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Original article

Cystatin C, kidney function, and cardiovascular risk factors in primary hypertension☆

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Objective: To investigate the clinical usefulness of serum cystatin C (Scys) and cystatin C-based equations for the screening of chronic kidney disease in primary hypertensive patients, and correlate these markers with risk factors for cardiovascular disease.

Methods: A cross-sectional study was performed in 199 middle-aged adults at a basic health unit. Kidney function assessment included measurements of serum creatinine (Scr) and Scys levels, 24-hour microalbuminuria (MA), as well as glomerular filtration rate (GFR) through Larsson and Modification of Diet in Renal Disease (MDRD) study equations. Bland-Altman plot analysis was used to calculate the agreement between equations.

Results: High levels of Scys were found in 22% of the patients, even with normal values of GFR estimated by MDRD study equation. Systolic blood pressure and MA correlated better with Scys than Scr, but there was no correlation between Scys and diastolic blood pressure. Gender, age ≥ 60 years, MA, and uric acid were significantly associated with high Scys levels. After multivariate analysis, only age ≥ 60 yrs (RR = 6.4; $p < 0.001$) and male gender (RR = 3.0; $p = 0.006$) remained associated with high Scys levels.

Conclusion: Cystatin C can be used as a screening marker both for detecting mild declines of renal function and for preventing the risk of cardiovascular events in hypertensive subjects with presumably normal renal function.

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Cistatina C, função renal e fatores de risco cardiovascular na hipertensão primária

R E S U M O

Palavras-chave:

Hipertensão primária
Doença renal crônica
Risco cardiovascular
Ritmo de filtração glomerular
Atenção primária
Cistatina C

Objetivo: Investigar a utilidade clínica da cistatina C sérica (Scys) e da equação baseada na cistatina C na triagem da doença renal crônica em pacientes com hipertensão primária e correlacionar esses marcadores com fatores de risco para doença cardiovascular.

Métodos: Foi realizado um estudo transversal com 199 adultos de meia-idade em uma unidade básica de saúde. A avaliação da função renal incluiu medidas dos níveis séricos da creatinina (Scr) e Scys, microalbuminúria de 24 h (MA), bem como da taxa de filtração glomerular (TFG) por meio das equações de Larsson e do estudo MDRD. Foi utilizada a análise Bland-Altman plot para calcular a concordância entre as equações.

Resultados: Foram encontrados níveis elevados de Scys em 22% dos pacientes, mesmo com valores normais da TFG estimada pela equação do estudo MDRD. A pressão sistólica e a MA correlacionaram-se melhor com a Scys do que com a Scr, mas não houve correlação entre Scys e pressão diastólica. Gênero, idade maior que 60 anos, MA e ácido úrico foram significativamente associados com valores elevados de Scys. Após análise multivariada, apenas idade maior que 60 anos (RR = 6.4; $p < 0.001$) e gênero masculino (RR = 3.0; $p = 0.006$) permaneceram associados a níveis aumentados de Scys.

Conclusão: A cistatina C pode ser utilizada como um marcador de triagem tanto para detectar leves declínios da função renal como para prevenir o risco de eventos cardiovasculares em sujeitos hipertensos com função renal presumivelmente normal.

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Introduction

Chronic kidney disease (CKD) is a worldwide public health problem, and frequently leads to end-stage kidney disease. Mild to moderate reductions in kidney function are relatively common in hypertensive patients, and are also associated with increased risk for cardiovascular events. The detection, monitoring, and treatment of CKD are now recognized as essential components of risk stratification for cardiovascular morbidity and mortality in hypertensive patients.^{1,2}

The first step for efficient prevention is early diagnosis, but serum creatinine (Scr) is affected by various nonrenal factors, particularly muscle mass, and may remain within the normal range even with a decrease in glomerular filtration rate (GFR) of $\geq 50\%$.³ The Modification of Diet in Renal Disease (MDRD) study equation is currently recommended because it overcomes, at least in part, some of the limitations of creatinine measurements.⁴

Cystatin C has been emerging as a marker of GFR that performs better than Scr, and even better than equations based on Scr. Similar to Scr, several equations have been elaborated to estimate GFR based on serum cystatin C (Scys). However, these equations must be vigorously evaluated in large, diverse populations and in multiple clinical situations.^{5,6} Particularly, the relation of cystatin C with GFR has not been studied in depth in a population of middle-aged adults with near normal kidney function.⁷ Hence, this study aimed to investigate the clinical usefulness of Scys and Scys-based equation for the screening of decreased renal function in middle-aged adults with essential hypertension. Furthermore, the study aimed to

correlate the Scys values with conventional risk factors for cardiovascular disease (CVD).

Methods

A cross-sectional study was performed in 297 patients attending the Registration and Monitoring System for Hypertension and Diabetes at Primary Care Unit (Sistema de Gestão Clínica da Hipertensão Arterial e Diabetes Mellitus da Atenção Básica – HiperDia, Brazilian Ministry of Health), between January and June 2008. Of the 297 individuals, 199 primary hypertensive patients on regular follow-up treatment and without diabetes mellitus (DM) were selected for this study. Study design details were previously described and published.^{8,9}

Patients were randomly allocated in this study. Exclusion criteria were as follows: age < 20 years, pregnancy, inability to give informed consent, severe cardiac and renal insufficiency, malnutrition, malignancy or infection, advanced dementia, thyroid dysfunction, use of glucocorticoids, and macroalbuminuric stage (urinary albumin excretion ≥ 300 mg/day).

The following clinical information was recorded for all eligible patients: age, gender, height, weight, duration of hypertension, and body mass index (BMI). BMI was calculated by dividing body weight in kilograms by the square of height in meters. Systolic (SBP) and diastolic (DBP) blood pressures were obtained by using a digital sphygmomanometer (Omron®), while patients were seated. The average of two blood pressure readings was considered.

Serum from whole blood was drawn into BD Vacutainer tubes (BD Systems – Basel, Switzerland) from the antecubital vein of fasting subjects early in the morning. The serum was isolated by centrifugation at 3,500 rpm for 15 min and analyzed on the same day. Serum concentrations of biochemical parameters were measured using immunoturbidimetry (ADVIA 1650, Bayer). Renal function was evaluated through the determination of Scr and Scys levels, quantification of urinary albumin excretion, and through the estimation of GFR based on both Scr and Scys levels. The urinary albumin excretion was determined in a 24-h urine sample using immunoturbidimetry (ADVIA 1650, Bayer). The urine samples were collected on the same day as the serum samples, and the patients had been previously instructed on a 24-h urine sample collection. Urinary albumin excretion < 30 mg/day was classified as normoalbuminuria and between 30-299 mg/day as microalbuminuria (MA).

Serum creatinine was measured by kinetic Jaffe method (ADVIA 1650, Bayer). Cystatin C was measured through a BNA nephelometer that employs a particle-enhanced immunonephelometric assay (N Latex Cystatin C, BN 100 system analyzer – Germany). The reference range of Scys and Scr for healthy individuals was reported as 0.53 to 0.95 mg/L and ≤ 1.2 mg/dL, respectively.¹⁰

Creatinine-based GFR ($eGFR_{cr}$) was estimated by the four-variable MDRD study equation: $eGFR_{cr}$ (mL/min/1.73 m²) = $186.3 \times (Scr [mg/dL])^{-1.154} \times (age [years])^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$.¹¹ Cystatin C-based GFR ($eGFR_{cys}$) was estimated using the Larsson formula: $eGFR_{cys}$ (mL/min) = $77.239 \times cystatin\ C [mg/L]^{-1.2623}$.¹² The GFR determined by the MDRD formula was used as the reference method.⁴

Statistical analysis was performed using Stata version 10.0. Comparisons between quantitative variables were assessed using Student's t-test or Mann-Whitney's test. Correlation analysis was performed using Spearman's rank test. MedCalc for Windows, version 10.4.3.0 (Medcalc Software – Belgium), was also used for a Bland-Altman analysis of the GFR estimates.¹³ The limits of agreement are given by the mean ± 1.96 SD, containing 95% of the values. The mean difference (bias) between methods is a measure of accuracy. The SD around the mean reflects the dispersion and precision of the equations. Statistical significance was inferred when $p < 0.05$.

Factors associated with Scys levels > 0.95 mg/L were identified using multivariate logistic regression analysis and the following were used as independent variables: gender, age ≥ 60 yrs, SBP > 150 mmHg, DBP > 80 mmHg, hypertriglyceridemia (TG > 150 mg/dL), and MA > 30 mg/day (urinary albumin excretion [UAE], 30-299 mg/day). The model included variables whose univariate analysis showed $p < 0.20$. Adjustment of the model was evaluated by the Hosmer & Lemeshow test. The study protocol was approved by the Institutional Review Board of the Universidade Federal do Maranhão (Protocol 1977/2007).

Results

A total of 297 hypertensive patients that participated in the HiperDia program were evaluated. The final sample consisted of 199 hypertensive individuals without DM.

Table 1 summarizes the clinical characteristics of these patients. The study was comprised predominantly of middle-aged adults with a mean age of 60.6 ± 11.8 years, females (73.4%), and overweight patients (27.5 ± 4.8 kg/m²). The subjects mean values of Scr and Scys levels were 0.87 ± 0.31 mg/dL and 0.89 ± 0.25 mg/L, respectively.

Table 1 – Clinical characteristics of the studied patients.

Clinical characteristics	n = 199
Age, years	60.6 \pm 11.8
Gender (female), %	73.4
BMI, kg/m ²	27.5 \pm 4.8
SBP, mmHg	149.9 \pm 24.2
DBP, mmHg	89.0 \pm 12.5
Hypertension duration, years	7.8 \pm 7.7
Fasting glucose, mg/dL	78.7 \pm 13.3
Total cholesterol, mg/dL	206.7 \pm 41.0
Triglycerides, mg/dL	135.8 \pm 68.2
LDL-cholesterol, mg/dL	133.4 \pm 34.5
HDL-cholesterol, mg/dL	46.1 \pm 11.3
Blood urea nitrogen, mg/dL	30.2 \pm 11.07
Uric acid, mg/dL	4.5 \pm 1.38
UAE, mg/day (normo/micro), %	86.4 / 13.6
Serum creatinine, mg/dL	0.87 \pm 0.31
Serum cystatin C, mg/L	0.89 \pm 0.25
$eGFR_{cr}$, mL/min/1.73 m ² ^a	88.1 \pm 23.0
$eGFR_{cys}$, mL/min. ^b	95.9 \pm 24.0
Data for continuous variables are mean \pm SD and for categorical variables are proportions. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; UAE, urinary albumin excretion; normo, normoalbuminuria; micro, microalbuminuria; eGFR, estimated glomerular filtration rate.	
^a Based on Modification of Diet in Renal Disease (MDRD) study equation;	
^b Based on Larsson formula.	

The levels of Scys in males were slightly higher than in females (0.95 ± 0.21 mg/L vs. 0.86 ± 0.25 mg/L; $p = 0.025$). The mean values of Scys in subjects aged ≥ 60 years were significantly higher than those aged < 60 years (0.97 ± 0.29 mg/L vs. 0.79 ± 0.13 mg/L; $p < 0.001$). Likewise, when renal function was assessed based on Scr, there was a significant difference between males and females (1.04 ± 0.22 mg/dL vs. 0.78 ± 0.31 mg/dL; $p < 0.001$). The patients aged ≥ 60 years had higher levels of Scr than those aged < 60 years (0.92 ± 0.39 mg/dL vs. 0.78 ± 0.16 mg/dL; $p = 0.002$)(Table 2). Of the patients with GFR ≥ 60 mL/min/1.73 m² estimated by MDRD equation, 22% presented elevated Scys levels (> 0.95 mg/L), while only 3% exceed normal values of Scr (>1.2 mg/dL). The same pattern was observed in patients with GFR ≥ 60 mL/min/1.73 m² estimated by Larsson formula. The percentage of elevated Scys levels (23%) was much higher than for those with elevated Scr levels (4%).

There was a close linear relationship between Scr and Scys levels ($r = 0.82$, $p < 0.001$). A moderately strong correlation was

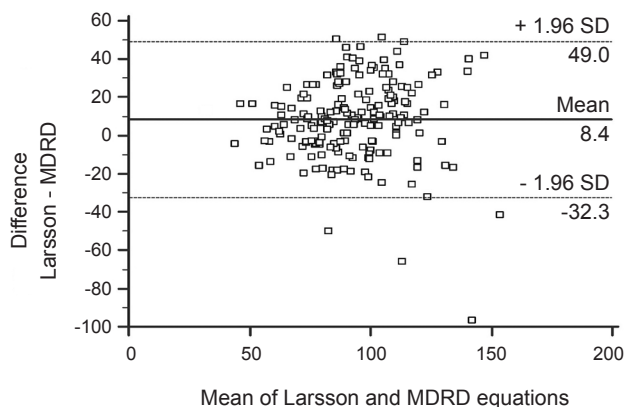
Table 2 - Mean levels of serum creatinine (mg/dL) and serum cystatin C (mg/L) according to the variables gender, age and glomerular filtration rate (GFR) estimated by the Modification of Diet in Renal Disease (MDRD) study equation.

Variable	Serum cystatin C			Serum creatinine	
	(%)	Mean \pm SD	p	Mean \pm SD	p
Gender			0.025		< 0.001
Male	(26.6)	0.95 \pm 0.21		1.04 \pm 0.22	
Female	(73.4)	0.86 \pm 0.25		0.78 \pm 0.31	
Age			< 0.001		0.002
≥ 60 years	(53.6)	0.97 \pm 0.29		0.92 \pm 0.39	
< 60 years	(46.4)	0.79 \pm 0.13		0.78 \pm 0.16	
eGFR _{cr} ^a			< 0.001		0.015
≥ 60 years	(92.5)	0.84 \pm 0.15		0.79 \pm 0.16	
< 60 years	(7.5)	1.38 \pm 0.54		1.38 \pm 0.11	

SD, standard deviation.
^a Estimated glomerular filtration rate based on MDRD study equation (mL/min./1.73 m²).

observed between eGFR_{cys} and eGFR_{cr} ($r = 0.61$, $p < 0.001$). In order to assess the correlation of different risk factors for hypertension with renal function, comparisons with Scr and Scys were performed. Scr and Scys levels correlated significantly but weakly with age ($r = 0.22$, $p = 0.001$ and $r = 0.37$, $p < 0.001$, respectively), SBP ($r = 0.19$, $p = 0.005$ and $r = 0.28$, $p < 0.001$, respectively) and MA ($r = 0.31$, $p < 0.001$ and $r = 0.38$, $p < 0.001$, respectively). The results demonstrated no significant correlation between Scys, DBP, and triglycerides. However, serum concentrations of uric acid displayed a highly significant correlation with Scr and Scys levels ($r = 0.50$, $p < 0.001$ and $r = 0.44$, $p < 0.001$, respectively).

The agreement analysis was calculated according to Bland and Altman.¹³ The mean difference between eGFR_{cys} and eGFR_{cr} equations was 8.4 mL/min. The maximum value of the mean \pm 1.96 SD difference attained +49 and the minimum -32.3 (CI 5.3 to 11.3). The precision of the eGFR_{cys} in relation to eGFR_{cr} was of 20.72 mL/min/1.73 m² (Fig. 1). After multivariate analysis, only age ≥ 60 years (RR = 6.4; 95% CI = 2.84-14.28; $p < 0.001$) and male gender (RR = 3.0; 95% CI = 1.38-6.55; $p = 0.006$) remained associated with increased Scys levels (> 0.95 mg/L).

**Fig. 1 - Agreement analysis.**

Discussion

The current study demonstrated that serum cystatin C levels had a significant association with conventional risk factors for cardiovascular disease and with traditional markers of GFR of hypertensive patients in primary health care. In accordance with previous studies,^{14,15} the results showed that Scys had a significant correlation with Scr, and that the Larsson formula presented a good agreement with the commonly used MDRD equation. In spite of this, 22% of the patients presented elevated levels of Scys, even with normal values of GFR estimated by MDRD equation. This suggests that Scys may recognize a certain degree of renal dysfunction that was not identified through the determination of Scr levels or eGFR_{cr}.¹⁶

As expected, the MDRD study equation showed lower mean values of GFR than the Scys-based equation. This observation could be explained by the fact that the MDRD equation was derived from participants having moderate or severe renal failure.¹¹ Herget-Rosenthal et al.¹⁷ suggested that a GFR calculated with the MDRD equation performs best when the GFR ranges between 20 and 60 mL/min. Hence, there are good reasons for believing that the MDRD equation underestimates GFR and thus overestimates CKD prevalence in subjects with near-normal creatinine values. Otherwise, the potential of cystatin C and eGFR_{cys} would lie in the recognition of CKD stage 2 (GFR 60-90 mL/min.), mainly in patients with low muscle mass.¹⁷ Furthermore, it has been reported that Scys is superior to other markers as a predictor of GFR in primary hypertension.¹⁸

In this research, the impacts of hypertension on Scys and Scr levels were found to be markedly different in subjects < 60 and ≥ 60 years of age. Despite weak correlation of the variable age with Scys, advanced age (≥ 60 years) was found to be an independent predictor of elevated Scys levels. Kottgen et al.¹⁹ demonstrated a strong and independent association of age with Scys concentrations in individuals over 60 years, even after adjustment for chronic health conditions. According to Galteau et al., Scys levels increase slightly in

males and females above the age of 60 years, probably due to physiological aging of renal function.²⁰ Although it has been reported that Scys is independent of age and gender,⁵ the present results reveal an association between male gender and elevated Scys levels. However, this finding might be explained by the lower prevalence of men with high mean age and, consequently, a mild reduction in GFR level.

The decline of kidney function in older adults is age-associated, and may represent usual ageing. The ageing of the kidney may be explained by cumulative exposure to subclinical risk factors and microvascular disease, biochemical decrements in kidney cells, loss of nephron units, and increased stress on the remaining nephrons over time.⁷ Despite the lack of a general agreement defining the lower limit of normal GFR in older people, it is known that a decrease in GFR is associated with less successful aging.^{21,22} Recently, it has been found that Scys has a better diagnostic performance than Scr to predict successful aging.²²

In the present study, high Scys levels correlated better with SBP than Scr. In contrast, there was no correlation between Scys levels and DBP. In the same way, some authors found that DBP was negatively²³ or not correlated²⁴ with Scys levels, whereas SBP showed good correlation with Scys levels.²³⁻²⁵ Noteworthy, these associations were found in patients without clinical CKD by the use of traditional creatinine-based methods. This implies that Scys may be an important link between kidney function and adequate SBP control.

Interestingly, microalbuminuria had a significantly better correlation with cystatin C concentrations than creatinine. MA indicates the presence of early signs of atherosclerotic vascular damage, serves as marker of severity of hypertension, and can predict the development of overt proteinuria and cardiovascular events.²⁵ A significant correlation was also observed between Scys and serum uric acid, which is also known to be closely associated with hypertension, metabolic syndrome, cardiovascular risk, and new-onset kidney disease.²⁶ Consistent with the present findings, several studies have reported a significant correlation of Scys with uric acid,²⁷ MA, age, and GFR in a hypertensive population.²⁸ It has been observed that both Scys and MA are independent risk factors for incident CKD stage 3 and could be useful as screening tools.²⁹ Moreover, it was displayed that cystatin C-based GFR is more sensitive than creatinine-based GFR for predicting MA development in the early stage of hypertension.³⁰

In a prospective study involving 4,663 elderly subjects with eGFR ≥ 60 mL/min/1.73 m², Shlipak et al. showed that Scys levels ≥ 1.0 mg/L were related to worse cardiovascular outcomes, and had a four-fold risk for progressing to CKD after four years of follow-up compared with those with lower Scys levels.³¹ Previous studies have also demonstrated an association between Scys and several established cardiovascular risk factors,³² heart failure,³³ coronary events,³⁴ atherogenic process,³⁵ and death,³⁶ especially in older people. Due to this, Scys has been suggested to be not only a marker of renal function, but also a marker of metabolic abnormalities, inflammation, atherogenesis, and an independent risk factor for cardiovascular diseases.^{27, 37}

In contrast to previous reports, Grubb et al.³⁸ have shown that inflammatory conditions of a patient do not influence

the role of Scys as a marker of GFR. Rodondi et al.³⁹ have also demonstrated that Scys is not associated with carotid atherosclerosis in a population-based random sample of 523 middle-aged adults. In this setting, increased levels of Scys may identify a state of "preclinical" kidney disease, which could prevent subsequent development of CKD in patients with hypertension and diabetes.^{40,41} The capacity of Scys to early identify an abnormal filtration may explain the fact that an increased Scys level indicates higher incidence of cardiovascular events and death even with normal GFR.³⁸

There are some limitations to this study. First, GFR was not measured with a gold standard method, and thus it was not possible to confirm which equation had the best estimate of GFR. However, the aim was not to establish the accuracy of the equations with a direct measurement of GFR, but rather to evaluate the clinical usefulness of cystatin C in primary care. Second, the present study's participants were mostly hypertensive women and with mixed ethnicity, which limits the generalization of the data. Nevertheless, only a few studies have compared Scys- and Scr-based equations for the screening of CKD in middle-aged adults with primary hypertension in basic health care.

Scys C measurement is being introduced in clinical practice around the world. However, despite its clear advantages over creatinine as a marker of GFR, cystatin C is still not widely used in routine clinical laboratory testing, especially in primary care. The major reasons for the lack of incorporation of cystatin C into daily clinical practice have been its higher reagent cost, the absence of standardized measurement procedures, and the need for further validation in large populations.

In public health, cystatin C can be used as a reliable marker for both detecting early kidney dysfunction and preventing the risk of cardiovascular morbimortality. Regardless of whether mildly reduced renal function represents pathology or normal ageing in older adults, adequate screening and follow-up are needed. In addition, cystatin C may provide new insights into the importance of the relationship between kidney disease and hypertension in subjects with presumably normal renal function.

Conflict of interest

All authors declare to have no conflict of interest.

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