Original Article

Serum Tartrate-resistant Acid Phosphatase-5b Levels are Associated with the Severity and Extent of Coronary Atherosclerosis in Patients with Coronary Artery Disease

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Aims: Tartrate-resistant acid phosphatase (TRACP)-5b and osteoprotegerin (OPG) are specific and sensitive markers of bone resorption in patients with rheumatoid arthritis (RA) and chronic kidney disease (CKD). The TRACP-5b level is associated with the severity of RA and CKD, while the OPG level is associated with the severity of coronary atherosclerosis and calcification, and can predict a poor outcome in patients with coronary artery disease (CAD). However, the impact of TRACP-5b on coronary atherosclerosis in CAD patients remains unclear.

Methods: A total of 71 CAD patients (57 men, 14 women; mean age: 69.0 ± 9.7 years) and 28 age- and gender-matched healthy subjects were investigated. The number of diseased vessels (a marker of the severity of coronary atherosclerosis) and the Gensini score (a marker of the extent of coronary atherosclerosis), as well as the OPG and TRACP-5b levels were measured in CAD patients. The TRACP-5b levels were classified into quartiles.

Results: The TRACP-5b levels were significantly higher in CAD patients than in healthy subjects. Patients with higher TRACP-5b levels had higher OPG levels and Gensini scores than those with lower TRACP-5b levels. Higher TRACP-5b levels were associated with an increased number of diseased vessels. A multivariate linear regression analysis showed that the OPG level and the number of diseased vessels or the Gensini score were significantly and independently associated with the TRACP-5b level.

Conclusions: These data indicate that the TRACP-5b level is significantly associated with the OPG level and with the severity and extent of coronary atherosclerosis in CAD patients.

Key words: Tartrate-resistant acid phosphatase-5b, Osteoprotegerin, Number of diseased vessels, Gensini score, Coronary artery disease

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known as type-5 acid phosphatase and purple acid phosphatase, is an iron-containing glycoprotein expressed in high amounts by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells\(^{17}\). Two forms of TRACP circulate in the human blood: TRACP-5a, which is derived from macrophages and dendritic cells, and TRACP-5b, which is derived from osteoclasts. It has been suggested that TRACP-5a is a biomarker of the systemic inflammatory burden in patients with chronic inflammatory diseases, including rheumatoid arthritis (RA) and sarcoidosis\(^{17,18}\). Recent studies have shown that the serum TRACP-5b activity is correlated with bone resorption markers and osteoclast numbers\(^{17}\). In addition, the serum TRACP-5b activity is a promising new marker with clinical relevance in diseases such as RA, chronic kidney disease (CKD), and bone metastasis in various cancers that are associated with increased bone resorption and destruction\(^{17,19}\). Thus, TRACP-5b is a sensitive and specific marker of bone resorption and bone remodeling in patients with RA, CKD, and cancer with bone metastasis.

However, no studies have investigated the effects of TRACP-5b on the severity and extent of coronary atherosclerosis in CAD patients. Thus, the aim of the present study was to investigate whether or not the serum levels of TRACP-5b are associated with the severity and extent of coronary atherosclerosis in CAD patients.

### Methods

#### Study Population

A total of 71 selected (nonconsecutive) CAD patients (57 men and 14 women; mean age: 69.0 ± 9.7 years) were enrolled in the current study. A total of 28 age- and gender-matched normal healthy subjects (20 men and 8 women; mean age: 69.2 ± 6.3 years) were included for comparison. All of the patients and normal healthy subjects were recruited from an outpatient clinic of Tama-Nagayama Hospital, Nippon Medical School, between October 2012 and December 2013. All of the patients underwent coronary angiography (CAG) prior to their enrollment in this study.

This study included CAD patients and normal healthy subjects who were ≥ 20 years of age. CAD was defined as stable angina with or without a history of myocardial infarction and found to have ≥ 70% stenosis in at least 1 branch of the main coronary arteries on CAG.

All patients presenting with the clinical signs of cardiogenic shock, acute decompensated heart failure, acute myocardial infarction (AMI) or unstable angina within the previous month, vasospastic angina, stroke, peripheral artery disease (PAD), or patients who had undergone percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) prior to enrollment, and osteoporosis patients were excluded from the present study. Patients with CAD and normal healthy subjects with clinical signs of acute infection, autoimmune disorders, severe renal (serum creatinine > 1.5 mg/dL), hepatic disease or suspected malignancies were excluded from the study. Normal healthy subjects with osteoporosis and those with a history of cardiovascular diseases, including coronary artery disease, heart failure, cardiomyopathy and valvular heart disease, or with stroke or PAD were excluded from this study. In addition, normal healthy subjects with a history of hypertension, dyslipidemia or diabetes who had been receiving these medications before enrollment were also excluded from the present study.

Eleven patients had a history of non-ST elevation myocardial infarction and did not undergo CAG or PCI in the acute phase because they had been admitted to other hospitals more than 12 h after the onset of AMI without chest pain. They were referred from these hospitals for further examinations, including CAG and noninvasive exercise tests. Therefore, we performed the exercise treadmill test or stress myocardial perfusion imaging to detect myocardial ischemia at an outpatient clinic of our hospital, where these patients were found to be positive for myocardial ischemia. They were therefore allowed to participate in this study and underwent CAG for the first time at our hospital after enrollment in the present study.

The left ventricular ejection fraction (LVEF) was measured in each patient by echocardiography using a LOGIQ 7 system (GE Healthcare, Milwaukee, WI, USA) within one week of the measurement of the biochemical markers and was analyzed by two blinded trained cardiologists.

The risk factors in the patient population were as follows: smoking, with subjects defined as active smokers if they smoked at least 1 cigarette per day for ≥ 1 year; hypertension, defined as a systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg or treatment with antihypertensive medication; diabetes, defined as a fasting plasma glucose (FPG) level of ≥ 126 mg/dL, a casual plasma glucose level of ≥ 200 mg/dL or a 2-h plasma glucose level during a 75-g oral glucose tolerance test of ≥ 200 mg/dL or treatment with anti-diabetic medication; and dyslipidemia, defined as an overnight fasting serum total cholesterol (TC) level of ≥ 220 mg/dL, a triglyceride (TG) level of ≥ 150 mg/dL, a low-density lipoprotein (LDL) cholesterol level of ≥ 140 mg/dL, a high-density lipoprotein (HDL) cholesterol level of ≤ 40 mg/dL, or treatment with lipid-modulating medications prior to enrollment in the study. The LDL cholesterol
The investigation protocol was approved by the Institutional Ethics Committee of Nippon Medical School, Tama-Nagayama Hospital. All patients with CAD and normal healthy subjects provided their written informed consent prior to enrollment in the study.

CAG

Patients underwent CAG for the first time at entry. The CAG findings were estimated at entry and were based on the consensus opinion of two experienced cardiologists. Coronary angiograms were scored by two techniques, as follows:

1) The number of diseased coronary vessels (severity score), including the coronary arteries with ≥70% (in diameter) stenosis, with scores ranging from 0 to 320.

2) The Gensini score is used to evaluate the extent of coronary atherosclerosis. According to this scoring system, the angiographic severity of the lesion was rated as follows: 1 point = 0%–25%, 2 points = 25%–50%, 4 points = 50%–75%, 8 points = 75%–90%, 16 points = 90%–99% and 32 points = 100% (a completely occluded vessel). The score was multiplied by the segment location multiplying factor (left main coronary artery = 5 points, proximal left descending branch and left circumflex artery lesion = 2.5 points, middle left ending artery lesion = 1.5 points, first diagonal branch and the obtuse marginal branches and right coronary artery lesion = 1 point, and second diagonal and left circumflex artery posterolateral branch lesion = 0.5 points), and the results were then summed to obtain the Gensini score for each patient.21)

Laboratory Measurements

At entry, the plasma levels of OPG were measured with a specific enzyme-linked immunosorbent assay (ELISA) using a commercially available system (R&D Systems). The intra- and inter-assay coefficients of variation were ≤5% for all of the ELISAs.

The serum levels of TRACP-5b were measured using a fragment-absorbed immune-capture enzyme assay (SRL Inc., Tokyo, Japan), and the intra- and inter-assay coefficients of variation were also ≤5% for all of the assays.

The high-sensitivity C-reactive protein (hsCRP) levels were measured using an immunoturbidimetry assay. The estimated glomerular filtration rate (eGFR) was calculated according to the following equation for Japanese subjects, as recommended by the Japanese Society of Nephology: eGFR (mL/min/1.73 m²) = 194 × serum creatinine⁻¹.₀⁹₄ (mg/dL) × age⁻₀.₂₈₇ (years) × 0.₇₃₉, if female. CKD was defined as eGFR < 60 mL/min/1.73 m². The serum levels of albumin, TC, TG, HDL cholesterol, glycated hemoglobin A1c (HbA1c; NGSP; National Glycohemoglobin Standardization Program) and calcium (Ca) were measured by routine techniques using an automated analyzer. The calcium concentration was adjusted using the following equation by Payne: adjusted calcium concentration (mg/dL) = measured serum calcium (mg/dL) +

Table 1. The demographic and clinical characteristics of the 71 CAD patients and 28 age and gender-matched normal control subjects.

|                      | CAD (n=71) | Normal (n=28) | P value |
|----------------------|------------|---------------|---------|
| Age (years)          | 69.0 ± 9.7 | 69.2 ± 6.3    | 0.931   |
| Gender (male, %)     | 57 (80.3)  | 20 (71.4)     | 0.422   |
| Body mass index (kg/m²) | 24.7 ± 4.0 | 23.3 ± 1.6    | 0.075   |
| Systolic BP (mmHg)   | 129 ± 19   | 122 ± 4       | 0.056   |
| Heart rate (beats/min) | 68 ± 9     | 70 ± 7        | 0.117   |
| TRACP-5b (mU/dL)     | 362.5 (285.0, 482.0) | 300.5 (248.0, 334.6) | 0.001   |
| hs-CRP (mg/L)        | 0.76 (0.28, 2.61) | 0.27 (0.18, 0.29) | <0.001  |
| Total cholesterol (mg/dL) | 162 ± 31   | 174 ± 12      | 0.053   |
| LDL-cholesterol (mg/dL) | 113 ± 33   | 100 ± 12      | 0.036   |
| HDL-cholesterol (mg/dL) | 52 ± 12    | 55 ± 6        | 0.227   |
| Triglycerides (mg/dL) | 115 (86, 149) | 96 (79, 108)   | 0.015   |
| FPG (mg/dL)          | 122 ± 30   | 92 ± 8        | <0.001  |
| eGFR (mL/min/1.73 m²) | 61.5 ± 15.9 | 61.5 ± 8.2    | 0.998   |

The data are expressed as the mean ± SD or median (interquartile range).

CAD, patients with coronary artery disease; Normal, normal healthy subjects; BP, blood pressure; TRACP, tartrate-resistant acid phosphatase; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate.
Table 2. The baseline demographic and clinical characteristics of the 71 CAD patients in the TRACP-5b quartiles

|                      | Q1: ≤285 (n = 18) | Q2: 286-362 (n = 16) | Q3: 363-482 (n = 20) | Q4: >482 (n = 17) | P value |
|----------------------|-------------------|----------------------|----------------------|-------------------|---------|
| Age (years)          | 65.2 ± 10.3       | 64.7 ± 8.3           | 70.3 ± 7.6           | 75.8 ± 9.0        | 0.001   |
| Gender (male, %)     | 15 (83.3)         | 14 (87.5)            | 17 (85.0)            | 11 (64.7)         | 0.318   |
| Systolic blood pressure (mmHg) | 129 ± 20    | 136 ± 19             | 143 ± 19             | 134 ± 19          | 0.157   |
| Heart rate (beats/min) | 70 ± 14       | 68 ± 7               | 60 ± 8               | 62 ± 9            | 0.007   |
| Body mass index (kg/m²) | 24.8 ± 3.5  | 27.1 ± 4.8           | 23.6 ± 2.5           | 22.9 ± 4.0        | 0.012   |
| Waist circumference (cm) | 91.3 ± 10.3 | 97.7 ± 11.7          | 86.5 ± 7.3           | 86.2 ± 9.8        | 0.003   |
| Active smoker (%)    | 15 (83.3)         | 13 (81.3)            | 18 (90.0)            | 11 (64.7)         | 0.271   |
| Hypertension (%)     | 17 (94.4)         | 16 (100)             | 18 (90.0)            | 17 (100)          | 0.364   |
| Diabetes (%)         | 11 (61.1)         | 8 (50.0)             | 8 (40.0)             | 5 (29.4)          | 0.270   |
| Hyperuricemia (%)    | 4 (22.2)          | 6 (43.8)             | 6 (30.0)             | 8 (47.1)          | 0.373   |
| Prior PCI/CABG/Stroke/PAD (%) | 0/0/0/0 (0) | 0/0/0/0 (0)         | 0/0/0/0 (0)         | 0/0/0/0 (0)       | 1.000   |
| History of myocardial infarction (%) | 2 (11.1)     | 3 (18.8)             | 3 (15.0)             | 3 (17.6)          | 0.928   |
| ACEI use (%)         | 1 (5.6)           | 2 (12.5)             | 4 (20.0)             | 3 (17.6)          | 0.599   |
| Angiotensin receptor blocker use (%) | 15 (83.3)   | 12 (75.0)            | 14 (70.0)            | 12 (70.6)         | 0.778   |
| Calcium channel blocker use (%) | 6 (33.3)    | 8 (50.0)             | 8 (40.0)             | 10 (45.1)         | 0.446   |
| Anti-platelet agents use (%) | 18 (100)   | 16 (100)             | 19 (95.0)            | 17 (100)          | 0.460   |
| Statin use (%)       | 14 (77.8)         | 11 (68.8)            | 15 (75.0)            | 13 (76.5)         | 0.936   |
| Osteoprotegerin (pmol/L) | 92.9 (72.2, 107.0) | 86.9 (51.4, 106.5)  | 106.5 (69.0, 124.0)  | 135.0 (92.0, 165.0) | 0.012 |
| High-sensitivity C-reactive protein (mg/L) | 0.57 (0.23, 0.93) | 1.05 (0.46, 3.47) | 0.61 (0.27, 7.43) | 0.90 (0.27, 1.62) | 0.393 |
| LDL cholesterol (mg/dL) | 98 ± 16         | 96 ± 28              | 89 ± 15              | 78 ± 27           | 0.035   |
| HDL cholesterol (mg/dL) | 53 ± 11        | 57 ± 14              | 57 ± 13              | 58 ± 15           | 0.620   |
| Triglycerides (mg/dL) | 138 (114, 211) | 92 (69, 108)         | 91 (75, 135)         | 114 (88, 163)     | 0.523   |
| Calcium (mg/dL)      | 9.2 ± 0.5         | 9.1 ± 0.4            | 9.0 ± 0.3            | 9.3 ± 0.7         | 0.238   |
| Albumin (g/dL)       | 4.3 ± 0.4         | 4.1 ± 0.4            | 4.0 ± 0.5            | 4.2 ± 0.5         | 0.215   |
| eGFR (mL/min/1.73 m²) | 59.3 ± 13.7       | 64.8 ± 20.9          | 58.3 ± 18.6          | 52.6 ± 17.8       | 0.286   |
| Chronic kidney disease (%) | 8 (44.4)   | 5 (31.3)             | 8 (40.0)             | 9 (52.9)          | 0.644   |
| Fasting plasma glucose (mg/dL) | 130 ± 29   | 128 ± 55             | 107 ± 27             | 109 ± 19          | 0.084   |
| HbA1c (%)            | 6.2 ± 0.8         | 6.4 ± 1.1            | 5.9 ± 0.5            | 6.1 ± 0.4         | 0.317   |
| Gensini score        | 38.0 (30.9, 52.0) | 44.8 (32.4, 57.1)   | 54.0 (35.6, 86.3)    | 67.0 (43.5, 96.0) | 0.038   |

Number of diseased vessels

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| 1 |   |   |   |   |   |
| 2 |   |   |   |   |   |
| 3 |   |   |   |   |   |
| 4 |   |   |   |   |   |
| 5 |   |   |   |   |   |
| 6 |   |   |   |   |   |

LVEF (%) 58.1 ± 11.7 57.4 ± 14.8 51.0 ± 14.9 60.0 ± 10.2 0.180

The data are expressed as the mean ± SD or median (interquartile range). TRACP, tartrate-resistant acid phosphatase (mU/dL); differences in TRACP-5b levels among the quartiles (Q1, Q2, Q3 and Q4); PCI, percutaneous coronary intervention, CABG, coronary artery bypass grafting; PAD, peripheral artery disease; ACEI, angiotensin-converting enzyme inhibitor; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.

(4 × serum albumin [g/dL]).

Statistical Analysis

The results are presented as the mean ± standard deviation or the median (interquartile range) for continuous variables and the percentage of the total number of patients for categorical variables. Student’s t-test for independent samples and the chi-squared test were used to compare the continuous and categorical variables, respectively. The TRACP-5b, OPG, TG and hsCRP levels had skewed distributions. Thus, the Mann–Whitney U test was used for unpaired comparisons between the groups, and Wilcoxon’s signed-rank test was used for paired comparisons within the groups. The bivariate correlation between the parameters was assessed with the Pearson or Spearman correlation (r) coefficients for a normal or skewed distribution, respectively. The TRACP-5b levels were compared across the Gensini score quartiles and the number of coronary vessels (one-, two- and three-vessel...
Table 3. The baseline demographic and clinical characteristics of the 71 CAD patients in the Gensini score quartiles

|                      | Q1: ≤35.0 (n = 20) | Q2: 35.0-48.5 (n = 16) | Q3: 48.6-71.0 (n = 17) | Q4: >71.0 (n = 18) | P value |
|----------------------|---------------------|------------------------|------------------------|-------------------|---------|
| Age (years)          | 69.1 ± 7.4          | 65.5 ± 10.3            | 69.8 ± 9.5             | 71.5 ± 11.2       | 0.340   |
| Gender (male, %)     | 19 (95.0)           | 12 (75.0)              | 14 (72.4)              | 12 (66.7)         | 0.160   |
| Systolic blood pressure (mmHg) | 129 ± 20               | 136 ± 19              | 143 ± 19               | 134 ± 19          | 0.140   |
| Heart rate (beats/min) | 70 ± 14               | 68 ± 7                | 60 ± 8                 | 62 ± 9            | 0.190   |
| Body mass index (kg/m²) | 24.8 ± 3.5           | 27.1 ± 4.8            | 23.6 ± 2.5            | 22.9 ± 4.0        | 0.525   |
| TRACP-5b (mU/dL)     | 315.5 (270.0, 407.3) | 349.0 (247.8, 461.8)  | 362.0 (285.0, 601.5)  | 422.5 (341.3, 601.9) | 0.038   |
| CRP (mg/L)           | 83.1 (57.0, 120.8)   | 112.0 (81.0, 148.0)   | 82.5 (72.8, 98.2)      | 98.3 (63.6, 111.0) | 0.403   |
| LDL cholesterol (mg/dL) | 111 ± 29              | 124 ± 42              | 108 ± 26               | 112 ± 34          | 0.520   |
| HDL cholesterol (mg/dL) | 53 ± 14                | 53 ± 9                | 51 ± 10                | 51 ± 14          | 0.869   |
| Calcium (mg/dL)      | 9.0 ± 0.3            | 9.1 ± 0.4             | 8.8 ± 1.6             | 8.8 ± 2.3        | 0.923   |
| eGFR (mL/min/1.73 m²) | 63.5 ± 10.6           | 65.9 ± 12.6           | 63.7 ± 18.8           | 53.1 ± 18.5      | 0.073   |
| HbA1c (%)            | 6.4 ± 1.2            | 6.1 ± 0.7            | 6.0 ± 0.5             | 5.9 ± 0.5        | 0.043   |
| Number of diseased vessels |                  |                        |                        |                  | <0.001  |
| 1                    | 6                   | 5                     | 1                     | 0                |         |
| 2                    | 12                  | 8                     | 7                     | 3                |         |
| 3                    | 2                   | 3                     | 9                     | 15               |         |
| LVEF (%)             | 58.9 ± 12.7          | 57.7 ± 13.9           | 53.6 ± 12.4           | 50.5 ± 15.2      | 0.041   |
| Hypertension (%)     | 18 (90.0)           | 15 (93.8)             | 17 (100)              | 18 (100)        | 0.340   |
| Diabetes (%)         | 12 (60.0)           | 8 (50.0)              | 6 (35.3)              | 6 (33.3)        | 0.306   |
| Active smoker (%)    | 17 (85.0)           | 12 (75.0)             | 12 (70.6)             | 16 (88.9)       | 0.491   |

The data are expressed as the mean ± SD or median (interquartile range). TRACP, tartrate-resistant acid phosphatase; differences in Gensini scores among the quartiles (Q1, Q2, Q3 and Q4); CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.

Results

The baseline demographic and clinical characteristics of the 71 CAD patients and 28 normal healthy subjects are shown in Table 1. The LDL-cholesterol, TG, FPG, hsCRP, and TRACP-5b levels were significantly higher in patients with CAD than in normal healthy subjects. The body mass index (BMI) and SBP tended to be higher in patients with CAD than in normal healthy subjects, whereas the TC levels tended to be lower in patients with CAD than in normal healthy subjects. However, there were no significant differences between the two groups in terms of the age, gender, heart rate, HDL cholesterol or eGFR values (Table 1).

The baseline demographic and clinical characteristics of the 71 CAD patients in the TRACP-5b quartiles are summarized in Table 2. The serum levels of TRACP-5b were divided into quartiles, as follows: first (Q1; n = 18), ≤285 mU/dL; second (Q2; n = 16), 286–362 mU/dL; third (Q3; n = 20), 363–482 mU/dL; and fourth (Q4; n = 17), >482 mU/dL.

Patients with higher TRACP-5b levels tended to be older and had higher OPG levels and Gensini scores than those with lower TRACP-5b levels, and were significantly associated with an increased number of diseased coronary vessels. Patients with higher TRACP-5b levels also had lower heart rate, BMI, waist circumference and LDL cholesterol values (Table 2), and...
tended to have lower FPG levels than those with lower TRACP-5b levels. There were no significant differences in the other variables among the quartiles, including the prevalence of a history of myocardial infarction (Table 2). In addition, neither group included any patients with a history of PCI, CABG, stroke, or PAD prior to enrollment (Table 2).

The demographic and clinical characteristics of the CAD patients in the Gensini score quartiles are summarized in Table 3. The Gensini scores were divided into quartiles, as follows: first (Q1; n = 20), ≤ 55.0; second (Q2; n = 16), 55.0–48.5; third (Q3; n = 17), 48.6–71.0; and fourth (Q4; n = 18), > 71.0. Patients with higher Gensini scores had higher TRACP-5b levels than those with lower scores and were significantly associated with an increased number of diseased coronary vessels. Patients with higher Gensini scores also had lower HbA1c and LVEF values (Table 3), and tended to have lower eGFR levels than those with lower scores. There were no significant differences in any other variables among the quartiles (Table 3).

The demographic and clinical characteristics of the patients in the three groups according to the number of diseased vessels (one-vessel, two-vessel, and three-vessel disease) are shown in Table 4. Patients with an increased number of diseased vessels had higher TRACP-5b and Gensini score values, and unexpectedly had a lower prevalence of diabetes than those with fewer diseased vessels. Those with an increased number of diseased vessels also tended to have lower HDL cholesterol and HbA1c values than those with fewer diseased vessels. There were no significant differences in any other variables among the three groups (Table 4).

The relationships between the TRACP-5b level and the number of diseased vessels and the Gensini scores are shown in Fig. 1A and 1B, respectively. Fig. 1A shows the differences in the TRACP-5b levels among the groups with one- (n = 12), two- (n = 30), and three-vessel (n = 29) disease. Higher TRACP-5b levels were significantly associated with an increased severity of CAD, as assessed by the number of diseased vessels (p = 0.018, Fig. 1A). Fig. 1B shows the differences in the TRACP-5b levels among the Gensini score quartiles (Q1, Q2, Q3, and Q4). Similarly, higher TRACP-5b levels were significantly associated with an increased extent of coronary atherosclerosis, as estimated by the Gensini scores (p = 0.038).

To elucidate the pathophysiological mechanisms by which the TRACP-5b level predicts the severity and extent of coronary atherosclerosis, we investigated the relationships between the TRACP-5b level and other variables, including the number of diseased vessels (model 1, Table 5) and the Gensini score (model

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Table 4. The baseline demographic and clinical characteristics of the 71 CAD patients according to the number of diseased vessels

|                               | One-vessel (n = 12) | Two-vessel (n = 30) | Three-vessel (n = 29) | P value |
|-------------------------------|---------------------|---------------------|----------------------|---------|
| Age (years)                  | 71.6 ± 7.8          | 66.3 ± 9.4          | 70.8 ± 10.2          | 0.115   |
| Gender (male, %)             | 9 (78.0)            | 25 (83.3)           | 23 (79.3)            | 0.817   |
| Systolic blood pressure (mmHg)| 129 ± 15            | 130 ± 18            | 127 ± 21             | 0.806   |
| Heart rate (beats/min)       | 67 ± 12             | 66 ± 8              | 70 ± 8               | 0.181   |
| Body mass index (kg/m²)      | 23.5 ± 2.3          | 25.5 ± 4.7          | 24.4 ± 3.8           | 0.307   |
| TRACP-5b (mU/dL)             | 356.0 (277.5, 407.3)| 295.0 (255.3, 449.3)| 430.0 (332.5, 612.0)| 0.018   |
| Osteoprotegerin (pmol/L)     | 113.5 (83.7, 153.3) | 92.0 (64.7, 113.8)  | 81.3 (56.7, 108.5)   | 0.126   |
| High-sensitivity CRP (mg/L)  | 0.36 (0.22, 0.60)   | 0.69 (0.27, 0.96)   | 1.11 (0.42, 3.27)    | 0.201   |
| LDL cholesterol (mg/dL)      | 104 ± 38            | 115 ± 33            | 115 ± 31             | 0.553   |
| HDL cholesterol (mg/dL)      | 58 ± 12             | 51 ± 12             | 51 ± 12              | 0.075   |
| Calcium (mg/dL)              | 9.1 ± 0.3           | 8.9 ± 1.3           | 8.8 ± 1.7            | 0.836   |
| eGFR (mL/min/1.73 m²)        | 60.7 ± 12.6         | 65.5 ± 15.0         | 57.6 ± 17.5          | 0.166   |
| HbA1c (%)                    | 6.5 ± 0.6           | 6.1 ± 1.1           | 5.9 ± 0.5            | 0.066   |
| Gensini score                | 35.5 (26.0, 46.3)   | 37.5 (31.9, 53.4)   | 71.0 (54.2, 102.5)   | < 0.001 |
| LVEF (%)                     | 54.4 ± 13.6         | 56.6 ± 13.4         | 54.1 ± 14.4          | 0.767   |
| Hypertension (%)             | 11 (91.7)           | 28 (93.3)           | 29 (100)             | 0.329   |
| Diabetes (%)                 | 9 (75.0)            | 14 (46.7)           | 9 (31.0)             | 0.035   |
| Active smoker (%)            | 25 (83.3)           | 22 (75.9)           | 22 (75.9)            | 0.739   |

The data are expressed as the mean ± SD or median (interquartile range). TRACP, tartrate-resistant acid phosphatase; differences in the number of diseased vessels among the three groups (one-vessel, two-vessel, three-vessel); CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.
arteries occur via an active process that resembles bone formation and bone remodeling. OPG is a glycoprotein and a member of the tumor necrosis factor (TNF)-related family. It is expressed by endothelial cells, vascular smooth muscle cells, and osteoblasts, and acts as a decoy receptor for the receptor activator of nuclear factor κB (RANK) ligand (RANKL). OPG can be detected in the atherosclerotic lesions of CAD patients. Elevated concentrations of circulating OPG have been significantly associated with aortic plaque as well as with the increased prevalence of, and severity of CAD.

Mangan et al. showed that the upregulation of OPG causes the TNF-α sensitization of the endothelial cells and the migration of the monocytes to the vascular intima, which are key processes in the development of vascular atherosclerosis and calcification. Thus, OPG may play important roles in the pathogenesis and development of coronary atherosclerosis and calcification in CAD patients.

Discussion

Atherosclerosis and calcification of the coronary arteries occur via an active process that resembles bone formation and bone remodeling. OPG is a glycoprotein and a member of the tumor necrosis factor (TNF)-related family. It is expressed by endothelial cells, vascular smooth muscle cells, and osteoblasts, and acts as a decoy receptor for the receptor activator of nuclear factor κB (RANK) ligand (RANKL). OPG can be detected in the atherosclerotic lesions of CAD patients. Elevated concentrations of circulating OPG have been significantly associated with aortic plaque as well as with the increased prevalence of, and severity of CAD. Mangan et al. showed that the upregulation of OPG causes the TNF-α sensitization of the endothelial cells and the migration of the monocytes to the vascular intima, which are key processes in the development of vascular atherosclerosis and calcification. Thus, OPG may play important roles in the pathogenesis and development of coronary atherosclerosis and calcification in CAD patients.

Tartrate-resistant acid phosphate (TRACP) is an iron-containing glycoprotein that is highly expressed by bone-resorbing osteoclasts, inflammatory macrophages, and dendritic cells. Two forms of TRACP are known: TRACP-5a and TRACP-5b. A multiple linear regression analysis showed that the OPG level (β coefficient = 0.355, p = 0.003), eGFR (β coefficient = −0.264, p = 0.021), and the number of diseased vessels (β coefficient = 0.365, p < 0.001) were significant and independent determinants of the TRACP-5b level (model 1, Table 5). Furthermore, the OPG level (β coefficient = 0.291, p = 0.021), the use of ACEIs (β coefficient = 0.248, p = 0.024), and the Gensini score (β coefficient = 0.240, p = 0.036) were significantly and independently associated with the TRACP-5b level (model 2, Table 6).

In addition, we also investigated the relationships between the TRACP 5-level and the OPG level, patient age, and eGFR. The TRACP 5b-level was significantly and positively associated with the OPG level (r = 0.353, p < 0.001, Fig. 2) and patient age (r = 0.428, p < 0.001, not shown in Figure). The TRACP 5b level was significantly and negatively associated with the eGFR (r = −0.256, p = 0.032, not shown in Figure).

Fig. 1. Relationships between the TRACP-5b levels and the number of diseased vessels and the Gensini scores. (A) The differences in the TRACP-5b levels among the groups with one- (n = 12), two- (n = 30) and three-vessel (n = 29) disease. Higher TRACP-5b levels were significantly associated with an increased severity of CAD, as assessed by the number of diseased vessels (p = 0.018). (B) The differences in the TRACP-5b levels among the Gensini score quartiles (Q1, Q2, Q3, and Q4). Similarly, higher TRACP-5b levels were significantly associated with an increased extent of coronary atherosclerosis as estimated by the Gensini score (p = 0.038). The box and whisker plots show the median levels and the 25th and 75th interquartile ranges (delineated by the bottom and top of each box), and the circles represent outliers (> 1.5% of the 75% confidence interval).
circulate in the human blood: TRACP-5a, which is derived from macrophages and dendritic cells, and TRACP-5b, which is derived from osteoclasts\(^\text{17, 18}\). Recent studies have shown that serum TRACP-5b activity is correlated with bone resorption markers and osteoclast numbers\(^\text{17}\). In addition, the serum TRACP-5b activity is a promising new marker with clinical relevance in diseases such as RA, CKD, and bone metastasis in various cancers with increased bone resorption and destruction\(^\text{17, 19}\). Thus, both OPG and TRACP-5b are sensitive and specific markers of bone resorption and bone remodeling.

However, no previous studies have investigated the relationship between the serum levels of TRACP-5b and the severity or extent of coronary atherosclerosis in CAD patients. The present study provides important evidence showing that the serum levels of TRACP-5b are significantly associated with the severity of CAD (as assessed by the number of diseased coronary vessels) and the extent of coronary atherosclerosis (as indicated by the Gensini score). The patients with higher TRACP-5b levels tended to be older and had higher OPG levels and lower BMI and LDL cholesterol values than those with lower TRACP-5b levels. In addition, we also showed that the serum levels of TRACP-5b were significantly higher in patients with CAD than in normal healthy subjects. Furthermore, the patients with higher Gensini scores had higher TRACP-5b levels, as did those with an increased number of diseased vessels.

A multivariate linear regression analysis showed that the OPG levels were significantly and independently correlated with the serum levels of TRACP-5b. These findings suggest that the serum levels of TRACP-5b are significantly associated with the plasma levels of OPG, which are a sensitive and specific marker of bone reabsorption and bone remodeling in CAD patients.

Finally, the present study demonstrated, for the first time that the serum levels of TRACP-5b are significantly and independently associated with the plasma levels of OPG and with the severity and extent of coronary atherosclerosis in CAD patients. These data provide important information for determining optimum therapeutic strategies for patients with CAD.

The mechanisms underlying the significant relationship between the serum levels of TRACP-5b and the severity and extent of coronary atherosclerosis in CAD patients are unknown. Epidemiological studies have shown that osteoporosis, which contributes to morbidity and mortality independently of age, often coexists with atherosclerosis, calcification and cardiovascular diseases, including CAD\(^\text{28-30}\).

Parham \textit{et al} \(^\text{31}\) showed that an atherogenic high-fat diet induced dyslipidemia and lipid oxidation, such as oxidized LDL cholesterol, which promoted atherosclerosis and reduced bone mineralization in mice. Tintut \textit{et al} \(^\text{32}\) showed that TNF-\(\alpha\) induced by a high-fat diet promoted \textit{in vitro} vascular calcination via the cyclic AMP pathway.

Tintut \textit{et al} \(^\text{33}\) also showed that the osteoclastic activity as indicated by the TRACP activity was also significantly higher in LDL receptor knockout mice, which showed elevated serum cholesterol levels and
CAD37). OPG is a key factor in bone remodeling and is a sensitive and specific marker of bone resorption2), while the serum TRACP-5b activity is a promising new marker with clinical relevance in diseases such as RA, CKD and bone metastasis in various cancers that are associated with increased bone resorption and destruction17, 19). These data suggest that both TRACP-5b and OPG levels are sensitive and specific markers of bone resorption and bone remodeling in patients with CKD and RA. However, the TRACP-5b levels may be superior to the OPG levels in predicting the severity and extent of coronary atherosclerosis in patients with CAD. These discrepancies may be related to differences in the length (short) of the follow-up period, the frequent uses of modern pharmacotherapy, including statins, ACEI or angiotensin receptor blocker, the evaluation of relatively low-risk patient populations (with or without CAD), or the use of a small sample size.

Finally, we hypothesize that elevated TRACP-5b activity may be associated with the OPG levels as well as with the severity and extent of coronary atherosclerosis in CAD patients.

**Study Limitations**

The present study is associated with several possible limitations. The most significant limitation is the small sample size. In addition, we only studied CAD...
patients; as such, our results may not be applicable to the general population. Second, the majority of CAD patients had been treated with various medications, including statins, prior to their enrollment in the present study. Thus, all of the eligible patients in the present study were stabilized with the above medications, suggesting that the TRACP-5b and OPG levels may have already been stabilized by these medications. Third, we did not estimate the coronary calcification using computed tomography. Fourth, we only measured the serum levels of TRACP-5b at entry.

Conclusion
In conclusion, the serum levels of TRACP-5b were significantly and independently associated with the OPG levels as well as with the severity and extent of coronary atherosclerosis in CAD patients. Further studies are needed to confirm these findings.

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Disclosures
The authors declare no conflicts of interest in association with the present study.

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