Observational Study

Medicine®

OPEN

Association of cystatin C with coronary artery calcification in patients undergoing multidetector computed tomography

Hui Xiong, MD[a,b], Li Wang, MD[a,b], Fulu Jin, MD[a], Bo Zhang, MD[c], Xiaozhong Wang, MD[a], Xiansong Chang, MD[a], Liang-Ping Zhao, MD[a,*]

Abstract

Cystatin C is associated with atherosclerosis, but the relationship between cystatin C and coronary artery calcification (CAC) is uncertain. The purpose of this study was to evaluate the predictive value of cystatin C on the occurrence and severity of CAC.

A total of 1447 hospitalized patients with coronary computed tomography angiography were selected in this study. According to the CAC score (CACS), patients were divided into calcification group (with CAC, n = 749) and control group (without CAC, n = 698). The calcification group was further divided into low calcification group (CACS < 100, n = 407), medium calcification group (CACS 100–400, n = 203), and high calcification group (CACS ≥ 400, n = 139).

Patients with CAC had higher cystatin C level than those in control group ($P < .05$). With the increase of calcification score, the cystatin C level showed an upward trend. The cystatin C level in the high calcification group was significantly higher than those in the low and medium calcification group ($P < .05$). ROC curve analysis showed that cystatin C had a high predictive value for the occurrence of CAC [area under the curve 0.640, 95% confidence interval (95% CI) 0.591–0.690, cut-off value 0.945 mg/L, sensitivity 0.683, specificity 0.558, $P < .05$] and severe CAC (area under the curve 0.638, 95% CI 0.550–0.762, cut-off value 0.965 mg/L, sensitivity 0.865, specificity 0.398, $P < .05$). Multivariate logistic regression analysis showed that cystatin C was an independent predictor of severe CAC (AOR 3.748, 95% CI 1.138–10.044, $P < .05$).

Cystatin C was significantly associated with the occurrence and severity of CAC, suggesting that cystatin C had the potential as a predictor of CAC.

Abbreviations: AOR = adjusted odd ratio, CAC = coronary artery calcification, CACS = coronary artery calcification score, CI = confidence interval, CysC = Cystatin C, eGFR = estimated glomerular filtration rate, IL-6 = interleukin-6, LAD = left anterior descending, LCX = circumflex, LM = left main, MDRD = modification of diet in renal disease trial, OPG = osteoprotegerin, p38MAPK = p38 mitogen-activated protein kinase, RANK = receptor activator of nuclear factor-κB, RANKL = RANK ligand, RCA = right coronary artery, ROC = receiver operating characteristic, TNF-α = tumor necrosis factor-α.

Keywords: coronary artery calcification, cystatin C, prediction

1. Introduction

Coronary artery disease is a common cause of morbidity and mortality worldwide. It is mainly caused by coronary atherosclerosis, which causes blockage or stenosis of blood vessels, ischemia and hypoxia of myocardium, and even myocardial necrosis.[1,2] At present, it is generally believed that coronary artery calcification (CAC) is a predetermined marker of subclinical atherosclerosis and an independent predictor of coronary artery

Editor: Antonino Tuttolomondo.

HX, LW, and FJ contributed equally to this work.

This study was supported by the Suzhou Science and Technology Development Program Guidance Project Fund (grant number SYSD2013093), Youth Natural Science Fund of Soochow University (grant number SDY2013A32), Research Fund of the Second Affiliated Hospital of Soochow University (grant number SDFEYGJ1405), and Xinxin Heart (SIP) Foundation (2019-CCA-ACCESS-058).

All authors declared no conflicts of interest.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Department of Cardiology, [b] Emergency Department of Xuguan District, [c] Radiology Department, The Second Affiliated Hospital of Soochow University, Suzhou City, China.

[*] Correspondence: Liang-Ping Zhao, Department of Cardiology, The Second Affiliated Hospital of Soochow University, No 1055, Sanxiang Road, Suzhou City 215004, China (e-mail: zhaoliangping1234@aliyun.com).

How to cite this article: Xiong H, Wang L, Jin F, Zhang B, Wang X, Chang X, Zhao LP. Association of cystatin C with coronary artery calcification in patients undergoing multidetector computed tomography. Medicine 2021;100:30(e26761).

Received: 20 March 2021 / Received in final form: 6 June 2021 / Accepted: 8 July 2021

http://dx.doi.org/10.1097/MD.0000000000026761

disease.[3,4] CAC is closely related to atherosclerotic plaque load and cardiovascular event incidence. Calcification usually occurs in the early stages of atherosclerosis, but the initial symptoms of calcification are not obvious. When symptoms occur, it may have progressed to atherosclerosis or even other cardiovascular events. Therefore, the early discovery of CAC has clinical value in preventing coronary artery disease and delaying its progression.

CAC is characterized by calcium deposition in the coronary artery wall, resulting in decreased elasticity of the vascular wall and abnormal vasoconstriction, which is a pathophysiological phenomenon related to atherosclerosis.[5] Some studies have shown that the pathogenesis of CAC mainly involves inflammatory response, genetic factors, and metabolic disorders, and is believed to be related to ectopic bone production.[6–8] Traditional risk factors for CAC include increased age, male, poor lifestyle, diabetes, hypertension, etc.[9] The common detection methods of CAC include X-ray, coronary angiography, and multidetector computed tomography.[10] X-ray and coronary angiography are less sensitive for the CAC evaluation. Computed tomography can quantify calcification, which is the main method for CAC detection. It also has the characteristics of high radiation and insufficient sensitivity to the detection of micro-calcification.[11]

Cystatin C (CysC), a lysosomal cysteine protease inhibitor, is widely expressed in all nucleated cells and most organs, with a constant production rate and distributed in all human body fluids. CysC is located on chromosome 20 and consists of about 120 amino acid residues with a molecular weight of 13.3 KD. It is a low molecular weight, basic non-glycosylated protein.[12–14] CysC is an ideal homologous marker to reflect changes in glomerular filtration rate, which was initially used to evaluate renal function. Recent studies have shown that CysC not only played a role of intracellular and extracellular activity regulator of lysosomal cysteine protease, but also was associated with other biological function disorders, such as cancer occurrence, bone remodeling, and cardiovascular disease risk stratification and prognosis assessment.[15,16] The increased CysC level in patients with coronary artery disease inhibited the secretion of cysteine protease and the degradation of extracellular matrix, leading to the occurrence of vascular wall remodeling.[17] There are few studies on the relationship between CysC and CAC. In this study, we selected patients who underwent coronary computed tomography angiography to assess CAC, and explored the predictive value of CysC for CAC.

2. Methods

2.1. Study subjects

This was a retrospective study conducted in an Asian country. We selected consecutive patients who were admitted to the Second Affiliated Hospital of Soochow University and examined coronary computed tomography angiography from January 2010 to September 2019. Patients with severe hepatic and renal insufficiency, infectious diseases and conditions, hemorrhagic disease, malignant tumors, not suitable for the use of iodine contrast agent, and unsuccessful coronary computed tomography angiography examination were excluded. This study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Soochow University. Clinical investigation was conducted according to the principle of the Declaration of Helsinki.

2.2. Coronary computed tomography angiography examination and study group

Coronary computed tomography angiography examination was performed using 64-slice spiral computed tomography. Coronary artery calcification score (CACS), including left main (LM), left anterior descending (LAD), circumflex (LCX), right coronary artery (RCA), was evaluated by smart Score 3.5 software. The number of diseased coronary arteries (luminal diameter narrowing ≥50%) was recorded, and patients with stenosis of the left main ≥50% were considered to have 2-vessel disease.

A total of 1447 eligible patients were selected in this study. According to the CACS, patients were divided into calcification group (with CAC, n = 749) and control group (without CAC, n = 698). The calcification group was further divided into low calcification group (CACS < 100, n = 407), medium calcification group (CACS 100–400, n = 203), and high calcification group (CACS≥400, n = 139).

2.3. Laboratory investigations and clinical data collection

Serum CysC was measured by immune turbidimetry (Shanghai Jingyuan Medical Instrument Co., LTD). White blood cell, red blood cell, platelets, fasting plasma glucose, blood urea nitrogen, creatinine, blood uric acid, blood calcium, blood phosphorus, C-reactive protein, and serum lipid profiles including triglyceride, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol were assessed using standard methods. Estimated glomerular filtration rate (eGFR) was calculated by modification of diet in renal disease trial (MDRD) formula. The demographic and clinical characteristics of the selected patients were collected from hospital case records. These included gender, age, smoking, drinking, hypertension, diabetes mellitus, stroke, systolic blood pressure, diastolic blood pressure, height, weight. The body mass index (kg/m^2) was calculated.

2.4. Statistical analyses

The continuous variables between the 2 groups were compared by independent-sample *t* test if the data were normally distributed, and Mann–Whitney *U* test if skewed. Analysis of variance snk test was used for comparison among three groups. Categorical variables were expressed as frequency (percentage), and compared by the Chi-square test. Logistic backward conditional regression analysis was applied to identify related factors of CAC occurrence and severe CAC, and adjusted odd ratio (AOR) and 95% confidence interval (95% CI) were presented. Receiver operating characteristic (ROC) curve was used to evaluate the predictive value of CysC in the occurrence and severity of CAC. SPSS 17.0 for Windows (SPSS Inc., IL) was used for all statistical analyses, and 2-sided $P < .05$ was considered statistically significant.

3. Results

3.1. Calcification score and distribution

Among the 749 calcification group patients, the calcification score ranged from 1 to 4055, with an average of 271.9 ± 500.2. They were distributed in the LM at 65.6 ± 104.0, the LAD at 151.0 ± 258.5, the LCX at 80.2 ± 136.5, and the RCA at 160.6 ± 293.4. There were 146 (19.5%) patients with LM calcification,

Table 1

Distribution of calcification score in patients with coronary artery calcification.

Characteristics	Low calcification (n = 407)	Medium calcification (n = 203)	High calcification (n = 139)	P
Total calcification score	32.3 ± 28.4	215.5 ± 81.8	1055.5 ± 742.9	<.001
LM score	15.0 ± 17.5	59.8 ± 52.2	135.4 ± 155.8	<.001
LAD score	25.7 ± 24.7	126.1 ± 79.5	483.1 ± 401.1	<.001
LCA score	14.2 ± 17.4	55.0 ± 57.3	175.4 ± 198.0	<.001
RCA score	16.0 ± 18.8	80.8 ± 69.4	429.3 ± 421.8	<.001
LM calcification (%)	57 (14.0)	44 (21.7)	45 (32.4)	<.001
LAD calcification (%)	313 (76.9)	185 (91.1)	132 (95.0)	<.001
LCX calcification (%)	123 (30.2)	113 (55.7)	115 (82.7)	<.001
RCA calcification (%)	158 (38.8)	138 (68.0)	126 (90.6)	<.001

LAD = left anterior descending, LCX = circumflex, LM = left main, RCA = right coronary artery.

630 (84.1%) patients with LAD calcification, 351 (46.9%) patients with LCX calcification, and 422 (56.3%) patients with RCA calcification.

In the calcification subgroup (low, medium, high calcification group), with the increase of calcification score, the incidence of calcification in LM, LAD, LCX, RCA, showed an upward trend. There were significant differences among the three groups about the incidence of the LM calcification (14.0% vs 21.7% vs 32.4%, $P < .001$), the LAD calcification (76.9% vs 91.1% vs 95.0%, $P < .001$), the LCX calcification (30.2% vs 55.7% vs 82.7%, $P < .001$), and the RCA calcification (38.8% vs 68.0% vs 90.6%, $P < .001$) (Table 1).

3.2. Baseline characteristics

The baseline demographic and clinical characteristics of patients are summarized in Tables 2 and 3. Patients with CAC had higher age and systolic blood pressure, and lower diastolic blood pressure than those without CAC. Male, hypertension, diabetes, and stroke were more common in the calcification group compared with the control group (Table 2).

There were significant differences in the age, systolic blood pressure, and prevalence of hypertension among the low, medium, high calcification groups. With the increase of calcification score, the age, systolic blood pressure, and prevalence of hypertension showed an upward trend (Table 3).

Table 2

Comparison of basic data between calcification group and control group.

Characteristics	Control group (n = 698)	Calcification group (n = 749)	P
Male (n, %)	350 (50.1)	452 (60.3)	<.001
Age, yr	59.2 ± 11.7	67.0 ± 9.8	<.001
Smoking (n, %)	148 (21.2)	169 (22.6)	.567
Drinking (n, %)	82 (11.7)	94 (12.6)	.288
Hypertension (n, %)	356 (51.0)	537 (71.7)	<.001
Diabetes mellitus (n, %)	78 (11.2)	176 (23.5)	<.001
Stroke (n, %)	34 (4.9)	65 (8.7)	.005
Systolic blood pressure, mm Hg	136.0 ± 19.5	140.1 ± 19.9	<.001
Diastolic blood pressure, mm Hg	79.8 ± 12.3	78.0 ± 11.7	.004
Height, m	1.64 ± 0.09	1.63 ± 0.08	.101
Weight, kg	66.7 ± 11.5	66.5 ± 11.3	.725
BMI, kg/m^2	24.6 ± 3.3	24.8 ± 3.4	.372

BMI = body mass index.

3.3. Blood results

The CysC level in the calcification group was significantly higher than that in the control group (1.05 ± 0.21 vs 0.94 ± 0.20 mg/L, $P < .001$). Patients with CAC had higher fasting blood glucose, blood urea nitrogen, creatinine, uric acid, and C-reactive protein levels, and lower red blood cell, platelet, and eGFR than those without CAC (Table 4).

The CysC levels among the low, medium, high calcification groups were 1.02 ± 0.19, 1.03 ± 0.20, 1.13 ± 0.25 mg/L, respectively. There were significant differences in the CysC, red blood cell, blood urea nitrogen, creatinine, eGFR, and C-reactive protein levels among the low, medium, high calcification groups. With the increase of calcification score, the CysC, blood urea nitrogen, creatinine, and C-reactive protein showed an upward trend, and red blood cell and eGFR showed a downward trend (Table 5).

3.4. CAC and diseased vessel number

Compared with the control group, 1-vessel disease (27.8% vs 10.3%), 2-vessel disease (16.4% vs 1.4%), and 3-vessel disease (13.5% vs 0.1%) were more common, and 0-vessel disease (42.3% vs 88.1%) was less common in the calcification group.

There were significant differences in the diseased vessel number among the low, medium, high calcification groups, including 0-vessel disease (55.5% vs 36.5% vs 12.2%), 1-vessel disease (28.3% vs 27.6% vs 26.6%), 2-vessel disease (11.5% vs 17.7% vs 28.8%), and 3-vessel disease (4.7% vs 18.2% vs 32.4%). High calcification group was more likely to have 2-vessel and 3-vessel disease.

3.5. Diseased vessel number and CysC level

The CysC levels in 0-vessel, 1-vessel, 2-vessel, 3-vessel disease were 0.96 ± 0.19, 1.02 ± 0.22, 1.06 ± 0.20, and 1.13 ± 0.26 mg/L, respectively, with significant difference ($P < .001$). With the increase of the diseased vessel number, the CysC level showed an upward trend.

3.6. ROC curve analysis results

ROC curve analysis showed that CysC had a high predictive value for the occurrence of CAC [area under the curve 0.640, 95% confidence interval (95% CI) 0.591–0.690, cut-off value 0.945 mg/L, sensitivity 0.683, specificity 0.558, $P = .001$] and severe CAC (area under the curve 0.638, 95% CI 0.550–0.762,

Table 3

Comparison of basic data among groups with different severity of calcification.

Characteristics	Low calcification (n = 407)	Medium calcification (n = 203)	High calcification (n = 139)	P
Male (n, %)	234 (57.5)	122 (60.1)	96 (69.1)	.055
Age, yr	65.3 ± 9.5	68.3 ± 9.8	70.7 ± 9.4	<.001
Smoking (n, %)	92 (22.6)	40 (19.7)	37 (26.6)	.323
Drinking (n, %)	50 (12.3)	20 (9.9)	24 (17.3)	.123
Hypertension (n, %)	276 (67.8)	149 (73.4)	112 (80.6)	.013
Diabetes mellitus (n, %)	89 (21.9)	50 (24.6)	37 (26.6)	.472
Stroke (n, %)	32 (7.9)	14 (6.9)	19 (13.7)	.063
Systolic blood pressure, mm Hg	138.5 ± 18.9	139.6 ± 20.1	145.5 ± 21.9	.002
Diastolic blood pressure, mm Hg	78.2 ± 11.6	78.4 ± 12.1	76.9 ± 11.3	.497
Height, m	1.63 ± 0.09	1.64 ± 0.09	1.63 ± 0.08	.923
Weight, kg	66.8 ± 11.4	66.5 ± 11.2	65.9 ± 11.0	.755
BMI, kg/m^2	24.9 ± 3.5	24.8 ± 3.4	24.6 ± 3.3	.671

BMI = body mass index.

cut-off value 0.965 mg/L, sensitivity 0.865, specificity 0.398, $P = .007$).

3.7. Multivariate logistic regression analysis results

The multivariate logistic regression analysis revealed that male (AOR = 1.878, 95% CI = 1.270–2.777, $P = .002$), age (AOR = 1.065, 95% CI = 1.020–1.112, $P = .005$), hypertension (AOR = 1.911, 95% CI = 1.280–2.851, $P = .002$), diabetes (AOR = 1.844, 95% CI = 1.344–2.529, $P = .001$), red blood cell (AOR = 0.691, 95% CI = 0.484–0.988, $P = .042$), and creatinine (AOR = 1.018, 95% CI = 1.003–1.031, $P = .016$) remained independent predictors of the occurrence of CAC (Table 6).

In the calcification subgroup, the multivariate logistic regression analysis revealed that male (AOR = 1.583, 95%CI = 1.022–2.451, $P = .040$), age (AOR = 1.051, 95%CI = 1.028–1.076, $P = .001$), hypertension (AOR = 1.826, 95%CI = 1.116–2.989, $P = .017$), CysC (AOR = 3.748, 95%CI = 1.138–10.044, $P = .034$), red blood cell (AOR = 0.560, 95%CI = 0.386–0.814, $P = .004$), and C-reactive protein (AOR = 1.017, 95%CI = 1.001–1.032, $P = .036$) were independent predictors of the severe CAC (CACS≥400) (Table 7).

4. Discussion

This study investigated the related factors of CAC. The results showed that patients with CAC had higher CysC level than those without CAC. With the increase of calcification score, the CysC showed an upward trend, and was revealed to be an independent risk factor of severe CAC, suggesting that CysC presented to be a potential predictor of the occurrence and severity of CAC.

As a member of the cysteine protease inhibitor family, CysC is considered to be the most important cysteine protease inhibitor. CysC is a non-glycosylated protein with a relatively low molecular weight of about 13.3 KD ubiquitous in body fluids and tissue cells. Its production is stable, independent of age, gender, and bilirubin. The main metabolic pathway of CysC is the kidney. It can be freely filtered by the glomeruli without tubular secretion and completely reabsorbed and catabolized in

Table 4

Comparison of blood indexes between calcification group and control group.

Characteristics	Control group (n = 698)	Calcification group (n = 749)	P
Cystatin C, mg/L	0.94 ± 0.20	1.05 ± 0.21	<.001
White blood cells, $\times 10^9$/L	6.39 ± 2.58	6.50 ± 2.24	.375
Red blood cells, $\times 10^{12}$/L	4.51 ± 0.50	4.43 ± 0.55	.006
Platelet, $\times 10^9$/L	205.9 ± 81.2	196.1 ± 64.3	.011
Fasting blood glucose, mmol/L	5.49 ± 1.44	5.78 ± 1.80	.001
Total cholesterol, mmol/L	4.71 ± 0.98	4.64 ± 1.09	.216
Triglycerides, mmol/L	1.68 ± 1.25	1.64 ± 1.37	.554
LDL-C, mmol/L	2.83 ± 0.84	2.78 ± 0.92	.315
HDL-C, mmol/L	1.18 ± 0.31	1.17 ± 0.38	.766
Urea nitrogen, mmol/L	5.06 ± 1.30	5.54 ± 1.82	<.001
Creatinine, μmol/L	65.7 ± 14.5	71.6 ± 22.4	<.001
Blood uric acid, μmol/L	327.6 ± 87.7	344.7 ± 99.6	.001
eGFR, mL/min/1.73 m^2	97.0 ± 18.1	89.1 ± 20.4	<.001
Blood calcium, mmol/L	2.26 ± 0.14	2.26 ± 0.13	.721
Blood phosphorus, mmol/L	1.16 ± 0.21	1.16 ± 0.19	.819
C-reactive protein, mg/L	6.60 ± 6.28	8.26 ± 13.27	.004

eGFR = estimated glomerular filtration rate, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

Table 5

Comparison of blood indexes among groups with different severity of calcification.

Characteristics	Low calcification (n = 407)	Medium calcification (n = 203)	High calcification (n = 139)	P
Cystatin C, mg/L	1.02 ± 0.19	1.03 ± 0.20	1.13 ± 0.25	.015
White blood cells, $\times 10^9$/L	6.39 ± 1.91	6.68 ± 2.91	6.61 ± 2.03	.260
Red blood cells, $\times 10^{12}$/L	4.51 ± 0.52	4.38 ± 0.56	4.30 ± 0.60	<.001
Platelet, $\times 10^9$/L	196.3 ± 56.4	196.8 ± 77.0	195.1 ± 66.2	.971
Fasting blood glucose, mmol/L	5.74 ± 1.74	5.97 ± 2.06	5.63 ± 1.57	.206
Total cholesterol, mmol/L	4.72 ± 1.02	4.57 ± 1.17	4.47 ± 1.16	.080
Triglycerides, mmol/L	1.75 ± 1.54	1.54 ± 1.17	1.41 ± 1.02	.055
LDL-C, mmol/L	2.84 ± 0.88	2.70 ± 0.94	2.68 ± 1.00	.155
HDL-C, mmol/L	1.15 ± 0.40	1.20 ± 0.36	1.20 ± 0.37	.317
Urea nitrogen, mmol/L	5.38 ± 1.51	5.53 ± 1.81	6.02 ± 2.49	.002
Creatinine, μmol/L	69.6 ± 18.0	70.7 ± 18.9	79.0 ± 34.2	<.001
Blood uric acid, μmol/L	343.1 ± 93.9	336.0 ± 101.1	361.8 ± 111.7	.056
eGFR, mL/min/1.73 m^2	91.2 ± 19.6	89.6 ± 21.4	82.4 ± 19.6	<.001
Blood calcium, mmol/L	2.26 ± 0.12	2.27 ± 0.12	2.25 ± 0.15	.546
Blood phosphorus, mmol/L	1.14 ± 0.19	1.16 ± 0.20	1.19 ± 0.19	.052
C-reactive protein, mg/L	7.26 ± 9.21	8.14 ± 12.38	11.30 ± 21.51	.011

eGFR = estimated glomerular filtration rate, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

Table 6

Multivariate logistic regression analysis results of coronary artery calcification occurrence.

Variables	Adjusted odds ratio (AOR)	95% confidence interval (95% CI)	*P*
Male	1.878	1.270–2.777	.002
Age	1.065	1.020–1.112	.005
Hypertension	1.911	1.280–2.851	.002
Diabetes	1.844	1.344–2.529	.001
Red blood cell	0.691	0.484–0.988	.042
Creatinine	1.018	1.003–1.031	.016

the proximal tubule, which means it reflects changes in glomerular filtration rate more accurately than creatinine. In addition, it is confined to the extracellular space and distributed throughout the body. Therefore, CysC is considered as a sensitive indicator for the assessment of renal insufficiency.[18–21]

Recent studies have shown that CysC is associated with atherosclerotic diseases.[22,23] The study by Maahs et al[22] showed that the increase of serum CysC level was correlated with the occurrence and development of subclinical atherosclerosis. Wang et al[23] reported that increased serum CysC level was associated with increased risk and poor prognosis in patients with coronary artery disease, and the higher serum CysC level was, the more severe coronary artery stenosis and the more diseased vessels. However, some studies have yielded different results, including that there was no significant difference in serum CysC level between patients with coronary artery disease and those without coronary artery disease,[24] and CysC level in patients with severe coronary artery disease was lower than that in patients with nonsevere coronary artery disease.[25]

Thus, the relationship between CysC and coronary artery disease is still controversial, but it is generally believed that the CysC level was positively associated with coronary artery disease. As was shown in this study, with the increase of the diseased vessel number, the CysC level showed an upward trend, and the CysC level in 3-vessel disease was significantly higher than that in the other 2 groups. Arterial calcification and atherosclerosis have the same influencing factors, including age, gender, lipids, hypertension, diabetes, chronic kidney disease, smoking, sleep status, and mood.[26,27] CAC is a marker of atherosclerosis, which is the result of the progression of coronary atherosclerosis to a certain stage. As a related factor of atherosclerosis, CysC may also be associated with CAC.

The mechanism of CAC is not completely clear at present. Vascular smooth muscle cells, inflammatory cytokines, and OPG/RANK-RANKL (osteoprotegerin/receptor activator of nuclear factor-κB/RANK ligand) signaling systems are involved in the formation of calcification.[28–32] Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are secreted by inflammatory cells, which can induce vascular smooth muscle cells to transform into osteogenic phenotype, and then promote calcification.[30] TNF-α promotes its DNA to combine with osteoblast differentiation related regulatory factors such as osteoblast specific factor-2 and cAMP-response element binding protein through cAMP signaling pathway, which induces osteoblast differentiation and regulates calcification.[31] After calcification, calcification crystals interact with macrophages to promote macrophages to secrete inflammatory factors, further promote calcification and form a vicious circle.[32] Parathyroid hormone, as an important regulatory protein in the body's calcium balance, can block vascular smooth muscle cells calcification by inhibiting alkaline phosphatase activity and inhibit the role of vascular calcification.[33]

Table 7

Multivariate logistic regression analysis results of severe coronary artery calcification.

Variables	Adjusted odds ratio (AOR)	95% confidence interval (95% CI)	*P*
Male	1.583	1.022–2.451	.040
Age	1.051	1.028–1.076	.001
Hypertension	1.826	1.116–2.989	.017
Cystatin C	3.748	1.138–10.044	.034
Red blood cell	0.560	0.386–0.814	.004
C-reactive protein	1.017	1.001–1.032	.036

Several studies had tried to find the relationship between CysC and CAC. Sugiyama et al[34] recruited 456 subjects without renal disease and manifestation of coronary artery disease to complete spiral computed tomography examination, and the results showed that serum CysC level in females was positively correlated with CAC severity, while that in males was not. Ruiz-salas et al[35] recruited 104 stable chest pain patients without kidney disease, and got that cystatin C was significantly correlated with CAC severity and the presence of coronary artery disease. Our study with larger sample showed that increased CysC level was associated with the occurrence and severity of CAC, and presented to be a potential predictor of CAC.

The mechanism of cystatin C in CAC is still unclear. CysC has a certain effect on the migration and phagocytosis of neutrophils and monocytes, and promotes the secretion of inflammatory cytokines such as IL-6 and TNF-α, and then induces vascular smooth muscle cells to transform into osteogenic phenotype.[30] As a cysteine protease inhibitor, the increased CysC could reduce the degradation of extracellular matrix in vascular wall, and then lead to vascular wall remodeling including calcification.[36] CysC is one of the ideal endogenous markers reflecting glomerular filtration rate. The CysC level in patients with chronic kidney disease is increased, which is involved in the disorder of calcium and phosphorus metabolism, leading to the occurrence of CAC.

The association of C-reactive protein with CAC has been reported in several studies.[37–39] As an inflammatory marker, C-reactive protein promotes the transformation of vascular smooth muscle cells into osteoblasts, which may be one of the intrinsic mechanisms of calcification formation. Henze et al[40] treated human aortic smooth muscle cells with recombinant human C-reactive protein, and found that after upregulation of C-reactive protein, the mRNA and protein expression of core binding factor a1 and vascular induction activity were significantly increased, and the expression and activity of non-specific alkaline phosphatase in osteogenic enzyme tissue were also increased, thus promoting the differentiation of vascular smooth muscle cells into osteoblasts and promoting the process of calcification. Henze et al[40] further studies showed that C-reactive protein can increase the expression of oxidative stress marker mRNA, activate p38 mitogen-activated protein kinase (p38MAPK) signal pathway, and then promote the differentiation of vascular smooth muscle cells into osteoblasts.

Little research has been done on red blood cell with coronary artery disease and CAC. Schaffer et al[41] performed coronary angiography in 3534 patients and collected fasting samples for

hematologic and lipids assessment, and the results showed high-density lipoprotein cholesterol level was directly associated with red blood cell count, and higher red blood cell level was associated with lower prevalence and severity of coronary artery disease. As was shown in our study, low red blood cell count presented to be an independent predictor of CAC occurrence and severe CAC. But the intrinsic mechanism of red blood cell with atherosclerosis and CAC is unclear. The decrease of red blood cell count may be related to higher inflammatory state, which affect the occurrence and degree of calcification. In addition, this study also showed that age, hypertension, diabetes mellitus, creatinine, and other factors were related to the CAC, suggesting that the occurrence and progression of CAC were the result of the joint action of multiple factors. High glucose environment and insulin resistance can promote the transformation of vascular smooth muscle cells into osteoblasts, and then lead to the occurrence of vascular calcification.

4.1. Limitations

Our findings must be interpreted in light of the study's limitations. For example, this is a single-center retrospective study, rather than multicenter expansion study, and the representativeness of the sample is relatively insufficient. In order to achieve a certain number of patients, and have more complete clinical data, the time span of selected patients was 9 years. In different time periods, the characteristics of patients may also change. This is a cross-sectional study, not a longitudinal study, and did not assess the long-term effect of CysC on CAC. Therefore, more multi-center studies with larger sample size and long-term follow-up are needed to investigate relationship between CysC and CAC.

5. Conclusion

CysC was associated with the CAC occurrence and severe CAC, suggesting that CysC presented to be a potential predictor of CAC.

Author contributions

LPZ and HX participated in the study design. LW, FJ, HX, and XW participated in acquisition of data and interpretation of the results. BZ participated in the collation and verification of CT data. XC participated in the data analysis. HX, LW, FJ, and LPZ contributed to writing and revising the manuscript. All authors read and approved the final manuscript.

Conceptualization: liang-ping zhao.
Data curation: Hui Xiong, Li Wang, Fulu Jin, Bo Zhang, Xiaozhong Wang.
Formal analysis: Xiansong Chang.
Methodology: Li Wang, Bo Zhang.
Writing – original draft: Hui Xiong, Fulu Jin.
Writing – review & editing: liang-ping zhao.

References

[1] Bansilal S, Castellano JM, Fuster V. Global burden of CVD: focus on secondary prevention of cardiovascular disease. Int J Cardiol 2015;201 (suppl 1):S1–7.
[2] Li H, Sun K, Zhao R, et al. Inflammatory biomarkers of coronary heart disease. Front Biosci (Schol Ed) 2018;10:185–96.
[3] Osawa K, Nakanishi R, Budoff M. Coronary artery calcification. Glob Heart 2016;11:287–93.
[4] El-Ashmawy HM, Roshdy HS, Saad Z, Ahmed AM. Serum endostatin level as a marker for coronary artery calcification in type 2 diabetic patients. J Saudi Heart Assoc 2019;31:24–31.
[5] Fathala A, Alreshoodi S, Rujaib MA, et al. Coronary artery calcium score in high-risk asymptomatic women in Saudi Arabia. Ann Saudi Med 2015;35:298–302.
[6] Gauss S, Klinghammer L, Steinhoff A, et al. Association of systemic inflammation with epicardial fat and coronary artery calcification. Inflamm Res 2015;64:313–9.
[7] Lee YA, Kang SG, Song SW, Rho JS, Kim EK. Association between metabolic syndrome, smoking status and coronary artery calcification. PLoS One 2015;10:e0122430.
[8] Vallin R, Wajih N, Greenwood GT, Sane DC. Arterial calcification: a review of mechanisms, animal models, and the prospects for therapy. Med Res Rev 2001;21:274–301.
[9] Kronmal RA, McClelland RL, Detrano R, et al. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 2007;115:2722–30.
[10] Huynh K. Coronary artery disease: coronary artery calcium testing. Nat Rev Cardiol 2017;14:634.
[11] Schoepf IC, Buechel RR, Kovari H, Hammoud DA, Tarr PE. Subclinical atherosclerosis imaging in people living with HIV. J Clin Med 2019;8: 1125.
[12] Barrett AJ, Davies ME, Grubb A. The place of human gamma-trace (cystatin C) amongst the cysteine proteinase inhibitors. Biochem Biophys Res Commun 1984;120:631–6.
[13] Abrahamson M, Olafsson I, Palsdottir A, et al. Structure and expression of the human cystatin C gene. Biochem J 1990;268:287–94.
[14] Fanos V, Mussap M, Plebani M, Cataldi L. Cystatin C in paediatric nephrology. Present situation and prospects. Minerva Pediatr 1999; 51:167–77.
[15] Leto G, Crescimanno M, Flandina C. On the role of cystatin C in cancer progression. Life Sci 2018;202:152–60.
[16] Johansson L, Grubb A, Abrahamson M, et al. A peptidyl derivative structurally based on the inhibitory center of cystatin C inhibits bone resorption in vitro. Bone 2000;26:451–9.
[17] Angelidis C, Deftereos S, Giannopoulos G, et al. Cystatin C: an emerging biomarker in cardiovascular disease. Curr Top Med Chem 2013;13: 164–79.
[18] Inker LA, Okparavero A. Cystatin C as a marker of glomerular filtration rate: prospects and limitations. Curr Opin Nephrol Hypertens 2011;20:631–9.
[19] Slort PR, Ozden N, Pape L, et al. Comparing cystatin C and creatinine in the diagnosis of pediatric acute renal allograft dysfunction. Pediatr Nephrol 2012;27:843–9.
[20] van der Laan SW, Fall T, Soumaré A, et al. Cystatin C and cardiovascular disease: a Mendelian randomization study. J Am Coll Cardiol 2016;68:934–45.
[21] Grubb A. Cystatin C is indispensable for evaluation of kidney disease. EJIFCC 2017;28:268–76.
[22] Maahs DM, Ogden LG, Kretowski A, et al. Serum cystatin C predicts progression of subclinical coronary atherosclerosis in individuals with type 1 diabetes. Diabetes 2007;56:2774–9.
[23] Wang GN, Sun K, Hu DL, Wu HH, Wang XZ, Zhang JS. Serum cystatin C levels are associated with coronary artery disease and its severity. Clin Biochem 2014;47:176–81.
[24] Vakili H, Mohamadian A, Naderian M, Khaheshi I. Cystatin C may not be a precious predictor for coronary artery disease and its severity: an area of uncertainty. Acta Biomed 2018;89:209–13.
[25] Doganer YC, Aydogan U, Aydogdu A, et al. Relationship of cystatin C with coronary artery disease and its severity. Coron Artery Dis 2013;24:119–26.
[26] Nicoll R, Wiklund U, Zhao Y, et al. Gender and age effects on risk factor-based prediction of coronary artery calcium in symptomatic patients: a Euro-CCAD study. Atherosclerosis 2016;252:32–9.
[27] Bensenor IM, Goulart AC, Santos IS, et al. Association between a healthy cardiovascular risk factor profile and coronary artery calcium score: results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Am Heart J 2016;174:51–9.
[28] Panh L, Lairez O, Ruidavets JB, et al. Coronary artery calcification: from crystal to plaque rupture. Arch Cardiovasc Dis 2017;110:550–61.

[29] Bucay N, Sarosi I, Dunstan CR, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260–8.
[30] Hénaut L, Sanchez-Nino MD, Aldamiz-Echevarría Castillo G, Galinier M, Carrié D, Ferrières J. Targeting local vascular and systemic consequences of inflammation on vascular and cardiac valve calcification. Expert Opin Ther Targets 2016;20:89–105.
[31] Tintut Y, Patel J, Parhami F, Demer LL. Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. Circulation 2000;102:2636–42.
[32] Nadra I, Mason JC, Philippidis P, et al. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? Circ Res 2005;96:1248–56.
[33] Jono S, Nishizawa Y, Shioi A, Morii H. 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. Circulation 1998; 98:1302–6.
[34] Sugiyama H, Miyoshi T, Osawa K, et al. Serum cystatin C levels are associated with coronary artery calcification in women without chronic kidney disease. J Cardiol 2017;70:559–64.
[35] Ruiz-Salas A, Cortés-Rodríguez M, Alegre-Bayo N, Algarra-García J, Galván EDT, Jiménez-Navarro M. Relationship between cystatin C and coronary artery calcification in patients with intermediate cardiovascular risk. Med Clin (Barc) 2014;143:535–8.
[36] Kampoli AM, Tousoulis D, Papageorgiou N, et al. Clinical utility of biomarkers in premature atherosclerosis. Curr Med Chem 2012;19: 2521–33.
[37] Gulcicek S, Zoccali C, Olgun DÇ, et al. Long-term progression of coronary artery calcification is independent of classical risk factors, C-reactive protein, and parathyroid hormone in renal transplant patients. Cardiorenal Med 2017;7:284–94.
[38] Puri R, Nicholls SJ, Shao M, et al. Impact of statins on serial coronary calcification during atheroma progression and regression. J Am Coll Cardiol 2015;65:1273–82.
[39] Rodriguez-Granillo GA, Carrascosa P, Bruining N. Progression of coronary artery calcification at the crossroads: sign of progression or stabilization of coronary atherosclerosis? Cardiovasc Diagn Ther 2016;6:250–8.
[40] Henze LA, Luong TTD, Boehme B, et al. Impact of C-reactive protein on osteo-/chondrogenic transdifferentiation and calcification of vascular smooth muscle cells. Aging (Albany NY) 2019;11:5445–62.
[41] Schaffer A, Verdoia M, Cassetti E, et al. Impact of red blood cells count on the relationship between high density lipoproteins and the prevalence and extent of coronary artery disease: a single centre study [corrected]. J Thromb Thrombolysis 2015;40:61–8.