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Creatinine- vs. cystatin C-based equations compared with ^{99m}TcDTPA scintigraphy to assess glomerular filtration rate in chronic kidney disease

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Abstract

Background: **In chronic kidney disease (CKD), accurate estimation of the glomerular filtration rate (GFR) is mandatory. Gold standard methods for its estimation are expensive and time-consuming. We compared creatinine- versus cystatin C–based equations to measure GFR, employing ^{99m}Tc-DTPA scintigraphy as the gold standard.**

Methods: **This was a prospective cross-sectional observational study including 300 subjects. CKD was defined according to K/DOQI guidelines, and patients were separated into groups: stage 1 (G1), n=26; stage 2 (G2), n=52; stage 3 (G3), n=90; stage 4 (G4), n=37; stage 5 (G5), n=60; and control group, n=35. Creatinine-based estimates were from 24-hour creatinine clearance using the Walser formula, Cockcroft-Gault, MDRD-4 and CKD-EPI; cystatin C equations used were Larsson, Larsson modified equation, Grubb and Hoek.**

Results: **Age and body mass index were different among groups; proteinuria, hypertension, diabetes and primary glomerulopathies significantly increased as CKD worsened. In the global assessment, CKD-EPI and Hoek gave the highest correlations with ^{99m}Tc-DTPA: ρ=0.826, p<0.001 and ρ=0.704, p<0.001, respectively. Most significant linear regressions obtained: CKD-EPI vs. ^{99m}Tc-DTPA, Hoek vs. ^{99m}Tc-DTPA and CKD-EPI vs. Hoek. However, important differences emerged when each group was analyzed separately. Best significant correlations obtained with ^{99m}Tc-DTPA: control group, creatinine clearance ρ=0.421, p=0.012; G1, Crockoft-Gault ρ=0.588, p=0.003; G2, CKD-EPI ρ=0.462, p<0.05; G3, CKD-EPI ρ=0.508, p<0.001; G4, Hoek ρ=0.618, p<0.001; G5, CKD-EPI ρ=0.604, p<0.001.**

Conclusions: **At GFR <60 ml/min, CKD-EPI and Hoek equations appeared to best correlate with ^{99m}TcDTPA. In controls and at early stages of CKD, creatinine-based equations correlated better with ^{99m}Tc-DTPA, with CKD-EPI being the one with the best degree of agreement.**

Key words: *Chronic kidney disease, Creatinine, Cystatin C, Glomerular filtration rate, ^{99m}Tc-DTPA scintigraphy*

Introduction

The assessment of kidney function is relevant and important in daily medical practice, and it is useful in knowing the health status of an individual, to interpret signs and symptoms, to choose the dose and kind of drug to administer, to prepare the patient for radiocontrast media and to detect, assess and monitor kidney disease. The glomerular filtration rate (GFR) is considered the best general index that reflects kidney function, both in health and in disease (1).

In turn, chronic kidney disease (CKD) is an important and growing public health problem worldwide. The correct estimation of GFR is critical for the correct assessment of CKD

patients. The Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, widely employed in clinical practice, stratify CKD into 5 stages according to the GFR estimated through the depuration of creatinine (2). In those guidelines, GFR reductions are defined as slight where there is only kidney damage (stages 1 and 2; GFR >60 ml/min per 1.73 m^2). Moderate (stage 3; GFR 59-30 ml/min per 1.73 m^2) and severe (stage 4; GFR 29-15 ml/ min per 1.73m^2) reductions of GFR and end-stage renal disease (stage 5; GFR <15 ml/ min per 1.73 m^2) are defined as CKD, independently of renal damage. To estimate GFR precisely, an ideal marker of filtration is required. An ideal marker of GFR is defined as an endogenous molecule that, produced at a constant rate, is freely disposed by the kidney only by glomerular filtration, without being either secreted or reabsorbed by tubular cells (3). The gold standard for the estimation of GFR is based on the clearance of exogenous substances such as inulin, iohexol, ^{51}Cr-EDTA, ^{99m}Tc-DTPA or [^{125}I]-iothalamate, which involve laborious and invasive, time-consuming techniques. During the last few decades, serum creatinine has been the most frequently employed marker to estimate GFR. Creatinine is completely filtered by the glomerular membrane and is not reabsorbed or metabolized by the kidney, although it is partially secreted by the proximal tubule. Tubular secretion raises creatinine clearance 10%-20%, reaching 50% in cases of advanced CKD (4). Creatinine is usually determined by the Jaffé reaction, which is based in a complex formation between alkaline picrate and creatinine. According to this method, other different chromogens besides creatinine are normally present in serum and absent in urine. Thus, creatinine concentration is overestimated in serum and the clearance underestimated in approximately 10%-20% of cases. Other factors such as age, sex, race, body surface area (BSA), muscular mass and kind of diet (protein intake), also affect creatinine concentration. Creatinine clearance offers a better estimation of GFR than serum creatinine alone, but 24-hour urine collection is needed, which is cumbersome and not precise. The K/DOQI guidelines emphasize the necessity to assess GFR employing equations based on serum creatinine, and not to rely on serum creatinine concentration alone (2). The most commonly used creatinine-based formulas include the Cockcroft-Gault equation, adjusted for age, weight and sex, which is focused on measuring creatinine depuration (5). Another widely used formula is the Modification of Diet in Renal Disease (MDRD) Study equation and its variants, which focuses on estimating GFR (5). Finally, the Chronic Kidney Disease Epidemiology (CKD-EPI) equation, published in 2009 appears to be more exact than the previous ones for estimating GFR (1). All these equations have not been properly validated at the GFR at which they were employed, and because the creatinine methods are not standardized among clinical centers, this gives rise to differences in creatinine measurements, demographic and anthropometric data (2, 6). Finally, the precision of these equations remains the subject of intense debate.

Recently, cystatin C has been proposed as a new endogenous marker of GFR. This low-molecular-weight cysteine-protease inhibitor (13,300 Da) is produced at a constant rate by all of the nucleated cells of the human body (7). Cystatin C appears to protect connective tissue from intracellular enzymatic destruction, and exerts antibacterial and antiviral effects (8). Cystatin C is freely filtered through the glomerular membrane and is reabsorbed and metabolized but not secreted by the proximal tubule (8). As only minute amounts of cystatin C are normally excreted in the urine, its urinary depuration cannot be measured under normal conditions. Consequently, serum cystatin C concentration depends almost exclusively on GFR (7). Serum cystatin C concentration appears to be independent of muscular mass, sex, age or nutritional status (7, 9), although recent studies have questioned these findings (10, 11). Serum cystatin C levels may not be altered by inflammation, fever or other agents (9). Moreover, it appears to be a better marker of GFR in special clinical conditions such as hepatic cirrhosis, diabetes mellitus and in the elderly (12, 13). Due to these properties, many have proposed cystatin C as a superior marker for GFR than creatinine (7). However, high glucocorticoid doses increase cystatin C plasmatic concentrations (14). Besides, cystatin C levels fall in hypothyroidism and increase in hyperthyroid states (15). In contrast to creatinine, cystatin C measurement presents no interference with other chromogens, with the exception of elevated titers of rheumatoid factor in vitro (16).

We assessed the estimation of GFR based on creatinine equations and compared them with cystatin C–based formulas, employing ^{99m}Tc-DTPA renal scintigraphy as the gold standard. We stratified CKD patients into the 5 stages of CKD according to radioisotopic determination, and those without renal disease were included, following K/DOQI guidelines (1).

Methods

Study design

A prospective cross-sectional observational study was undertaken between October 2009 and September 2010 at the British Hospital of Buenos Aires, Argentina. Three hundred adult patients were included.

Regulatory aspects

The study was approved by the institutional review board of the hospital. Cystatin C kits were donated by Gentian Inc., Oslo, Norway. Local permission from the Ministry of Health and ANMAT-INAME were obtained (form 788/0509, May 13th 2009).

Population

Three hundred white adult outpatients between 18 and 80 years were included: 174 men (58%) and 126 women (42%). CKD and its stages were defined according to K/DOQI guidelines (1): criteria number 1: renal damage >3 months, as established by structural or functional damage, with or without decrease in GFR, shown by histopathological anomalies and renal damage markers including those found in blood, urine or images; or criteria number 2: GFR <60 ml/min per 1.73 m^2 for >3 months, with or without renal damage. In turn, National Kidney Foundation K/DOQI guidelines divide CKD into 5 stages (1); we also included a control group, which was defined as subjects without hypertension, diabetes mellitus, thyroid disease, single kidney, cancer or previous episodes of renal disease, microhematuria or proteinuria, and with a normal renal sonogram.

Studies performed

The following studies were performed: fasting serum creatinine and cystatin C, 24-hour creatinine clearance, 24-hour proteinuria, ^{99m}Tc-DTPA scintigraphy and renal sonogram. Blood sampling and radiorenograms were all completed at the British Hospital facilities by the same professionals.

GFR was estimated by serum creatinine, 24-hour creatinine clearance and the following equations: Cockcroft-Gault (17), MDRD-4 (18), CKD-EPI (2), plus serum cystatin C, and Larsson (19), Larsson modified equation according to DuBois BSA (proposed by our group), Grubb (20) and Hoek (21) equations. Dynamic gammagraphy with ^{99m}Tc-DTPA was used as the gold standard (22).

Creatinine was determined by the dry chemistry sarcosine oxidase method with traceable calibration to mass spectrometry isotopic dilution using a Vitros 5.1 FS autoanalyzer (Johnson & Johnson, NJ, USA). Total error of creatinine determination was 9.8% (total error recommended: <10% according to the: National Kidney Disease Education Program [NKDEP] http://www.nkdep.nih.gov/labprofessionals/reporting_eGFR.htm). Method bias was 0.0056 (recommended method bias: <0.05). Normal levels of serum creatinine in males are 0.71-1.12 mg/dL; in females, 0.57-1.02 mg/dL.

Creatinine clearance was determined adjusted for age, weight and height according to DuBois BSA equation (23). The correct urine collection was tested by Walser equation (24). GFR was also estimated by the Cockcroft-Gault, MDRD-4 and CKD EPI formulas.

Twenty-four hour urine creatinine clearance:

$$GFR = \frac{\text{urinary creatinine}}{\text{serum creatinine}} \times \frac{\text{daily urinary output}}{1{,}440} \times \frac{\text{DuBois BSA}}{1.73\ m^2}$$

where urinary and serum creatinine is measured in mg/dL.

Cockcroft-Gault:

$$GFR = \frac{(140 - age) \times weight}{72 \times \text{serum creatinine}} \times (0.85, \text{if female}) \text{ per } 1.73\ m^2\ BSA$$

where weight is in kg.

MDRD-4: $GFR = 186 \times (creatinine/88.4)^{-1.154} \times (age)^{-0.203} \times (0.742, \text{if female})$

CKD EPI: In males, if creatinine <0.9

$$GFR = 141 \times \left(\frac{\text{plasmatic creatinine}}{0.9}\right)^{-0.411} \times 0.993^{age}$$

In males, if creatinine >0.9

$$GFR = 141 \times \left(\frac{\text{plasmatic creatinine}}{0.9}\right)^{-1.209} \times 0.993^{age}$$

In females, if creatinine <0.7

$$GFR = 144 \times \left(\frac{\text{plasmatic creatinine}}{0.7}\right)^{-0.329} \times 0.993^{age}$$

In females, if creatinine >0.7

$$GFR = 144 \times \left(\frac{\text{plasmatic creatinine}}{0.7}\right)^{-1.209} \times 0.993^{age}$$

DuBois equation for BSA calculation:

$$BSA = 0.007184 \times (\text{weight kg})^{0.425} \times (\text{height cm})^{0.725}$$

Walser formula:

Males: 28.2 – (0.172 x age); Females; 21.9 – (0.115 x age)

Cystatin C was determined by immunoturbidimetry (Gentian Laboratory, Oslo, Norway), Vitros 5.1 FS (Johnson & Johnson, New Jersey, USA). Normal levels are 0.57-1.09 mg/L.

Equations

Larsson (A):

$GFR = 99.43 \times (\text{cystatin C})^{-1.5837}$

Larsson modified equations according to DuBois BSA formula:

$\text{Larsson (A)} \times 0.007184 \times \text{weight}^{0.425} \times \text{height}^{0.725}$

where weight is in kg and height in cm.

Grubb:

$GFR = 84.69 \times (\text{cystatin C})^{-1.680}$ (x 0.948, if female)

Hoek:

$$GFR = -4.32 + 80.35 \times \frac{10}{\text{Cystatin C}}$$

^{99m}Tc-DTPA gammagraphy was performed in all 300 subjects as the gold standard method to assess GFR and consequently stratify CKD (22).

Statistical analysis

Results are expressed as means ± 2 standard deviations. Intergroup comparisons were analyzed with the chi-square (χ^2) test, 1-way ANOVA, and Mann-Whitney test for paired comparisons. Correlations between variables are expressed by Spearman coefficient. The degree of agreement between methods was analyzed by Kappa coefficient. Linear regression analysis was performed between ^{99m}Tc-DTPA (dependent variable) and other independent variables, and between CKD-EPI (dependent) versus Hoek. Results were considered significant if the p value was ≤ 0.05. Bland-Altman plots were used to compare the different estimates of the GFR.

Results

Global analysis

Subjects were included in the different groups based on GFR measured by ^{99m}Tc-DTPA scintigraphy. The control group displayed a lower but nonsignificant GFR compared with stage 1 (81.53 ± 13.94 vs. 95.28 ± 15.80 ml/min) but significantly superior to stage 2 (70.05 ± 11.30 ml/min). As for age, it was different among groups, showing a significant progressive increase from stage 1 to 4 and a nonsignificant decrease with regard to stage 5. The control group presented a mean age between stages 2 and 3, albeit the difference was nonsignificant (Tab. I). Body mass index (BMI) showed a progressive, significant increase from stages 1 to 4, and a significant decrease in stage 5 to similar levels as stage 3. The control group showed the lowest BMI (Tab. I).

With respect to the marker of renal damage employed, 24-hour proteinuria, it was statistically different and raised as CKD worsened, being <150 mg/day in the control group (0.049 ± 0.11 g/day) (Tab. I).

Hypertension (χ^2=80.3; p<0.0001), diabetes mellitus (χ^2=26.08; p<0.0001) and primary glomerulopathies (χ^2=20.65; p=0.024) also increased significantly as CKD worsened (Tab. II).

CKD-EPI and Hoek equations showed the best correlations with ^{99m}Tc-DTPA: ρ=0.826, p< 0.001, and ρ=0.704, p<0.001, respectively. Moreover, there was a significant correlation among all creatinine-based equations, a phenomenon that also occurred among cystatin C–based formulas (Tab. III). Using univariate linear regression analysis, the highest results with respect to the gold standard were CKD-EPI versus ^{99m}Tc-DTPA, r=0.826, r^2=0.682; Hoek versus ^{99m}Tc-DTPA, r=0.704, r^2=0.496; and between themselves, CKD-EPI versus Hoek: r=0.811, r^2=0.658 (Figs 1-3). The highest kappa coefficients were ^{99m}Tc-DTPA: Hoek: 0.585, p<0.001; and ^{99m}Tc-DTPA: CKD-EPI: 0.505, p<0.001 (Tab. IV).

However, important GFR differences were reported when each group was analyzed separately (Tab. V). The most significant correlations were control group, creatinine clearance, ρ=0.421, p = 0.012; G1, Cockcroft-Gault, ρ=0.588, p=0.003; CKD-EPI, ρ=0.460, p<0.05; G2, CKD-EPI, ρ=0.462, p<0.05; G3, CKD-EPI, ρ=0.508, p<0.001; MDRD-4, ρ=0.506, p<0.001; Hoek, ρ=0.475, p<0.001; G4, Hoek, ρ=0.618, p<0.001; creatinine clearance, ρ=0.507, p=0.04; CKD-EPI, ρ=0.463, p=0.02; G5, CKD-EPI, ρ=0.604, p<0.001; and Hoek, ρ=0.592, p<0.001. Bland-Altman plots were employed to compare different estimates of the GFR between ^{99m}Tc-DTPA and the equations that gave the best correlations (Figs. 4-7).

Discussion

To our knowledge this is the first prospective study in which GFR was assessed comparing creatinine- and cystatin C–based equations, employing ^{99m}Tc-DTPA as the gold standard. With respect to the global population under consideration, which included a control group, age and BMI showed a trend toward increasing as CKD function worsened, displaying the peak of both variables at CKD stage 4 (Tab. I).

TABLE I
GENERAL DATA OF CERTAIN VARIABLES AT DIFFERENT STAGES OF CKD

			Confidence interval for the mean at 95%			
	Group	**Median**	**Lower limit**	**Upper limit**	**Standard deviation**	**Interquartile amplitude**
BMI	Control	23.95	22.690	25.217	3.68	2.63
	1	25.74	23.982	27.503	4.36	7.56
	2	25.95	24.764	27.133	4.26	5.47
	3	26.95	26.007	27.888	4.49	6.45
	4	29.29	27.170	31.418	6.37	6.51
	5	26.38	25.160	27.593	4.71	6.12
Age	Control	48.63	44.25	53.006	12.74	16.00
	1	42.89	37.48	48.294	13.39	17.00
	2	45.23	41.41	49.051	13.72	18.75
	3	54.44	51.28	57.610	15.12	24.00
	4	63.22	58.47	67.963	14.24	14.50
	5	61.33	56.90	65.771	17.18	16.75
Proteinuria	Control	.049	.012	.087	.11	.00
	1	.37	.135	.598	.57	.41
	2	.31	.183	.426	.43	.40
	3	1.39	.708	2.075	3.26	1.14
	4	1.87	.808	1.560	.99	.71
	5	2.48	1.516	3.440	3.724	2.83
^{99m}Tc-DTPA	Control	81.53	73.308	89.753	13.94	29.57
	1	95.26	88.605	101.944	15.80	15.77
	2	70.05	66.903	73.194	11.30	14.33
	3	45.59	43.624	47.556	9.39	14.27
	4	22.60	20.653	24.547	5.75	6.91
	5	11.18	8.989	13.364	8.40	9.79
Serum creatinine	Control	.791	.749	.833	.122	.130
	1	.879	.792	.967	.216	.290
	2	1.02	.932	1.102	.306	.360
	3	1.42	1.285	1.560	.656	.690
	4	2.42	1.859	2.872	.769	1.045
	5	6.88	6.056	7.700	3.183	4.423
Cystatin C	Control	.748	.717	.780	.092	.120
	1	.824	.724	.925	.249	.172
	2	.935	.846	1.024	.320	.364
	3	1.32	1.199	1.437	.569	.645
	4	1.98	1.532	2.035	.755	.850
	5	4.03	3.666	4.397	1.414	2.268

BMI = body mass index; CKD = chronic kidney disease.

Hypertension, diabetes mellitus, primary glomerulopathies and proteinuria increased significantly as CKD moved to stage 5 (Tabs. I and II). It is interesting to note that the control group had a lower GFR assessed by ^{99m}Tc-DTPA than that of the group with stage 1 CKD (Tab. I). This could be due to the fact that controls were nonsignificantly older than CKD stage 1 patients (48.63 ± 12.74 vs. 42.89 ± 13.39 years, p=ns), and mean GFR physiologically corresponded to age (81.53 ± 13.94 ml/min, 95% CI, 73.3-89.8). This may explain why in the control group the best method that correlated with scintigraphy was 24-hour creatinine clearance evaluated by Walser equation, which requires a steady stage to be carried out. Moreover, tubular creatinine excretion may have been lower due to this being a population without evidence of renal disease. In CKD stage 1, the presence of proteinuria could also have contributed to a certain degree of hyperfiltration, increasing GFR with respect to the control group (Tab. I). In this global 300-patient initial evaluation, CKD-EPI and Hoek equations displayed the highest statistically significant correlations and the best linear regressions with respect to ^{99m}Tc-DTPA (Tab. III; Figs. 1-3), as well as the highest significant kappa agreement constants (Tab. IV). Finally, the different creatinine-based equations showed a high and significant correlation among themselves; the same phenomenon was reported with cystatin C–based formulas (Tab. III).

Additionally, as GFR approaches 60 ml/min or lower, both serum creatinine and cystatin C concentrations, and their respective derived equations, converge to a better correlation among themselves, reaching a total significant correlation at CKD stage 5, meaning any equation would be valid to employ (Tab. IV). In CKD stages 3 and 4, the CKD-EPI and Hoek equations were the ones to best correlate with ^{99m}Tc-DTPA.

However, in the healthy population and in the initial stages of CKD, only creatinine-based equations significantly correlated with ^{99m}Tc-DTPA. In the control group, only with daily endogenous creatinine clearance adjusted for age and weight and estimated by the Walser formula, could a certain correlation with ^{99m}Tc-DTPA be obtained (Tab. V).

With respect to creatinine equations to estimate GFR, the most classically employed are the Cockcroft-Gault since 1976 and the MDRD-4 since 1999 (17, 18). This may be due to an easy access to the variables employed in such equations. However, those variables present many limitations with respect to their accuracy and the stage of GFR in which they are used. This reality may also explain why creatinine-based equations are still being developed, employing newer mathematical models.

Recently published (in 2009), the CKD-EPI equation to study GFR is based on the National Health and Nutrition Examination Survey (NHANES), measuring creatinine with Jaffé kinetic model and employing a database grouped in 10 studies (2). The development of this equation is the answer to the criticism that exists with respect to the stratification system of CKD, which is based on MDRD-4 results. The authors, extrapolating another 16 studies, validated CKD-EPI and recommend using it instead of MDRD-4 (2). The necessity for a new equation is mainly due to the lack of precision in estimating GFR at levels >60 ml/min. In both equations, age, race and sex are included – variables all associated with muscle mass, the main source of creatinine (25). The inaccuracy in determinations of GFR suggests that other variables may partially determine creatinine variations, independently of GFR (2). In the present work we demonstrate that in CKD stage 2, CKD-EPI was the best equation to adjust to a real GFR as assessed by ^{99m}Tc-DTPA, being also superior to cystatin C–based equations. Our findings

TABLE II
MOST FREQUENT CAUSES OF CKD

	Group						
CKD etiology	**0**	**1**	**2**	**3**	**4**	**5**	**Total χ^2 p Value**
Hypertension	0	10	28	56	33	48	17,580.30 0.0001
Diabetes	0	5	5	12	14	17	5,326.77 0.0001
Glomerulonephritis	0	9	18	30	10	18	8,520.65 0.024
PKD	0	2	3	11	2	9	278.54 0.13

CKD = chronic kidney disease; PKD = polycystic kidney disease.

TABLE III
GLOBAL CORRELATIONS OF THE DIFFERENT VARIABLES WITH RESPECT TO ^{99m}TC-DTPA AND AMONG THEMSELVES

		^{99m}Tc-DTPA	Creatinine clearance	Creatinine	Cystatin C	Larsson	Grubb	Hoek	Larsson BSA	MDRD-4	Cockcroft-Gault
^{99m}Tc-DTPA	Pearson coefficient	1	.059	-.637	-.695	.621	.603	**.704**	.631	**.816**	**.722**
	p Value	.	.312	.000	.000	.000	.000	**.000**	.000	**.000**	**.000**
Creatinine clearance	Pearson coefficient	.059	1	-.061	-.161	.139	.136	.150	.124	.088	.078
	p Value	.312	.	.291	.005	.016	.018	.009	.032	.129	.175
Creatinine	Pearson coefficient	-.637	-.061	1	.858	-.487	-.464	-.607	-.495	-.729	-.648
	p Value	.000	.291	.	.000	.000	.000	.000	.000	.000	.000
Cystatin C	Pearson coefficient	-.695	-.161	.858	1	-.612	-.588	-.741	-.623	-.783	-.698
	p Value	.000	.005	.000	.	.000	.000	.000	.000	.000	.000
Larsson	Pearson coefficient	.621	.139	-.487	-.612	1	**.997**	**.951**	**.979**	.703	.606
	p Value	.000	.016	.000	.000	.	**.000**	**.000**	**.000**	.000	.000
Grubb	Pearson coefficient	.603	.136	-.464	-.588	**.997**	1	**.944**	**.979**	.684	.589
	p Value	.000	.018	.000	.000	**.000**	.	**.000**	**.000**	.000	.000
Hoek	Pearson coefficient	**.704**	.150	-.607	-.741	**.951**	**.944**	1	**.939**	.806	.694
	p Value	**.000**	.009	.000	.000	**.000**	**.000**	.	**.000**	.000	.000
Larsson modified by BSA	Pearson coefficient	.631	.124	-.495	-.623	**.979**	**.979**	**.939**	1	.717	.678
	p Value	.000	.032	.000	.000	**.000**	**.000**	**.000**	.	.000	.000
MDRD-4	Pearson coefficient	**.816**	.088	-.729	-.783	.703	.684	.806	.717	1	**.867**
	p Value	**.000**	.129	.000	.000	.000	.000	.000	.000	.	**.000**
Cockcroft-Gault	Pearson coefficient	**.722**	.078	-.648	-.698	.606	.589	.694	.678	**.867**	1
	p Value	**.000**	.175	.000	.000	.000	.000	.000	.000	**.000**	.
CKD-EPI	Pearson coefficient	**.826**	.084	-.718	-.778	.716	.695	.811	.729	**.992**	**.876**
	p Value	**.000**	.146	.000	.000	.000	.000	.000	.000	**.000**	**.000**

Bold: Significant correlations with ^{99m}Tc-DTPA.
BSA = body surface area.

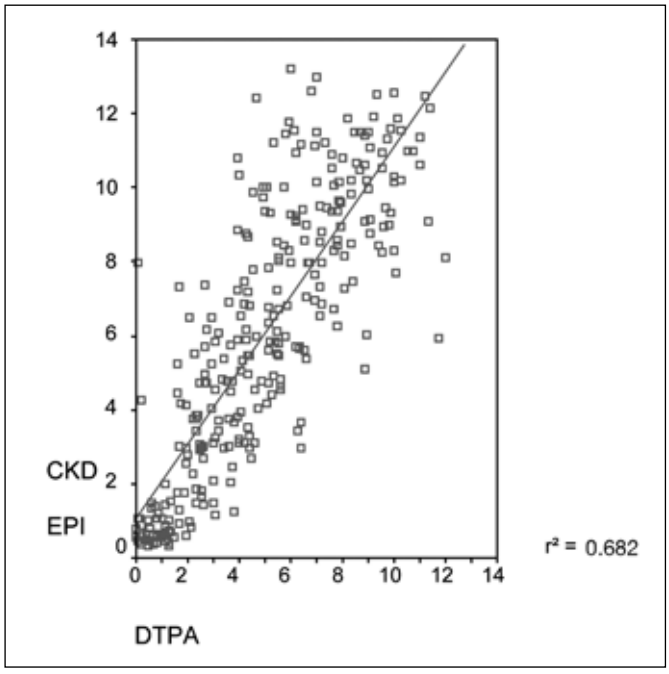

Fig. 1 - Linear regression between CKD-EPI and DTPA, global assessment.

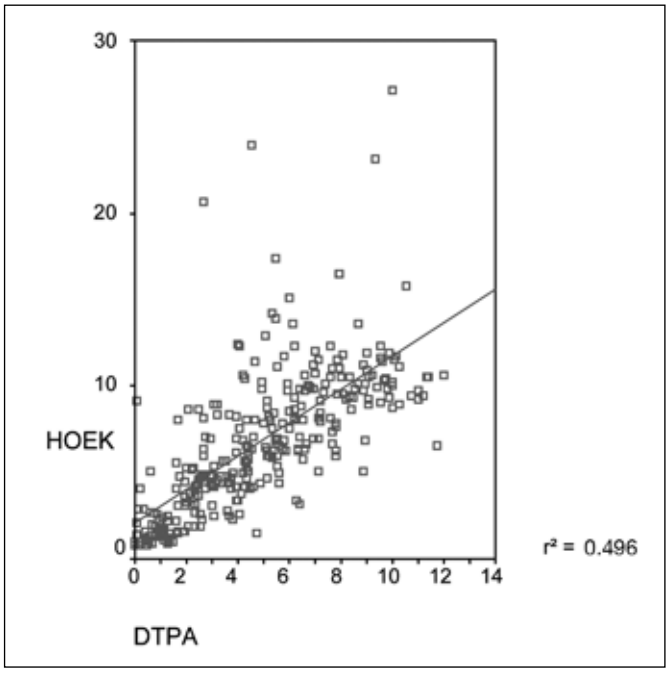

Fig. 2 - Linear regression between HOEK and DTPA, global assessment.

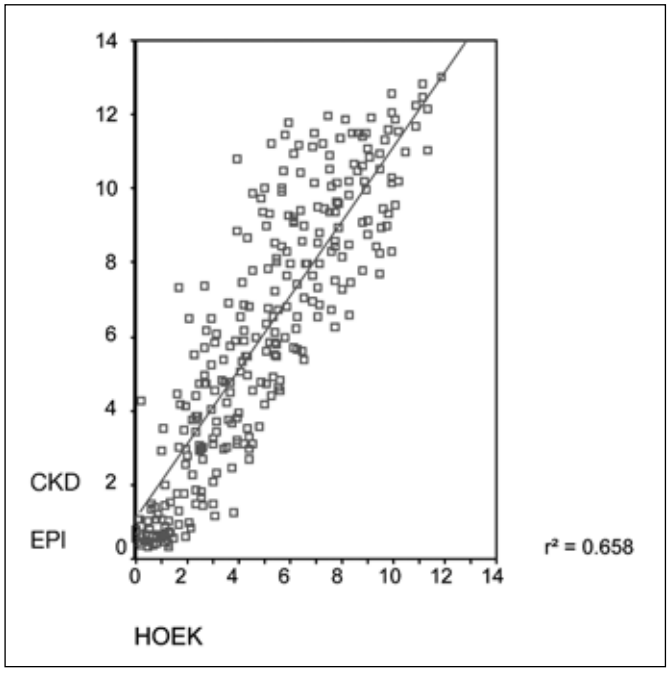

Fig. 3 - Linear regression between CKD-EPI and HOEK, global assessment.

agree with those reported by Stevens et al, who recently compared CKD-EPI with MDRD-4 in subjects with GFR >60 ml/min (26). They observed that the trend to overestimate GFR decreased from 11.9 ml/min with MDRD-4 to 4.2 ml/min with the CKD-EPI equation. This finding is important to improve the real detection of CKD at early stages in patients with lower risk. It is estimated that diagnosed CKD cases at stages 1 and 2 comprise approximately 20 million people in the United States, and a similar number would include people who ignore their clinical situation regarding CKD (26).

Regarding CKD stage 1, all 3 creatinine-based equations significantly correlated with ^{99m}Tc-DTPA scintigraphy, with the CKD-EPI equation at that stage the one with a significance trend, but below that of the Cockcroft-Gault formula (Tab. V). This may be because in our work the CKD stage 1 population was younger and had a lower BMI (Tab. I), and because of the design of the equation itself, as CKD-EPI could underestimate the GFR, as suggested by Stevens (26). In addition, this could explain why in the control group Walser-estimated creatinine clearance was the best method to calculate renal function when compared with the scintigraphy, as all of the equations are designed to measure GFR in CKD subjects, independently of the GFR. This may also explain why NKDEP warns that due to the lack of precision and the bias of the methods that measure serum creatinine, these inaccuracies exert a great impact on the estimated GFR as creatinine levels approach <1 mg/dL. Therefore, GFR is overestimated, and CKD cases underdiagnosed. In this setting, the NKDEP recommendation suggests that for an estimated GFR >60 ml/min, the result should be reported as ">60 ml/min" and not by the exact number obtained with the equation (http://

TABLE IV
MOST IMPORTANT AGREEMENT COMPARISONS (KAPPA >0.3)

Comparison	Kappa coefficient	p Value
HOEK and ^{99m}Tc-DTPA	0.585	0.0001
CREATININE CLEARANCE and ^{99m}Tc-DTPA	0.508	0.0001
CKD-EPI and ^{99m}Tc-DTPA	0.505	0.0001
Larsson and ^{99m}Tc-DTPA	0.429	0.0001
MDRD-4 and ^{99m}Tc-DTPA	0.399	0.0001
Hoek and ^{99m}Tc-CKD EPI	0.393	0.0001
Grubb and ^{99m}Tc-DTPA	0.322	0.0001
Cockcroft-Gault and ^{99m}Tc-DTPA	0.321	0.0001

TABLE V
SIGNIFICANT CORRELATIONS OR STRONG TREND TO SIGNIFICANCE ACCORDING TO EACH GROUP

Method	Control group	Group 1	Group 2	Group 3	Group 4	Group 5
	^{99m}Tc-DTPA	^{99m}Tc-DTPA	^{99m}Tc-DTPA	^{99m}Tc-DTPA	^{99m}Tc-DTPA	^{99m}Tc-DTPA
Creatinine clearance	0.421* 0.012†				0.507 0.04†	0.594 0.0001†
Cockcroft-Gault		0.588* 0.003†	0.660 0.06	0.466 0.0001†	0.370 0.1	0.496 0.0001†
MDRD-4		0.346* 0.09	0.414 0.09	0.506 0.0001†	0.409 0.09	0.567 0.0001†
CKD-EPI		0.460* 0.08	0.462 0.05	0.508 0.0001†	0.463 0.02†	0.604 0.0001†
Larsson				0.314 0.003†	0.390 0.08	0.511 0.0001†
Larsson modified					0.401 0.08	0.454 0.0001†
Grubb					0.488 0.04†	0.500 0.0001†
Hoek				0.475 0.0001†	0.618 0.0001†	0.532 0.0001†

*Pearson correlation; †p<0.05.

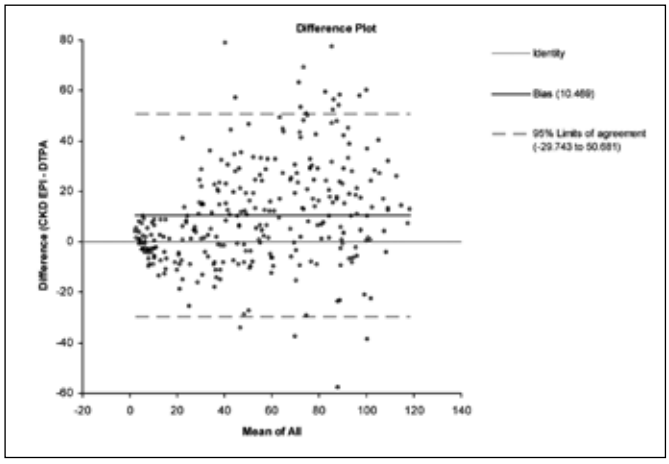

Fig. 4 - Bland-Altman plot between CKD-EPI and ^{99m}Tc-DTPA.

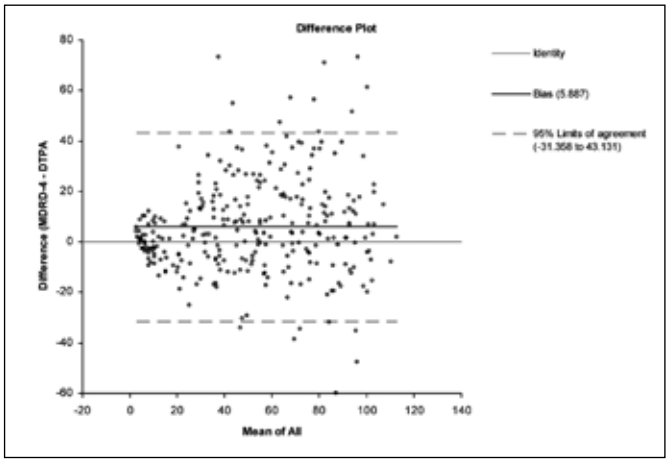

Fig. 5 - Bland-Altman plot between MDRD-4 and ^{99m}Tc-DTPA.

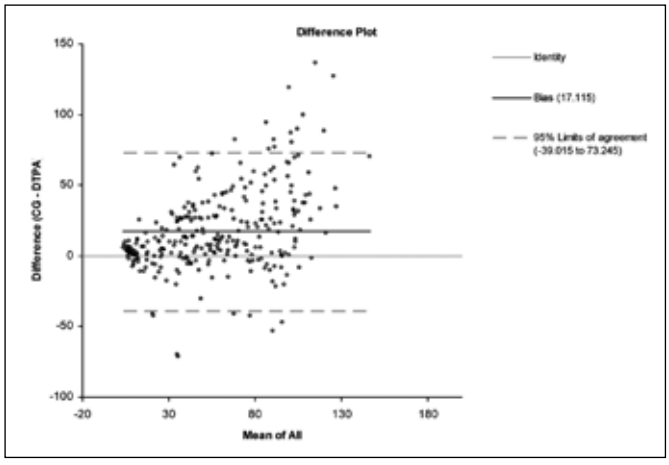

Fig. 6 - Bland-Altman plot between Cockcroft-Gault (CG) and ^{99m}Tc-DTPA.

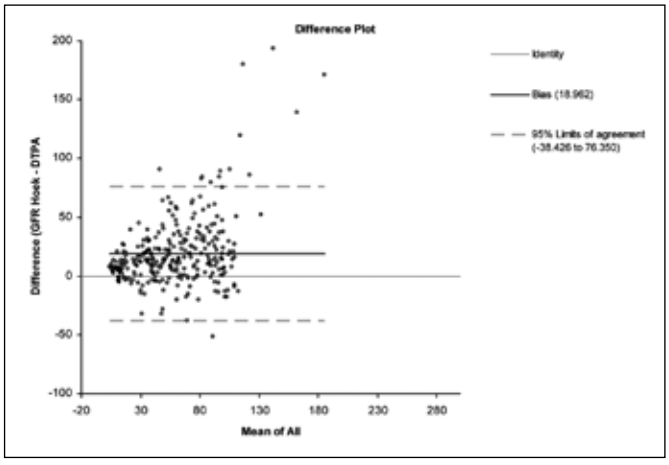

Fig. 7 - Bland-Altman plot between Hoek and ^{99m}Tc-DTPA.

www.nkdep.nih.gov/labprofessionals/reporting_eGFR.htm). For CKD stages 3, 4 and 5, the CKD-EPI equation remained the best one that correlated with ^{99m}Tc-DTPA scintigraphy, in agreement with the results of the study by Stevens et al (26). Moreover, Stevens et al propose CKD-EPI as the equation to be employed in all CKD stages, based on both global and stratified analyses, as our results also show. At these stages, NKDEP states that due to higher serum creatinine levels, the inaccuracy and bias of the method are less important, and as a result, the estimated GFR is much more precise.

To our knowledge, this is the first prospective study that assesses cystatin C and some cystatin C–based equations in comparison with ^{99m}Tc-DTPA scintigraphy. From 1985, cystatin C has been proposed as a promising endogenous marker of GFR. The use of cystatin C may be capable of identifying the declining rate of renal function at initial stages of CKD with GFR >60 ml/min, also known as preclinical kidney disease, as some publications have shown abnormally high cystatin C levels (≥1.0 mg/L) (27). In this setting, some cross-sectional studies have shown that cystatin C presents a higher sensitivity than creatinine to detect mild kidney disease (28). Cystatin C concentration has been reported to increase when GFR ranges between 70 and 90 ml/min – in the so-called creatinine-blind range zone. In our study, this statement was partially confirmed: group 1: cystatin C, 0.824 ± 0.249 mg/L (95% CI, 0.724-0.925); group 2: cystatin C, 0.935 ± 0.320 mg/L (95% CI, 0.846-1.024) (Tab. I). However, the phenomenon that occurs with creatinine is repeated with cystatin C: bare cystatin C levels, without an appropriate equation, lack the required sensitivity to detect false-negative cases, considerably increasing the failure to identify real cases of CKD, particularly at the initial stages (20, 21, 29). While some authors have reported significant differences employing cystatin C–based equations com-

pared with creatinine ones to determine the correct GFR in CKD, others have found no differences (20, 21, 29). Finally, some equations have recently been developed in which both creatinine and cystatin C have been used simultaneously, but the results are scant and contradictory (30-34), which is why we have not included them in our work.

Eriksen et al remark, in agreement with our results, that cystatin C equations were not superior to those based on creatinine to estimate GFR in 1,621 normal subjects, using iohexol clearance as reference (35). In that study, 24-hour creatinine clearance was not determined, which showed better results in our control population (Tab. V). To give more credit to this classical method of renal function, we employed the Walser equation to certify that the urine collection was correct. It is noteworthy that the K/DOQI guidelines suggest that the equations should be applied to assess GFR in CKD, and not in the general population (1). Eriksen et al report that the best creatinine-based correlations were obtained with MDRD-4 (ρ=0.56, 95% CI, 0.52-060) and CKD-EPI (ρ=0.55, 95% CI, 0.52-0.59), while the Grubb, Hoek and Larsson equations were the best cystatin C–based equations (35).

Although cystatin C appears to present a higher sensitivity than creatinine to detect early decreases in GFR in CKD, more studies are needed for a correct interpretation of these increases, and better equations with more practical applications and more adequate for the different stages of CKD, particularly at stages 1 and 2. The same situation occurred with creatinine itself, with which new and more accurate equations are still being developed. Although new data suggest that cystatin C could be considered a more precise marker of GFR in subjects with mild reductions of GFR when compared with creatinine (36), these studies are not only scarce, but also contradictory with small numbers of patients included (3, 9, 21, 37).

Why is it important to focus on a correct GFR estimation at these early stages of CKD? It is important due to the large number of false-negative CKD cases that are apparently being reported using creatinine-based equations, particularly with the Cockcroft-Gault and MDRD-4 equations (5, 36). Moreover, this is the end of the spectrum of CKD with the greatest potential degree of recovery or preservation of renal function.

In the present work, cystatin C–derived equations were not superior to creatinine-based ones. Among creatinine equations, only Cockcroft-Gault for stage 1 and CKD-EPI for stage 2 had significant correlations with ^{99m}Tc-DTPA (Tab. V). In the global analysis (Tab. III) and from stage 3 downwards (Tab. V), the Hoek equation was the one to show the best correlation and precision with respect to ^{99m}TC-DTPA and to the CKD-EPI equation (Fig. 3).

Cystatin C determination is more expensive, and without any evidence of significant advantages compared with creatinine, it is logical that its use in clinical practice is limited. The role of cystatin C in nephrology and as a useful tool to measure GFR in CKD has not yet been established, and more clinical research is needed. Some studies have demonstrated that cystatin C appears to better identify CKD patients with a higher risk of cardiovascular complications at GFR <60 ml/min when estimated by the CKD-EPI equation (38). Moreover, it has recently been published that in the Asian population, an elevated serum cystatin level could also be considered as an independent predictor of cardiovascular events in subjects with normal renal function, as has been demonstrated for age and hypertension (39).

In renal studies based on creatinine determinations, it is a critical point for drawing valid conclusions that there be a uniform calibration and a homogeneous technical assay of the creatinine method (40, 41). Our study was performed at a single center, employing a better method for creatinine measurement than the Jaffé method (40) with the same protocol for scintigraphy, resulting in a more homogenous output. Finally, on clinical grounds, we have employed equations that have been extrapolated from previous works, as is the case of CKD-EPI, that have validated this promising equation as well as the rest of them.

With regard to the limitations of the present study, the cohorts were not matched according to sex or age, and BMI varied significantly among the stages (Tab. I), which could certainly have influenced on the results. Moreover, ^{99m}Tc-DTPA scintigraphy was used as the gold standard for GFR measurement, but it is not used routinely due to its cost, and the fact that it is time-consuming and laborious (22). If another method had been employed as the gold standard (i.e., inulin clearance, iohexol, ^{51}Cr-EDTA or [^{125}I]-iothalamate), the results could possibly have been different. Finally, the small number of patients included and their ethnic characteristics must be taken into account when conclusions or extrapolations are to be made.

In conclusion, we recommend that for the general population without risk factors for CKD, 24-hour creatinine clearance adjusted for age, sex and weight and evaluated by Walser formula should be employed to assess GFR. Both creatinine- and cystatin C–based equations have not yet been validated for the general population. For CKD patients with GFR >60 ml/min, NKDEP recommendations must not be forgotten. However, among creatinine-based equations, the CKD-EPI equation appears to be more accurate for estimating GFR at these early stages, but probably fewer patients would have CKD when compared with those found by the MDRD-4 or Cockcroft-Gault equations (26, 42). The role that cystatin C–

based equations should play in this group with GFR >60 ml/min is still under intense development. With respect to CKD patients with GFR <60 ml/min, creatinine- and cystatin C–based equations appear to be adequate to measure GFR. Among those based on cystatin C, the Hoek formula presents the best correlation and degree of agreement with the CKD-EPI equation (Tab. IV; Fig. 3). The potential biological and theoretical advantages that cystatin C presents when compared with creatinine should be shown in mathematical equations to better estimate GFR. New cystatin C–based equations must be developed and validated for each CKD stage, standardizing the assays and reducing its cost. The resolution of these important issues, as has been occurring with creatinine, will demand time.

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