Research Article

Estimation of Glomerular Filtration Rate Based on Serum Cystatin C versus Creatinine in a Uruguayan Population

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Background. Estimation of glomerular filtration rate (eGFR) from biomarkers has evolved and multiple equations are available to estimate renal function at bedside. *Methods.* In a random sample of 119 Uruguayans (54.5% women; 56.2 years (mean)), we used Bland and Altman's method and Cohen's kappa statistic to assess concordance on a continuous or categorical (eGFR < 60 *versus* ≥60 mL/min/1.73 m^2) scale between eGFR$_{cys}$ (reference) and eGFR derived from serum creatinine according to the Modification of Diet in Renal Disease (eGFR$_{mdrd}$) or the Chronic Kidney Disease Epidemiology Collaboration equations (eGFR$_{epi}$) or from both serum cystatin C and creatinine (eGFR$_{mix}$). *Results.* In all participants, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ were, respectively, 9.7, 11.5, and 5.6 mL/min/1.73 m^2 higher ($P < 0.0001$) than eGFR$_{cys}$. The prevalence of eGFR <60 mL/min/1.73 m^2 was the highest for eGFR$_{cys}$ (21.8%), intermediate for eGFR$_{mix}$ (11.8%), and the lowest for eGFR$_{mdrd}$ (5.9%) and eGFR$_{epi}$ (3.4%). Using eGFR$_{cys}$ as reference, we found only fair agreement with the equations based on creatinine (Cohen's kappa statistic 0.15 to 0.23). *Conclusion.* Using different equations we reached clinically significant differences in the estimation of renal function. eGFR$_{cys}$ provides lower estimates, resulting in higher prevalence of eGFR <60 mL/min/1.73 m^2.

1. Introduction

The glomerular filtration rate (GFR) is the most widely used indicator of overall renal function. The GFR can be measured by clearance of an ideal, usually exogenous, filtration marker such as inulin, iothalamate, EDTA, diethylene triamine pentaacetic acid, or iohexol. The clearance of endogenous markers such as creatinine or blood urea nitrogen can overestimate or underestimate the GFR. All these methods have the drawback to be complex and require 24-hour urine collection, which is not always practicable in day-to-day clinical practice. A more pragmatic approach is to estimate GFR from equations based on serum creatinine [1, 2].

More recently, experts proposed cystatin C as an alternative to creatinine [3]. Cystatin C is a nonglycosylated protein with low molecular mass (13.3 kDa) generated by all nucleated cells of the body at a constant rate, is freely filtered by the glomerulus, and is not secreted by renal tubules

but completely reabsorbed with subsequent degradation by proximal tubular cells [4, 5]. For those properties cystatin C is an endogenous surrogate of GFR. Compared with serum creatinine, cystatin C levels are less dependent on ethnicity, sex, age, and muscle mass or protein intake. Moreover, as serum creatinine, cystatin C is an independent predictor of cardiovascular and overall mortality [6].

Compared to GFR measurement based on the renal clearance of exogenous markers, estimated glomerular filtration rate (eGFR) is more precise if derived from both cystatin C and creatinine levels in serum [3]. This observation was consistent across sex, age, and diabetes mellitus [7]. On the other hand, measurement of cystatin C is costly and eGFR based on cystatin C needs further validation across a broad spectrum of populations with or without chronic kidney disease. The purpose of our current study was to compare estimates of eGFR based on cystatin C and creatinine in randomly recruited Uruguayans, using eGFR derived from cystatin C as reference.

2. Materials and Methods

2.1. Study Population. GEnotipo Fenotipo y Ambiente de la HiperTension en UruguaY (GEFA-HT-UY) is a prospective cohort study started in April 2012 conducted by the Unidad de Hipertensión Arterial, Hospital de Clínicas Dr. Manuel Quintela, Universidad de la República, Montevideo, Uruguay [8]. The Ethics Committee of the University Hospital approved the study protocol and all participants gave informed written consent. The aim of the study is to explore the relation of blood pressure with genetic and environmental factors in a representative Uruguayan population sample. Nuclear families were randomly recruited from the inhabitants of a geographically defined area, the Juana de América housing project, located about 10 km from downtown Montevideo. A nuclear family had to include at least one parent and two siblings. The minimum age was 18, without upper age limit. Family members living at the same address or within a distance of no more than 10 km were eligible. We invited participants by telephone. The participation rate among eligible subjects was 72.7%. In November 2013, 149 people had participated, of whom we excluded 30 from the present analysis, because either cystatin C or creatinine had not been measured ($n = 22$) or because they had not yet completed the physical examination ($n = 8$). Thus, the number of participants analysed statistically totalled 119.

2.2. Field Work. The examinations took place at a field centre located within the neighbourhood. Trained observers administered a standardised questionnaire inquiring into each participant's medical history, smoking and drinking habits, and intake of medications. They measured blood pressure according to the European guidelines. After participants had rested for 5 minutes in the sitting position, the observers obtained five consecutive blood pressure readings (phase V diastolic pressure) to the nearest 2 mm Hg, using mercury sphygmomanometers. Standard cuffs had a 12×24 cm inflatable portion, but if upper arm girth exceeded 31 cm, larger cuffs with 15×35 cm bladders were used. Pulse pressure is the difference of systolic minus diastolic blood pressure. Mean arterial pressure is diastolic pressure plus one-third of pulse pressure. For analysis, the five blood pressure readings were averaged. Hypertension was an office blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or use of antihypertensive drugs. The observers measured body height to the nearest 0.5 cm with a pliable measurer and the participant standing against the wall. Participants wore light indoor clothing without shoes for body weight measurements. Body mass index was weight in kilograms divided by square of height in meters. Venous blood samples were obtained after at least 12 hours fasting and were kept at 4°C and within 2 hours period were analysed for serum levels of cystatin C, creatinine, cholesterol, and glucose. Diabetes mellitus was the use of anti-diabetic drugs or a fasting glucose ≥ 126 mg/dL (7 mmol/L).

2.3. Arterial Phenotypes. After the participants had rested 15 min in the supine position, we recorded during an 8 s period the radial waveforms at the right side by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Houston, TX) interfaced with a computer running SphygmoCor software, version 8.2 (AtCor Medical, West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV. We calibrated the radial pulse wave on the brachial blood pressure [9]. From the radial signal, the SphygmoCor software calculates the aortic pulse wave by means of a validated generalised transfer function [10, 11]. The augmentation index was the ratio of the second to the first peak of the pressure wave expressed as a percentage.

Aortic pulse wave velocity was measured by sequential ECG-gated recordings of the arterial pressure waveform at the carotid and femoral arteries [12]. We measured the distances from the suprasternal notch to the carotid sampling site (distance *A*) and from the suprasternal notch to the femoral sampling site (distance *B*). Pulse wave travel distance was calculated as distance *B* minus distance *A*. Pulse transit time was the average of 10 consecutive beats. Pulse wave velocity was the distance in meters divided by the transit time in seconds [12].

2.4. Laboratory Methods. Serum cystatin C was measured by a particle-enhanced turbidimetric immunoassay (PETIA), (COBAS, Roche diagnostics, Germany). The latex enhanced particles coated with anticystatin C antibodies in the reagent agglutinate with the human cystatin C in the sample. The degree of the turbidity caused by the aggregate was determined turbidimetrically at 546 nm. This assay has a detection limit of 0.4 mg/L and a coefficient of variation of 1.3%. Serum creatinine was measured by modified kinetic Jaffé methods with the modifications described elsewhere [13, 14]. The detection limit is 0.17 mg/dL and the coefficient of variation was 1.6%. We use a creatinine method that has calibration traceable to an IDMS reference measurement procedure according to present recommendations [15, 16].

2.5. Estimated Glomerular Filtration Rate. We estimated GFR using four equations. First, we computed eGFR from serum cystatin C eGFR$_{cys}$, as described by Inker and colleagues [3]. Next, we calculated eGFR from serum creatinine according to the IDMS-traceable MDRD Study Equation (MDRD) [17, 18] formula (eGFR$_{mdrd}$) or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [2] equation (eGFR$_{epi}$). Finally, as proposed by Inker and colleagues, [3] we also derived eGFR from both serum cystatin C and serum creatinine (eGFR$_{mix}$). All aforementioned estimates [1–3] account for sex and age and with the exception of eGFR$_{cys}$ also consider ethnicity (black versus nonblack). This particular characteristic was irrelevant for our current study as our participants only included Whites mainly of European descent. Table S1 in the online data supplement provides detailed information on each formula (see Table S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/837106). In our current analyses, we compared findings based on the various methods to estimate GFR against eGFR$_{cys}$ as the referent method. Low glomerular filtration rate (L-GFR) was an eGFR < 60 mL/min/1.73 m^2 based on a single determination of each biomarker.

2.6. Statistical Analysis. For database management and statistical analysis, we used SAS software, version 9.3 (SAS Institute, Cary, NC). First, in exploratory analyses, we assessed the characteristics of participants by fourths of the distribution of eGFR$_{cys}$. For comparison of means and proportions, we applied Student's t-test (or ANOVA) and the χ^2 statistic, respectively. We assessed agreement between paired measurements on a continuous scale by Bland and Altman's method [19]. To allow comparison with literature data, we also computed correlation coefficients. The National Kidney Foundation KDOQI guideline proposes a threshold of 60 mL/min/1.73 m^2 to diagnose chronic kidney disease [20]. In categorical analyses, we, therefore, also assessed the agreement between equations to dichotomize subjects in L-GFR or not-L-GFR using Cohen's kappa statistic [21]. A kappa value of 0.20 or less indicates slight agreement, 0.20 to 0.40 fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 1.00 almost perfect agreement. We studied the association between the four definitions of eGFR dichotomized at 60 mL/min/1.73 m^2 in hypertensive and diabetic subjects by McNemar's test for paired comparisons of proportions. Because of the low frequencies in some cells, we applied exact statistics in two-by-two tables. Finally, we assessed the added capacity of eGFR$_{cys}$ to differentiate between normotension versus hypertension or between people with or without diabetes mellitus, using the integrated discrimination improvement (IDI) and the net reclassification improvement (NRI) [22, 23]. Statistical significance was an α level of 0.05.

3. Results

3.1. Characteristics of Participants. The 119 participants included 68 women (57.1%) and 53 (44.5%) hypertensive

patients, of whom 35 (66.0%) were on antihypertensive drug treatment. Among 68 women and 51 men, 11 (16.2%) and 5 (9.8%) were smokers; 21 women (30.1%) and 32 men (62.7%) reported intake of alcohol. In smokers, median tobacco use was 10 cigarettes per day (interquartile range, 6–15). In drinkers, the median alcohol consumption was 8 g per day (interquartile range, 4–54). In the whole study population, age (SD) averaged was 56.5 (17.3) years and systolic and diastolic blood pressure 126.5 (19.6) mm Hg and 79.6 (11.7) mm Hg, respectively. Based on a self-report of the main maternal and paternal background, 37 participants (31.1%) reported a mixture of Caucasian, African, or Native-American, while 82 participants (68.9%) reported coincident Caucasian background.

Among all participants, serum cystatin C and serum creatinine averaged from 0.99 (0.22) mg/L to 0.81 (0.21) mg/dL with no difference between women and men for cystatin C (0.99 versus 1.00 mg/L; P = 0.77), whereas women had lower serum creatinine than men had (0.72 versus 0.93 mg/dL; P < 0.0001). In all participants, mean values were 80.0 (23.8) mL/min/1.73 m^2 for eGFR$_{cys}$, 89.7 (22.5) mL/min/1.73 m^2 for eGFR$_{mdrd}$, 91.5 (19.0) mL/min/1.73 m^2 for eGFR$_{epi}$, and 85.6 (20.2) mL/min/1.73 m^2 for eGFR$_{mix}$, with no sex differences ($P \geq 0.23$).

Table 1 provides the characteristics of participants by fourths of the distribution of eGFR$_{cys}$, which was used as reference. The prevalence of hypertension (P = 0.027), age (P < 0.0001), and systolic blood pressure (P = 0.0097), but not diastolic blood pressure (P = 0.79) or mean arterial pressure (P = 0.18) increased (P = 0.027) with lower eGFR$_{cys}$ category. The central systolic augmentation index and aortic pulse wave velocity also rose (P < 0.0001) across decreasing fourths of the eGFR$_{cys}$ distribution. Trends in eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ ran in parallel with the distribution of eGFR$_{cys}$.

3.2. Concordance between Estimates of GFR on a Continuous Scale. Figure 1 shows the Bland and Altman plots comparing eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$, with eGFR$_{cys}$ as the referent method. Table 2 shows the mean deviations of eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ from eGFR$_{cys}$. In all participants, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ were, respectively, 9.7, 11.5, and 5.6 mL/min/1.73 m^2 higher than eGFR$_{cys}$. The corresponding ±2 SD intervals expressed in mL/min/1.73 m^2 (Figure 1) ranged from −38.5 to +57.9 for eGFR$_{mdrd}$, −25.5 to +48.5 for eGFR$_{epi}$, and −10.2 to +21.4 for eGFR$_{mix}$, and the corresponding correlation coefficients were −0.04 (P = 0.69), −0.26 (P = 0.0046), and −0.40 (P < 0.0001), respectively. Analyses stratified according to sex, age, normotension versus hypertension, and absence versus presence of diabetes mellitus were consistent with those in all participants (Table 2). Figure 2 shows that across fourths of the distribution of eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ were consistently higher (P < 0.002) than eGFR$_{cys}$ except in the highest category of eGFR$_{cys}$ (P = 0.25).

3.3. Concordance between Estimates of GFR on a Categorical Scale. The prevalence of L-GFR was the highest for eGFR$_{cys}$,

TABLE 1: Characteristics of 119 participants by fourths of the distribution of eGFR$_{cys}$.

Characteristic	Categories of eGFR$_{cys}$				P
eGFR$_{cys}$ limits, mL/min/1.73 m^2	>93.7	93.7–78.7	78.7–61.5	≤61.5	$\cdots$
Number of subjects (%)	30	30	30	29	$\cdots$
Women	18 (60.0)	22 (73.3)	12 (40.0)	16 (53.3)	0.063
Smokers	4 (13.3)	5 (16.7)	3 (10.0)	4 (13.8)	0.90
Drinking alcohol	16 (53.3)	13 (43.3)	12 (40.0)	12 (41.4)	0.73
Hypertension	8 (26.7)	11 (36.7)	16 (53.3)	18 (62.1)	0.027
Antihypertensive treatment	3 (10.0)	8 (26.7)	12 (40.0)	12 (66.6)	0.32
Cardiovascular disease	2 (6.7)	1 (3.3)	3 (10.0)	2 (6.9)	0.87
Diabetes mellitus	4 (13.3)	1 (3.3)	7 (23.3)[*]	8 (27.6)	0.058
Mean (SD) of characteristic					
Age, years	41.3 (14.0)	52.1 (14.7)[†]	64.2 (13.9)[†]	68.9 (11.8)	<0.0001
Body mass index, kg/m^2	27.6 (4.4)	29.2 (5.1)	29.1 (5.3)	29.5 (4.6)	0.44
Office blood pressure					
Systolic pressure, mmHg	118.5 (15.1)	123.3 (16.6)	130.9 (21.1)	133.6 (22.0)	0.0097
Diastolic pressure, mmHg	79.3 (10.6)	78.2 (11.9)	79.8 (12.1)	81.4 (12.7)	0.79
Pulse rate, beats per minute	71.3 (7.1)	69.1 (9.9)	75.4 (8.9)[*]	71.0 (9.8)	0.054
Central augmentation index, %	14.7 (14.8)	19.6 (11.3)	28.7 (9.8)[†]	23.6 (10.9)	<0.0001
Aortic pulse wave velocity, m/s	7.6 (1.8)	8.6 (2.5)	11.1 (3.2)[†]	10.0 (2.9)	<0.0001
Biochemical measurements					
Total cholesterol (mg/dL)	204.2 (30.2)	194.6 (28.8)	216.5 (38.6)[*]	225.3 (34.9)	0.004
Glucose (mg/dL)	100.0 (35.3)	96.6 (12.7)	101.8 (21.6)	101.9 (15.6)	0.88
Serum cystatin C (mg/L)	0.75 (0.15)	0.94 (0.05)[§]	1.03 (0.07)[§]	1.27 (0.14)[§]	<0.0001
Serum creatinine, mg/dL	0.75 (0.14)	0.81 (0.15)	0.77 (0.28)	0.92 (0.23)[*]	0.0077
eGFR$_{mdrd}$, mL/min/1.73 m^2	103.2 (21.6)	92.9 (16.1)[*]	90.1 (24.5)	71.9 (5.4)[†]	<0.0001
eGFR$_{epi}$, mL/min/1.73 m^2	107.1 (14.2)	96.4 (12.8)[†]	88.6 (17.1)[*]	73.4 (14.1)[‡]	<0.0001
eGFR$_{mix}$, mL/min/1.73 m^2	110.4 (13.1)	90.7 (7.0)[§]	78.6 (8.6)[§]	61.8 (9.2)[§]	<0.0001

eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ indicate estimated glomerular filtration rate derived from serum cystatin C, from serum creatinine according to the IDMS-traceable Modification of Diet in Renal Disease (MDRD) Study Equation or the Chronic Kidney Disease Epidemiology Collaboration equations, or from both serum cystatin C and creatinine. Office blood pressure was the average of five consecutive readings. Hypertension was an office blood pressure of ≥140 mmHg systolic or ≥90 mmHg diastolic or use of antihypertensive drugs. Diabetes mellitus was a fasting glucose level of ≥126 mg/dL or use of antidiabetic drugs. The central augmentation index was standardised to a heart rate of 75 beats/minute. Conversion factors: creatinine from mg/dL to μmol/L, multiply by 88.4; cholesterol from mg/dL to mmol/L, multiply by 0.0259. P values denote the significance of the differences in prevalence or means across quartiles of eGFR$_{cys}$. Significance of the difference with the adjacent lower fourth:[*]$P \leq 0.05$; [†]$P \leq 0.01$; [‡]$P \leq 0.001$; [§]$P \leq 0.0001$.

intermediate for eGFR$_{mix}$, and the lowest for eGFR$_{mdrd}$ and eGFR$_{epi}$ (Table 3). Using eGFR$_{cys}$ as reference, Cohen's kappa statistic was 0.230 (95% confidence interval [CI], 0.036 to 0.427; $P = 0.0005$) for eGFR$_{mdrd}$, 0.151 (CI, −0.021 to 0.322; $P = 0.032$) for eGFR$_{epi}$, and 0.587 (CI, 0.399 to 0.775; $P < 0.0001$) for eGFR$_{mix}$.

3.4. Association between Chronic Kidney Disease and Response Variables. Among 53 hypertensive patients, the prevalence of L-GFR was higher ($P < 0.0001$) if patients were categorized based on eGFR$_{cys}$ (17 patients, 32.1%) compared with eGFR$_{mdrd}$ (4 patients, 7.5%), eGFR$_{epi}$ (3 patients, 5.7%), or eGFR$_{mix}$ (8 patients, 15.1%). Among 20 diabetic patients, we observed a similar trend. The prevalence of L-GFR was 8 patients (40.0%) based on eGFR$_{cys}$, 2 patients (10.0%) based on eGFR$_{mdrd}$, 2 patients (10.0%) based on eGFR$_{epi}$, and 3 patients (15%) based on eGFR$_{mix}$. However, the differences

with eGFR$_{cys}$ did not reach formal statistical significance ($P \geq 0.075$).

Finally, we explored whether an eGFR$_{cys}$ below 60 mL/min/1.73 m^2 improved the differentiation between normotension *versus* hypertension or between people without or with diabetes mellitus based on the other estimates of GFR. However, the classification based on eGFR$_{cys}$ did not improve IDI ($P \geq 0.53$) or NRI ($P \geq 0.24$) for hypertension or IDI ($P \geq 0.37$) or NRI ($P \geq 0.24$) for diabetes mellitus.

4. Discussion

In our current analysis, we compared the performance of the equations based on cystatin C and creatinine to estimate GFR in a Uruguayan population sample. The Uruguayan population has been considered as mainly European descent, with a negligible Native American or African contributions.
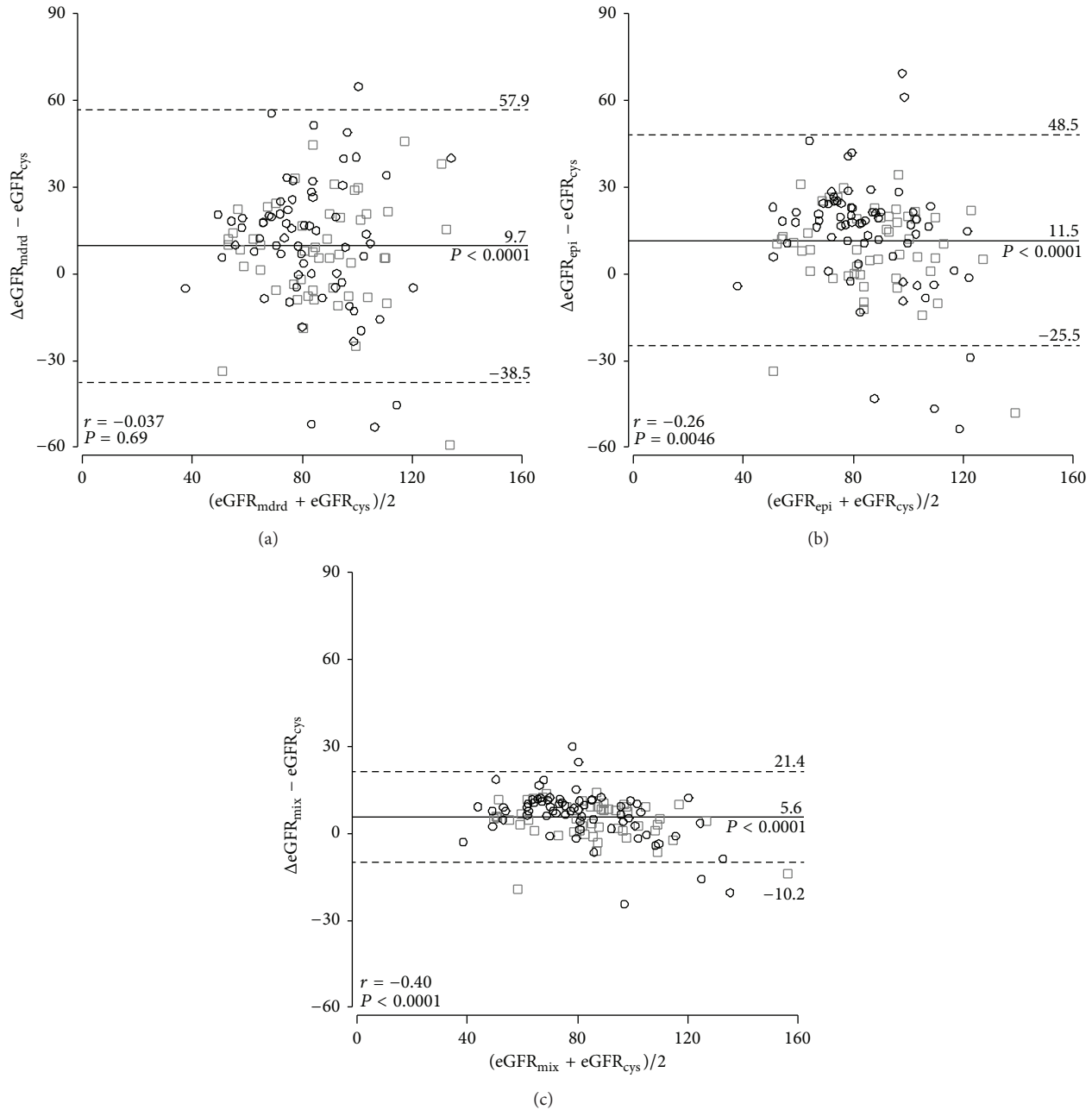
(a)

(b)

(c)

FIGURE 1: Bland and Altman plots comparing eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$, with eGFR$_{cys}$ as the referent method. eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ indicate estimated glomerular filtration rate derived from serum cystatin C, from serum creatinine according to the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations, or from both serum cystatin C and creatinine. R indicate the intraclass correlation coefficient. P denotes the significance level.

However, based on serological and molecular markers, recent studies demonstrate that Native American and African had an important influence in the conformation of the present one [24–26].

The key finding was that eGFR$_{cys}$ provides lower estimates in comparison with creatinine based equations (eGFR$_{mdrd}$ and eGFR$_{epi}$). Mean eGFR was 80.0, 89.7, 91.5, and 85.6 mL/min/1.73 m^2 for eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$, respectively. Thus, the prevalence of L-GFR, was higher when derived from equations involving cystatin

C (eGFR$_{cys}$ and eGFR$_{mix}$) than when derived from creatinine based equations. The prevalence of L-GFR using eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ was 21.8%, 5.9%, 3.4%, and 11.8%, respectively. In categorical analysis, the agreement between cystatin-based (eGFR$_{cys}$) equations and creatinine-based equations (eGFR$_{mdrd}$ and eGFR$_{epi}$) to detect eGFR under 60 mL/min/1.73 m^2 was low.

Over the past, many reports highlighted the ability of cystatin C to detect renal disease early in different settings [27–31]. Several researchers reported cystatin C outperforms

TABLE 2: Differences between various estimates of GFR with eGFR derived from serum cystatin C as referent method.

Group	N	Difference in estimates of glomerular filtration rate (mL/min/1.73 m^2)		
		eGFR$_{mdrd}$	eGFR$_{epi}$	eGFR$_{mix}$
All participants	119	9.7 (5.3–14.0)§	11.5 (8.2–14.9)§	5.6 (4.1–7.0)§
Women	68	11.3 (4.7–17.9)‡	13.7 (8.7–18.6)§	6.4 (4.3–8.5)§
Men	51	7.5 (2.1–12.9)§	8.7 (4.3–12.9)‡	4.5 (2.6–6.3)§
<60 years	57	3.2 (−4.1–10.4)	9.6 (3.9–15.3)†	4.5 (2.1–6.8)‡
≥60 years	62	15.6 (10.8–20.5)§	13.3 (9.4–17.2)§	6.6 (4.9–8.3)§
Normotension	66	8.1 (0.9–15.3)*	10.4 (4.9–15.8)‡	4.9 (2.6–7.3)§
Hypertension	53	11.6 (7.3–15.9)§	12.9 (9.5–16.4)§	6.3 (4.8–7.7)§
No diabetes	99	9.5 (4.7–14.4)‡	11.9 (8.3–15.7)§	5.7 (4.1–7.3)§
Diabetes	20	10.3 (−0.7–21.3)	9.4 (0.7–18.1)*	4.9 (1.4–8.4)†

eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ indicate estimated glomerular filtration rate from serum creatinine according to the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations or from both serum cystatin C and creatinine. Differences were computed as eGFR$_{mdrd}$, eGFR$_{epi}$, or eGFR$_{mix}$ minus GFR estimated from serum cystatin C (eGFR$_{cys}$). The values between brackets were the 95% confidence intervals (mean ± 1.96 standard errors). N indicates the number of participants. Significance of the difference with eGFR$_{cys}$: $^*P \leq 0.05$; $^\dagger P \leq 0.01$; $^\ddagger P \leq 0.001$; $^\S P \leq 0.0001$.
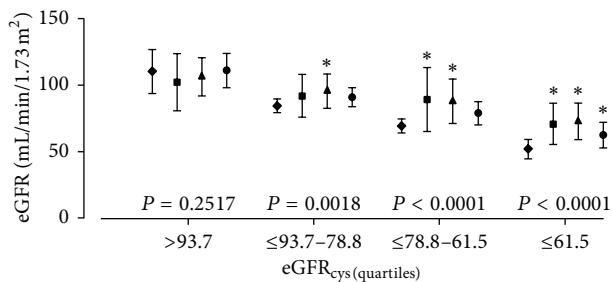

FIGURE 2: Mean and mean ±1 standard deviation interval of eGFR$_{cys}$ (◆), eGFR$_{mdrd}$ (■), eGFR$_{epi}$ (▲), and eGFR$_{mix}$ (●) across fourths of the distribution of eGFR$_{cys}$. The abbreviations eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ indicate estimated glomerular filtration rate derived from serum cystatin C, from serum creatinine according to the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations, or from both serum cystatin C and creatinine. eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ were consistently higher ($P < 0.0001$) than eGFR$_{cys}$ except in the highest category of eGFR$_{cys}$ ($P = 0.25$).

serum creatinine in the early diagnosis of acute kidney injury [32, 33] or its prognosis [34]. In line with our results, other investigators reported lower estimates of eGFR using cystatin C as biomarker [6, 35, 36]. In contrast, in a Belgian population sample [$n = 4,189$], Delanaye and colleagues report that the prevalence of eGFR below 60 mL/min/1.73 m^2 using eGFR$_{cys}$, eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ was 4.7%, 13.0%, 9.8%, and 5.0%, respectively [37]. In a recent report, among 394 old (>74 y) subjects and patients from England, Kilbride and colleagues describe lower eGFR$_{cys}$ (55.2 mL/min/1.73 m^2) in comparison with creatinine based equations (eGFR$_{mdrd}$, 57.6 mL/min/1.73 m^2; eGFR$_{epi}$, 57.0 mL/min/1.73 m^2) [38]. Grams and colleagues [36], analyzing representative subsamples of the adult participants in the National Health and Nutrition Examination Surveys (NHANES; 1988–1994 [$n = 15,133$] and 1999–2002 [$n = 8,238$]), observed slightly higher eGFR using cystatin C than creatinine equations (102.9 versus

99.4 mL/min/1.73 m^2). However, in line with our results, the proportion of subjects with eGFR below 60 mL/min/1.73 m^2 was larger if derived from cystatin C than creatinine (5.5% versus 4.7%). More recently, Tsai and colleagues [35] reported similar results in diabetic patients enrolled in NHANES. The mean eGFR$_{cys}$ was 100.7 mL/min/1.73 m^2 and the eGFR$_{epi}$ was 95.1 mL/min/1.73 m^2 while the proportions of patients with eGFR below 60 mL/min/1.73 m^2 were 22.0% and 16.5%, respectively. Pattaro and colleagues also found similar results in a cross-sectional analysis of a population from three alpine villages [39]. Finally, in a recent meta-analysis Shlipak and colleagues noticed similar mean eGFR$_{cys}$ and eGFR$_{epi}$ estimates while the proportion of subjects under the threshold to define CKD was 13.7 and 9.7 mL/min/1.73 m^2 using cystatin and creatinine based equations, respectively [6].

In line with our current results, Delanaye described low agreement between creatinine and cystatin C based formulas (kappa statistic 0.32 [eGFR$_{cys}$ versus eGFR$_{mdrd}$] and 0.39 [eGFR$_{cys}$ versus eGFR$_{epi}$]) [37]. In this Belgian study, the percentage of discordant subjects estimated by eGFR$_{mdrd}$, eGFR$_{epi}$, and eGFR$_{mix}$ was 11.2%, 8.29%, and 2.71%, taking eGFR$_{cys}$ as reference.

How to explain the discrepancies between cystatin C and creatinine based equations? We first discarded all potential sources of preanalytical and analytical errors. We analysed creatinine in fresh serum samples and cystatin C in samples that were kept frozen at −80°C. Over a period of 10 years, a decay in cystatin C levels occurs using a particle-enhanced nephelometric assay (PENIA) [40]. Such decay is not observed if one uses the more robust particle-enhanced turbidimetric assay (PETIA). Furthermore, our samples were analysed within one year after blood collection and were processed with calibration each time. We used a validated method [40, 41] and reagents (Tina-quant Cystatin C Gen. 2) standardized to the international reference material ERM-DA 471/IFCC, as currently recommended for the use of CKD-EPI equations [3, 7]. Studies of bias (mean difference from reference method) usually overestimated GFR compared

TABLE 3: Prevalence by categories of eGFR according to the stages of chronic kidney disease, for equation to estimate the GFR.

Stage of kidney disease	Estimated glomerular filtration rate				P
	$eGFR_{cys}$	$eGFR_{mdrd}$	$eGFR_{epi}$	$eGFR_{mix}$	
<60 mL/min/1.73 m^2	26 (21.8)	7 (5.9)‡	4 (3.4)§	14 (11.8)*	<0.0001
60–89 mL/min/1.73 m^2	56 (47.1)	59 (49.6)	51 (42.8)	58 (48.7)	0.73
≥ 90 mL/min/1.73 m^2	37 (31.1)	53 (44.5)*	64 (53.8)‡	47 (39.5)	0.004

$eGFR_{cys}$, $eGFR_{mdrd}$, $eGFR_{epi}$, and $eGFR_{mix}$ indicate estimated glomerular filtration rate derived from serum cystatin C, from serum creatinine according to the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations or from both serum cystatin C and creatinine. Values are number of participants (%). P values are for the overall difference across four estimates of glomerular filtration rate. Significance of the difference with $eGFR_{cys}$: $^{*}P \leq 0.05$; $^{\dagger}P \leq 0.01$; $^{\ddagger}P \leq 0.001$; $^{\S}P \leq 0.0001$.

with the reference when using MDRD (range −1.0 to +3.5) or CKD-EPI (range −0.23 to +4.4) and underestimated when using cystatin C based equations (range −5.7 to −1.2) [38, 42–44]. Fewer studies showed a lower positive bias of cystatin C than CKD-EPI based equations [7, 45].

Several epidemiological studies showed that cystatin C is a better predictor of outcomes in coronary heart disease, acute coronary syndrome, and heart failure, independently of serum creatinine and GFR estimation [6, 46–49]. Furthermore, Peralta et al. demonstrated in a large and ethnically diverse population that subjects with decreased $eGFR_{cys}$ had elevated risk of death, cardiovascular disease, and heart failure and had an elevated risk of kidney failure [50, 51]. In keeping with these studies, our population sample had a high cardiovascular risk profile. We found a higher prevalence of L-GFR among hypertensive subjects ($P < 0.001$) if they were categorized based on $eGFR_{cys}$ (32.1%) than on $eGFR_{epi}$ (5.7%) with a similar but not significant trend among the few diabetics patients. However, when computing IDI and NRI we did not observe significant differences between the various estimates of eGFR for hypertension or diabetes ($P \geq 0.24$). In accordance with previous reports [48, 52–54], the prevalence of hypertension ($P = 0.27$) and age ($P < 0.0001$), systolic blood pressure ($P < 0.01$), cholesterol ($P = 0.004$), aortic pulse wave velocity ($P < 0.0001$), and the central augmentation index ($P < 0.0001$) increased with lower categories of $eGFR_{cys}$ (Table 1).

Our results should be interpreted within the limitation of the study. First, we did not have a reliable "*gold standard*" due to the variability in 24 h urine collections. Performing inulin or iothalamate clearance implies invasive and tedious procedures that are not suitable for our population study. Second, participants of the study may not be representative of the Uruguayan population, because we randomly sampled a neighbourhood and our participants had a higher cardiovascular risk profile than the general population of Uruguay [55]. Finally, the small sample size of our population is a limiting factor to analyse specific subgroups of participants. However, the number of participants is large enough to describe the difference between eGFR estimating equations.

In conclusion, to our knowledge, this is the first report based on a population from South America comparing to the performance of eGFR equations based on cystatin C and creatinine. We confirm discrepancies in eGFR using equation based on different biomarkers, particularly in the range of GFR under 60 mL/min/1.73 m^2. Generally, the equation based on cystatin C, compared with creatinine, results in lower eGFR values and, therefore, higher estimates of the prevalence of eGFR below 60 mL/min/1.73 m^2.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

References

[1] A. S. Levey, J. P. Bosch, J. B. Lewis, T. Greene, N. Rogers, and D. Roth, "A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation," *Annals of Internal Medicine*, vol. 130, no. 6, pp. 461–470, 1999.

[2] A. S. Levey, L. A. Stevens, C. H. Schmid et al., "A new equation to estimate glomerular filtration rate," *Annals of Internal Medicine*, vol. 150, no. 9, pp. 604–612, 2009.

[3] L. A. Inker, C. H. Schmid, H. Tighiouart et al., "Estimating glomerular filtration rate from serum creatinine and cystatin C," *New England Journal of Medicine*, vol. 367, no. 1, pp. 20–29, 2012.

[4] J. Kyhse-Andersen, C. Schmidt, G. Nordin et al., "Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine

for glomerular filtration rate," *Clinical Chemistry*, vol. 40, no. 10, pp. 1921–1926, 1994.

[5] E. Premaratne, R. J. MacIsaac, S. Finch, S. Panagiotopoulos, E. Ekinci, and G. Jerums, "Serial measurements of cystatin c are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes," *Diabetes Care*, vol. 31, no. 5, pp. 971–973, 2008.

[6] M. G. Shlipak, K. Matsushita, J. Ärnlöv et al., "Cystatin C versus creatinine in determining risk based on kidney function," *The New England Journal of Medicine*, vol. 369, pp. 932–943, 2013.

[7] L. Fan, L. A. Inker, J. Rossert et al., "Glomerular filtration rate estimation using cystatin C alone or combined with creatinine as a confirmatory test," *Nephrology Dialysis Transplantation*, vol. 29, no. 6, pp. 1195–1203, 2014.

[8] L. Luzardo, I. Lujambio, M. Sottolano et al., "Cohorte GEFA-HT-UY (GEnotipo, Fenotipo y Ambiente de la HiperTensión Arterial en UruguaY)," *Revista Médica del Uruguay*, vol. 29, pp. 103–113, 2013.

[9] L. M. Van Bortel, E. J. Balkestein, J. J. van der Heijden-Spek et al., "Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking," *Journal of Hypertension*, vol. 19, no. 6, pp. 1037–1044, 2001.

[10] C. H. Chen, E. Nevo, B. Fetics et al., "Estimation of Central aortic pressure waveform by mathematical transformation of radial tonometry pressure: validation of generalized transfer function," *Circulation*, vol. 95, no. 7, pp. 1827–1836, 1997.

[11] A. L. Pauca, M. F. O'Rourke, and N. D. Kon, "Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform," *Hypertension*, vol. 38, no. 4, pp. 932–937, 2001.

[12] L. M. van Bortel, S. Laurent, P. Boutouyrie et al., "Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity," *Journal of Hypertension*, vol. 30, no. 3, pp. 445–448, 2012.

[13] L. D. Bowers and E. T. Wong, "Kinetic serum creatinine assays. II. A critical evaluation and review," *Clinical Chemistry*, vol. 26, no. 5, pp. 555–561, 1980.

[14] M. Peake and M. Whiting, "Measurement of serum creatinine—current status and future goals," *The Clinical Biochemist Reviews*, vol. 27, no. 4, pp. 173–182, 2006.

[15] G. L. Myers, W. G. Miller, J. Coresh et al., "Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program," *Clinical Chemistry*, vol. 52, no. 1, pp. 5–18, 2006.

[16] "Laboratory evaluation—Creatinine Standardization Recommendations," Internet source, http://nkdep.nih.gov/.

[17] A. S. Levey, J. Coresh, T. Greene et al., "Expressing the modification of diet in renal disease study equation for estimating glomerular filtration rate with standardized serum creatinine values," *Clinical Chemistry*, vol. 53, no. 4, pp. 766–772, 2007.

[18] A. S. Levey, J. Coresh, T. Greene, L. A. Stevens, Y. Zhang, and S. Hendriksen, "Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the Modification of Diet in Renal Disease study equation for estimating glomerular filtration rate (Annals of Internal Medicine (2006) 145, (247–254))," *Annals of Internal Medicine*, vol. 145, pp. 247–254, 2008.

[19] J. M. Bland and D. G. Altman, "Statistical methods for assessing agreement between two methods of clinical measurement," *The Lancet*, vol. 1, no. 8476, pp. 307–310, 1986.

[20] KDOQI Guidelines, http://www.kidney.org/.

[21] J. R. Landis and G. G. Koch, "The measurement of observer agreement for categorical data," *Biometrics*, vol. 33, no. 1, pp. 159–174, 1977.

[22] M. J. Pencina, S. D'Agostino, R. B. D'Agostino, and R. S. Vasan, "Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond," *Statistics in Medicine*, vol. 27, no. 2, pp. 157–172, 2008.

[23] M. J. Pencina, R. B. D'Agostino Sr., and E. W. Steyerberg, "Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers," *Statistics in Medicine*, vol. 30, no. 1, pp. 11–21, 2011.

[24] B. Bertoni, L. Jin, R. Chakraborty, and M. Sans, "Directional mating and a rapid male population expansion in a hybrid Uruguayan population," *American Journal of Human Biology*, vol. 17, no. 6, pp. 801–808, 2005.

[25] C. Gascue, A. Mimbacas, M. Sans et al., "Frequencies of the four major Amerindian mtDNA haplogroups in the population of Montevideo, Uruguay," *Human Biology*, vol. 77, no. 6, pp. 873–878, 2005.

[26] M. Sans, "'Race', ethnic and genetic adscription in Uruguay," *Runa*, vol. 30, no. 2, pp. 163–174, 2009.

[27] V. R. Dharnidharka, C. Kwon, and G. Stevens, "Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis," *American Journal of Kidney Diseases*, vol. 40, no. 2, pp. 221–226, 2002.

[28] E. Coll, A. Botey, L. Alvarez et al., "Serum cystatin C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment," *American Journal of Kidney Diseases*, vol. 36, no. 1, pp. 29–34, 2000.

[29] H. Mangge, P. Liebmann, H. Tanil et al., "Cystatin C, an early indicator for incipient renal disease in rheumatoid arthritis," *Clinica Chimica Acta*, vol. 300, no. 1-2, pp. 195–202, 2000.

[30] L. Pucci, S. Triscornia, D. Lucchesi et al., "Cystatin C and estimates of renal function: searching for a better measure of kidney function in diabetic patients," *Clinical Chemistry*, vol. 53, no. 3, pp. 480–488, 2007.

[31] K. Nitta, T. Hayashi, K. Uchida et al., "Serum cystatin C concentration as a marker of glomerular filtration rate in patients with various renal diseases," *Internal Medicine*, vol. 41, no. 11, pp. 931–935, 2002.

[32] K. Soto, S. Coelho, B. Rodrigues et al., "Cystatin C as a marker of acute kidney injury in the emergency department," *Clinical Journal of the American Society of Nephrology*, vol. 5, no. 10, pp. 1745–1754, 2010.

[33] Z. Zhang, B. Lu, X. Sheng, and N. Jin, "Cystatin C in prediction of acute kidney injury: a systemic review and meta-analysis," *American Journal of Kidney Diseases*, vol. 58, no. 3, pp. 356–365, 2011.

[34] A. Spahillari, C. R. Parikh, K. Sint et al., "Serum cystatin C- versus creatinine-based definitions of acute kidney injury following cardiac surgery: a prospective cohort study," *The American Journal of Kidney Diseases*, vol. 60, no. 6, pp. 922–929, 2012.

[35] C. W. Tsai, M. E. Grams, L. A. Inker, J. Coresh, and E. Selvin, "Cystatin C- and creatinine-based estimated glomerular filtration rate, vascular disease, and mortality in persons with diabetes in the U.S.," *Diabetes Care*, vol. 37, no. 4, pp. 1002–1008, 2014.

[36] M. E. Grams, S. P. Juraschek, E. Selvin et al., "Trends in the prevalence of reduced GFR in the United States: a comparison

of creatinine- and cystatin c-based estimates," *American Journal of Kidney Diseases*, vol. 62, no. 2, pp. 253–260, 2013.

[37] P. Delanaye, E. Cavalier, O. Moranne, L. Lutteri, J. Krzesinski, and O. Bruyère, "Creatinine-or cystatin C-based equations to estimate glomerular filtration in the general population: impact on the epidemiology of chronic kidney disease," *BMC Nephrology*, vol. 14, no. 1, article 57, 2013.

[38] H. S. Kilbride, P. E. Stevens, G. Eaglestone et al., "Accuracy of the MDRD (Modification of Diet in Renal Disease) study and CKD-EPI (CKD Epidemiology Collaboration) equations for estimation of GFR in the elderly," *American Journal of Kidney Diseases*, vol. 61, no. 1, pp. 57–66, 2013.

[39] C. Pattaro, P. Riegler, G. Stifter, M. Modenese, C. Minelli, and P. P. Pramstaller, "Estimating the glomerular filtration rate in the general population using different equations: effects on classification and association," *Nephron—Clinical Practice*, vol. 123, no. 1-2, pp. 102–111, 2013.

[40] N. V. Voskoboev, T. S. Larson, A. D. Rule, and J. C. Lieske, "Analytic and clinical validation of a standardized cystatin C particle enhanced turbidimetric assay (PETIA) to estimate glomerular filtration rate," *Clinical Chemistry and Laboratory Medicine*, vol. 50, no. 9, pp. 1591–1596, 2012.

[41] M. Conde-Sánchez, E. Roldán-Fontana, N. Chueca-Porcuna, S. Pardo, and J. Porras-Gracia, "Analytical performance evaluation of a particle-enhanced turbidimetric cystatin C assay on the Roche COBAS 6000 analyzer," *Clinical Biochemistry*, vol. 43, no. 10-11, pp. 921–925, 2010.

[42] L. A. Stevens, C. H. Schmid, T. Greene et al., "Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m2," *American Journal of Kidney Diseases*, vol. 56, no. 3, pp. 486–495, 2010.

[43] I. Masson, N. Maillard, I. Tack et al., "GFR estimation using standardized cystatin C in kidney transplant recipients," *American Journal of Kidney Diseases*, vol. 61, no. 2, pp. 279–284, 2013.

[44] J.-F. Feng, L. Qiu, L. Zhang et al., "Multicenter study of creatinine- and/or cystatin C-based equations for estimation of glomerular filtration rates in Chinese patients with chronic kidney disease," *PLoS ONE*, vol. 8, no. 3, Article ID e57240, 2013.

[45] M. Zhang, Y. Chen, L. Tang et al., "Applicability of chronic kidney disease epidemiology collaboration equations in a Chinese population," *Nephrology, Dialysis, Transplantation*, vol. 29, no. 3, pp. 580–586, 2014.

[46] M. G. Shlipak, R. Katz, M. J. Sarnak et al., "Cystatin C and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease," *Annals of Internal Medicine*, vol. 145, no. 4, pp. 237–246, 2006.

[47] M. G. Shlipak, M. J. Sarnak, R. Katz et al., "Cystatin C and the risk of death and cardiovascular events among elderly persons," *The New England Journal of Medicine*, vol. 352, no. 20, pp. 2049–2060, 2005.

[48] W. Koenig, D. Twardella, H. Brenner, and D. Rothenbacher, "Plasma concentrations of cystatin C in patients with coronary heart disease and risk for secondary cardiovascular events: more than simply a marker of glomerular filtration rate," *Clinical Chemistry*, vol. 51, no. 2, pp. 321–327, 2005.

[49] T. Jernberg, B. Lindahl, S. James, A. Larsson, L.-O. Hansson, and L. Wallentin, "Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome," *Circulation*, vol. 110, no. 16, pp. 2342–2348, 2004.

[50] C. A. Peralta, R. Katz, M. J. Sarnak et al., "Cystatin C identifies chronic kidney disease patients at higher risk for complications," *Journal of the American Society of Nephrology*, vol. 22, no. 1, pp. 147–155, 2011.

[51] C. A. Peralta, M. G. Shlipak, S. Judd et al., "Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality," *The Journal of the American Medical Association*, vol. 305, no. 15, pp. 1545–1552, 2011.

[52] M. Madero, C. L. Wassel, C. A. Peralta et al., "Cystatin C associates with arterial stiffness in older adults," *Journal of the American Society of Nephrology*, vol. 20, no. 5, pp. 1086–1093, 2009.

[53] M. Odaira, H. Tomiyama, C. Matsumoto et al., "Association of serum cystatin C with pulse wave velocity, but not pressure wave reflection, in subjects with normal renal function or mild chronic kidney disease," *American Journal of Hypertension*, vol. 23, no. 9, pp. 967–973, 2010.

[54] S. H. Song, I. S. Kwak, Y. J. Kim et al., "Serum cystatin C is related to pulse wave velocity even in subjects with normal serum creatinine," *Hypertension Research*, vol. 31, no. 10, pp. 1895–1902, 2008.

[55] Censo Nacional 2011, http://www.ine.gub.uy/censos2011/index.html.