

## Original Articles

### **Serum cystatin C levels are associated with coronary artery calcification in women without chronic kidney disease**

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**Keywords:** cystatin C, coronary artery calcification, chronic kidney disease, sex

**Word count - excluding title page, abstract, figure legend, figures and tables:** 2709 words

## **Abstract**

**Background:** Chronic renal disease (CKD) is a determinant of coronary artery calcification (CAC), which is a predictor of cardiovascular events. However, in a population without CKD, the association between CAC and renal function is unclear. CAC is affected by sex. This study aimed to determine whether serum cystatin C, a sensitive marker of kidney function, or sex differences are associated with CAC in patients without CKD.

**Methods:** We evaluated 456 consecutive patients ( $61 \pm 13$  years, 42% women) without CKD and evidence of coronary artery disease. The CAC (Agatston) score was examined by multidetector computed tomography.

**Results:** When patients were categorized into three CAC groups based on the Agatston score, mild ( $<10$ ), moderate (11–399), and severe ( $\geq 400$ ) in each sex, serum cystatin C levels were gradually increased by severity of CAC in women, but not men. Receiver operating characteristic curve analysis showed that, in women, a cutoff value of 0.97 mg/l for cystatin C discriminated patients with severe CAC with a sensitivity of 71% and specificity of 77% (area under the curve, 0.74; 95% CI: 0.62–0.86;  $P < 0.01$ ). Multivariate logistic analysis showed that serum cystatin C was not associated with severe CAC in all patients and men, but this association was observed in women (OR: 7.80 for cystatin C  $\geq 0.97$  mg/l, 95% CI: 1.76–34.6,  $p < 0.01$ ).

**Conclusion:** Higher serum cystatin C levels are associated with greater CAC in women without CKD. Measurement of cystatin C may be useful for identifying women who are at high risk for cardiovascular disease.

## **Introduction**

Chronic kidney disease (CKD) is a worldwide health problem that carries a substantial risk for cardiovascular morbidity and death [1, 2]. Serum creatinine concentrations or creatinine-based estimating equations have served as the primary tool for evaluating kidney function in clinical practice [3]. However, the creatinine-based Modification of Diet and Renal Disease study formula for estimating glomerular filtration rate (GFR) still has substantial inaccuracy when applied to healthy persons and older individuals [4]. Recent studies have shown that cystatin C is a more reliable index of renal function [5]. Cystatin C is a small serine protease inhibitor that plays pleiotropic roles in human vascular pathophysiology [6]. Cystatin C is also a strong predictor of future coronary artery disease (CAD), ischemic stroke, and heart failure. [7-11]

Coronary artery calcification (CAC) is an established marker of subclinical atherosclerosis and an independent predictor of future coronary heart disease [12-14]. Previous studies have demonstrated a relation between CKD and greater CAC [15-19]. However, the effect of serum cystatin C levels on CAC in a population without CKD has not fully determined. Furthermore, several studies have shown that the severity of CAC is affected by sex [20-22].

Accordingly, this study aimed to evaluate the association between cystatin C levels and CAC in patients without evidence of CKD and CAD. We also aimed to evaluate the effect of sex on the association between cystatin C levels and CAC.

## **Material and Methods**

### ***Study population***

Patients without evidence of CKD and CAD who underwent multidetector row computed tomography (CT) for CAC and a blood test within 7 days were retrospectively included. First, patients who underwent multidetector row CT to examine CAC for suspected CAD at Okayama University Hospital between August 2011 and July 2013 were screened (n=1273). Patients were excluded if they had CKD defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m<sup>2</sup> (n=398) and a history of coronary artery disease or coronary stents (n=63), Patients with no cystatin C measurements within 7 days (n=342) and no evaluation of coronary ischemia (n=14) were also excluded. Finally, a total of 456 patients were included in the analysis.

This study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (Okayama, Japan). All of the patients provided written, informed consent. The study was conducted in accordance with the latest version of the Declaration of Helsinki.

### ***Laboratory tests***

Serum cystatin C levels were measured by an immunological turbid metric assay (Nescoat GC Cystatin C; Alfresa Pharma, Osaka, Japan) as previously described [11]. Other laboratory parameters were measured using standard laboratory techniques with an automatic analyzer. eGFR was calculated by the following equation:  $eGFR \text{ (ml/min/1.73 m}^2\text{)} = 194 \times Cr^{-1.094} \times \text{age}^{-0.287} \times (0.739 \text{ if female})$  [23] CKD was defined as eGFR <60 ml/min/1.73 m<sup>2</sup>.

### ***CT protocol***

For CAC imaging, a 64-slice non-enhanced CT scan was obtained with a diagnostic CT scanner (Somatom Definition Flash; Siemens Medical Solutions, Germany), as previously

described [24]. The parameters were as follows: detector collimation was  $64 \times 0.6$  mm equaling a slice acquisition of  $128 \times 0.6$  mm using the flying focal spot technique; table pitch was adapted to heart rate (0.17–0.38); rotation time was 275 ms; the tube current–time product was 360 mA; and tube voltage was 120 kVp. CAC was quantified using the standard Agatston calcium scoring algorithm [25]. The cohort was stratified into four groups according to the CAC score: CAC score of 0–9 (n=204), CAC score of 10–99 (n=111), CAC score of 100–399 (n=125), and CAC score  $\geq 400$  (n=158). For coronary CT angiography, the initial bolus of contrast agent (Omnipaque 350; Daiichi Sankyo, Tokyo, Japan) was calculated as body weight  $\times 0.07$  ml and was injected over 10 s. This was followed by a second bolus consisting of 80% of the initial volume of contrast medium diluted to 50% with normal saline and then a compensatory 20% bolus of normal saline. The patients arrived at the hospital 60 min before the CT scan and received oral metoprolol and/or intravenous landiolol hydrochloride (0.125 mg/kg) until the heart rate was less than 60 beats/min [26]. Coronary artery stenosis was evaluated on axial and curved multiplanar reformatted images using commercially available cardiac reconstruction software (Virtual Place, Raijin; AZE Inc., Tokyo, Japan). Evaluations were performed on a per-segment basis, using 16 segments as established in the American Heart Association segment model [27]. One experienced and trained senior cardiologist and two senior CT technologists performed the analyses.

### ***Statistical analysis***

Continuous variables with normal distribution are shown as mean  $\pm$  SD and continuous variables with skewed distribution are presented as median (interquartile range). ANOVA or the Kruskal–Wallis test was applied to compare continuous variables among study groups. The chi-square test was applied to compare categorical variables among the groups. The optimal value of

cystatin C that best separated the patients with severe CAC  $\geq 400$  was determined by constructing a receiver operating characteristic curve. Multivariate logistic regression analysis included all variables used in the univariate analysis. A p value  $< 0.05$  was considered statistically significant. Statistical analysis was performed using SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

## Results

Patients' characteristics according to sex are shown in Table 1. A total of 456 consecutive participants (61±13 years, 58% men) were included. Men had a higher proportion of current smokers. With regard to biochemical parameters, women had higher levels of total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. Serum levels of creatinine and cystatin C in men were significantly higher than those in women. Table 2 shows the patients' characteristics according to CAC scores of mild (0–9), moderate (10–399), and severe ( $\geq 400$ ). Age, the proportion of men, the prevalence of diabetes mellitus, and serum cystatin C levels were significantly higher in the highest CAC group. Furthermore, the association between CAC and serum cystatin C levels was analyzed according to sex. In women, serum cystatin C levels in the mild, moderate, and severe CAC groups gradually increased as the severity of CAC increased (0.87 [0.16], 0.89 [0.21], and 1.00 [0.15] mg/l, expressed as median [interquartile range], respectively,  $p < 0.01$ ), but they did not increase in men (0.95 [0.28], 0.96 [0.22], and 0.97 [0.17] mg/l, respectively,  $p = \text{N.S.}$ )

Patients were then classified into tertiles (T1–T3) based on cystatin C levels. In all patients, T1 was 0.56–0.85 mg/l (n=150), T2 was 0.86–0.99 mg/l (n=158), and T3 was 1.00–1.70 mg/l (n=148). In men, T1 was 0.58–0.89 mg/l (n=97), T2 was 0.90–1.03 mg/l (n=83), and T3 was 1.04–1.70 mg/l (n=85). In women, T1 was 0.56–0.82 mg/l (n=61), T2 was 0.83–0.93 mg/l (n=67), and T3 was 0.94–1.30 mg/l (n=63). A gradual increase in the Agatston score was observed in women, but not in all patients and men (Figure 1).

Receiver operating characteristic curve analyses were performed. In all patients, a cutoff value of 0.88 mg/l for cystatin C could discriminate patients with severe CAC with a sensitivity of 76% and specificity of 44% (area under the curve, 0.60; 95% CI: 0.53–0.66,  $P = 0.02$ ). In men,

a cutoff value of 0.78 mg/l for cystatin C had a sensitivity of 96% and specificity of 17% (area under the curve, 0.51; 95% CI: 0.43–0.59, P=0.58). In women, a cutoff value of 0.97 mg/l for cystatin C showed a sensitivity of 71% and specificity of 77% (area under the curve, 0.74; 95% CI: 0.62–0.86, P<0.01).

To confirm the association between cystatin C levels and CAC, logistic analyses were performed (Table 3). In all patients and men, cystatin C was not a factor involved in severe CAC. In women, cystatin C > 0.97mg/l was an independent factor associated with severe CAC, and this association remain significant after adjusting for confounding factors (odds ratio: 7.80, 95% confidence interval [CI]: 1.76–34.6, p<0.01).

Furthermore, to eliminate the effect of diabetes mellitus on the association between cystatin C and CAC, receiver operating characteristic curve analysis and logistic analyses in patients without diabetes mellitus were performed. In all patients, a cutoff value of 0.95 mg/l for cystatin C discriminated patients with severe CAC with a sensitivity of 68% and specificity of 42% (area under the curve, 0.59; 95% CI: 0.50–0.69, P=0.09). In men, a cutoff value of 0.95 mg/l for cystatin C had a sensitivity of 67% and specificity of 54% (area under the curve, 0.50; 95% CI: 0.38–0.63, P=0.94). In women, a cutoff value of 0.98 mg/l for cystatin C showed a sensitivity of 71% and specificity of 22% (area under the curve, 0.74; 95% CI: 0.59–0.89, P=0.03).

Multivariate logistic analyses showed that, in all patients, age >65 years (odds ratio: 65.1, 95% CI: 3.55–77.1, p<0.01) and male sex (odds ratio: 3.18, 95% CI: 1.08–9.37, p=0.03), but not cystatin C levels >0.95 mg/l (odds ratio: 1.49, 95% CI: 0.55–3.98, p=0.43), were factors involved in severe CAC. In men, age (odds ratio: 15.77, 95% CI: 3.12–79.66, p<0.01), but not cystatin C levels >0.95 mg/l (odds ratio: 0.79, 95% CI: 0.25–2.61, p=0.71), was involved in severe CAC. In



women, only cystatin C levels  $>0.97$  mg/l were an independent factor associated with severe CAC (odds ratio: 12.31, 95% CI: 1.12–134.50,  $p=0.04$ ).

## **Discussion**

This study demonstrated that higher serum cystatin C levels were independently associated with greater CAC in women, but not in men, without CKD. Measurement of serum cystatin C levels could be useful for identifying individuals at risk of cardiovascular disease in women without CKD.

This study clearly showed an effect of sex on the association between serum cystatin C levels and CAC. One potential explanation for this finding is that traditional risk factors in men affect CAC more than those in women. In this study population, diabetes mellitus was an independent factor associated with CAC  $>400$  in men, but not in women. Therefore, the effect of diabetes mellitus on CAC in men may be stronger than that in women, which leads to a differential effect of cystatin C on CAC. Another explanation is that cystatin C may be involved in development of atherosclerosis in the early stage. An increase in CAC in women by the female hormone estrogen is slower than that in men, indicating that cystatin C is directly involved in development of atherosclerosis. Imai et al. reported that higher serum cystatin C concentrations were correlated with early-stage coronary atherosclerotic plaques among patients determined by CT coronary angiography without established chronic kidney dysfunction [28]. In fact, the Agatston score in men was much greater than that in women in study. Furthermore, our results of subanalysis without diabetes mellitus showed that age had a significant effect on severe CAC in men, but not in women, in subjects without CKD.

Smoking is a risk factor of progression of CAC [29]. In our study, the prevalence of being a

current smoker in men was greater than that in women. However, our findings showed that smoking was not a significant factor associated with CAC in men and women. A previous study reported that CAC in former smokers was more severe than that in current smokers [30]. In our study, data regarding current or former smokers were not available. Furthermore, cystatin C levels were not different between non-smoking men (n=153) and smoking men (n=112) (0.96 [0.24] mg/l and 0.96 [0.22] mg/l, p=0.93). This finding suggested that smoking did not affect cystatin C levels. Further analysis including a parameter reflecting burden of smoking exposure, such as pack-years of smoking, is needed to clarify the effect of smoking on CAC in subjects without CKD.

Previous studies have demonstrated that serum cystatin C levels are a useful marker for predicting worsening or new onset of cardiovascular disease in a variety of populations [7-10]. There is some biological plausibility for the role of cystatin C in the pathogenesis of vascular disease [6]. However, whether cystatin C plays a causal role in the etiology of cardiovascular disease in the clinical setting remains unclear. Recently, van der Laan et al. [31] reported that Mendelian randomization analyses did not support a causal role of cystatin C in the etiology of cardiovascular disease in Caucasians. This finding suggests that cystatin C is a marker, but not a target, for intervention. However, predicting patients at high risk of events is critically important for clinical practice. Cystatin C remains important for risk assessment.

There are several limitations in this study. First, serum cystatin C levels appear to be elevated in patients with hypothyroidism and depressed in those with hyperthyroidism[32]. However, this study did not examine thyroid hormones in all patients. Therefore, an effect of thyroid function on cystatin C levels cannot be denied in this study. Second, this study included patients with suspected CAD. Therefore, our results cannot be applied to the general population. Third, CKD was only

defined by eGFR in this study because data on proteinuria or microalbuminuria were not available. Albuminuria is a marker of kidney damage. Therefore, our results need to be interpreted with caution in patients with an eGFR  $\geq 60$  ml/min/1.73 m<sup>2</sup> and albuminuria.

In conclusion, serum cystatin C is an independent factor associated with CAC as a marker of subclinical atherosclerosis in women, but not in men, without CKD and CAD. Serum cystatin C could be useful for identifying female patients at high risk of cardiovascular disease without CKD.

**Funding:** This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

**Disclosures:** All authors declare that they have no competing financial interests.

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## Figure legend

### Figure 1. Box plot showing Agatston scores according to tertiles of cystatin C.

All patients: T1, 0.56–0.85 mg/l (n=150); T2, 0.86–0.99 mg/l (n=158); and T3, 1.00–1.70 mg/l (n=148). Men: T1, 0.58–0.89 mg/l (n=97); T2, 0.90–1.03 mg/l (n=83); and T3, 1.04–1.70 mg/l (n=85). Women: T1, 0.56–0.82 mg/l (n=61); T2, 0.83–0.93 mg/l (n=67); and T3, 0.94–1.30 mg/l (n=63).

\* $p < 0.05$  vs. T1 group; † $p < 0.05$  vs. T2 group.