Correlation Between Serum Cystatin C and Markers of Subclinical Atherosclerosis in Hypertensive Patients

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Abstract

Background: Serum cystatin C (s-CC), an endogenous marker of kidney function, has also been proposed as a cardiovascular risk marker. However, it is unknown whether it is a direct marker of atherosclerosis, independently of kidney function.

Objective: The aim of this study was to correlate s-CC with two surrogate markers of subclinical atherosclerosis.

Methods: This is a cross-sectional study involving 103 middle-aged (57.49 ± 11.7 years) hypertensive outpatients, being 60 female (58.25%), most with preserved kidney function. S-CC was correlated with carotid intima media thickness (IMT) and flow-mediated dilation of brachial artery (FMD), both assessed by ultrasound, as well as with measured creatinine clearance and established cardiovascular risk factors.

Results: S-CC was neither significantly correlated with IMT (r = –0.024; p = 0.84) nor with FMD (r = –0.050; p = 0.687) and no significant association was observed with conventional risk factors and inflammatory markers. In univariate analysis, s-CC was correlated with measured creatinine clearance (r = -0,498; p < 0,001), age (r = 0,408; p < 0,001), microalbuminuria (r = 0,291; p = 0,014), uric acid (r = 0,391; p < 0,001), ratio E/e' (r = 0,242; p = 0,049) and Framingham score (r = 0,359; p = 0,001). However, after multiple regression analysis, only the association with measured creatinine clearance remained significant (r = -0,491; p < 0,001).

Conclusion: In middle-aged hypertensive outpatients, s-CC correlated with measured creatinine clearance, as expected, but no association was observed with markers of atherosclerosis neither with established cardiovascular risk factors. (Arq Bras Cardiol 2012;99(4):899-906)

Keywords: Cystatin C; subclinical atherosclerosis; hypertension; creatinine.

Introduction

Cystatin C, a novel endogenous marker of kidney function, has been proposed also as a marker of cardiovascular risk[1]. It is a member of the superfamily of cysteine proteinase inhibitors produced by all nucleated cells. Because of its low molecular weight, it is freely filtered by the glomeruli and almost completely reabsorbed and catabolized in the tubules, without suffering tubular secretion, characteristics that make it more sensitive and accurate than creatinine[2].

Many studies have demonstrated association between this serum protein and various established cardiovascular risk factors and markers[1,3-9], incidence of coronary events[10,11] and heart failure[12,13] and, more importantly, the risk of cardiovascular death, particularly in the elderly[14-16].

Some authors have argued that, as cystatin C is involved in the protein catabolism, inhibiting elastolitic proteases, which are augmented in degenerative and inflammatory processes like atherosclerosis, it could function as a direct marker of the atherogenic process itself[1,10,17].

However, at the present time, it is not established whether s-CC is merely a better marker of kidney function, which would be truly implicated in the cardiovascular risk, or if it could be considered a direct marker of atherosclerosis, independent of kidney function[18].

Therefore, this study aimed to correlate s-CC levels in hypertensive patients without overt cardiovascular disease with two surrogate markers of subclinical atherosclerosis at the same time, namely the carotid intima media thickness (IMT) and the flow-mediated dilation of brachial artery (FMD), as well as with traditional risk factors and inflammatory markers.

Methods

This is a cross-sectional study involving hypertensive outpatients attended at a university service, chosen at random. We considered exclusion criteria for the enrollment in the research: a history of thyroid disease and/or current use of thyroxine, macroproteinuria (≥ 300 mg/ 24h), current use

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Manuscript received November 17, 2011; manuscript revised November 17, 2011; accepted January 17, 2012.

of anti-inflammatory agents or high sensitivity C-reactive protein > 10 mg/l and a history and/ or clinical evidence of atherosclerotic disease.

All participants underwent a structured medical history interview, including the search for cardiovascular risk factors, and physical examination, which included measure of waist circumference, height and weight. Body mass index was calculated as weight in kilograms divided by the square of height in meters (Kg/m^2). Blood pressure was defined as the average of the last two of three measurements with a mercury sphygmomanometer taken at intervals ≥ 2 minutes after the participants had been seated for at least 5 minutes. Smoking was defined as current smoking of ≥ 1 cigarette/ day. Those participants who reported to practice < 30 minutes three times/ week of physical exercise were considered sedentary.

Fasting (12-hour) venous samples were drawn and biochemical parameters were assayed using an automatic analyzer. The following measurements were obtained: glucose, urea, creatinine, potassium, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, leukocyte count, uric acid, high sensitivity C-reactive protein, fibrinogen and s-CC. Each participant collected his or her own urine for 24 h for the determination of microalbuminuria and creatinine clearance. High sensitivity C-reactive protein and microalbuminuria were measured by immunoturbidimetry. Measured creatinine clearance was standardized by the body surface area and was used as an index of glomerular filtration rate, being considered low when < 60 ml/min/1.73m^2. Creatinine clearance was also estimated by Cockcroft-Gault and Modification in Diet in Renal Disease (MDRD) formulas[19]. S-CC was measured by a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring) with a nephelometer (BNII). The range of detection of the assay is 0,195 to 7,330 mg per liter, with the reference range for healthy persons reported as 0,53 to 0,95 mg per liter[14].

Diabetes mellitus was defined as fasting plasma glucose ≥ 126 mg/dL, using any hypoglycaemic medication and/ or a 2h glucose ≥ 200 mg/dL in an oral glucose tolerance test. Dyslipidemia was defined as elevated total cholesterol (≥ 200 mg/dL), decreased HDL-cholesterol (< 40 mg/dl in males and < 50 mg/dL in females), elevated LDL-cholesterol (≥ 160mg/dL), elevated triglycerides (≥ 150 mg/dL) and/or using hypolipemiant agents[20].

The individual's absolute 10-year risk of coronary heart disease was estimated by means of the Framingham score[20]. Metabolic syndrome was identified according to the International Diabetes Federation criteria[21].

All participants underwent transthoracic echocardiography, measurement of carotid IMT and assessment of FMD of the brachial artery with high-resolution Doppler B-mode ultrasound equipment (General Electric). Examinations were performed by two experts operators, blinded to the patient's clinical and laboratory findings.

Echocardiography was performed using a 3,5 MHz transducer. Left ventricular mass (g) was estimated using the formula of Devereux and Reichek and the left ventricular mass index was calculated by dividing left ventricular mass by body surface area (g/m^2). Left ventricular hypertrophy was defined as an increase in left ventricular mass index ≥ 115 g/m^2 for men and > 95 g/m^2 for women[22]. The left ventricular ejection fraction was estimated by Teichholz's method and was used as the parameter of left ventricular systolic function[22]. Left ventricular diastolic function was assessed by the ratio E/e' (the early diastolic transmitral flow velocity divided by the early diastolic mitral annular velocity obtained from tissue Doppler), which has been used to estimate left ventricular filling pressure[23].

A 7.5-MHz linear array transducer, with a frequency range of 7-10 MHz, was used to measure common carotid IMT. Thickness measurement was performed at the posterior artery wall, 1 cm proximal to carotid bifurcation, as the distance between two echogenic lines corresponding to the lumen-intima and media-adventitia interfaces of the artery wall, according to current recommendations[24]. It was considered normal a carotid IMT ≤ 1mm.

A 10- MHz linear array transducer was used to assess the FMD of the brachial artery, according to technique published previously[25]. After assessment of baseline diameter of the right brachial artery (D1), a cuff was inflated to 50 mm Hg above the participant's systolic blood pressure to occlude the artery and kept inflated for 5 minutes. Images of the right brachial artery were then obtained 60-90 seconds after cuff deflation and artery diameter was measured again (D2), in diastole (gated with ECG R wave). %FMD was computed with the formula D2-D1/D1 x 100 and adopted as an estimation of endothelial function. Acceptable normal values vary from 10 to 15%[25].

Association between s-CC levels (mg/L), used as a continuous or as a categorical variable (< or ≥ 50th percentile), and each of the following variables was searched: age (years), smoking (yes/no), diabetes mellitus (yes/no), sedentary lifestyle (yes/no), waist circumference (cm), body mass index (kg/m^2), systolic and diastolic blood pressure (mmHg), total cholesterol (mg/dL), HDL-cholesterol (mg/dL), LDL-cholesterol (mg/dL), triglycerides (mg/dL), glucose (mg/dL), glycosylated hemoglobin (%), urea (mg/dL), creatinine (mg/dL), measured creatinine clearance (ml/min/1.73m^2), estimated creatinine clearance by Cockcroft-Gault and MDRD formulas (ml/min/1.73m^2), uric acid (mg/dL), microalbuminuria (mg/24h), high sensitivity C-reactive protein (mg/dL), fibrinogen (mg/dL), left ventricular ejection fraction (%), ratio E/e', left ventricular mass index (g/m2), FMD (%), carotid IMT (mm), Framingham score (%) and metabolic syndrome (yes/no).

The project was previously approved by the Institution's Research Ethics Committee. Informed consent was obtained from all participants.

Categorical variables were expressed as percentage (%) and continuous variables, as mean ± standard deviation (SD). To compare means it was used the Student's t-test and categorical variables, the chi-square test ($\chi 2$). Pearson's correlation coefficient, or Spearman's coefficient for nonparametric variables, was calculated to analyze the association between two continuous variables, being adopted a 95 % confidence interval. All statistical analysis were conducted using the SPSS package for Windows, version 16.0. Two-tailed p values < 0,05 were considered as expressing a statistically significant association. Associations presenting a p < 0.10 at the univariate analysis were included in the final multiple regression analysis model.

Results

One hundred and three hypertensive outpatients were recruited for this research, with a mean age of 57.49 ± 11.7 years and predominance of female and non-whites. The sample was characterized also by an average measured creatinine clearance within normal range and a glomerular filtration rate < 60 ml/min/1.73 m² in less than 1/5 of the participants. Clinical and laboratory data of the study sample are shown in Table 1.

With respect to risk assessment, it was observed a mean Famingham score of 9.0 ± 8.4%, being 58.9% of the participants classified as low, 25% as medium and 16% as high risk. Mean s-CC was 0.75 ± 0.16 mg/L, following a normal distribution, being significantly higher in male (0.80 ± 0.15 *vs.* 0.72 ± 0.16 mg/L, p = 0.039), with no difference between whites and non-whites (0.78 ± 0.16 *vs.* 0.73 ± 0.15 mg/L, p = 0.15). It was observed a significant correlation with age (r = 0.408, p < 0.001) and a trend in relation to body mass index (r = -0.193, p = 0.091).

As can be seen in Table 2, s-CC was significantly correlated with all other kidney function parameters. The inverse correlation observed between s-CC and measured creatinine clearance, considered to be the reference marker of glomerular filtration rate in this study, remained significant even after adjustment for age (r = -0.369, p=0.001), gender (r = -0.412, p < 0.001), race (r = -0.419, p < 0.001), body mass index (r = -0.383, p = 0.001) and all these variables jointly (r = -0.365, p = 0.002). As shown in Table 3, all other estimated kidney function parameters except urea were also significantly correlated with measured creatinine clearance.

No significant association was observed between s-CC levels and diabetes mellitus (p = 0.915), smoking (p = 0.881), sedentary lifestyle (p = 0.115), dyslipidemia (p = 0.865) and metabolic syndrome (p = 0.526).

A strong inverse correlation between the two subclinical atherosclerosis markers evaluated in this work, namely carotid IMT and FMD of brachial artery, was observed (r = -0.480, p < 0.001). However, as shown in Table 4, s-CC was not significantly correlated with any of these markers. In the same way, there was no correlation with the inflammatory markers studied here, namely leukocyte count, high sensitivity C-reactive protein and fibrinogen. On the other hand, s-CC correlated significantly with microalbuminuria, uric acid, ratio E/e' and Framingham score.

However, after multiple linear regression analysis including all associations with p < 0.10, only the correlation with measured creatinine clearance remained significant, being observed a borderline significance for the association with uric acid (Table 5).

Discussion

In the current study we evaluated hypertensive outpatients free of manifested cardiovascular disease, predominantly of middle age, with preserved renal function and classified as of low and medium risk according to Famingham score. These characteristics correspond to a profile of patient commonly found in the daily clinical practice, for which there is yet uncertainty about risk assessment.

As expected, s-CC correlated significantly with all kidney function parameters evaluated, including measured creatinine clearance. Taking measured creatinine clearance as a reference, s-CC performance was roughly equivalent to Cockcroft-Gault formula. However, as creatinine clearance, even measured, cannot be considered a gold standard of kidney function, it is possible that s-CC sensitivity is being underestimated in this analysis.

In this research, s-CC was not significantly correlated with markers of atherosclerosis, inflammatory markers, left ventricular mass index, neither with traditional risk factors

Table 1 – Baseline characteristics of study population (n= 103)

Age, mean (± SD), years	**57.49 (11.7)**
Woman, n (%)	60 (58.2)
Non-white, n (%)	66 (64.1)
Cigarette smoking, n (%)	10 (9.8)
Physically inactive, n (%)	44 (42.7)
Diabetes mellitus, n (%)	17 (16.5)
Family history of premature coronary heart disease, n (%)	50 (48.5)
Body mass index, mean (± SD), Kg/m²	28.0 (5.0)
Waist circumference, mean (± SD), cm	
Men	92.9 (9.6)
Woman	92.5 (10.0)
Metabolic syndrome, n (%)	53 (51.5)
Dyslipidemia, n (%)	75 (72.8)
Systolic blood pressure, mean (± SD), mmHg	145.9 (21.7)
Diastolic blood pressure, mean (± SD), mmHg	87.0 (12.7)
Antihypertensive agents use, n (%)	100 (97.0)
Total cholesterol, mean (± SD), mg/dL	206.0 (44.3)
HDL-cholesterol, mean (± SD), mg/dL	
Men	42.5 (9.3)
Woman	48.9 (11.8)
LDL-cholesterol, mean (± SD), mg/dL	132.8 (38.3)
Triglyceride, mean (± SD), mg/dL	153.05 (66.0)
Creatinine, mean (± SD), mg/dL	0.84 (0.22)
Creatinine clearance, mean (± SD), ml/min/1.73 m²	
Measured	94.8 (43.3)
Estimated by Crockcroft-Gault formula	88.36 (30.28)
Estimated by MDRD formula	95.5 (26.25)
Measured creatinine clearance < 60 ml/min/1,73 m², n (%)	19 (18.4)
Glucose, mean (± SD), mg/dL	103.15 (26.0)
Urea, mean (± SD), mg/dL	30.25 (9.4)
Carotid IMT, mean (± SD), mm	1.06 (0.22)
High carotid IMT, n (%)	46 (44.6)
Left ventricular mass index, mean (± SD), g/m²	103.55 (26.17)
Left ventricular hypertrophy, n (%)	52 (50.48)

except age. On the other hand, the significant associations observed with uric acid, microalbuminuria, ratio E/e' and Framingham score, as well as with age, disappeared after multiple regression analysis, remaining significant only the correlation with measured creatinine clearance.

Carotid IMT and FMD of brachial artery have been considered as, respectively, structural and functional markers of subclinical atherosclerosis[26-29]. As expected, in the current study, a strong inverse linear correlation between these two markers was observed, corroborating the fact that they reflect different aspects of the same pathophysiologic process. The few studies that aimed to correlate s-CC with these markers have reported conflicting findings. While two small cross-sectional studies involving middle-aged hypertensive patients reported significant correlation between s-CC and carotid IMT[3,30], larger cross-sectional studies did not confirm it, neither in middle-aged individuals, with preserved kidney function[4,31], nor in the elderly, with discrete to moderate kidney function impairment[32]. The Australian study conducted by Potter et al.[33] was the only one, before ours, to correlate s-CC with either IMT as FMD at the same time. Indeed, it is a cross-sectional analysis of a subgroup of elderly stroke participants (n = 173) of the Vitamins to Prevent Stroke Trial (VITATOPS trial), whose primary objective was to evaluate the effectiveness of homocysteine lowering with B-vitamins supplementation in the reduction of incidence of major vascular events. The authors observed no correlation with IMT; with regard to FMD, they found a significant correlation, but clearly influenced by kidney function.

In short, our findings corroborate the role of s-CC as marker of kidney function but do not demonstrate independent association with markers of atherosclerosis neither with established risk factors. Therefore, based on our findings, as well as others', we believe that the widely reported association between s-CC and cardiovascular risk is fundamentally connected to its role as kidney function marker and reject in principle the hypothesis that it could reflect independently an intrinsic vascular damage and consequently the atherogenic process itself.

Among other authors, the postulated role of s-CC as a marker of inflammation and atherosclerosis has been controversial too. Despite innumerous publications reporting significant association between s-CC and various inflammatory markers[1,5-8,10,14,34-36], it is not established yet if this association is independent of kidney function. Singh et al.[36] for instance, in a cross-sectional analysis of the elderly cohort Heart and Soul Study, verified that the significant correlation observed among s-CC and high sensitivity C-reactive protein and fibrinogen simply disappeared after adjustment for measured creatinine clearance. If one considers that renal impairment itself, even slight, is associated to inflammatory markers elevation, s-CC could simply reflect, with more sensitivity than creatinine or estimated creatinine clearance, this association.

With respect to its association with markers of atherosclerosis, beyond the divergent findings regarding carotid IMT and FMD, including ours, it must be added the absence of significant association with coronary calcification evaluated by computed tomography, as demonstrated in a cross-sectional analysis of the large Multi-Ethnic Study of Atherosclerosis (MESA)[6]. In the same way, a significant correlation with microalbuminuria was reported by some authors[3,4,8], but not by other[7].

In the European cohort Prospective Epidemiological Study of Myocardial Infarction (PRIME Study), that included only healthy middle-aged men, s-CC was significantly associated with the occurrence of the first coronary event, independently of classical factors, but the significance of this association disappeared after adjusting for high sensitivity C-reactive protein[35].

Table 2 – Univariate correlation between s-CC and conventional kidney function parameters

Variable	r value	p value
Urea	0.507	< 0.001
Creatinine	0.515	< 0.001
Measured creatinine clearance	- 0.498	< 0.001
Estimated creatinine clearance by:		
Cockcroft-Gault formula	- 0.518	< 0.001
MDRD formula	- 0.520	< 0.001

Table 3 - Correlation between the different estimated kidney function parameters and measured creatinine clearance

Variable	r value	p value
Cystatin C	- 0.498	< 0.001
Urea	- 0.111	0.304
Creatinine	- 0.388	< 0.001
Cockcroft-Gault formula	0.487	< 0.001
MDRD formula	0.385	< 0.001

Table 4 – Univariate correlation between s- CC and different clinical and laboratory continuous variables evaluated

Variable	r value	p value
Systolic blood pressure	0.211	0.064
Diastolic blood pressure	0.086	0.455
Waist circumference	- 0.052	0.655
Glucose	- 0.026	0.817
Glycosylated hemoglobin	0.071	0.552
Microalbuminuria	0.291	0.014
Uric acid	0.391	< 0.001
Potassium	0.095	0.446
Leukocyte count	0.047	0.685
High sensitivity C- reactive protein	- 0.139	0.228
Fibrinogen	0.123	0.339
Total cholesterol total	0.008	0.945
HDL- cholesterol	0.040	0.729
LDL- cholesterol	- 0.087	0.462
Triglycerides	0.152	0.184
Left ventricular mass index	0.026	0.824
Ratio E/e'	0.242	0.049
Left ventricle ejection fraction	0.003	0.981
Carotid IMT	- 0.024	0.844
FMD of brachial artery	- 0.050	0.687
Framingham score	0.359	0.001

Table 5 – Results of multiple linear regression including all associations with p < 0,10

Variable	β value	p value
Measured creatinine clearance	- 0.491	< 0.001
Uric acid	0.229	0.059
Age	0.111	0.377
Microalbuminuria	- 0.115	0.343
Rate E/e'	0.066	0.594
Framingham score	0.002	0.990
Body mass index	- 0.032	0.805
Systolic blood pressure	0.029	0.811

In a recently published cohort, theTromsø Study, which studied a general population, involving more than 6.000 adult men and women, cystatin C was a risk factor for all-cause mortality in women but was not independently associated with fatal and non-fatal myocardial infarction or ischemic stroke in the whole population.[37] Even in the large elderly cohorts, in which s-CC presented strong correlation with cardiovascular mortality, its association specifically with atherosclerotic events, namely acute myocardial infarction and stroke, did not become defined, being demonstrated in the Cardiovascular Health Study[14], but not in the Health ABC study[15]. The Health ABC's authors speculate that individuals with renal impairment, even slight or moderate, may have a disproportionally higher risk of fatal events rather than nonfatal acute myocardial infarction or stroke. This could be due to the greater incidence of ventricular arrhythmias and sudden death in this setting, not necessarily associated to atherosclerotic events, once these individuals have a greater prevalence of structural abnormalities of the conduction system, myocardial fibrosis and hypertrophy, ventricular

dysfunction and heart failure[15]. So, the apparent superiority of s-CC over creatinine based kidney function assessment, regarding cardiovascular risk prediction, as showed in these cohorts, could be fundamentally due to its greater sensitivity to detect smaller degrees of renal impairment, which would be the true cardiovascular risk predictor. In accordance with this, in patients with advanced chronic kidney disease, Menon et al.[38] demonstrated that the strength of s-CC regarding cardiovascular risk prediction was not clearly superior to that of measured glomerular filtration rate with iothalamate.

On the other hand, it is possible that in the setting of myocardial injury, in which there is intense extracellular matrix remodeling, s-CC levels keep correlation with the extension of the injury and, consequently, with the future risk of cardiac events. It is why in this particular situation there is a great augmentation of elastolytic proteases, which can be counterbalanced for elevation of its main inhibitor, cystatin C. This could likely explain the predictive capacity of s-CC, regarding secondary cardiac events, independent of kidney function, as demonstrated by Koenig et al.[10] in patients with recent acute coronary syndrome, most with acute myocardial infarction, as well as its absence in the setting of chronic stable coronary heart disease as reported by the Heart and Soul Study[39]. The same explanation could be proposed for the association between s-CC and the risk of development of heart failure, a condition characterized also by intense extracellular matrix remodeling[40].

As said before, the role of s-CC as an independent marker of atherosclerosis seems to be not yet established, a point of view that has been endorsed by some of the main researchers in this area[1,39]. So we believe that renal impairment, even slight, is the true cardiovascular risk predictor, and s-CC, just a more sensitive marker of this impairment.

Finally, the present study was limited by its cross-sectional design. Furthermore, as our study evaluated a specific profile of patients, our results cannot automatically be extrapolated to the general population. On the other hand, our work presents some positive aspects that should be emphasized. To our knowledge, it is the first study to correlate s-CC with these two markers of subclinical atherosclerosis, at the same time, in individuals without manifested cardiovascular disease. Furthermore, we also correlated s-CC with inflammatory markers, 24-hour microalbuminuria and left ventricular mass index, beyond traditional risk factors, as well as we worked with measured rather than estimated creatinine clearance.

Conclusion

In middle-aged hypertensive outpatients, most with preserved kidney function, s-CC correlated with measured creatinine clearance, as expected, but no association was observed with two markers of atherosclerosis neither with established cardiovascular risk factors.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was partially funded by Fundação de Amparo a Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA).

Study Association

This article is part of the thesis of doctoral submitted by Francisco das Chagas Monteiro Junior , from Universidade Federal de São Paulo.

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