Identification of Adipsin as a Novel Prognostic Biomarker in Patients With Coronary Artery Disease

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**Background**—Circulating proteins are exposed to vascular endothelial layer and influence their functions. Among them, adipsin is a member of the trypsin family of peptidases and is mainly secreted from adipocytes, monocytes, and macrophages, catalyzing the rate-limiting step of the alternative complement pathway. However, its pathophysiological role in cardiovascular disease remains to be elucidated. Here, we examined whether serum adipsin levels have a prognostic impact in patients with coronary artery disease.

**Methods and Results**—In 370 consecutive patients undergoing diagnostic coronary angiography, we performed a cytokine array analysis for screening serum levels of 50 cytokines/chemokines and growth factors. Among them, classification and regression analysis identified adipsin as the best biomarker for prediction of their long-term prognosis (median 71 months; interquartile range, 55–81 months). Kaplan–Meier curve showed that higher adipsin levels (≥2400 ng/mL) were significantly associated with all-cause death (hazard ratio [HR], 4.2; 95% CI, 1.7–10.6 [<0.001]) and rehospitalization (HR, 2.4; 95% CI, 1.7–3.5 [<0.001]). Interestingly, higher high-sensitivity C-reactive protein levels (≥1 mg/L) were significantly correlated with all-cause death (HR, 3.2; 95% CI, 1.7–5.9 [<0.001]) and rehospitalization (HR, 1.5, 95% CI, 1.1–1.9 [<0.001]). Importantly, the combination of adipsin (≥2400 ng/mL) and high-sensitivity C-reactive protein (≥1 mg/L) was more significantly associated with all-cause death (HR, 21.0; 95% CI, 2.9–154.1 [<0.001]). Finally, the receiver operating characteristic curve demonstrated that serum adipsin levels predict the death caused by acute myocardial infarction in patients with coronary artery disease (C-statistic, 0.847).

**Conclusions**—These results indicate that adipsin is a novel biomarker that predicts all-cause death and rehospitalization in patients with coronary artery disease, demonstrating the novel aspects of the alternative complementary system in the pathogenesis of coronary artery disease. ([J Am Heart Assoc. 2019;8:e013716. DOI: 10.1161/JAHA.119.013716.])

**Key Words:** atherosclerosis • biomarker • coronary artery disease • prognosis

Cardiovascular risk factors, such as hypertension, diabetes mellitus (DM), dyslipidemia, and smoking, have been used for “risk classification” of patients with coronary artery disease (CAD). The assessment of these factors is important for predicting future cardiovascular events. In particular, acute myocardial infarction (AMI) is a fatal cardiovascular event and is the most common cause of sudden cardiac death. AMI has become an important social issue because of the increasing number of patients with ischemic cardiomyopathy and resultant heart failure. It is well known that the levels of hsCRP (high sensitivity C-reactive protein), brain natriuretic peptide (BNP), D-dimer, and fibrinogen can predict the incidence of future cardiovascular events. However, these biomarkers are also increased in patients with inflammatory diseases. Thus, it is important to develop a novel biomarker for prediction of CAD and cardiovascular events. Circulating cytokines/chemokines and growth factors regulate endothelial functions and promote the development of vascular diseases. Indeed, vascular diseases are characterized by endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation, and inflammatory cell accumulation in the adventitial adipose tissues. Cardiovascular risk factors (eg, hypertension, DM, dyslipidemia, smoking, and aging) induce vascular oxidative stress and trigger a variety of vascular disorders including CAD. Enhanced vascular oxidative stress promotes the secretion of cytokines/chemokines and growth factors from vascular wall.
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Clinical Perspective

What Is New?

- Serum adipsin levels predict all-cause death in patients with coronary artery diseases.
- Serum adipsin levels predict future incidences of acute myocardial infarction in patients with coronary artery diseases.
- Elevating serum adipsin levels are associated with cancer death in patients with coronary artery diseases.

What Are the Clinical Implications?

- Adipsin plays an important role in the pathogenesis of coronary artery diseases and is also useful as a novel biomarker and therapeutic target.
- Serum adipsin level is a useful biomarker to predict future incidences of acute myocardial infarction and to predict new onset of cancer.

Thus, thrombin activatable fibrinolysis inhibitor may play a crucial role in the regulation of the complement system, which causes endothelial dysfunction, vascular smooth muscle cell proliferation, and adventitial inflammation.27 When considering the role of adipsin in the alternative complement pathway and its strong expression in the adventitial adipose tissue, we hypothesized that adipsin may potentially be involved in the pathogenesis of CAD. In the present study, we thus tested our hypothesis that serum levels of adipsin are a useful prognostic biomarker in those patients.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

The ethical review board of Tohoku University approved the study protocol, and written informed consent was obtained from all patients (No. 2008-470). From January 2008 to June 2011, a total of 377 patients were referred for diagnostic cardiac catheterization for evaluation of chest pain and/or ECG abnormalities at our Tohoku University Hospital. The patients enrolled had evidence of myocardial ischemia on exercise ECG and/or myocardial radionuclide imaging. Patients with unstable angina or AMI were excluded from the present study. Seven of the patients were lost to follow-up attributable to the Great East Japan earthquake disaster, March 11, 2011.

Evaluation of Coronary Artery Stenosis

Two experienced cardiologists, who were blinded to the patients’ data, including serum levels of cytokines/chemokines and growth factors, evaluated the coronary angiograms. The severity of coronary stenosis was assessed according to the American Heart Association standards. A narrowing of the lumen by >51% of the diameter was considered as a clinically significant stenosis.

Baseline Measurements

In all patients, the medical history was recorded, including details of any previous myocardial infarction, previous revascularization, angina pectoris, hypertension, previous stroke or transient ischemic attacks, DM, and smoking status. Patients with hypertension were regarded as being at risk if their blood pressure was ≥140/90 mm Hg or if they had a history of antihypertensive drug use. Patients with DM were regarded as being at risk if their fasting glucose level was ≥126 mg/dL or...
if they had a history of hypoglycemic drug or insulin use. Patients with dyslipidemia were regarded as being at risk if their low-density lipoprotein cholesterol level was ≥140 mg/dL or their high-density lipoprotein cholesterol level was ≤40 mg/dL, or if they were taking a lipid-lowering drug. Fasting blood samples were collected for measurement of cytokines/chemokines and growth factors immediately before coronary angiogram from the antecubital vein with patients in the supine position. Serum samples were collected and were centrifuged for 10 minutes at 2500g within 30 minutes of blood collection, and aliquots were stored at −80°C. Serum levels of hsCRP were measured using the sandwich technique (Roche Diagnostics). Values of other laboratory parameters were obtained with an autoanalyzer at the Tohoku University Hospital.

Measurement of Cytokines/Chemokines and Growth Factors

Serum levels of cytokines/chemokines and growth factors were measured with a Bioplex system (Bio-Rad) according to the manufacturer’s instructions. Human cytokines/chemokines and growth factors were measured with commercially available kits (Bio-Rad, 27-Plex, #M50-0KCAF0Y and 21-Plex, #MFO-005KMI, #171A7002M).

Immunofluorescence Staining

For immunofluorescence staining, coronary arteries obtained from patients who died of AMI were fixed with 4% phosphate-buffered paraformaldehyde and were embedded in optimal cutting temperature. For immunostaining, we used the following primary antibodies: adipsin (200:1, Santa Cruz Biotechnology, Inc., sc-47683) and α-smooth muscle antibody (400:1, Sigma-Aldrich, 113200). Tissue sections were mounted using ProLong Diamond Antifade Mountant with 4’,6-diamidino-2-phenylindole (Thermo Fisher Scientific) and was visualized on an LSM780 confocal microscope (Carl Zeiss).

Follow-Up

Information on death, rehospitalization, and revascularization was obtained annually using follow-up questionnaires, telephone interviews, and medical records. The primary end point was the composite of all-cause death, rehospitalization, and revascularization, while the secondary end points consisted of all-cause death, rehospitalization, and revascularization. The primary and secondary end points were analyzed based on the time to the first occurrence. Composite cardiovascular death was defined as death caused by AMI, heat failure, stroke, sudden death, and other cardiovascular death. Rehospitalization was defined as hospital admission for any cause after enrollment in the present study. Revascularization was defined as undergoing percutaneous coronary intervention or coronary artery bypass grafting after enrollment in the study.

Statistical Analysis

Categorical variables were presented as numerals and percentages. Continuous variables were presented as means±SDs. Correlations between serum adipsin levels and age, BNP, or hsCRP levels were analyzed using the Spearman correlation coefficient. Differences in serum adipsin levels in terms of sex, smoking status, and the presence of hypertension, DM, or dyslipidemia were analyzed by Fisher exact test. To determine the most appropriate cutoff points for high and low serum adipsin levels, we performed classification and regression tree (CART) analysis, which is an empirical, statistical technique based on recursive partitioning of the data space to predict the response. Briefly, the models were obtained by binary splitting of the data by the value of predictors, and the split variable and split point were automatically selected from possible candidate predictor values to achieve the best fit. Then, one or both ‘‘child nodes’’ were split into 2 regions recursively, and the process continued until some stopping rule was applied. Finally, the result of this process has been represented as a binary decision tree. We also performed the CART analysis to determine the most appropriate cutoff point for levels of BNP and hsCRP. Kaplan–Meier curves were plotted based on the cutoff points of primary end point for each biomarker. Kaplan–Meier curves were plotted for the primary end point, all-cause death, rehospitalization, and revascularization relative to the cutoff points. The log-rank test was applied to compare event-free survival between groups. Cox proportional hazards model was used for univariable and multivariable analyses. To assess the nonlinear and time-varying effects in hazard ratio (HR) of the primary end point, an HR plot was plotted. All P values were 2-tailed, and a P<0.05 was considered to be statistically significant. All analyses were performed with R, version 3.1.3 (R Foundation for Statistical Computing, Vienna, http://www.R-project.org/). Additionally, an HR plot was illustrated in using the “rms” package in R.

Results

Identification of Adipsin by Multiple Screening

First, we performed cytokines array analyses to evaluate the serum levels of cytokines/chemokines and growth factors. Importantly, cytokines array showed that many cytokines were significantly elevated in patients with CAD compared with those without it. Then, we performed the CART analysis to evaluate the best biomarker for the prediction of the
primary end point, ie, the composite of all-cause death, rehospitalization, and revascularization. Importantly, among the 50 cytokines/chemokines and growth factors examined, serum levels of adipsin were identified as the best biomarker for poor prognosis in patients with CAD (Figure S1). Baseline characteristics of the 370 patients are shown in Table 1. The mean age was 64±13 years, 66% of them were men, and 49 (13%) died after a mean of 3.2 years (range, 1.5–4.8 years). Among them, 62% had hypertension, 34% had DM, 50% had dyslipidemia, and 30% had a history of smoking. The cutoff point of serum adipsin level was 400 ng/mL as determined by the CART analysis. Baseline characteristics of the groups with higher (≥400 ng/mL) and lower (<400 ng/mL) levels of serum adipsin are shown in Table 1. Additionally, serum levels of cytokines/chemokines and growth factors in the groups with higher (≥400 ng/mL) and lower (<400 ng/mL) levels of

Table 1. Baseline Patient Characteristics

|                      | All Patients (N=370) | Adipsin ≥400 ng/mL (n=260) | Adipsin <400 ng/mL (n=110) | P Value |
|----------------------|----------------------|----------------------------|---------------------------|---------|
| Age, y               | 63.9±13.1            | 66.5±11.9                  | 61.2±13.8                 | <0.001  |
| Men, %               | 66.4                 | 67.5                       | 65.2                      | 0.582   |
| Family history of IHD, % | 5.1            | 4.9                        | 5.3                       | 0.846   |
| Medical history, %   |                      |                            |                           |         |
| Hypertension         | 62.0                 | 66.0                       | 58.0                      | 0.060   |
| DM                   | 34.2                 | 39.6                       | 28.8                      | 0.010   |
| Dyslipidemia         | 50.1                 | 55.5                       | 44.7                      | 0.015   |
| Smoking, %           | 30.2                 | 34.3                       | 25.9                      | 0.037   |
| Angiographic findings, % |                |                            |                           |         |
| No coronary stenosis | 58.1                 | 54.4                       | 67.0                      |         |
| 1-Vessel disease     | 21.5                 | 23.0                       | 17.9                      |         |
| 2-Vessel disease     | 11.7                 | 13.2                       | 8.0                       |         |
| 3-Vessel disease     | 8.7                  | 9.4                        | 7.1                       |         |
| eGFR, mL/min per 1.73/m² | 56.6±28.8         | 48.3±26.8                  | 65.0±28.4                 | <0.001  |
| LVEF, %              | 38.9±34.0            | 36.1±33.8                  | 41.7±34.1                 | 0.058   |
| Total cholesterol, mg/dL | 156.4±67.3      | 147.2±69.1                 | 165.6±64.5                | 0.002   |
| LDL, mg/dL           | 88.0±46.6            | 85.9±45.1                  | 90.1±48.1                 | 0.302   |
| HDL, mg/dL           | 43.1±20.9            | 42.0±20.3                  | 44.3±21.2                 | 0.211   |
| Triglycerides, mg/dL | 120.5±110.5          | 113.8±109.8                | 127.3±111.0               | 0.158   |
| Body weight, kg      | 55.2±23.2            | 53.5±23.3                  | 56.9±22.9                 | 0.098   |
| BMI                  | 19.0±10.1            | 18.3±10.2                  | 19.6±9.9                  | 0.152   |
| Systolic BP, mm Hg   | 106.5±50.3           | 107.2±50.1                 | 105.8±50.6                | 0.743   |
| Diastolic BP, mm Hg  | 61.6±29.6            | 61.1±28.9                  | 62.1±30.3                 | 0.681   |
| HbA1c                | 5.9±1.4              | 6.0±1.3                    | 5.8±1.4                   | 0.005   |
| hsCRP, mg/L          | 2.0±3.2              | 2.2±3.2                    | 1.8±3.2                   | 0.221   |
| Medication, %        |                      |                            |                           |         |
| Aspirin              | 26.3                 | 34.3                       | 18.2                      | <0.001  |
| β-Blockers           | 32.3                 | 34.7                       | 29.9                      | 0.432   |
| Statins              | 45.6                 | 46.8                       | 44.3                      | 0.129   |
| ACEIs                | 17.0                 | 21.5                       | 12.5                      | 0.001   |
| ARBs                 | 17.2                 | 21.1                       | 13.3                      | 0.021   |
| CCBs                 | 69.2                 | 57.4                       | 81.1                      | <0.001  |

Values are expressed as average±SD unless otherwise indicated. ACEIs indicates angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; BP, blood pressure; CCBs, calcium channel blockers; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IHD, ischemic heart disease; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction.
serum adipin are shown in Figure S2. Interestingly, serum adipin levels were not associated with classic risk factors for atherosclerosis, including smoking, DM, dyslipidemia, and aging except hypertension (Figure S3).

Long-term Outcomes

We also examined the causes of death during the follow-up period in this population. During the median follow-up period of 71 months (interquartile range, 55-81 months), 226 patients (61%) reached the primary end point, mainly all-cause death (Figure 1A). Regarding the serum adipin levels, 45 of 260 patients (17%) died in the higher group, while 6 of 110 (5%) died in the lower group (Figure 1B). In the present study, among the 199 patients who were admitted to hospitals, 61 underwent coronary artery bypass grafting or percutaneous coronary intervention. In particular, in the higher and lower adipin level groups, 158 (61%) and 41 (37%) were admitted to hospitals (Figure 1C), and 49 (19%) and 12 (11%) underwent coronary revascularization (Figure 1D), respectively. Notably, 4 deaths (8.9%) attributable to AMI occurred in the higher adipin level group, whereas there was no AMI in the lower adipin level group (Figure S4A). Thus, in this study population, serum adipin levels had a significantly higher specificity for predicting death caused by AMI. Regarding “other cardiovascular death” (aneurysm rupture, peripheral ischemia, aortic dissection, and cerebrovascular events excluding AMI), 7 patients (3%) with higher adipin levels and 3 (3%) with lower adipin levels died (Table 2). In addition, 12 (5%) and 3 (3%) cancer deaths were noted in the higher and lower groups, respectively (Table 2). Importantly, serum adipin levels were significantly elevated in patients with cancer death compared with those without it (Figure S5). Regarding “other causes of death (eg, accident, pneumonia, respiratory failure, and renal failure),” 10 patients (4%) with higher adipin levels and no patients (0%) with lower adipin levels died (Table 2). Detailed causes of death were not available in the remaining 9 (4%) (Table 2).

Prognostic Impacts of Serum Adipin Levels

We next examined whether plasma levels of BNP and serum levels of hsCRP and adiponectin could predict all-cause death, rehospitalization, and revascularization. The cutoff points of

Figure 1. Kaplan–Meier curves in patients with coronary artery disease. Higher adipin levels were significantly associated with (A) primary end point, (B) all-cause death, and (C) rehospitalization, but not with (D) revascularization. The primary end point was a composite of all-cause death, rehospitalization, and revascularization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting during the follow-up period.
BNP, hsCRP, and adiponectin were determined as 100 pg/mL, 1 mg/L, and 9373 ng/mL, respectively, by the CART analysis. Higher plasma BNP levels were associated with the primary end point (Figure S6A), all-cause death (Figure S6B), and rehospitalization (Figure S6C), but not with revascularization (Figure S6D). This was also the case with serum hsCRP levels (Figure S7) and serum adiponectin levels (Figure S8).

Next, we examined the ability of combinations of serum adipin levels with plasma BNP or serum hsCRP levels to predict prognosis compared with each level alone. Serum adipin levels were significantly correlated with plasma BNP and adiponectin levels (Figure S9A and S9B), but not those of hsCRP levels (Figure S9C). We further compared the performance of the combinations of adipin and these biomarkers in predicting prognosis compared with each level alone. The combination of higher serum adipin levels and higher plasma BNP levels was more significantly associated with the primary end point (Figure 2A), all-cause death (Figure 2B), and rehospitalization (Figure 2C), but not with revascularization (Figure 2D), than lower serum adipin levels or lower plasma BNP levels alone. Additionally, the combination of higher serum levels of adipin and hsCRP levels was more significantly associated with the primary end point (Figure 3A), all-cause death (Figure 3B), and rehospitalization (Figure 3C), but not with revascularization (Figure 3D), than lower serum levels of adipin or hsCRP alone. Moreover, we fitted univariable Cox proportional hazard model utilizing 50 proteins as the independent variables. The significance was assessed using Bonferroni correction. As a result, the

Table 2. Causes of Death

|                      | All Patients (N=370) | Adipsin ≥400 ng/mL (n=260) | Adipsin <400 ng/mL (n=110) | P Value |
|----------------------|----------------------|---------------------------|---------------------------|---------|
| AMI                  | 1.1 (4)              | 1.5 (4)                   | 0 (0)                     | 0.323   |
| Other cardiovascular cause | 2.7 (10) | 2.7 (7) | 2.7 (3) | 1.000 |
| Cancer               | 4.1 (15)             | 4.6 (12)                  | 2.7 (3)                   | 0.567   |
| Pneumonia            | 0.8 (3)              | 1.1 (3)                   | 0 (0)                     | 0.558   |
| Other causes         | 2.7 (10)             | 3.8 (10)                  | 0 (0)                     | 0.037   |
| Unknown              | 2.4 (9)              | 3.5 (9)                   | 0 (0)                     | 0.063   |

Values are expressed as percentage (absolute number). Other cardiovascular cause, cardiovascular death excluding acute myocardial infarction (AMI); other causes, known cause of death excluding AMI, other cardiovascular cause, cancer, and pneumonia.

Figure 2. Kaplan–Meier curves based on the levels of adipin and brain natriuretic peptide (BNP). The combination of adipin levels (≥400 ng/mL) and BNP levels (≥100 pg/mL) was more significantly associated with (A) primary end point, (B) all-cause death, and (C) rehospitalization than the combination of adipin levels (<400 ng/mL) and BNP levels (<100 pg/mL).
proportional hazard assumption was satisfied in all candidate covariates except for TNF-related apoptosis-inducing ligand (TRAIL). The additive Cox proportional hazard model with spline representation was fit for TRAIL, although it was not significant with $P = 0.876$. Adipsin and cyclophilin A were still significant with adjusted $P < 0.001$ by Bonferroni correction.

Finally, we used Cox proportional hazards model for multivariable analysis. In multivariable analyses including age, sex, body mass index, glycated hemoglobin, presence of hypertension, dyslipidemia, DM, smoking status, aspirin, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, estimated glomerular filtration rate, BNP levels, and hsCRP levels, serum adipsin levels remained associated with the primary end point and rehospitalization, but not with all-cause death or revascularization (Table 3). Next, we analyzed the nonlinear and time-varying effects in HRs of the primary end point by an HR plot (Figure S10). HR plot revealed that serum adipsin levels were an accurate biomarker for predicting the primary end point in patients with CAD. There was a slightly worsening trend in prognosis, but the prognostic effect of adipsin level according to time was not significant and independent of the length of follow-up period. Additionally, to assess adipsin’s performance of prediction, we conducted simulation study as follows. First, we randomly separated the data set into the training set with three fourths of the total observations, and the validation set with one fourth of the observations. Next, we fit the CART model using the training set to identify the protein for prediction of the primary end point and its threshold. Finally, we defined an indicator to classify the risk strata using the detected protein and its threshold, and we fit the Cox proportional hazard model using the indicator in the training and the validation sets. We repeated this simulation for 1000 runs and calculated the median (interquartile range) of the cutoff of adipsin and the HRs in the training and the validation sets of the iterations. Of the 1000 runs of the simulation, adipsin was selected 945 times as the predictor of the primary end point with the cutoff of $395.2326$ (395.2326–396.3145) and the HR of $2.52$ (2.35–2.72) in the training set and $2.32$ (1.87–2.88) in the validation set.

**Serum Adipsin Levels Predict Deaths Caused by AMI**

During the follow-up period, 5 patients had an incidence of AMI, and 4 of them died. Importantly, all of them were in the...
Table 3. Cox Hazard Model for Univariate and Multivariate Analysis

| Primary end point | HR (95% CI) | P Value | Adjusted HR (95% CI) | P Value |
|-------------------|-------------|---------|----------------------|---------|
| Adipsin ≥400 ng/mL| 2.5 (1.8 to 3.5) | <0.001 | 2.0 (1.2 to 3.2) | 0.007 |
| Men, %            | 0.9 (0.7 to 1.2) | 0.673  | ...                 | ...    |
| Age, y            | 1.0 (1.0 to 1.0) | <0.001 | 1.0 (1.0 to 1.0) | 0.163  |
| BNP ≥100 pg/mL    | 2.1 (1.6 to 2.6) | <0.001 | 1.8 (1.2 to 2.8) | 0.006  |
| hsCRP ≥1 mg/L     | 1.6 (1.2 to 2.1) | <0.001 | 1.1 (0.7 to 1.6) | 0.734  |
| BMI               | 1.0 (0.9 to 1.0) | 0.007  | 1.0 (0.9 to 1.0) | 0.138  |
| Hypertension, %   | 1.3 (0.9 to 1.7) | 0.114  | ...                 | ...    |
| DM, %             | 1.5 (1.1 to 2.0) | 0.003  | 1.2 (0.7 to 1.9) | 0.520  |
| Smoke, %          | 1.0 (0.7 to 1.3) | 0.931  | ...                 | ...    |
| Dyslipidemia, %   | 1.2 (0.8 to 1.3) | 0.973  | ...                 | ...    |
| HbA1c, %          | 1.0 (0.7 to 1.3) | 0.005  | 1.3 (1.1 to 1.5) | 0.009  |
| ACEIs, %          | 1.0 (0.7 to 1.4) | 0.922  | ...                 | ...    |
| ARBs, %           | 1.5 (1.1 to 2.1) | 0.014  | 1.4 (0.9 to 2.1) | 0.120  |
| CCBs, %           | 0.9 (0.7 to 1.1) | 0.305  | ...                 | ...    |
| Statins, %        | 1.2 (0.9 to 1.5) | 0.267  | ...                 | ...    |
| Aspirin, %        | 1.7 (1.3 to 2.1) | <0.001 | 1.4 (1.0 to 1.8) | 0.045  |
| eGFR, ml/min per 1.73/m² | 1.0 (1.0 to 1.0) | 0.001  | 1.0 (1.0 to 1.0) | 0.397  |

All-cause death

| Adipsin ≥400 ng/mL | 4.2 (1.7 to 10.6) | <0.001 | 2.8 (0.9 to 9.0) | 0.082 |
| Men, %             | 2.0 (1.0 to 4.0)  | 0.052  | ...             | ...   |
| Age, y             | 1.0 (1.0 to 1.1)  | 0.001  | 1.0 (1.0 to 1.1) | 0.354 |
| BNP ≥100 pg/mL     | 3.6 (2.0 to 6.7)  | <0.001 | 3.8 (1.6 to 9.2) | 0.003 |
| hsCRP ≥1 mg/L      | 3.2 (1.7 to 5.9)  | <0.001 | 1.8 (0.8 to 4.1) | 0.131 |
| BMI                | 1.0 (0.9 to 1.0)  | 0.177  | ...             | ...   |
| Hypertension, %    | 1.4 (0.7 to 2.6)  | 0.318  | ...             | ...   |
| DM, %              | 1.5 (0.9 to 2.7)  | 0.139  | ...             | ...   |
| Smoke, %           | 1.5 (0.9 to 2.7)  | 0.154  | ...             | ...   |
| Dyslipidemia, %    | 1.3 (0.7 to 2.3)  | 0.412  | ...             | ...   |
| HbA1c, %           | 0.9 (0.7 to 1.2)  | 0.555  | ...             | ...   |
| ACEIs, %           | 1.8 (1.1 to 2.9)  | 0.032  | 1.5 (0.7 to 3.2) | 0.273 |
| ARBs, %            | 0.6 (0.3 to 1.2)  | 0.169  | ...             | ...   |
| CCBs, %            | 0.6 (0.4 to 1.0)  | 0.053  | ...             | ...   |
| Statins, %         | 1.1 (0.7 to 2.0)  | 0.618  | ...             | ...   |
| Aspirin, %         | 1.0 (0.6 to 1.7)  | 0.927  | ...             | ...   |
| eGFR, ml/min per 1.73/m² | 1.0 (1.0 to 1.0) | 0.016  | 1.0 (1.0 to 1.0) | 0.993 |

Rehospitalization

| Adipsin ≥400 ng/mL | 2.4 (1.7 to 3.5) | <0.001 | 2.1 (1.2 to 3.7) | 0.006 |
| Men, %             | 0.9 (0.8 to 1.2) | 0.508  | ...             | ...   |
| Age, y             | 1.0 (1.0 to 1.1) | <0.001 | 1.0 (1.0 to 1.0) | 0.219 |
| BNP ≥100 pg/mL     | 2.0 (1.5 to 2.6) | <0.001 | 2.0 (1.3 to 3.1) | 0.003 |
| hsCRP ≥1 mg/L      | 1.5 (1.1 to 1.9) | 0.007  | 0.9 (0.6 to 1.4) | 0.697 |

Continued
group with higher serum adipsin levels (Table 2). Here, we performed receiver operating characteristic analysis, which showed that serum adipsin levels were useful for predicting death caused by AMI (C-statistic, 0.84 [sensitivity, 64.8%, specificity, 100%]) (Figure S11A). Furthermore, serum adipsin levels predicted the future incidence of AMI in patients with CAD (C-statistic, 0.809 [sensitivity, 64.9%, specificity, 100%]) (Figure S11B). Moreover, receiver operating characteristic curve showed that plasma BNP levels effectively predicted death caused by AMI (C-statistic, 0.806; 95% CI, 0.708–0.877 [sensitivity, 68.9%, specificity, 100%]) (Figure S12A), and future incidence of AMI in patients with CAD (C-statistic, 0.793; 95% CI, 0.708–0.877 [sensitivity, 68.9%, specificity, 100%]) (Figure S12B). However, serum hsCRP levels may not be useful for predicting death caused by AMI (C-statistic, 0.506; 95% CI, 0.188–0.905 [sensitivity, 52.4%, specificity, 75.0%]) (Figure S12C), or for predicting future incidence of AMI in patients with CAD (C-statistic, 0.589; 95% CI, 0.298–0.879 [sensitivity, 52.5%, specificity, 80.0%]) (Figure S12D). Thus, in this study population, serum adipsin levels had a significantly higher specificity for predicting the death caused by AMI (Figure S11A).

**Table 3. Continued**

|                     | HR   | 95% CI      | P Value | Adjusted HR | 95% CI      | P Value |
|---------------------|------|-------------|---------|-------------|-------------|---------|
| BMI                 | 1.0  | 0.9 to 1.0  | 0.010   | 1.0         | 0.9 to 1.0  | 0.246   |
| Hypertension, %     | 1.1  | 0.8 to 1.6  | 0.382   | ...         | ...         | ...     |
| DM, %               | 1.5  | 1.1 to 2.0  | 0.006   | 1.0         | 0.6 to 1.7  | 0.930   |
| Smoke, %            | 0.9  | 0.7 to 1.2  | 0.616   | ...         | ...         | ...     |
| Dyslipidemia, %     | 0.9  | 0.7 to 1.2  | 0.632   | ...         | ...         | ...     |
| HbA1c, %            | 1.2  | 1.0 to 1.4  | 0.001   | 1.3         | 1.1 to 1.6  | 0.001   |
| ACEIs, %            | 0.9  | 0.6 to 1.3  | 0.561   | ...         | ...         | ...     |
| ARBs, %             | 1.8  | 1.3 to 2.5  | 0.001   | 1.7         | 1.2 to 2.6  | 0.008   |
| CCBs, %             | 0.9  | 0.8 to 1.5  | 0.418   | ...         | ...         | ...     |
| Statins, %          | 1.1  | 0.9 to 1.5  | 0.418   | ...         | ...         | ...     |
| Aspirin, %          | 1.7  | 1.3 to 2.2  | <0.001  | 1.5         | 1.1 to 2.0  | 0.016   |
| eGFR, mL/min per 1.73/m² | 1.0  | 1.0 to 1.0  | 0.003   | 1.0         | 1.0 to 1.0  | 0.550   |

Revascularization

|                     | HR   | 95% CI      | P Value | Adjusted HR | 95% CI      | P Value |
|---------------------|------|-------------|---------|-------------|-------------|---------|
| Adipsin ≥400 ng/mL  | 1.8  | 1.0 to 3.4  | 0.062   | ...         | ...         | ...     |
| Men, %              | 1.2  | 0.7 to 2.1  | 0.451   | ...         | ...         | ...     |
| Age, y              | 1.0  | 1.0 to 1.1  | 0.011   | 1.0         | 1.0 to 1.0  | 0.818   |
| BNP ≥100 pg/mL      | 1.3  | 0.8 to 2.2  | 0.285   | ...         | ...         | ...     |
| hsCRP ≥1 mg/L       | 1.4  | 0.8 to 2.3  | 0.186   | ...         | ...         | ...     |
| BMI                 | 1.0  | 1.0 to 1.1  | 0.757   | ...         | ...         | ...     |
| Hypertension, %     | 2.4  | 1.3 to 4.7  | 0.008   | 1.4         | 0.6 to 3.3  | 0.459   |
| DM, %               | 3.5  | 2.0 to 5.9  | <0.001  | 2.0         | 0.9 to 4.4  | 0.069   |
| Smoke, %            | 1.4  | 0.8 to 2.4  | 0.186   | ...         | ...         | ...     |
| Dyslipidemia, %     | 2.0  | 1.1 to 3.5  | 0.018   | 0.8         | 0.3 to 2.4  | 0.659   |
| HbA1c, %            | 1.4  | 1.2 to 1.6  | <0.001  | 1.2         | 1.0 to 1.6  | 0.093   |
| ACEIs, %            | 1.5  | 0.9 to 2.4  | 0.136   | 1.2         | 0.7 to 2.1  | 0.482   |
| ARBs, %             | 1.4  | 0.8 to 2.4  | 0.272   | ...         | ...         | ...     |
| CCBs, %             | 0.6  | 0.4 to 1.0  | 0.040   | 0.9         | 0.5 to 1.5  | 0.616   |
| Statins, %          | 2.4  | 1.5 to 4.0  | <0.001  | 2.6         | 1.0 to 6.7  | 0.040   |
| Aspirin, %          | 2.4  | 1.7 to 3.5  | <0.001  | 1.6         | 1.0 to 2.6  | 0.047   |
| eGFR, mL/min per 1.73/m² | 1.0  | 1.0 to 1.0  | 0.001   | 1.0         | 1.0 to 1.0  | 0.064   |

ACEIs indicates angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; BNP, brain natriuretic peptide; CCBs, calcium channel blockers; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein.
Enhanced Adipsin Expression in Atherosclerotic Unstable Plaque

Adipsin is secreted from adipocytes, monocytes, and macrophages. Moreover, serum adipsin levels were elevated in patients with CAD and predicted death caused by AMI. Thus, we hypothesized that adipsin is also expressed in coronary atherosclerotic plaque in patients with CAD. To test this hypothesis, we performed immunostaining for adipsin in coronary arteries obtained from patients who died of AMI. Importantly, expression of adipsin was especially higher in the atherosclerotic plaque and adventitial adipocyte tissue in a patient (Figure 4), suggesting that adipsin is potentially involved in the unstabilization of atherosclerotic plaque in patients with CAD.

Discussion

The major findings of the present study are that: (1) the rates of all-cause death and rehospitalization were significantly higher in patients with higher serum adipsin levels than in those with lower serum adipsin levels; (2) serum adipsin levels, when combined with BNP or hsCRP levels, had better predicting capabilities; and (3) serum adipsin levels predicted the incidences and deaths caused by AMI in patients with CAD. These results indicate that serum adipsin levels have prognostic impacts for all-cause death and rehospitalization in patients with CAD and that combination of the adipsin levels and other established biomarkers (hsCRP and BNP) further enhances the prognostic impacts in those patients.

Adipsin in the Pathogenesis of Atherosclerosis

Adipokines are secreted from adipose tissue and may promote the development of CAD. In a clinical study, it was reported that plasma adipsin levels predict the occurrence of ischemic stroke. However, no study has ever addressed the role of adipsin in the pathogenesis of atherosclerosis in animals or humans. In the present study, by using a cytokines array, we demonstrated that serum levels of adipsin predict the long-term prognosis of patients with CAD. Indeed, higher adipsin levels were significantly associated with all-cause death and rehospitalization compared with lower adipsin levels. Thus, adipsin could be a potentially novel biomarker that reflects the development of atherosclerosis, although further analyses using genetically modified animal models are needed. It is widely known that circulating levels of BNP, homocysteine, oxidized low-density lipoprotein cholesterol, and CRP can predict future revascularization. However, circulating levels of these biomarkers are also elevated in patients with other diseases. Thus, serum adipsin levels could be a more specific biomarker that reflects the presence of atherosclerosis. Interestingly, adipsin is secreted from adipocytes and also from monocytes and macrophages, catalyzing the rate-limiting step of the alternative complement pathway. Additionally, adipsin is closely associated with the metabolic state and insulin resistance in mice. In contrast, adipsin is mechanistically important for pancreatic β-cell function in patients with type 2 DM. Thus, the interpretation of these reports is complex but indicates the potential role of adipsin in the pathogenesis of atherosclerosis.

Recently, Meloche et al. reported that bromodomain protein 4 is a trigger not only for pulmonary arterial hypertension but also for calcification and remodeling processes in patients with CAD. Bromodomain protein 4 is an important regulator of the proliferation/apoptosis balance by modulating gene expression and plays crucial roles in post-DNA damage events. In the present study, the cancer deaths were significantly increased in the group with higher serum adipsin levels compared with the group with lower serum adipsin levels. This may suggest that adipsin is involved in the pathogenesis of atherosclerosis and cancer.

In addition to adipsin, adipose tissues also secrete various inflammatory cytokines (eg, tumor necrosis factor-α) and chemokines (eg, monocyte chemoattractant protein), which leads to insulin resistance, dyslipidemia, and adipose tissue dysfunction and unstabilizes coronary atherosclerotic plaques. Increased levels of serum adipsin are closely
related to the increased metabolic disturbances. Moreover, adipin promotes lipid accumulation and adipocyte differentiation. Consistently, we found a strong expression of adipin in the coronary atherosclerotic plaque of patients with AMI. Moreover, in the present study, 4 deaths caused by AMI were in the group with higher adipin levels, whereas there were no deaths in the group with lower adipin levels. Indeed, receiver operating characteristic curves clearly showed that serum adipin levels predicted the incidence of and deaths caused by AMI in patients with CAD. Thus, adipin may be involved in unstabilization of coronary atherosclerotic plaque and could be a novel biomarker to predict the future incidence of AMI.

Comparison Between Adipsin and Other Biomarkers

In the present study, we compared serum levels of adipin with other biomarkers (BNP and hsCRP). It is well known that higher plasma BNP levels predict adverse prognosis, including the risk of cardiovascular death. Additionally, plasma levels of processed forms of BNP (N-terminal pro-BNP) can predict the incidence of revascularization. Moreover, higher serum levels of hsCRP (>1 mg/L) predict future cardiovascular events. Similarly, in the present study, higher serum hsCRP levels were associated with all-cause death and rehospitalization. However, serum hsCRP levels are elevated not only in patients with CAD but also in those with infectious diseases, which limit the usefulness of hsCRP as a specific biomarker for cardiovascular events. In the present study, there was no correlation between serum levels of adipin and those of hsCRP. Thus, we consider that adipin is secreted from adipose tissue while CRP is produced mainly by the liver and indirectly reflects the levels of circulating inflammatory cytokines. We also examined whether combination of adipin and other biomarkers could better predict prognosis than each biomarker alone. Indeed, the combination of adipin and BNP levels significantly better predicted the incidence of all-cause death and rehospitalization than each biomarker alone. This was also the case with hsCRP. These results suggest that the combination of adipin levels and other biomarkers enhances the prognostic impacts for patients with CAD. BNP and hsCRP levels are frequently used as useful biomarkers in the clinical practice. Thus, additional use of adipin could further improve clinical practice with biomarkers.

Adipsin Levels Predict Cancer Death

Cardiovascular diseases are the major cause of death in patients with CAD. However, the causes of death in patients with CAD are often noncardiac, especially after the establishment of medical therapies and coronary interventions. Recently, it has been noted that patients with cardiovascular diseases are at increased risk of cancer death. In the present study, 15 patients died as a result of cancer during the follow-up. Interestingly, serum adipin levels were significantly elevated in patients who died of cancer. Indeed, the risk factors for atherosclerosis and cancer are common, such as advanced age, DM, dyslipidemia, and smoking, all of which induce systemic inflammation and enhanced oxidative stress. In the present study, cancer death was also a major cause of death, demonstrating that patients with CAD are at increased risk of cancer death. Some medications for cardiovascular diseases may have influenced the incidence of cancer. Indeed, patients with higher adipin levels had a higher frequency of use of aspirin, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers than those with lower adipin levels. Here, it has been reported that angiotensin receptor blockers could be associated with a modestly increased risk of cancer, whereas a recent study has denied this notion. Additionally, calcium channel blockers and statins do not increase the new occurrence of cancer, and aspirin reduces cancer deaths. Thus, medications in patients with higher adipin levels may not have had a strong impact on the occurrence of cancer death.

Atherosclerotic diseases are closely associated with vascular inflammation. Recently, it has been reported that anti-inflammatory therapy targeting the interleukin (IL) 1β innate immunity pathway with canakinumab significantly decreased the occurrences of cardiovascular events and the new onset of cancers (CANTOS [Canakinumab Anti-Inflammatory Thrombosis Outcomes Study]). This study has directly demonstrated that inflammation promotes atherosclerosis and cancers. In the present study, serum adipin levels were significantly elevated in patients with cancer death compared with those without it. Indeed, adipin-mediated effects on alternative complement pathway may have augmented coagulation and inflammation in patients with CAD. Here, we consider the potential role of higher levels of circulating cytokines/chemokines and growth factors to promote atherosclerosis and cancer. In the present study, serum levels of IL-1α, IL-15, IL-17, leukemia inhibitory factor, macrophage colony-stimulating factor, tumor necrosis factor-β, granulocyte macrophage colony-stimulating factor, and monocyte chemotactic protein-1 were significantly elevated in patients with lower serum adipin levels compared with those with higher adipin levels. In contrast, serum levels of IL-16, cutaneous T-cell-attracting chemokine, adiponectin, and cyclophilin A were significantly elevated in patients with higher serum adipin levels compared with those with lower adipin levels. Indeed, it has been reported that inflammatory cytokines are associated with cancer development.
elevated levels of cytokines/chemokines in patients with higher adipsin may have contributed to the development of atherosclerosis and cancer in the present study.

**Alternative Complimentary System and Atherosclerosis**

Adipsin is mainly secreted from adipocytes, monocytes, and macrophages, catalyzing the rate-limiting step of the alternative complement pathway. In this pathway, adipsin cleaves complement factor B and catalyzes the formation of C3 convertase, which leads to a hydrolysis cascade that produces various complement fragments including C3a, C3b, C5a, and C5b. The complimentary system stimulates macrophages to phagocytose bacterium and induces inflammation.

In the present study, serum adipsin levels predicted future incidence of AMI in patients with CAD. Consistently, it has been reported that higher serum levels of adipsin were associated with an increased 10-year risk of ischemic stroke among middle-aged men. Thus, the present study shows the new aspects of the alternative complementary system that promotes atherosclerosis.

These days, it has been reported that lower circulating adiponectin levels increase insulin resistance, further develop arteriosclerosis, and contribute to the development of cardiovascular disease. As shown in Figure S2, serum adiponectin levels are positively correlated with serum adipsin levels in the present study. This result is in contrast to the previous reports. Considering the previous reports, it is easy to understand whether adipsin and adiponectin are inversely correlated, but it is conceivable that the present findings suggest a new aspect of the relationship between adipsin and adiponectin. Adipsin is also known as complement factor D, playing central roles in the complement system alternative pathway. The definitive difference between adipsin and adiponectin is thought to be a difference in the involvement in the immune system. However, at this time, there are no data that are sufficient for proof, which is a subject to be studied in future studies.

**Study Limitations**

Several limitations should be mentioned for the present study. First, the detailed mechanisms of adipsin-mediated development of atherosclerosis remain to be elucidated. Second, the number of patients in the present study was relatively small. However, despite the small sample size, serum adipsin levels had a significant prognostic impact to predict AMI. Additionally, this limitation did not affect the validity of the main result of the present study. Future prospective analysis in a large cohort will further elucidate the importance of serum levels of adipsin. Next, the association between adipsin and other complement factors is important. However, in the present study, we did not measure other complement factors. This point remains to be addressed in future studies.

**Conclusions**

In the present study, we were able to demonstrate, for the first time, that higher serum adipsin levels in patients with CAD predict all-cause death and rehospitalization. Combining adipsin levels with other established biomarkers (BNP or hsCRP levels) would further enhance the prognostic impacts of serum adipsin levels in patients with CAD. Importantly, the present study demonstrates that higher adipsin levels in patients with CAD can predict future death caused by AMI or cancer. Thus, serum adipsin levels could be a novel and useful biomarker in the super-aging society.

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36. Vasilenko MA, Kirienkova EV, Skuratovskaia DA, Zatolokin PA, Mironyuk NI, Litinova NL. The role of production of adipin and leptin in the development of insulin resistance in patients with abdominal obesity. Dokl Biochem Biophys. 2017;475:271–276.

37. Meloche J, Lampron MC, Nadeau V, Maltais M, Potus F, Lambert C, Tremblay E, Vitry G, Breuls-Bonnet S, Boucherat O, Charbonneau E, Provencher S, Paulin R, Bonnet S. Implication of inflammation and epigenetic readers in coronary artery remodeling in patients with pulmonary arterial hypertension. Artery. 2017;37:1513–1523.

38. Meloche J, Potus F, Vaillancourt M, Bourgeois A, Johnson I, Deschamps L, Chabot S, Ruffenach G, Henry S, Breuls-Bonnet S, Tremblay E, Nadeau V, Lambert C, Paradis R, Provencher S, Bonnet S. Bromodomain-containing protein 4: the epigenetic origin of pulmonary arterial hypertension. Circ. 2015;117:525–535.

39. Belkina AC, Denis GV. BET domain co-regulators in obesity, in insulin resistance in patients with abdominal obesity. Circ. 2015;117:525–535.

40. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science. 1993;259:89–91.

41. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci USA. 2003;100:7265–7270.

42. Nasu K, Tsuchikane E, Katoh O, Fujita H, Surmely JF, Ehara M, Kinoshita Y, Tobe K, Nagai R, Tobe K, Moriyama K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson ME, Shapiro S. Calcium channel blockers and the risk of cancer. JAMA. 1998;279:1004–1009.

43. Gursoy Calan O, Calan M, Yesil Senses P, Unal Kocabas G, Ozden E, Sari KR, Kocar M, Imamoglu C, Senses YM, Bozkaya G, Bilgir O. Increased adipin is associated with carotid intima media thickness and metabolic disturbances in polycystic ovary syndrome. Clin Endocrinol (Oxf). 2016;85:910–917.

44. Song NJ, Kim S, Jang BH, Chang SH, Yun UJ, Park KM, Waki H, Li DY, Tontonoz P, Park KW. Small molecule-induced complement factor D (adipsin) promotes lipid accumulation and adipocyte differentiation. PLoS One. 2016;11:e0152228.

45. Horwich TB, Hamilton MA, Fonarow GC. B-type natriuretic peptide levels in patients with type 2 diabetes. Circ. 2008;94:429–433.

46. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Hall Long K, Shah ND, Tontonoz P, Waki H, Li DY, Tontonoz P, Park KM, Waki H, Li DY, Tontonoz P, Park KW. Small molecule-induced complement factor D (adipsin) promotes lipid accumulation and adipocyte differentiation. PLoS One. 2016;11:e0152228.

47. Dunlay SM, Redfield MM, Weston SA, Theueme TM, Hall Long K, Shah ND, Roger VL. Hospitalizations after heart failure diagnosis and metabolic disturbances in patients with type 2 diabetes. Circ. 2008;94:429–433.

48. Hasin T, Gerber Y, Weston SA, Jiang R, Killian JM, Manienn SM, Gerhan JR, Roger VL. Patients with heart failure have an increased risk of incident cancer. J Am Coll Cardiol. 2013;62:881–886.

49. Hasin T, Gerber Y, Weston SA, Jiang R, Killian JM, Manienn SM, Gerhan JR, Roger VL. Hasin T, Gerber Y, Weston SA, Jiang R, Killian JM, Manienn SM, Gerhan JR, Roger VL. Patients with heart failure have an increased risk of incident cancer. J Am Coll Cardiol. 2016;62:265–271.

50. Ocampo NV, Taferesi J, Hauschild CL, Pai R. Cardiovascular medications and risk of cancer. Am J Cardiol. 2011;108:1045–1051.

51. Sipahi I, Debanne SM, Rowland DY, Simon D, Fang J. Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. Lancet. 2010;11:627–636.

52. Bhaskaran K, Douglas I, Evans S, van Staa T, Smeeth L. Angiotensin receptor blockers and risk of cancer: cohort study among people receiving antihypertensive drugs in UK General Practice Research Database. BMJ. 2012;344:e2697.

53. Rosenberg L, Rao RS, Palmer JR, Strom BL, Stolley PD, Zauber AG, Warshauer ME, Shapiro S. Calcium channel blockers and the risk of cancer. JAMA. 1998;279:1000–1004.

54. Dale KM, Coleman CH, Honyan KN, Kluger J, White CM. Statins and cancer risk: a meta-analysis. JAMA. 2006;295:74–80.

55. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377:31–41.

56. Ridker PM. A test in context: high-sensitivity C-reactive protein. J Am Coll Cardiol. 2016;67:712–723.

57. Ridker PM, Everett BM, Truern T, MacFadyen JG, Chang WH, Ballantyne C, Fosseca F, Frontini J, Koeng W-M, for the KAINJIAN JIP, Cornel HJ, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Koba Vala Z, Vidozmi L, Flax M, Shimokawa H, Ogawa H, Dellborg M, Ross Par LF, Troquet R, Libby P, Grinn RJ. An anti-inflammator therapy with canakinumab for atheroscerotic disease. Br J Med. 2017;377:1119–1131.

58. Grinberg-Bleyer Y, Ghosh S. A novel link between inflammation and cancer. Cancer Cell. 2016;30:279–830.

59. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: a prospective study and meta-analysis. Circulation. 2006;114:623–629.

60. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsutsumi-Ya K-saoka A, Ezaki O, Akamuta Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Farguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotoxicity and obesity. Nat Med. 2001;7:941–946.

61. Chouglane D, Nadakar M, Venkataraman K, Rajadhyaksha A, Hase N, James T, Kini S, Khadikar P, Andar V, Madkaikar M, Pradhan V. Adipokine interactions promote the pathogenesis of systemic lupus erythematosus. Cytokine. 2018;11:20–27.

62. Martinez HR, Escamilla-Ocasas CE, Camara-Lemarroy CR, Gonzalez-Garza MT, Tenorio-Pedraza JM, Hernandez-Torres M. Cytokine A concentration of adipin and adiponectin in patients with amyotrophic lateral sclerosis. Acta Neurol Belg. 2017;117:879–883.

63. Natarajan R, Hageman S, Hamalainen M, Leppanen T, Dastidar P, Molianen E. Adipokine balance and multiple sclerosis: a follow-up study of adiponectins. Mult Scler Int. 2015;946:271.
Supplemental Material
CART analyses were performed to determine the best biomarker for predicting prognosis in patients with coronary artery disease.
Figure S2. Boxplots of Serum Levels of Cytokines/Chemokines and Growth Factors.
Serum levels of IL-15, IL-16, IL-17, cutaneous T-cell-attracting chemokine (CTACK), leukemia inhibitory factor (LIF), granulocyte macrophage colony-stimulating factor (GM-CSF), adiponectin and Cyclophilin A (CyPA) were elevated in patients with higher serum levels of adipsin.
Figure S3. Serum Levels of Adipsin and Cardiovascular Risk Factors.

Distribution of serum adipsin levels based on the advanced age (>66 years), hypertension, smoking, diabetes mellitus, dyslipidemia, and sex. Results are expressed by box-plots analyses.
Serum adipsin levels were not associated with AMI death (A) or with composite cardiovascular death (B). Cardiovascular death was considered if the death was caused by acute myocardial infarction. Composite cardiovascular death was defined if the death was caused by acute myocardial infarction, heart failure, stroke, or other cardiovascular causes.
Serum adipsin levels were elevated in patients with future occurrences of cancer death.
Higher BNP levels were significantly associated with (A) primary endpoint, (B) all-cause death, and (C) rehospitalization, but not with (D) revascularization. The primary endpoint was a composite of all-cause death, rehospitalization, and revascularization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting.
Higher hsCRP levels were significantly associated with (A) primary endpoint, (B) all-cause death, (C) rehospitalization but not with (D) revascularization. The primary endpoint was a composite of all-cause death, rehospitalization, and revascularization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting.
Higher adiponectin levels were significantly associated with (A) primary endpoint, (B) all-cause death, (C) rehospitalization but not with (D) revascularization. The primary endpoint was a composite of all-cause death, rehospitalization, and revascularization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting.
Figure S9. Scatter Plots Showing the Relation Between Serum Adipsin Levels and Other Variables.

Scatter plots showed that serum levels of adipsin were correlated with (A) plasma levels of BNP and (C) those of adiponectin, but not those of hsCRP (B) in Spearman’s correlation analysis.
Hazard ratio plot revealed that serum adipsin levels were an accurate biomarker for predicting primary endpoint in CAD patients, independent of the length of follow-up period. Solid, broken, dotted and red lines indicate hazard ratio plot, 95% confidence intervals, smoothed control line and log(hazard ratio) =0, respectively. Blue bars show histogram.
ROC curve revealed that serum adipsin levels were an accurate biomarker for predicting (A) death due to AMI and (B) AMI incidence in CAD patients.
ROC curve revealed that plasma BNP levels were an accurate biomarker of predicting (A) death due to AMI and (B) AMI incidence in CAD patients, whereas serum hsCRP levels were not useful for predicting (C) death due to AMI or (D) AMI incidence in CAD patients.