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Original article

Association of calciprotein particles measured by a new method with coronary artery plaque in patients with coronary artery disease: A cross-sectional study

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ABSTRACT

Background: Calciprotein particles (CPPs) have been suggested to be associated with the degree of coronary atherosclerosis, and have also been established as a molecular marker for clinical outcome in patients with chronic kidney disease (CKD). However, there are several concerns with regard to conventional measurement of CPPs. We therefore developed a new CPP measurement system that can detect both smaller and lower-density CPPs.

Methods: We analyzed 71 consecutive patients who underwent percutaneous coronary intervention for acute coronary syndrome (ACS, $n = 29$) and/or stable angina pectoris (AP, $n = 42$) who did not have CKD of stage 4 or greater. CPP measurement was made using an infrared fluorescent bisphosphonate (OsteoSense, PerkinElmer, Waltham, MA, USA) and a gel filtration method. The coronary artery plaque was analyzed by gray-scale intravascular ultrasound (IVUS) and integrated backscatter (IB)-IVUS.

Results: The median CPP level (interquartile range) was 40,953 (19,171–74,131) arbitrary units (AU). When we divided the CPP level into quintiles, the total and lipid plaque volume were incrementally higher with increasing quintile from lowest to highest (both $p < 0.02$). After adjustment by age, body mass index, and estimated glomerular filtration rate, which factors were correlated with the above-described plaque components, the top quintile of CPP (>86,751 AU) had significantly higher total plaque (263 mm^3 vs. 161 mm^3; $p = 0.001$) and lipid plaque volume (156 mm^3 vs. 89 mm^3; $p < 0.001$) than the other quintiles. However, these associations were not found for the fibrous or calcified plaque volume. The CPP level was higher in the ACS group than the stable AP group ($p = 0.02$), and the total and lipid plaque volume were also higher in the ACS group than the stable AP group (both $p < 0.05$).

Conclusions: The results suggested that a high CPP level, as measured by the novel assay, is a surrogate marker for coronary atherosclerosis, especially in lipid-rich plaques, contributing to an increased risk of plaque vulnerability.

Introduction

Coronary artery disease (CAD) is the most common cause of mortality worldwide. CAD is largely due to coronary artery plaque. Coronary artery plaque consists of diverse components, i.e. lipid-rich plaque, fibrous plaque, and calcified plaque [1]. The mechanisms of plaque formation are considered to be multifactorial, and to include endothelial dysfunction, dyslipidemia, inflammatory, and immunologic factors [2–7].

Calciprotein particles (CPPs) are tiny nano-aggregates containing calcium-phosphate and fetuin-A, which form complexes with circulating calcium and phosphorus, resulting in increased solubility of these minerals and the suppression of crystal growth and mineral deposition [8]. As a result, CPPs have been considered a marker of high calcification propensity. CPPs are also thought to be an endogenous pathogen causing non-infective inflammation in patients with chronic kidney disease (CKD) [9]. Previous clinical studies have reported that higher serum CPP levels were positively correlated with coronary calcification assessed by a coronary

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computed tomography (CT) scan [10–12]. However, the value of those studies was limited by an important key point. The CPP levels in those studies were evaluated indirectly by measuring the fetuin-A reduction rate. This method is unreliable at lower levels of CPPs, because the coefficient of variation of the human fetuin-A enzyme-linked immunosorbent assay (ELISA) kits is 2–5%. Moreover, this assay depends on validated human fetuin-A ELISA kits, limiting its direct application to experimental animals. Recently, Miura and colleagues developed a novel CPP assay using a bisphosphonate conjugated with an infrared fluorescent dye (OsteoSense, PerkinElmer, Waltham, MA, USA) and a gel filtration spin column, which is more sensitive, rapid, and less expensive than previous CPP measurements using the fetuin-A method. More importantly, the new method revealed the presence of CPPs that are smaller in size and lower in density than those measured by the fetuin-A method and the flow cytometric method [13]. However, to date there has been no study on the association between the CPP level evaluated by the new method and the degree of atherosclerosis.

Integrated backscatter-intravascular ultrasound (IB-IVUS) is able to detect the characteristics of coronary artery plaque [14,15]. This use of IB-IVUS has mostly been applied to evaluate the progression or regression of plaque in patients at high risk of cardiovascular events [16]. The aim of the present study was to investigate the relationship between serum CPP levels measured by a new gel filtration method and coronary plaque components detected by IB-IVUS in patients who underwent percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris (AP) and did not have chronic kidney disease (CKD) greater than stage 4.

Material and methods

Study population

This is a cross-sectional study designed to evaluate the association between serum CPPs and coronary plaque measured by IB-IVUS. From January 2016 to July 2017, 307 consecutive patients with ACS or AP (119 ACS patients and 188 AP patients) underwent PCI at Okinawa Chubu Hospital. Cardiology specialists diagnosed ACS or AP according to the available guidelines [17–19]. Thirty-seven patients with CKD of stage 4 or greater—i.e. those with an estimated glomerular filtration rate (e-GFR) under 30 ml/min/1.73 m^2—were excluded. eGFR was calculated using the Japanese GFR equation [20]. Nineteen patients with cardiogenic shock and cardiac arrest at hospital arrival were also excluded. Ninety-four patients consented to participate after a full explanation of the purpose of this study. Patients with stable AP provided written informed consent before PCI, while the ACS patients consented after PCI. Patients with chronic total occlusion (n = 2), a lesion located in a stent (n = 5), bypass artery disease (n = 3), and inadequate IVUS imaging (n = 13) were excluded from this study. A total of 71 patients with ACS or AP who underwent PCI using IB-IVUS were prospectively recruited for this study. This study was approved by the institutional review board of Okinawa Chubu Hospital and carried out according to the guidelines of the Declaration of Helsinki.

Study protocol

All patients in this study underwent PCI for ACS or stable AP, and data on the patients' gender, body mass index, history of myocardial infarction and stroke, coronary risk factors such as hyperlipidemia, diabetes mellitus, hypertension and smoking history, episodes of atrial fibrillation, and peripheral arterial disease were collected from medical records.

Blood sampling and measurement of serum CPP levels

Samples for the measurement of serum CPP levels were collected from the patients during hospitalization. In patients with stable AP, we collected blood samples before PCI procedure. On the other hand, in patients with ACS, we collected blood samples around 1 week after PCI. Serum samples were obtained by centrifugation of clotted blood samples at 3000 rpm for 10 min within 60 min after blood sampling. They were stored at −80 °C until CPP measurement by the novel CPP assay using OsteoSense as previously reported and validated in detail [13]. Briefly, 5 μl of serum was added to 45 μl of Dulbecco's Modified Eagle Medium (DMEM) containing 100 mM HEPES and 0.5 μM OsteoSense. After incubation at 25 °C for 60 min, 30 μl of the mixture was subjected to gel filtration using a spin column (Bio-Rad, Hercules, CA, USA; molecular weight cut-off 40 kDa). Fifty μl of the flow-through was mixed with 50 μl of 2% sodium dodecyl sulfate (SDS) and 100 mM ethylenediaminetetraacetic acid (EDTA), and OsteoSense fluorescence images were taken (excitation at 685 nm, emission at 700 nm) using an infrared fluorescence scanner (Odyssey CLx, LI-COR, Lincoln, NE, USA). The fluorescence intensity of OsteoSense was defined as the total CPP level [13]. The coefficient of variation (CV) of this assay was 2.2% [13]. We sent all blood samples to Jichi Medical University for CPP measurement. Observers of serum CPPs were blinded to the subject demographics and the clinical information.

IVUS imaging

IVUS imaging was performed before intervention. To prevent coronary spasm, an optimal intracoronary dose of nitroglycerin (2 mg) was administered via catheter before measurement. Studies were performed with a 40-MHz, 5 Fr IVUS imaging catheter (ViewIT™; Terumo, Tokyo, Japan) and the VISIWAVE imaging system (Terumo). IVUS and IB-IVUS images were captured at a speed of 0.5 mm/s using a motorized pull-back system. We characterized tissue from coronary plaques in culprit lesions in patients with AP or ACS.

IVUS analysis

Plaque analysis was performed in the range of culprit lesions at 0.5 mm axial intervals. The plaque area was calculated as the external elastic membrane (EEM) cross-sectional area (CSA) – lumen CSA [21]. The total plaque volume was calculated as the sum of the plaque areas in each measured image [22]. IB values were defined for each histological category by comparison with the histologic images reported in the previous study [14,23]. IB-IVUS analysis was used to classify the color-coded tissue into four major components based on the IB scores calculated from the back-scattered signals from tissue: blue (lipid), green (fibrous), yellow (dense fibrotic), and red (calcified). The volumes of the lipid, fibrous, dense fibrotic, and calcified plaque were calculated as the sum of the areas of the respective tissue type in each cross-sectional area.

Statistical analysis

Continuous variables are presented as the means ± standard deviations (SD) and categorical variables as counts (%). Because the distributions of the high-sensitive C-reactive protein (hsCRP), CPP, and fibroblast growth factor 23 (FGF23) were highly skewed, these were log-transformed before the statistical analysis and expressed as the geometric mean (interquartile range [IQR]). Continuous variables were compared using the unpaired Student's t-test, and categorical variables were

Table 1
Baseline characteristics.

	All (n = 71)
Age, years	68 ± 12
Male	54 (76)
Body mass index, kg/m^2	26 ± 4
Hypertension	63 (89)
Diabetes mellitus	32 (45)
Insulin-treated	8 (11)
Dyslipidemia	70 (99)
Chronic kidney disease[a]	29 (41)
Smoker	11 (15)
Atrial fibrillation	8 (11)
History of myocardial infarction	37 (52)
History of stroke	8 (11)
Peripheral arterial disease	8 (11)
Chronic heart failure	9 (13)
eGFR, ml/min/1.73 m^2	68.8 ± 19.5
Hb A1c, %	6.5 ± 1.1
LDL-C, mg/dl	101 ± 32
HDL-C, mg/dl	49 ± 12
TG, mg/dl	141 ± 68
Calcium, mg/dl	9.1 ± 0.5
Phosphate, mg/dl	3.3 ± 0.6
Magnesium, mg/dl	2.0 ± 0.2
High-sensitive CRP, mg/l	1.8 (0.5–4.0)
CPP, AU	40,953 (19,171–74,131)
FGF23, pg/ml	44.6 (34.0–63.6)
1,25(OH)$_2$D$_3$, pg/ml	57.7 ± 33.4
Clinical presentation	
Stable ischemic heart disease	42 (59)
Acute coronary syndrome	29 (41)
Concomitant medications	
RAA inhibitors	47 (66)
β-blockers	33 (46)
Diuretics	16 (23)
Statins	68 (96)

Values are the number (%), mean ± SD, or median (IQR).
eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CRP, C-reactive protein; CPP, calciprotein particle; AU, arbitrary units; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; 1,25(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D$_3$; RAA, renin angiotensin aldosterone.
p-values represent differences between groups.
[a] Chronic kidney disease was defined as 30 < eGFR < 60.

compared using chi-square test or Fisher's tests, according to the data distribution. One-way analysis of variance was performed to detect differences in coronary plaque components among quintiles of CPP level.

Pearson's correlation coefficient was performed to investigate the relationship between clinical characteristics and coronary plaque components, which was statistically significant among quintiles of CPP level. Analysis of covariance (ANCOVA) was performed to detect the groups with a difference in coronary plaque components. This model included covariates that correlated marginally with coronary plaque components determined by Pearson's correlation test ($p < 0.1$). We also performed an ANCOVA model analysis including conventional cardiovascular risk factors [age, gender, body mass index, smoking, prevalent hypertension and diabetes, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and eGFR] as covariates and another ANCOVA model analysis including conventional cardiovascular risk factors and log-transformed FGF23 or 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$).

Differences/associations with a p-value less than 0.05 were considered statistically significant. Statistical analyses were performed using JMP version 9.0 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

The clinical characteristics, laboratory values, and medication use of patients at baseline are summarized in Table 1. The mean ± SD age of the patients was 68 ± 12 years, and 76% were male. Most of the patients had cardiovascular risk factors (dyslipidemia 99%, hypertension 89%, diabetes mellitus 45%, CKD 41%, and history of myocardial infarction 52%). The median (IQR) CPP level was 40,953 (range: 19,171–74,131) arbitrary units (AU). Online Figure 1 shows the distribution of CPP levels.

Association between coronary plaque components and serum CPP

Fig. 1 shows the association between the quintiles of serum CPP level and coronary plaque components. The total plaque volume and lipid plaque volume were incrementally higher with increasing quintile from lowest to highest. However, no such associations were found for the fibrous and calcified plaque volume.

The correlations between clinical variables and total plaque or lipid plaque volume are shown in Table 2 and Fig. 2. Simple linear regression analysis revealed that the serum log-transformed CPP level was positively correlated with the total plaque volume ($r = 0.24$, $p = 0.046$) and lipid plaque volume ($r = 0.23$, $p = 0.049$). Age was negatively associated with both total and lipid plaque volume. Body mass index (BMI) and eGFR were positively associated with both total and lipid plaque volume. When we used ANCOVA to compare the top quintile of CPP with other quintiles for total and lipid plaque volume after adjustment by age, BMI, and eGFR, the top quintile of CPP had significantly higher total plaque volume (263 mm^3 vs. 161 mm^3; $p = 0.001$) and lipid plaque volume (156 mm^3 vs. 89 mm^3; $p < 0.001$) than the other quintiles (Fig. 3). Even after adjustment for age, gender, BMI, smoking, prevalent hypertension and diabetes, LDL-cholesterol, HDL-cholesterol, and eGFR, the association was similar (Online Table 1). When we added log-transformed FGF23, the significant association was attenuated. However, the association remained highly significant when we added log-transformed 1,25(OH)$_2$D$_3$ (Online Table 1).

Association between CPP levels and clinical parameters

The top quintile of CPP had a higher prevalence of chronic heart failure (CHF) and higher hsCRP and FGF23 levels than the other quintiles. The HDL-cholesterol level and 1,25(OH)$_2$D$_3$ level were lower in the top quintile of CPP than in the other quintiles (Table 3).

Serum CPP levels in patients with ACS or stable AP

Patient characteristics in the ACS and stable AP groups are presented in Table 4. No statistically significant differences existed between the two groups, except that the patients with ACS had a higher prevalence of current smoking (31% vs. 5%, $p = 0.003$) and previous myocardial infarction (79% vs. 33%, $p < 0.001$). Serum CPP was higher in the ACS group than the stable AP group (60,545 [20,430–105,248] AU vs. 31,024 [17,899–61,281] AU, $p = 0.02$). Table 5 shows the characteristics of coronary lesions and IVUS in the ACS and stable AP groups. The ACS group had higher plaque volume (215.1 ± 123.9 mm^3 vs. 158.9 ± 100.4 mm^3, $p = 0.04$) and lipid plaque volume (128.1 ± 85.1 mm^3 vs. 85.0 ± 57.2 mm^3, $p = 0.01$) than the stable AP group.

When we divided the patients into an ACS and stable AP group and repeatedly performed ANCOVA on the association between CPPs and total plaque or lipid plaque volume after adjustment by age, BMI, and eGFR, the top quintile of CPP exhibited both higher

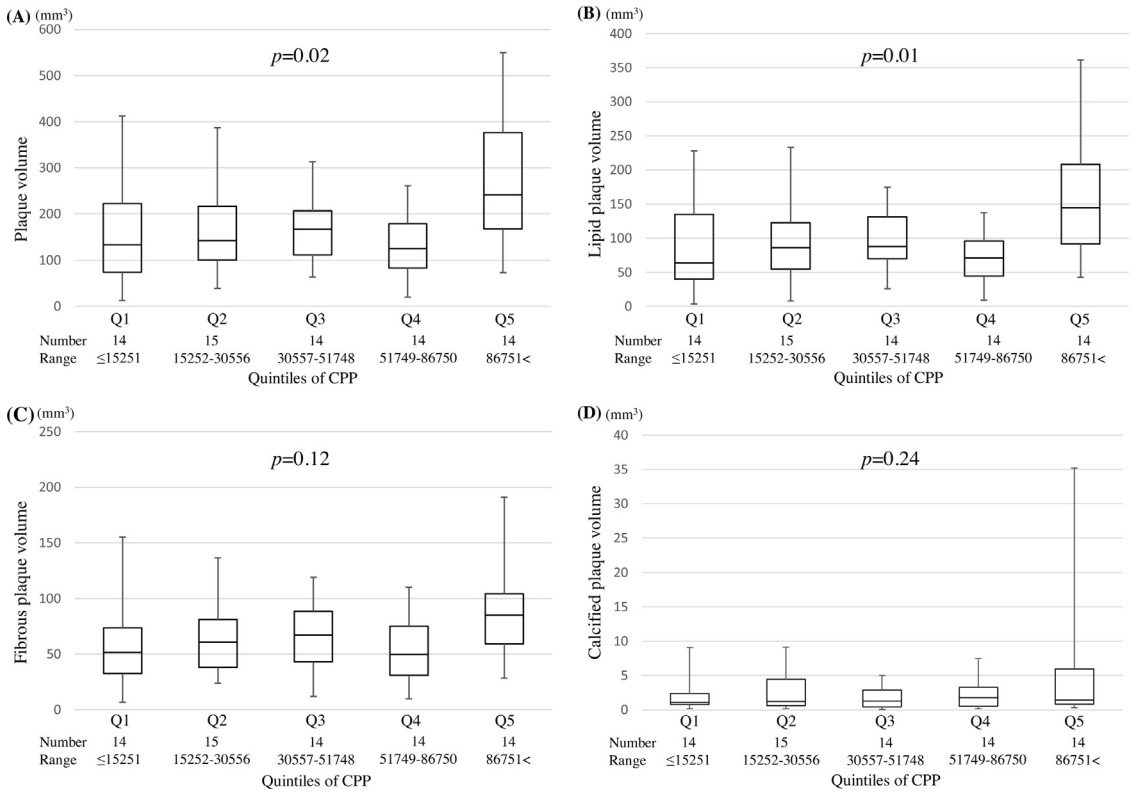

Fig. 1. Association between plaque components of coronary artery and quintiles of CPPs. The bottom line of the box-plot indicates the 25th percentile; the middle line, the median; the top line, the 75th percentile; and error bars, the minimum and maximum. CPPs, calciprotein particles.

total and lipid plaque volume than the other quintiles in the ACS group. However, this association was not found in the stable AP group (Online Figure 2).

Discussion

In the present study we reported for the first time that increased serum CPP levels measured by the gel filtration method were associated with higher total plaque volume and lipid plaque volume in patients with CKD of stage 1–3 or non-CKD who underwent PCI for ACS or stable AP. Moreover, the CPP levels were higher in patients with ACS than those with stable AP.

Conventional and novel method of measuring CPP

Previous papers used the fetuin-A method of measuring CPP levels which employs the fetuin-A reduction rate determined using human fetuin-A ELISA kits [10], and its reduction rates generated using these kits cannot be considered unreliable. Moreover, the fetuin-A method and the flow cytometric method [24] cannot detect CPPs smaller than 100 nm in diameter. In the present study, we measured the total CPP level by means of a new method using OsteoSense and a gel filtration spin column [13]. This novel method can measure a new kind of CPP (L-CPP), which is smaller in size and lower in density than the CPPs described previously. Miura and colleagues had shown that the CPPs in fresh plasma were almost exclusively L-CPP, and that the new method was available to detect CPPs even in the patients with mild CKD (CKD stage 1–3) or no CKD. In a previous study, CPP levels measured by the fetuin-A method were correlated with coronary calcification measured by computed tomography (CT) scan [25]. However, in our study we did not detect a correlation between the serum CPP level and calcified plaque volume measured by IB-IVUS. In the literature,

Table 2
Association of total and lipid plaque with clinical variables.

	Total plaque volume		Lipid plaque volume	
	r	*p*-Value	*r*	*p*-Value
Age	−0.31	0.01	−0.31	0.009
BMI	0.24	0.04	0.26	0.03
eGFR	0.23	0.055	0.26	0.03
LDL-C	−0.04	0.76	−0.08	0.50
HDL-C	−0.16	0.17	−0.15	0.22
TG	−0.03	0.83	−0.10	0.41
Calcium	0.03	0.80	−0.006	0.96
Phosphate	−0.04	0.72	−0.08	0.50
Magnesium	−0.16	0.20	−0.12	0.34
High sensitive CRP	0.10	0.42	0.13	0.29
CPP	0.24	0.045	0.23	0.049
FGF23	0.11	0.39	0.07	0.62
$1,25(OH)_2D_3$	−0.02	0.27	−0.04	0.75

BMI, body mass index; eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; CRP, C-reactive protein; CPP, calciprotein particle; FGF23, fibroblast growth factor 23; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3.
p-values represent differences between groups.

CPP, especially secondary CPP, which was detected in vitro [8], was considered to contribute to vascular calcification [26]. In our study, serum CPP contained L-CPP, and thus was different from the CPP determined by the fetuin-A method [13]. Moreover, because we excluded advanced CKD patients with e-GFR < 30 ml/min/1.73 m^2, there were fewer patients with secondary CPP.

Association between CPP and coronary plaque

In our study, CPP levels were associated with total and lipid plaque. The mechanisms of plaque formation have been consid-

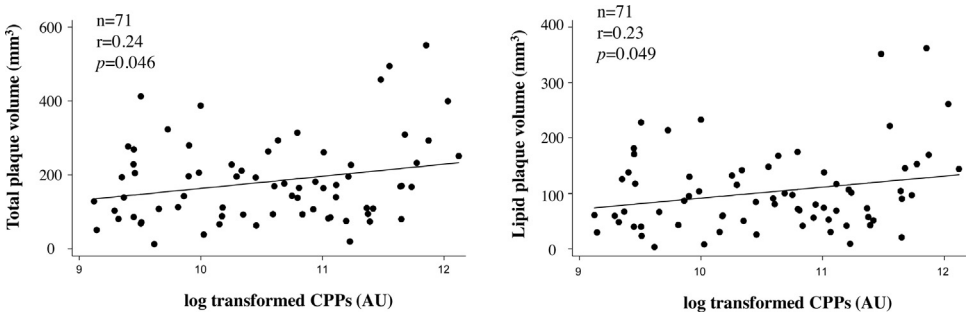

Fig. 2. Scatterplot displaying the association between log-transformed CPPs and total plaque volume, lipid plaque volume. AU, arbitrary unit; CPPs, calciprotein particles.

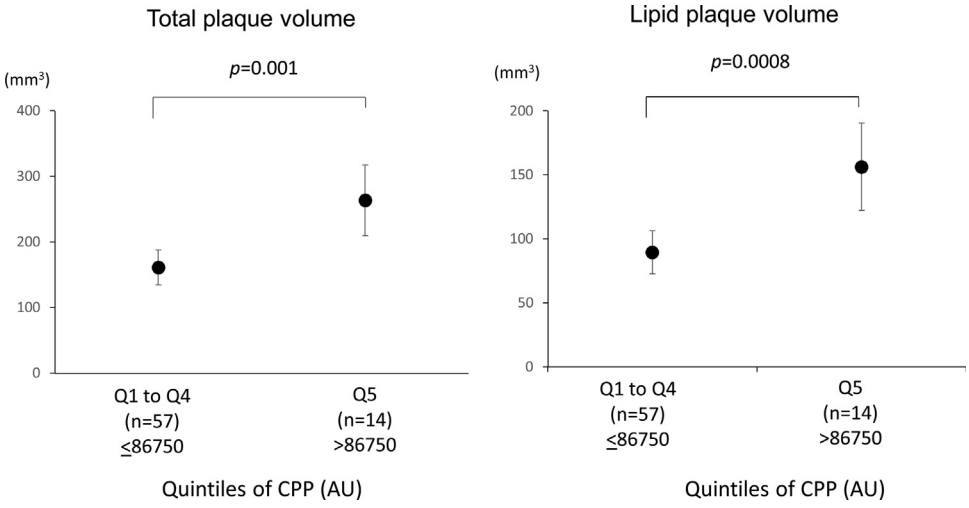

Fig. 3. Association between total or lipid plaque volume and quintiles of CPP. Black circles indicate mean values. Error bars indicate 95% confidence intervals. Analysis of covariance was performed with adjustment by age, body mass index, and estimated glomerular filtration rate. AU, arbitrary unit; CPPs, calciprotein particles.

ered to include endothelial dysfunction, dyslipidemia, and inflammatory and immunologic factors [2–7]. In this regard, it is understandable that chronic inflammation has been considered the cornerstone of the pathogenesis of atherosclerosis [3–5]. Oxidation of LDL (ox-LDL) is a requirement for the initiation and progression of atherosclerosis and is promoted by macrophages, endothelial cells, and smooth muscle cells [27,28]. Moreover, macrophages have been demonstrated within culprit lesions in autopsy studies of patients presenting with ACS [28,29]. Based on the above facts, macrophages are considered central to atherosclerosis. In previous clinical studies, serum CPP was independently correlated with levels of hsCRP and ox-LDL [25]. In addition, an in vitro study showed that calcium phosphate crystal stimulating macrophages and high levels of serum CPP provoked a pro-inflammatory response from macrophages [30]. Thus, CPPs may stimulate ox-LDL and macrophages in the vessel wall, which would promote the formation of lipid-rich plaque.

Association between CPP and atherosclerotic risk factors

Serum CPP was also strongly correlated with hsCRP. Inflammatory biomarkers, e.g. hsCRP, have been associated with the progression of atherosclerotic plaques [31,32]. However, in this study, hsCRP was not correlated with plaque volume or lipid plaque volume. It has been recognized that statin therapy lowers CRP levels independently of LDL reduction [31]. In this study, 96% of the patients were treated by statin therapy, which might explain the lack of correlation between lipid plaque and hsCRP. These findings indicate that CPPs of smaller size and lower density, which were successfully measured by the novel method used herein, may directly induce inflammation in the vessel wall, in the manner of lipoproteins.

In our study, high serum CPP levels were associated with low HDL-cholesterol. HDL particles have multiple potentially anti-atherogenic properties and inhibit inflammation [33,34]. According to these findings, low HDL-cholesterol leads to the formation of atherosclerotic plaque. Therefore, it was thought that high serum CPP was correlated with total and lipid plaque.

Association between CPP and FGF23, vitamin D

In this study, adding FGF23 attenuated the significant association between CPP and total and lipid plaque volume. This suggests that the relationship between CPP and total and lipid plaque formation may be mediated by FGF23. FGF23 is a phosphoregulatory hormone produced in bone [35,36]. Recent studies have noted an association between hyperphosphatemia and cardiovascular events, and many clinical data on serum FGF23 have been reported. These FGF23 may cause fat and lipid metabolism abnormalities [35,37]. CPP may be produced as a collection of calcium phosphate crystals when excessive phosphorus intake causes an increase in serum phosphorus, and then the resulting phosphorus excretion would decrease with a decrease in renal

Table 3
Clinical characteristics of Q1-4 vs. Q5.

	Q1-4 ($n=57$)	Q5 ($n=14$)	p-Value
Age, years	68 ± 12	67 ± 12	0.68
Male	43 (75)	11 (79)	0.81
Body mass index, kg/m^2	26 ± 4	27 ± 4	0.64
Hypertension	51 (89)	12 (86)	0.69
Diabetes mellitus	25 (44)	7 (50)	0.68
Insulin-treated	7 (12)	1 (7)	0.59
Dyslipidemia	56 (98)	14 (100)	0.62
Chronic kidney disease[a]	24 (42)	5 (36)	0.66
Smoker	6 (11)	5 (36)	0.02
Atrial fibrillation	7 (12)	1 (7)	0.59
History of myocardial infarction	27 (47)	10 (71)	0.11
History of stroke	8 (14)	0 (0)	0.14
Peripheral arterial disease	5 (9)	3 (21)	0.18
Chronic heart failure	4 (7)	5 (36)	0.004
eGFR, ml/min/1.73 m^2	65.7 ± 16.6	72.3 ± 25.3	0.32
Hb A1c, %	6.5 ± 1.1	6.6 ± 0.8	0.63
LDL-C, mg/dl	103 ± 31	94 ± 35	0.38
HDL-C, mg/dl	51 ± 12	43 ± 9	0.02
TG, mg/dl	145 ± 71	125 ± 53	0.33
Calcium, mg/dl	9.2 ± 0.4	9.0 ± 0.8	0.18
Phosphate, mg/dl	3.3 ± 0.5	3.3 ± 0.8	0.98
Magnesium, mg/dl	2.0 ± 0.2	2.0 ± 0.3	0.75
High sensitive CRP, mg/l	1.2 (0.5–2.9)	3.5 (1.5–8.9)	0.03
CPP, AU	30,806 (15,312–53,164)	116,386 (95,310–141,144)	<0.001
FGF23, pg/ml	42.9 (32.3–59.3)	57.2 (39.9–98.4)	0.03
1,25(OH)$_2$D$_3$, pg/ml	60.7 ± 22.2	45.5 ± 15.0	0.02
Clinical presentation			
Stable ischemic heart disease	38 (67)	4 (29)	0.01
Acute coronary syndrome	19 (33)	10 (71)	
Concomitant medications			
RAA inhibitors	38 (67)	9 (64)	0.87
β-blockers	24 (42)	9 (64)	0.14
Diuretics	13 (23)	3 (21)	0.91
Statins	54 (95)	14 (100)	0.38

Values are the number (%), mean ± SD, or median (IQR).
eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; CRP, C-reactive protein; CPP, calciprotein particle; AU, arbitrary units; FGF23, fibroblast growth factor 23; 1,25(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D$_3$; RAA, renin angiotensin aldosterone.
p-values represent differences between groups.
[a] Chronic kidney disease was defined as 30 < eGFR < 60.

function. In this scenario, FGF23 could be secreted from bone to prevent excessive production of CPP.

Although we expected to find that vitamin D plays an important role in the balance between circulating calcium and phosphorus for coronary plaque formation via CPP, our results showed that 1,25(OH)$_2$D$_3$ did not function as a mediator between CPP and coronary plaque. In a cohort study, 1,25(OH)$_2$D$_3$ deficiency was associated with incident cardiovascular disease [38]. Studies in the literature have suggested that serum 1,25(OH)$_2$D$_3$ levels affect the proliferation of vascular smooth muscle cells, and calcification [39]. In in vitro and animal studies, 1,25(OH)$_2$D$_3$ deficiency has been shown to activate the inflammatory and immune processes of atherosclerosis. Moreover, 1,25(OH)$_2$D$_3$ has been demonstrated to be an effective inhibitor of foam cell formation [40]. Based on the above facts, it is considered that serum CPP indirectly promotes vessel atherosclerosis.

Association between CPP and acute coronary syndrome

In the ACS group, the serum CPP level was significantly higher than in the stable AP group. Not surprisingly, the plaque volume and lipid plaque volume were also higher in the ACS group. In addition, the top quintile of CPP had significantly higher total and lipid plaque volumes than those of the other quintiles in the ACS group. These findings suggested that high CPP levels resulted in more lipid plaque and calcified plaque. In the literature, a high volume of lipid and spotty calcification can lead to vulnerable plaques [7] and ultimately to the development of ACS.

Study limitations

This study has several limitations. First, a relatively small number of patients in a single center were enrolled. However, the patient group included patients with ACS, which was useful for examining the acute phase reaction of CPP. Second, it was difficult to detect macrophages on the plaque by gray-scale IVUS and IB-IVUS, thus we could not consider the relationship between serum CPP and macrophages in culprit plaque. However, previous studies have shown that optical coherence tomography (OCT) can detect macrophages in plaque [41,42]. By using OCT, correlations between CPP levels and macrophages can be detected. Third, data on cardiac events should be collected in future studies, because we used a cross-sectional study design. Finally, in this study, to explore the pathological threshold of CPP for coronary plaque, we divided the participants into quintiles. Although the threshold of CPP was 86,750 AU, future studies will be needed to determine whether this value is generalizable to other populations. Clearly, additional

Table 4
Patient characteristics in ACS or stable AP.

Variables	Stable AP ($n=42$)	ACS ($n=29$)	p-Value
Age, years	69 ± 11	66 ± 13	0.35
Male	31 (74%)	23 (79%)	0.59
Body mass index, kg/m^2	26 ± 4	26 ± 4	0.88
Hypertension	37 (88%)	26 (90%)	0.84
Diabetes mellitus	19 (45%)	13 (45%)	0.97
Insulin-treated	8 (19%)	0 (0%)	0.01
Dyslipidemia	41 (98%)	29 (100%)	0.40
Chronic kidney disease[a]	19 (45%)	10 (34%)	0.36
Smoker	2 (5%)	9 (31%)	0.003
Atrial fibrillation	5 (12%)	3 (10%)	0.84
History of myocardial infarction	14 (33%)	23 (79%)	<0.001
History of stroke	5 (12%)	3 (10%)	0.84
Peripheral arterial disease	6 (14%)	2 (7%)	0.33
Chronic heart failure	6 (14%)	3 (10%)	0.62
eGFR, ml/min/1.73 m^2	65.3 ± 16.1	71.4 ± 21.5	0.18
Hb A1c, %	6.6 ± 1.3	6.3 ± 0.7	0.14
LDL-C, mg/dl	98 ± 32	105 ± 31	0.35
HDL-C, mg/dl	52 ± 13	46 ± 11	0.06
TG, mg/dl	141 ± 73	140 ± 61	0.93
Calcium, mg/dl	9.2 ± 0.5	9.0 ± 0.6	0.04
Phosphate, mg/dl	3.4 ± 0.5	3.2 ± 0.6	0.08
Magnesium, mg/dl	2.0 ± 0.2	2.1 ± 0.2	0.21
High sensitive CRP, mg/l	1.0 (0.5–3.4)	2.0 (0.7–6.7)	0.10
CPP, AU	31,024 (17,899–61,281)	60,545 (20,430–105,248)	0.02
FGF23, pg/ml	43.3 (32.5–63.4)	49.8 (35.5–77.3)	0.46
1,25$(OH)_2D_3$, pg/ml	59.5 ± 21.6	55.1 ± 22.1	0.41
Concomitant medications			
RAA inhibitors	26 (62%)	21 (72%)	0.36
β-blockers	17 (41%)	16 (55%)	0.22
Diuretics	11 (26%)	5 (17%)	0.38
Statins	40 (95%)	28 (97%)	0.79

Values are the number (%), mean ± SD, or median (IQR).
ACS, acute coronary syndrome; AP, angina pectoris; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; CRP, C-reactive protein; CPP, calciprotein particle; AU, arbitrary units; FGF23, fibroblast growth factor 23; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; RAA, renin angiotensin aldosterone.
[a] Chronic kidney disease was defined as 30 < eGFR < 60.

Table 5
Lesion and IVUS characteristics in ACS or stable AP.

Variables	Stable AP ($n=42$)	ACS ($n=29$)	p-Value
Target vessels			
LAD, N (%)	22 (53%)	16 (55%)	0.89
LCX, N (%)	6 (14%)	3 (10%)	
RCA, N (%)	14 (33%)	10 (35%)	
Lesion length (mm)	26.4 ± 14.1	25.6 ± 11.1	0.80
EEM volume (mm^3)	279.9 ± 156.8	355.9 ± 183.3	0.07
Plaque volume (mm^3)	158.9 ± 100.4	215.1 ± 123.9	0.04
Lipid volume (mm^3)	85.0 ± 57.2	128.1 ± 85.1	0.01
Fibrous volume (mm^3)	61.9 ± 37.2	77.2 ± 42.2	0.11
Dense fibrous volume (mm^3)	8.0 ± 7.8	7.5 ± 4.9	0.75
Calcification volume (mm^3)	3.4 ± 5.7	2.4 ± 3.0	0.39

Values are the number (%) or mean ± SD.
ACS, acute coronary syndrome; AP, angina pectoris; IVUS, intravascular ultrasound; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; EEM, external elastic membrane.
p-values represent differences between groups.

investigations will be needed to fully clarify the relation between CPPs and coronary artery plaque.

Conclusions

We demonstrated that higher CPP levels measured by a new method were positively correlated with total plaque volume and lipid plaque volume in coronary plaque detected by IB-IVUS. Together with previously reported findings, our results suggest that high CPP levels are a surrogate marker for coronary atherosclerosis, especially in lipid-rich plaques, contributing to an increased risk of plaque vulnerability.

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Conflicts of interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jjcc.2019.04.008.

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