first goal of this study was a sample of 40 stable renal transplanted patients being followed up at our out-patient clinic. All patients who came first to our clinic were classified according to the estimated glomerular filtration rate (eGFR) (Cockcroft–Gault) within the chronic kidney disease (CKD) stages [26] (stage 1: ≥90 ml/min/1.73 m^2, stage 2: 60–89 ml/min/1.73 m^2, stage 3: 30–59 ml/min/1.73 m^2 and stage 4: 15–29 ml/min/1.73 m^2) and invited to participate until approximately six to 11 patients in each stage were recruited. The first 44 patients (to allow for 10% drop-outs) who agreed to sign the informed consent were enrolled into the study. Exclusion criteria were diabetes mellitus, obstructive uropathy, edemas and CKD stage 5 (eGFR < 15 ml/min/1.73 m^2).

To determine the ^{51}Cr-EDTA-Cl within-subject CV, we planned a sample of 22 (to allow for 10% drop-outs) live kidney-donors. The same strategy to invite the first 22 who return to the out-patient clinic and accept to enter the study was carried out. At our hospital, donors are invited to return every 1–2 years for routine analysis.

A negative pregnancy test was required for women during childbearing age not using a regular contraceptive method.

Inulin renal clearance

For In-Cl determination, Sinistrin, an inulin-like polyfructosan (Sinistrin – INUTEST 25%; Fresenius Kabi Austria GmbH, Linz, Austria) with improved clinical properties over inulin, was employed. Sinistrin has the advantage of a higher solubility, which facilitates its administration without having to heat the solution [27].

A priming dose of 1500 mg/m^2 of inulin diluted in 100 ml of saline solution was given as a bolus followed by a constant infusion of 12 mg/m^2/min diluted in 500 ml of saline to achieve a stable plasma concentration of 20–40 mg/dl. Patients with an eGFR lower than 40 ml/min/1.73 m^2 received a reduced dose of inulin (2/3 of the above mentioned dose).

After a 90-min equilibrium interval, the first clearance started and was repeated every 60 min for four consecutive times.

Urine was carefully collected by spontaneous emptying of the bladder carefully inspected by a nurse and plasma samples obtained at the same time-points. The final clearance was calculated as the mean of the four tests.

Inulin concentration was determined by the anthrone method. Plasma was deproteinized and diluted to 1:11 with perchloric acid. The urine was diluted to 1:33 with distilled water. Urine and plasma samples were heated in constant

temperature water, at 52 °C, for 10 min and the absorbance was read at 620 nm [28,29]. The renal clearance was calculated using the equation $U \cdot V/P$, where U is the urinary inulin concentration, V is the volume of urine in ml/min and P is the inulin plasma concentration. The clearance was then corrected for 1.73 m^2 of body surface area.

Plasma clearance of ^{51}Cr-EDTA

A single dose of 3.7MBq (100 μCi) of the ^{51}Cr-EDTA tracer, in a volume of 1 ml was injected intravenously in the opposite arm of the inulin infusion. The exact injected dose was determined by weighting the syringe before and after the injection on a high precision analytic balance. The catheter was flushed through with 10 ml of saline. Accurately timed, 10 ml blood-samples were drawn into a heparinized tube from the opposite arm at 2, 4, 6 and 8 h after the injection. The plasma disappearance curve was constructed using the results of these four time-points.

To measure the radioisotope activity, the blood samples were centrifuged at 1738 g for 10 min and 3 ml of plasma measured in a well-counter calibrated for the energy of chromium-51 (320 keV). Each sample, including a 3 ml radioisotope control, taken as an aliquot from 3.7MBq (100 μCi) ^{51}Cr-EDTA diluted to 500 ml in saline, was counted for 5 min.

Plasma clearance rate was calculated by the slope-intercept method with single-compartment model, which assumes that the tracer has spread out immediately after injection in its volume of distribution. The Brochner–Mortensen's method was used for correcting systematic error of the slope-intercept technique according to the equation [30]:

$$Cl_1 = 0.99 \times Cl_2 - 0.0012 \times Cl_2^2,$$

where Cl_1 is the clearance corrected for the first exponential and Cl_2 is the noncorrected clearance.

Analyses of the different possibilities using the slope-intercept method (with two or more time-points) were also compared with the single sample technique proposed by Groth [31–33]. For the calculation of GFR with a single sample technique, the Christensen–Groth method was used, according to the following formula:

$$Cl = -In\ (ECV/V_t) \times ECV/(t \times g(t)),$$

where $ECV = 8116.6 \times A - 28.2$ is the extracellular volume, t (min) the time of drawing of the blood sample, A (m^2) the body surface area, and $g\ (t)$ is the function $g\ (t) = (0.0000017 \times t - 0.00120) \times Cl - 0.00075 \times t + 1.31$.

Plasma clearance of ^{51}Cr-EDTA was calculated using combinations of four blood-time samples (2, 4, 6 and 8 h), three blood-time samples (2, 4 and 6 h; 2, 4 and 8 h; 2, 6 and 8 h; 4, 6 and 8 h), two blood-time samples (2 and 4 h; 2 and 6 h; 2 and 8 h; 4 and 6 h; 4 and 8 h and 6 and 8 h) and one blood-time sample (2 h; 4 h; 6 h and 8 h) totaling 15 possible combinations. ^{51}Cr-EDTA-Cl was corrected for 1.73 m^2 body surface.

Estimated GFR

Estimated GFR was calculated with equations that used serum creatinine [34–37] as described below:

1 MDRD abbreviated formula (aMDRD) = $186 \times$ [serum creatinine (mg/dl)]$^{-1.154} \times$ [age]$^{-0.203} \times$ [0.742 if patient is female] $\times$ [1.21 if patient is black].

2 Cockcroft-Gault formula (CG) = ([140 − age(*years*)] × weight (kg))/72 × serum creatinine (mg/dl) × [0.85 if patient is female].

3 Nankivell formula = 6700/[serum creatinine (mg/dl) × 88.4] + [weight (kg)/4] − [serum urea (mmol/l)/2] − [100 height (meters)^2] + 35(if male) or 25(if female).

Statistics

Comparisons of values were tested by paired *t*-test. To check the Gaussian distribution, data were previously evaluated by Kolmogorov–Smirnov test.

The Pearson correlation coefficient and linear regression were applied to assess association between renal In-Cl and the 15 possible combinations obtained for the ^{51}Cr-EDTA-Cl.

Bland and Altman (B&A) statistical analysis, based on plotting differences between methods against the mean of the two-methods, was used to quantify the degree of agreement between In-Cl and ^{51}Cr-EDTA-Cl. The mean of the differences represents the estimated bias, the systematic difference between the methods, and the standard deviation of these differences measure random fluctuations around this mean. Ninety-five percent of differences (95% CI) lie between two limits defining the 'limits of agreement': the lower limit, which is the mean difference minus 1.96 standard deviations, and the upper one, which is the mean difference plus 1.96 standard deviations. The degree of agreement was based on mean bias and limits of agreement [38–41].

All the GFR measurements using the 15 possible ^{51}Cr-EDTA-Cl that do not statistically differ from In-Cl proceeded to further analysis.

The bias, precision and accuracy were calculated as recommended in the National Kidney Foundation guidelines on CKD; *bias* was defined as the mean difference between the In-Cl and ^{51}CrEDTA-Cl or eGFR; precision was defined as standard deviation (SD) of the difference between the In-Cl and ^{51}CrEDTA-Cl or eGFR. Accuracy

was defined as the percentage of ^{51}Cr-EDTA-Cl or eGFR lying within 30% of the Inulin clearance.

The within-subject repeatability of the ^{51}Cr-EDTA-Cl was evaluated by paired *t*-test to compare means in repeated measures (named test 1 and test 2).

The CV was calculated as 100 times the ratio of the standard deviation over the mean.

A P-value < 0.05 was considered significant. Body surface was calculated by DuBois equation:

$$\text{BSA } (\text{m}^2) = 0.007184 \times \text{Ht}^{0.725} \times \text{Wt}^{0.425},$$

where patient's body height (Ht) in centimeters and their weight (Wt) in kilograms [42].

Results

Patients

Forty-four renal transplant recipients signed the informed consent and were enrolled. None of them dropped-out from the study and all were analyzed. The baseline characteristics of the patients are described in Table 1.

Inulin renal clearance

The mean of the In-Cl was 44.5 ± 17.9 ml/min/1.73 m^2. According to In-Cl, 10 patients (23%) presented the CKD stage 2; 23 (52%) the stage 3 and 11 (25%) the stage 4 range. None of them was in the stage 1.

Table 1. Demographic characteristics of renal transplant recipients at baseline.

Characteristics	*n* = 44
Age (mean ± SD; years)	42 ± 11
Gender, male/female, *n* (%)	32/12 (73/27)
Race, white/nonwhite, *n* (%)	20/24 (45/55)
Body surface (mean ± SD; m^2)	1.69 ± 0.17
Height (mean ± SD; cm)	164 ± 8
Weight (mean ± SD; kg)	65 ± 12
Median time after transplantation; months (min–max)	22 (5–160)
Imunosuppression, *n* (%)	
Tacrolimus	24 (55)
Cyclosporin	10 (22.5)
Without CNIs	10 (22.5)
Chronic kidney disease classification – eGFR [*n* (%)]/median time after transplantation (months)	
Stage 1 (≥90)	6 (14)/14
Stage 2 (60–89)	12 (27)/16
Stage 3 (30–59)	20 (45)/33
Stage 4 (15–29)	6 (14)/59
ACE inhibitors and/or ARA II, *n* (%)	13 (29.6)

eGFR, Cockcroft–Gault equation (ml/min/1.73 m^2); ACE, angiotensin-converting enzyme; ARA II, angiotensin II receptor antagonists.

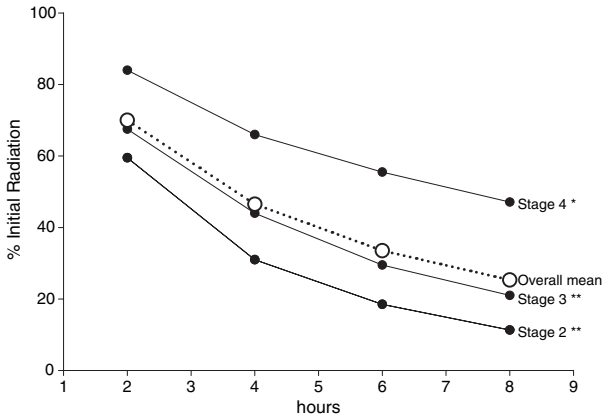

Figure 1 Percentage of decrease in initial radiation at all time-points in kidney transplanted patients. Dotted line with open circle represents the overall mean values. Stage 4 (15–29 ml/min/1.73 m^2), stage 3 (30–59 ml/min/1.73 m^2) and stage 2 (60–89 ml/min/1.73 m^2) according to the inulin clearance. *P < 0.001 comparing stage 4 to others stages. **P < 0.002 comparing stage 2 and 3.

Plasma clearance of ^{51}Cr-EDTA

Figure 1 shows the decrease, in percentage, of the initial plasma ^{51}Cr-EDTA radioactivity throughout the 8 h after the bolus injection in the three stages of CKD according to In-Cl results. The profile of the plasma radioactivity shows that after 2 h following tracer injection, the elimination of the radioisotope is possibly related to glomerular filtration only. There is a different radioactivity profile between stage 4 and all the others stages of CKD ($P < 0.001$) for all time-points. There was also a statistical difference between the stage 3 and stage 2 ($P < 0.002$).

Correlation between In-Cl and plasma clearance of ^{51}Cr-EDTA-Cl

Table 2 shows the results of In-Cl and all the possible combinations of the ^{51}Cr-EDTA-Cl. All these measured clearance showed a normal distribution.

The four time-points ^{51}Cr-EDTA-Cl (^{51}Cr-EDTA-Cl$_{2,4,6,8}$) highly correlated with In-Cl ($R = 0.94$). The mean ^{51}Cr-EDTA-Cl$_{2,4,6,8}$ was 47.0 ± 16.9 ml/min/1.73 m^2 and not statistically different from the mean In-Cl that was 44.5 ± 17.9 ml/min/1.73 m^2 ($P = 0.5$). The bias of calculating In-Cl using four time-points ^{51}Cr-EDTA-Cl$_{2,4,6,8}$ according to the B&A analysis was 2.5 ± 6.1 ml/min/1.73 m^2 (Fig. 2a).

For all two to three time-points combinations shown in Table 2, there was a high correlation with In-Cl. Specifically, for the two time-points combinations, those taken at 4 and 6 h as well as those taken at 4 and 8 h, or

Table 2. Descriptive statistics of inulin renal clearance and plasma clearance of ^{51}Cr-EDTA in renal transplant recipients.

Variable n = 44	Mean ± SD ml/min/1.73 m^2	Paired t-test P-value	R	R^2	B&A mean ± SD ml/min/1.73 m^2	Accuracy 30% (%)
Inulin Cl	44.5 ± 17.9	–	–	–	–	
^{51}Cr EDTA-Cl time-points (hours)						
2, 4, 6, 8	47.0 ± 16.9	0.50	0.94	0.89	2.5 ± 6.1	90.9
2, 4, 6	47.7 ± 16.5	0.39	0.93	0.87	3.2 ± 6.4	88.6
2, 4, 8	46.7 ± 16.7	0.56	0.95	0.90	2.2 ± 5.8	88.6
2, 6, 8	46.7 ± 16.5	0.56	0.93	0.87	2.2 ± 6.4	90.9
4, 6, 8	47.6 ± 18.8	0.43	0.94	0.88	3.1 ± 6.5	93.2
2, 4	48.6 ± 15.9	0.26	0.92	0.85	4.1 ± 6.9	79.5
2, 6	47.3 ± 16.3	0.45	0.93	0.86	2.8 ± 6.8	88.6
4, 6	47.2 ± 18.0	0.48	0.95	0.90	2.7 ± 5.9	93.2
4, 8	47.3 ± 18.4	0.47	0.95	0.90	2.8 ± 5.8	93.2
6, 8	47.6 ± 21.0	0.45	0.84	0.71	3.1 ± 11.2	81.8
2, 8	46.0 ± 16.2	0.67	0.94	0.89	1.5 ± 6.0	90.9
2	49.1 ± 18.1	0.23	0.76	0.58	4.6 ± 12.4	75.0
4	50.1 ± 17.1	0.14	0.92	0.84	5.6 ± 7.2	77.3
6	51.3 ± 17.8	0.04	–	–	–	
8	53.2 ± 18.6	0.03	–	–	–	

R^2, coefficient of determination; LR, linear regression (for all variables $P < 0.0001$); B&A, Bland and Altman analysis – mean ± SD of the differences between In-Cl and ^{51}Cr-EDTA-Cl.

three time-points taken at 2, 4 and 8 h presented the highest correlation ($R = 0.95$) with a minor bias (Fig. 2b,c).

The ^{51}Cr-EDTA-Cl measured taken at 4 and 6 h, 4 and 8 h or for three time-points at 4, 6 and 8 h had the best accuracy of 93.2%.

The results obtained with just a single time-point were either statistically different from the In-Cl or had a poorer correlation and a much higher bias.

Table 3 shows the B&A analysis of ^{51}Cr-EDTA-Cls for the different stages of CKD, using the various abbreviated time-point combinations. Only the time-points that did not differ statistically from the In-Cl were analyzed. The ^{51}Cr-EDTA-Cl with two time-points taken either at 4 and 6 h or at 4 and 8 h as well as the three time-points taken at 2, 4 and 8 h presented the lowest bias when all stages of CKD are regarded together. The bias, precision and accuracy for these time-points are presented again in Table 4 with the performance of the GFR estimated by three equations. The median serum creatinine was 1.55 mg/dl ranging from 0.75 to 4.5 mg/dl. Considerable overestimation of In-Cl was observed for all creatinine based equations and it was 15.6 ± 13.0 ml/min/1.73 m^2 ($P < 0.001$) for Nankivell, 14.4 ± 11.7 ml/min/1.73 m^2 ($P < 0.001$) for Cockcroft–Gault and it was 11.9 ± 15.3 ml/min/1.73 m^2 ($P < 0.001$) for aMDRD. Compared to ^{51}Cr-EDTA-Cl, the equations Nankivell, Cockcroft–Gault and aMDRD had a poor accuracy (39%, 54.5% and 59.1%, respectively).

Interday coefficient of variation of ^{51}Cr-EDTA-Cl in live kidney-donors

None of the 22 live kidney-donors dropped out from the study and all were analyzed. They had donated their kidney for 12–53 months before the test. This population comprises 10 females and 12 males, with a mean age of 38.6 ± 8.2 years (27–56 years). Their BMI ranged from 20 to 33. The two repeated ^{51}Cr-EDTA-Cl measurements were performed with a mean difference time of 15.4 ± 3.3 days.

Table 5 shows the mean of the differences between test 1 and test 2. The mean ^{51}Cr-EDTA-Cl$_{2,4,6,8}$ at test 1 was 69.1 ± 7.3 and the mean ^{51}Cr-EDTA-Cl$_{2,4,6,8}$ of test 2 was 69.1 ± 11.2 ml/min/1.73 m^2. No statistical differences were observed between replicate measurements in all ^{51}Cr-EDTA-Cl combinations shown in Table 5. The CV ranged from 6.1% to 10.1%.

Discussion

In this study, we have demonstrated that plasma ^{51}Cr-EDTA-Cl can replace inulin clearance in renal transplanted patients with a high concordance and minor error. We have also found that the strategy of collecting blood at two late time-points is very accurate and facilitating the logistics of blood sampling.

We have chosen to validate the plasma ^{51}Cr-EDTA-Cl because it does not require urine collection making it

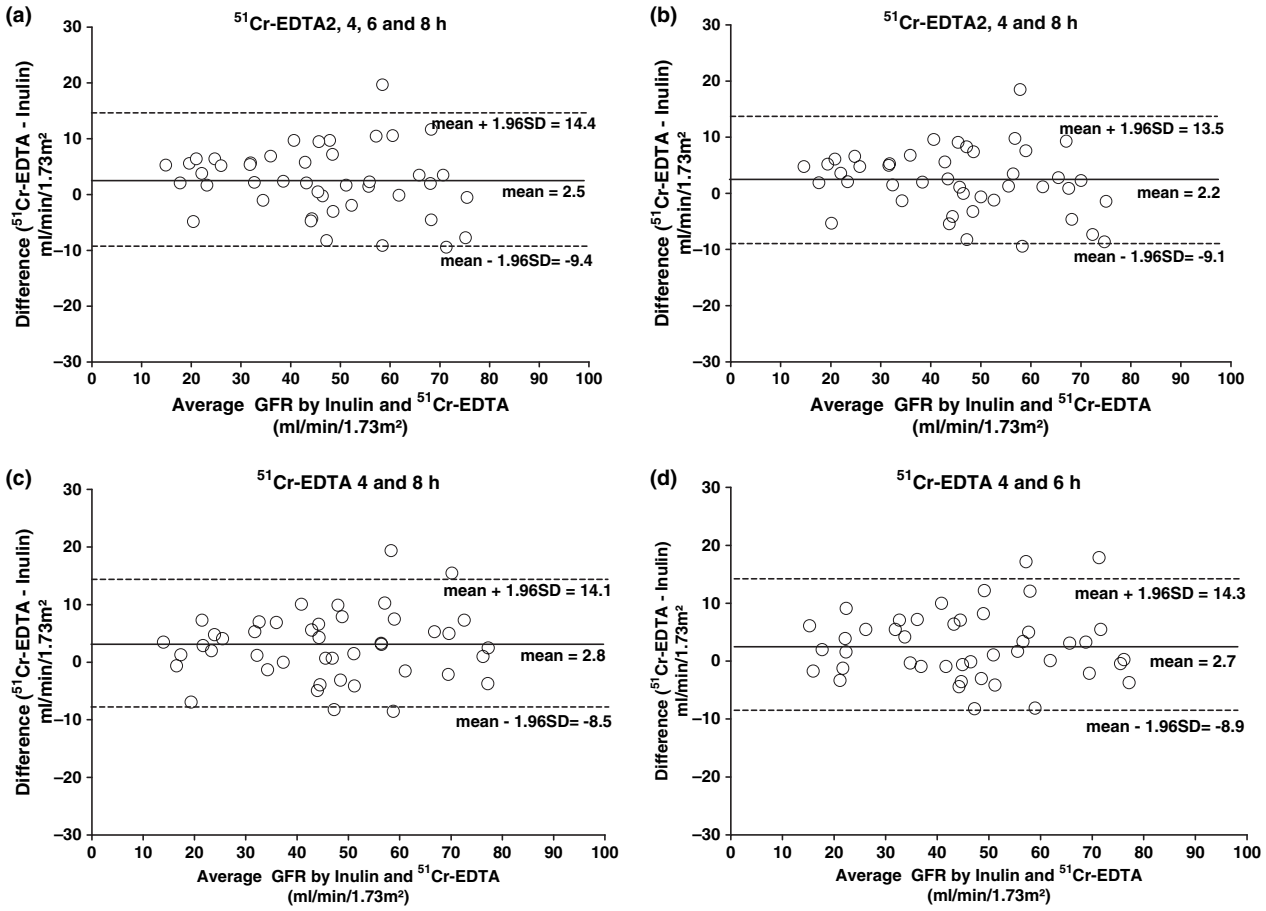

Figure 2 Bland and Altman analysis of the ^{51}Cr-EDTA-Cl and inulin clearance. Mean + 1.96 SD and mean – 1.96 SD are the upper and lower limits of the interval of agreement, respectively.

simpler to execute on daily practice without the interference of the patient. Besides, ^{51}Cr-EDTA is available in many centers worldwide. Although plasma ^{51}Cr-EDTA-Cl is being used by transplant centers, there are no studies in transplanted patients.

Therefore, the validation of plasma ^{51}Cr-EDTA versus In-Cl in a transplant population was demanding and had never been done before.

The measure of GFR with a single injection of ^{51}Cr-EDTA is a simple procedure that can be repeated regularly and with a lower cost when compared with others exogenous markers such as Iothalamate, iohexol and Inulin (polyfructosan).

The ^{51}Cr-EDTA-Cl measurement implies an exposure to radiation. However, the radiation burden is approximately 0.0077 mSv for a single clearance measurement, thus, it is lower than received from many radiographic tests, like a plain chest radiograph (0.02 mSv) [43,44]. The annual effective dose limit for individual members of the public from all radiation sources is 1 mSv that is a numerical value 133 higher than the effective dose for patients undergoing an GFR evaluation by ^{51}Cr-EDTA.

The need of such simple and easy-to-repeat method to measure GFR in a transplant population is illustrated in our study where the estimated GFR based on Cockcroft–Gault equation showed a large overestimation of renal function. At enrollment, none of the patients registered by Cockcroft-Gault equation as a stage 1 CKD (GFR ≥ 90 ml/min) presented In-Cl > 79 ml/min, which is quite predictable as a function of a single transplanted kidney.

Our data showed that plasma clearance of ^{51}Cr-EDTA only slightly overestimated renal clearance of inulin according to previous reports [17,20,45,46]. Extra-renal elimination of the tracer has been suggested as a possible reason for this finding.

We found a correlation of 0.95 between ^{51}Cr-EDTA-Cl and In-Cl. Previous studies in a nontransplanted population have described values about 0.92–0.97 [16,19,21,22]. However, the correlation coefficient is just a measure of association and does not determine whether the two

Table 3. Bland and Altman (B&A) analysis of ^{51}Cr-EDTA-Cl evaluated using various time-points according to the stage of chronic kidney disease (CKD) measured by the inulin clearance (In-Cl).

Stage of CKD (In-Cl ml/min/1.73 m^2)	Stage 2 60–89 (n = 10)	Stage 3 30–59 (n = 23)	Stage 4 15–29 (n = 11)
^{51}Cr EDTA-Cl time-points (hours)	B&A (mean ± SD)	B&A (mean ± SD)	B&A (mean ± SD)
2, 4, 6, 8	1.1 ± 6.7	3.4 ± 6.4	3.9 ± 3.3
2, 4, 6	1.0 ± 6.7	4.0 ± 6.7	5.3 ± 3.5
2, 4, 8	1.5 ± 6.0	3.1 ± 6.1	3.7 ± 3.3
2, 6, 8	2.4 ± 7.1	3.3 ± 6.5	3.9 ± 3.2
4, 6, 8	2.4 ± 8.1	3.6 ± 6.9	2.6 ± 3.8
2, 4	0.4 ± 6.7	4.2 ± 13.8	7.6 ± 5.6
2, 6	2.1 ± 7.3	3.9 ± 7.0	5.0 ± 3.3
4, 6	1.6 ± 6.9	3.0 ± 6.3	3.2 ± 4.0
4, 8	2.1 ± 6.7	3.2 ± 6.3	2.8 ± 4.0
2, 8	3.4 ± 6.0	2.7 ± 6.1	3.5 ± 3.2
6, 8	2.5 ± 18.5	3.9 ± 9.8	2.0 ± 4.6
2	0.2 ± 8.8	4.2 ± 13.8	9.9 ± 11.1
4	3.7 ± 6.7	5.0 ± 7.8	8.7 ± 5.7

Mean ± SD, mean ± standard deviation of the difference between inulin and ^{51}Cr-EDTA clearance, in ml/min/1.73 m^2.

methods agree sufficiently to be used interchangeably. Instead, we evaluated the agreement between the two methods based on the mean bias and limits of agreement, a more appropriated analysis to compare methods. When all patients were analyzed together, most of ^{51}Cr-EDTA-Cl, in various abbreviated time-point combinations, presented a good agreement with In-Cl.

Transplanted patients present a wide range of renal function. In 1996, a committee on renal clearance indicated different sampling strategies according to the estimated renal function. In these guidelines, an eGFR above 30 ml/min, could be evaluated by the single-sample technique. For eGFR between 15 and 30 ml/min, a later sampling was advised at 3 and 5 h after injection [47]. Others recommended different strategies. Fleming *et al.* [48] suggested two, three or four venous samples taken at between 2 and 5 h postinjection, or one sample at 3 or 4 h postinjection for adults, or at 2 h for children.

Table 5. Comparison between repeated ^{51}Cr-EDTA-Cl measurements in 22 healthy kidney donors 2 weeks apart.

51Cr-EDTA-Cl time-points (hours)	Mean ± SD of the difference (test 1–test 2)	Paired t-test (P-value)	Coefficient of variation (%)
2, 4, 6, 8	0.0 ± 8.7	1.00	6.5
2, 6, 8	0.6 ± 8.5	0.76	6.6
2, 4, 8	1.0 ± 8.4	0.59	6.1
2, 4	2.3 ± 9.6	0.27	7.3
2, 8	0.3 ± 7.8	0.85	6.4
4, 8	0.4 ± 15.5	0.91	10.1
4, 6	2.9 ± 12.7	0.30	9.7
4	2.0 ± 10.1	0.38	7.6

Mean ± standard deviation of the difference between test 1 and test 2 in ml/min/1.73 m^2.

In our opinion, the recommendation of a single strategy for the measurement of the ^{51}Cr-EDTA-Cl in all ranges of GFR is more adequate. Many different routine samplings according to the eGFR could be an error source because of its overestimation of GFR. Besides, in transplanted patients, there is a possibility of rapid change in GFR during the request of the test and its performance. Finally, different strategies could cause confusion to the laboratory logistics.

Our data have shown that the ^{51}Cr-EDTA-Cl with two time-points taken at 4 and 6 h can be used for this purpose because it presents a narrow limit of agreement with a minor bias and high accuracy and presents a simple workload logistics for the patient and the laboratory, although it does not have the best precision compared to the others combinations (Table 5).

This strategy with two time-points taken at 4 and 6 h after injection is in accordance with the rationale that kidney transplanted patients require a later sampling possibly related to the lower GFR. In 1969, Maisey *et al.* [49] reported that the tracer ^{51}Cr-EDTA is substantially equilibrated by 2 h in the normal subjects and with a GFR below 40 ml/min it may be necessary later sample to obtain a reliable result. Our own data are in accordance with this rationale (Fig. 1).

Table 4. Accuracy, bias and precision for measure GFR by ^{51}Cr-EDTA and estimated GFR by equations based on serum creatinine in kidney transplant patients.

GFR (n = 44)	Mean ± SD (ml/min/1.73 m^2)	Accuracy 30% (%)	Bias (ml/min/1.73 m^2)	Precision (ml/min/1.73 m^2)
Inulin	44.5 ± 17.9	–	–	–
^{51}CrEDTA 2, 4, 8	46.7 ± 16.7	88.6	2.2	5.8
^{51}CrEDTA 4, 6	47.2 ± 18.0	93.2	2.7	5.9
^{51}CrEDTA 4, 8	47.3 ± 18.4	93.2	2.8	5.8
eGFR				
Nankivell	60.1 ± 25.6	39.0	15.6	13.0
Cockcroft–Gault	58.9 ± 24.7	54.5	14.4	11.7
aMDRD	56.5 ± 25.9	59.1	11.9	15.3

Our results demonstrate that GFR estimated by Nankivell, Cockcroft–Gault and aMDRD equations does not reach a satisfying agreement with GFR measured by a standard method and although the aMDRD presents a better accuracy (59.1%) than the other equations, none of these allows an accurate measurement of renal function in kidney transplant recipient. Other authors found a poor accuracy (within 30% of the true GFR) for aMDRD equation in kidney transplant; Zahran *et al.* [50] reported 52.1% and 68.7% for GFR above and below 60 ml/min/1.73 m^2, respectively; Pöge *et al.* [8] found an accuracy of 60% for aMDRD and it was 33.7% for Cockcroft–Gault equation.

In clinical renal transplant practice, there is a constant need to monitor for GFR changes. Therefore, the determination of the intra-individual coefficient of variation of ^{51}Cr-EDTA-Cl is important to distinguish between real changes in GFR from the day-to-day changes in the subjects and in laboratory procedures. For this purpose, we have selected live-kidney donors. This population is not expected to change GFR over a short period of time.

We found a CV ranging from 6.1% to 10.1% between two repeated measurements of ^{51}Cr-EDTA-Cl. This range seems to be a very reasonable variation. The In-Cl, assessed by standard technique, has a CV of 7.5% [2,51].

Other authors described the same variation for ^{51}Cr-EDTA-Cl ranging from 3.9% to 11.6% [17,52–54].

In summary, we showed that plasma ^{51}Cr-EDTA-Cl is a very precise method to measure GFR in renal transplanted recipients. Our study also showed that an abbreviated strategy with blood sampling collected at later time-points (4 and 6 h) provides an accurate and convenient strategy to measure GFR for all stages of renal function in renal transplanted patients.

Authorship

FSRM: collected data, conducted data analysis, and wrote the manuscript. EDN: helped in designing the study and assisted with interpretation of the data. ESP and FA: helped with collected data. MTS and MHMS: helped with laboratory experiments. FBCL, CAB and LEI: reviewed the manuscript.

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