Original Article

Prognostic Impacts of Plasma Levels of Cyclophilin A in Patients With Coronary Artery Disease

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Objective—Cyclophilin A (CyPA) is secreted from vascular smooth muscle cells, inflammatory cells, and activated platelets in response to oxidative stress. We have recently demonstrated that plasma CyPA level is a novel biomarker for diagnosing coronary artery disease. However, it remains to be elucidated whether plasma CyPA levels also have a prognostic impact in such patients.

Approach and Results—In 511 consecutive patients undergoing diagnostic coronary angiography, we measured the plasma levels of CyPA, high-sensitivity C-reactive protein (hsCRP), and brain natriuretic peptide and evaluated their prognostic impacts during the follow-up (42 months, interquartile range: 25–55 months). Higher CyPA levels (≥12 ng/mL) were significantly associated with all-cause death, rehospitalization, and coronary revascularization. Higher hsCRP levels (≥1 mg/L) were also significantly correlated with the primary end point and all-cause death, but not with rehospitalization or coronary revascularization. Similarly, higher brain natriuretic peptide levels (≥100 pg/mL) were significantly associated with all-cause death and rehospitalization, but not with coronary revascularization. Importantly, the combination of CyPA (≥12 ng/mL) and hsCRP (≥1 mg/L) was more significantly associated with all-cause death (hazard ratio, 21.2; 95% confidence interval, 4.9–92.3,; $P<0.001$) than CyPA (≥12 ng/mL) or hsCRP (≥1 mg/L) alone.

Conclusions—The results indicate that plasma CyPA levels can be used to predict all-cause death, rehospitalization, and coronary revascularization in patients with coronary artery disease and that when combined with other biomarkers (hsCRP and brain natriuretic peptide levels), the CyPA levels have further enhanced prognostic impacts in those patients. (*Arterioscler Thromb Vasc Biol*. 2017;37:00-00. DOI: 10.1161/ATVBAHA.116.308986.)

Key Words: atherosclerosis ■ biomarker ■ brain ■ coronary artery disease ■ prognosis

Cardiovascular risk factors, including hypertension, diabetes mellitus, dyslipidemia, and smoking, have been used for "risk classification" of coronary artery disease (CAD).[1] The assessment of these factors is important for predicting future cardiovascular events.[2] In particular, acute myocardial infarction (AMI) is a fatal cardiovascular event that is the most common cause of sudden cardiac death.[3] Nowadays, AMI has become an important social issue because of the increasing number of patients with heart failure.[4] Consequently, it is becoming increasingly important to develop a useful biomarker to predict cardiovascular events, including AMI, in outpatient clinics. It has been reported that the plasma levels of high-sensitivity C-reactive protein (hsCRP),[5–7] brain natriuretic peptide (BNP),[8] D-dimer,[9] and fibrinogen[10] can predict the incidence of cardiovascular events. However, the plasma levels of these biomarkers are increased in inflammatory diseases, in general, in addition to arteriosclerotic diseases.[11] Indeed, useful biomarker that can effectively predict the risk of future AMI still remains to be developed.

Cyclophilin A (CyPA) was identified as the intracellular target of the immunosuppressive drug, cyclosporine.[12] Intracellular CyPA is a chaperon protein that has several functions[13] and plays important roles in protein folding and trafficking of extracellular signal–regulated kinase and apoptosis-inducing factor.[14,15] Recently, it has been reported that CyPA is secreted from endothelial cells,[16] vascular smooth muscle cells (VSMCs),[17,18] cardiac fibroblasts,[19] adipocytes,[20] macrophages,[19] and activated platelets.[13,21] It has been demonstrated that the secretion of CyPA is induced by excessive activation of Rho-kinase and oxidative stress specifically in VSMCs.[22] We have recently demonstrated that the plasma levels of CyPA are significantly higher in patients with CAD in proportion to the severity of the disorder.[22,23] It was previously reported that the plasma levels of CyPA are significantly higher in CAD patients with type 2 diabetes mellitus.[24] Indeed, several studies demonstrated the role of CyPA as a biomarker of CADs.[25–29] Huang et al[25] reported that the plasma levels of CyPA one month after AMI predict the prognosis of

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Nonstandard Abbreviations and Acronyms	
AMI	acute myocardial infarction
BNP	brain natriuretic peptide
CAD	coronary artery disease
CyPA	cyclophilin A
hsCRP	high-sensitivity C-reactive protein
VSMCs	vascular smooth muscle cells

the patients. Zuern et al[26] also reported that CyPA expression in myocardial biopsy specimens is an independent predictor of clinical outcome in patients with heart failure. In addition, urinary CyPA levels were positively correlated with the severity of diabetic nephropathy.[27] Furthermore, plasma levels of CyPA are useful as a prognostic factor in patients with ruptured intracranial aneurysm.[28] Interestingly, an SNP in CyPA (rs6850A/G) was associated with the elevation of plasma CyPA levels in patients with CAD.[29] These findings suggest that the plasma levels of CyPA could predict long-term prognosis of patients with CAD. In this study, we thus tested our hypothesis that the plasma levels of CyPA predict all-cause death, rehospitalization, and revascularization in patients with CAD.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Baseline Patient Characteristics

Baseline characteristics of the 511 patients are shown in Table. The mean age was 64±13 years, 65% of them were male, and 44 (8.6%) died after a mean age of 1.9 years (range 0.1–4.9 years). Among the participants, 65% had hypertension, 36% had diabetes mellitus, 51% had dyslipidemia, and 25.7% of the participants had a history of smoking. The mean value of left ventricular ejection fraction was 65%. The cut-off point of plasma CyPA level was 12.0 (ng/mL) as determined by the CART analysis. Baseline characteristics of the groups with higher (≥12 ng/mL) and lower (<12 ng/mL) plasma CyPA levels are shown in Table. Plasma CyPA levels were elevated in patients with advanced age, hypertension, smoking, diabetes mellitus, and dyslipidemia (Figure I in the online-only Data Supplement).

Long-Term Outcomes

During the median follow-up period of 42 months (interquartile range: 25–55 months), 44 patients (8.6%) reached the primary end point, predominantly due to all-cause death. Twenty-eight of 163 patients died in the group with higher plasma CyPA levels, whereas 16 of 338 patients died in the group with lower plasma CyPA levels. Notably, 3 deaths (10%) were due to AMI in the group with higher levels of CyPA, whereas there was no such case in the group with lower levels of CyPA. In this study population, plasma CyPA levels had a significantly high specificity in predicting the future occurrence of AMI. In contrast, 4 patients with higher

CyPA levels and 3 patients with lower CyPA levels died due to cerebrovascular events during the follow-up (no significant difference between the groups). Regarding "other cardiovascular death" (aneurysm rupture, peripheral ischemia, and aortic dissection excluding AMI and cerebrovascular events), there were 1 and 3 death cases in the groups with higher and lower plasma CyPA levels, respectively. In addition, deaths due to cancer were similarly noted in the groups with higher and lower CyPA levels (n=8 each), respectively. Regarding "other causes of death (eg, accident, pneumonia, respiratory failure, and renal failure)", 12 and 2 death cases were noted in the groups with higher and lower plasma CyPA levels, respectively. "Cardiovascular death" was considered if the death was caused by AMI or heart failure. "Composite cardiovascular death" was defined if the death was caused by AMI, heart failure, stroke, and other cardiovascular causes. Kaplan–Meier curve showed that higher plasma CyPA levels were associated with cardiovascular death (Figure IIA in the online-only Data Supplement), but not with composite cardiovascular death (Figure IIB in the online-only Data Supplement). Twelve patients with higher plasma CyPA levels and 2 patients with lower plasma CyPA levels died due to "other causes." In this study, 159 patients were admitted to hospitals and 54 underwent CABG or PCI. In particular, 64 with higher plasma CyPA levels and 95 with lower plasma CyPA levels were admitted to hospitals. Among them, 31 with higher plasma CyPA levels and 23 with lower plasma CyPA levels underwent coronary revascularization.

Prognostic Predictive Impacts of Plasma CyPA Levels

Patients with higher plasma CyPA levels had adverse prognosis of the primary end point, all-cause death, rehospitalization, and revascularization. Patients with higher plasma CyPA levels indicated adverse prognosis of the primary end point compared with those with lower plasma CyPA levels (Figure 1A). Similarly, higher plasma CyPA levels were significantly associated with all-cause death (Figure 1B), rehospitalization (Figure 1C), and revascularization (Figure 1D). Thus, plasma CyPA levels predicted all-cause death, rehospitalization, and revascularization. Next, we examined whether the plasma levels of BNP and hsCRP could predict all-cause death, rehospitalization, and revascularization. The cut-off point of plasma levels of BNP and hsCRP was determined to be 100 pg/mL and 1 mg/L, respectively, by CART analysis. Higher plasma BNP levels were associated with the primary end point (Figure IIIA in the online-only Data Supplement), all-cause death (Figure IIIB in the online-only Data Supplement), and rehospitalization (Figure IIIC in the online-only Data Supplement), but not with revascularization (Figure IIID in the online-only Data Supplement). Higher plasma hsCRP levels were associated with the primary end point (Figure IVA in the online-only Data Supplement) and all-cause death (Figure IVB in the online-only Data Supplement), but not with rehospitalization (Figure IVC in the online-only Data Supplement) or revascularization (Figure IVD in the online-only Data Supplement).

Next, we examined the ability of combinations of plasma CyPA levels with plasma BNP or hsCRP levels to predict

Table 1.   Baseline Patient Characteristics

	All Patients (n=511)	CyPA≥12 (n=163)	CyPA<12 (n=348)	P Value
Age, y	64.0±13.1	67.6±10.7	61.7±13.8	<0.001
Male sex, %	65.3	70	64	0.114
Family history of IHD, %	8.4	7.9	9.3	0.611
Medical story (%)				
Hypertension	64.8	75	58	<0.001
Diabetes mellitus	35.8	46	29	<0.001
Dyslipidemia	51	61	46	0.001
Cardiovascular diseases	14	26	11	0.007
Cerebral vascular diseases	3	5	1	0.018
Smoker, %	25.7	47.3	28.3	<0.001
eGFR, mL/min per 1.73 m²	56.6±20.6	51.2±21.4	59.4±14.1	<0.001
LVEF, %	64.7±12.0	64.4±11.0	64.9±12.3	0.667
Total cholesterol, mg/dL	177.2±38.7	173.5±39.8	178.5±37.4	0.187
LDL, mg/dL	101.9±33.4	98.2±32.9	103.7±33.1	0.076
HDL, mg/dL	49.4±13.5	50.0±13.5	49.3±13.2	0.576
TG, mg/dL	140.2±110.1	127.9±77.8	145.2±120.9	0.055
BMI	23.7±3.6	22.8±3.4	24.2±3.6	<0.001
Systolic BP, mm Hg	127.8±19.2	129.6±21.0	126.9±17.9	0.177
Diastolic BP, mm Hg	74.0±12.2	72.6±11.9	74.5±12.2	0.114
HbA1c, %	6.0±1.1	6.0±1.1	5.9±1.0	0.232
hsCRP, mg/dL	0.197±0.316	0.209±0.307	0.191±0.320	0.544

Plus-minus values are average±SD. ACE indicates angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; BMI, body mass index; BP, blood pressure; CyPA, cyclophilin A; HDL, high-density lipoprotein; hsCRP, high-sensitive C-reactive protein; IHD, ischemic heart disease; LDL, low-density lipoprotein; and LVEF, left ventricular ejection fraction.

prognosis compared with each level alone. Plasma CyPA levels were not correlated with plasma BNP (Figure VA in the online-only Data Supplement) or hsCRP levels (Figure VB in the online-only Data Supplement). Thus, we compared the performance of the combinations of CyPA and these biomarkers (BNP and hsCRP) in predicting prognosis compared with each level alone. The combination of higher plasma CyPA levels and higher plasma BNP levels was more significantly associated with the primary end point (Figure 2A), all-cause death (Figure 2B), rehospitalization (Figure 2C), and revascularization (Figure 2D) than lower plasma CyPA levels or lower plasma BNP levels alone. In addition, the combination of higher plasma CyPA levels and higher plasma hsCRP levels was more significantly associated with the primary end point (Figure 3A), all-cause death (Figure 3B), and revascularization (Figure 3D) than lower plasma CyPA levels or lower plasma hsCRP levels alone. Furthermore, the Kaplan–Meier curve showed that the combinations of CyPA levels with BNP and hsCRP levels further enhanced the prognostic impacts compared with each level alone. The combination of higher plasma levels of CyPA and BNP was more significantly associated with the primary end point (Figure 4A), all-cause death (Figure 4B), and rehospitalization (Figure 4C) than higher plasma levels of CyPA or BNP alone. Moreover,

the combination of higher plasma levels of CyPA and hsCRP was more strongly associated with all-cause death (Figure 4B) and revascularization (Figure 4D) compared with higher plasma levels of CyPA or hsCRP alone. In addition, we used Cox's proportional hazards model for multivariate analysis. In multivariable analysis including age, sex, body mass index (BMI), presence of hypertension, dyslipidemia, and diabetes mellitus, past cardiovascular events, past cerebrovascular events, smoking status, BNP level, hsCRP level, and plasma CyPA levels remained associated with the primary end point (Figure 4A) and all-cause death (Figure 4B), but not with rehospitalization (Figure 4C) or revascularization (Figure 4D). In addition, we examined correlation between plasma CyPA levels and serum cholesterol levels. Because serum uric acid levels predict mortality,[30] we examined whether plasma CyPA levels were correlated with the serum levels of uric acid. However, plasma CyPA levels were not correlated with serum uric acid levels (Figure VC in the online-only Data Supplement). In contrast, plasma CyPA levels were correlated with advanced ages (Figure IIID in the online-only Data Supplement). Finally, the plasma levels of CyPA were not correlated with the serum levels of total cholesterol, low-density lipoprotein, and high-density lipoprotein (Figure VI in the online-only Data Supplement).
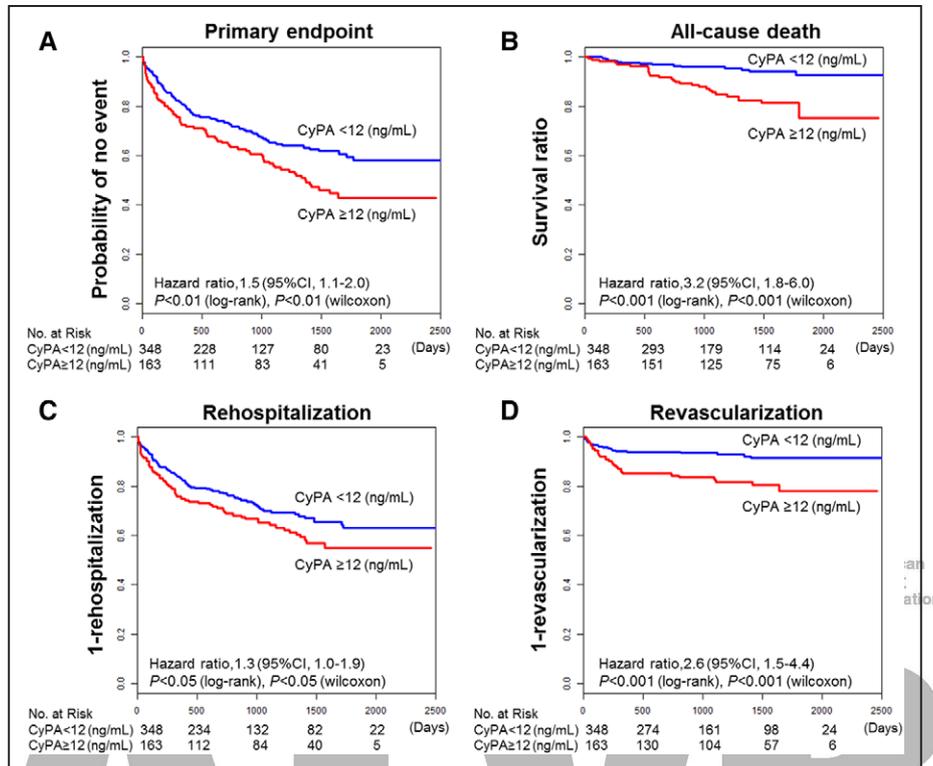
Figure 1. Kaplan–Meier curves in patients with CAD. Higher CyPA levels were significantly associated with (**A**) primary end point, (**B**) all-cause death, (**C**) rehospitalization, and (**D**) revascularization. The primary end point was a composite of all-cause death, rehospitalization, and revascularization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting during the follow-up period. CI indicates confidence interval.

Discussion

The major findings of this study are that (1) the rates of all-cause death, rehospitalization, and revascularization were higher in patients with higher plasma CyPA levels than in those with lower plasma CyPA levels, (2) plasma CyPA levels had a significantly high specificity in predicting future occurrence of AMI, and (3) plasma CyPA levels combined with BNP or hsCRP levels had better predicting capability. These results indicate that CyPA levels have prognostic impacts for all-cause death, rehospitalization, and coronary revascularization in patients with CAD and that combining them with other biomarkers (hsCRP and BNP) further enhances the prognostic impacts of CyPA levels in those patients.

CyPA and Arteriosclerosis

It is widely recognized that mechanical stretching, hypertension, angiotensin II, diabetes mellitus, smoking, dyslipidemia, and advanced age induce oxidative stress.[31,32] CyPA is secreted from VSMCs in response to oxidative stress.[17,18,33–36] Moreover, it has been reported that CyPA is also secreted from activated macrophages, lymphocytes, and platelets, all of which are important sources of extracellular CyPA.[21,37–39] Also, it has been reported that platelet-bound CyPA was associated with cardiovascular events.[21,37–39] The previous experimental studies using genetically modified mice showed that CyPA induces endothelial dysfunction, VSMC proliferation, and inflammatory cell migration, promoting the development of atherosclerosis.[31,32,40] We have recently demonstrated that

plasma CyPA levels are increased proportionally to the severity of CAD in humans.[23]

On the basis of these results, in this study, we examined whether plasma CyPA levels could predict long-term prognosis of patients with CAD. Importantly, we were able to demonstrate that the incidence of all-cause death, rehospitalization, and revascularization was higher in patients with higher plasma CyPA levels than in those with lower plasma CyPA levels. In particular, plasma CyPA levels had a strong prognostic impact for future revascularization. It has been reported that the plasma levels of BNP,[41] homocysteine,[42] oxidized low-density lipoprotein cholesterol,[43] and CRP[44] can predict future revascularization. This study clearly demonstrates the superiority of plasma CyPA levels over the biomarkers mentioned above. More importantly, plasma CyPA levels had a significantly high specificity in predicting the primary end point. Plasma CyPA levels are a biomarker that exactly reflects the severity of developing arteriosclerosis. Indeed, we have previously demonstrated that CyPA plays crucial roles in the development of arteriosclerosis in various animal models.[31,32,40] It is conceivable that we may be able to predict the severity of arteriosclerosis in patients with CAD by measuring plasma CyPA levels in the future.

Comparison Between CyPA and Other Biomarkers

In this study, we compared the plasma levels of CyPA with the plasma levels of BNP and hsCRP. It was previously reported that higher plasma BNP levels lead to adverse prognosis. For
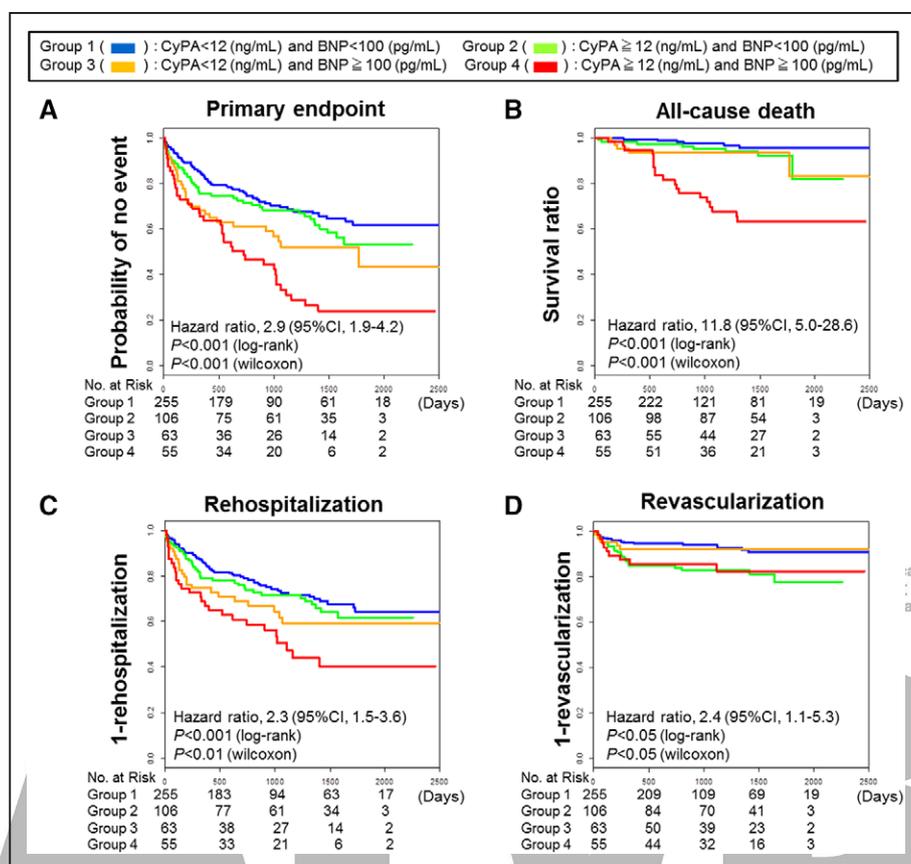
Figure 2. Kaplan–Meier curves based on the plasma levels of CyPA and brain natriuretic peptide (BNP). The combination of CyPA levels (≥12 ng/mL) and BNP levels (≥100 pg/mL) was more significantly associated with (**A**) primary end point, (**B**) all-cause death, (**C**) rehospitalization, and (**D**) revascularization than the combination of CyPA levels (<12ng/mL) and BNP levels (<100 pg/mL). CI indicates confidence interval.

example, it was reported that plasma BNP levels can predict the risk of cardiovascular death,[8] that the plasma levels of processed forms of BNP can predict the incidence of revascularization,[41] and that the plasma levels of hsCRP >1 mg/L predict future cardiovascular events.[6] In this study, higher plasma hsCRP levels were associated with all-cause death. However, higher plasma hsCRP levels could not predict the need for future revascularization. Importantly, plasma hsCRP levels are elevated not only in CAD but also in acute inflammatory diseases in general, such as infectious diseases.[6] This limits the use of hsCRP level as a biomarker in predicting future cardiovascular events. Indeed, although plasma hsCRP levels are currently measured in many hospitals in the world, the levels are not used to diagnose CAD itself. Importantly, there was no correlation between the plasma levels of CyPA and hsCRP. As to the reason for the lack of correlation, we consider that CyPA is secreted from the vasculature, whereas CRP is produced mainly by the liver and indirectly reflects the levels of circulating inflammatory cytokines.[45] Thus, the present results are important, demonstrating that plasma CyPA levels are closely associated with all-cause death and the need for future revascularization.

Prognostic Impacts of the Combination of CyPA With Other Biomarkers

In this study, we demonstrated that the plasma levels of CyPA were not correlated with those of BNP or hsCRP. Thus, we

examined whether the combination of CyPA and other biomarkers could better predict prognosis than each level alone. Indeed, the combination of CyPA and BNP levels significantly better predicted the incidence of all-cause death, rehospitalization, and revascularization than each level alone. Similarly, the combination of CyPA and hsCRP levels was more effective in predicting the incidence of all-cause death and revascularization than each level alone. These results suggest that the combination of CyPA levels and other biomarkers (BNP or hsCRP levels) enhances the prognostic impacts for patients with CAD. BNP and hsCRP levels play important roles in clinical practice as major biomarkers. Future use of CyPA level as an additional biomarker in clinical practice may significantly contribute to predicting CAD. The availability of biomarkers that can predict the incidence of cardiovascular events preclinically in patients with CAD will allow efficient identification of patients that require more stringent management of arteriosclerosis. It is expected that measuring the plasma levels of CyPA will become a routine test in clinical practice in the near future.

Future Perspectives

Although the treatment for AMI and ischemic heart diseases has been recently improved, it is still difficult to predict AMI before the onset. Importantly, half of the patients with AMI die before hospitalization.[46] In this study, three patients developed
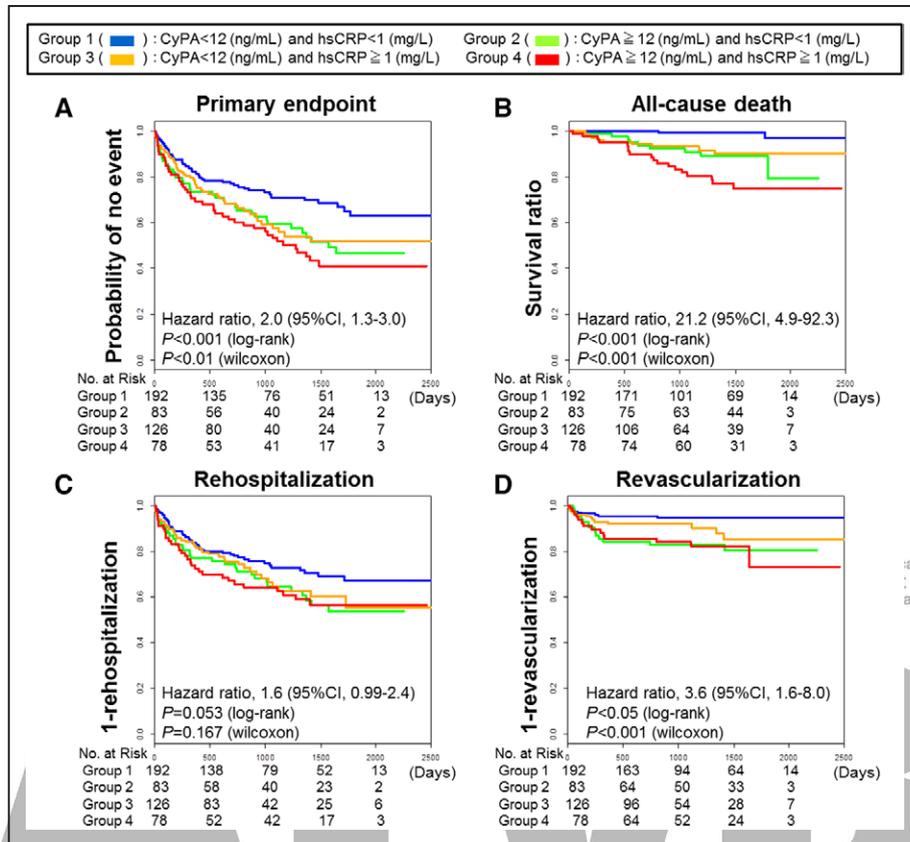
Figure 3. Kaplan–Meier curves based on the plasma levels of CyPA and high-sensitivity C-reactive protein (hsCRP). The combination of CyPA levels (≥12 ng/mL) and hsCRP levels (≥1 mg/L) was more significantly associated with (**A**) primary end point, (**B**) all-cause death, and (**D**) revascularization than the combination of CyPA levels (<12 ng/mL) and hsCRP levels (<1 mg/L). CI indicates confidence interval.

AMI during the follow-up, and all of them died. Thus, the development of a biomarker for the diagnosis of AMI is necessary. Importantly, all of the patients who developed AMI during the follow-up period were in the group with higher plasma CyPA levels. This suggests that it could be possible to identify patients at high risk of developing AMI with plasma CyPA levels.

We also were able to demonstrate that plasma CyPA levels were able to predict the need for future revascularization. Moreover, we found that the predicting ability of CyPA level was higher than that of other biomarkers. In addition, there is currently no biomarker in clinical practice to predict coronary artery restenosis. Thus, the plasma levels of CyPA are potentially useful as a biomarker for the prediction of restenosis after PCI or CABG as well.
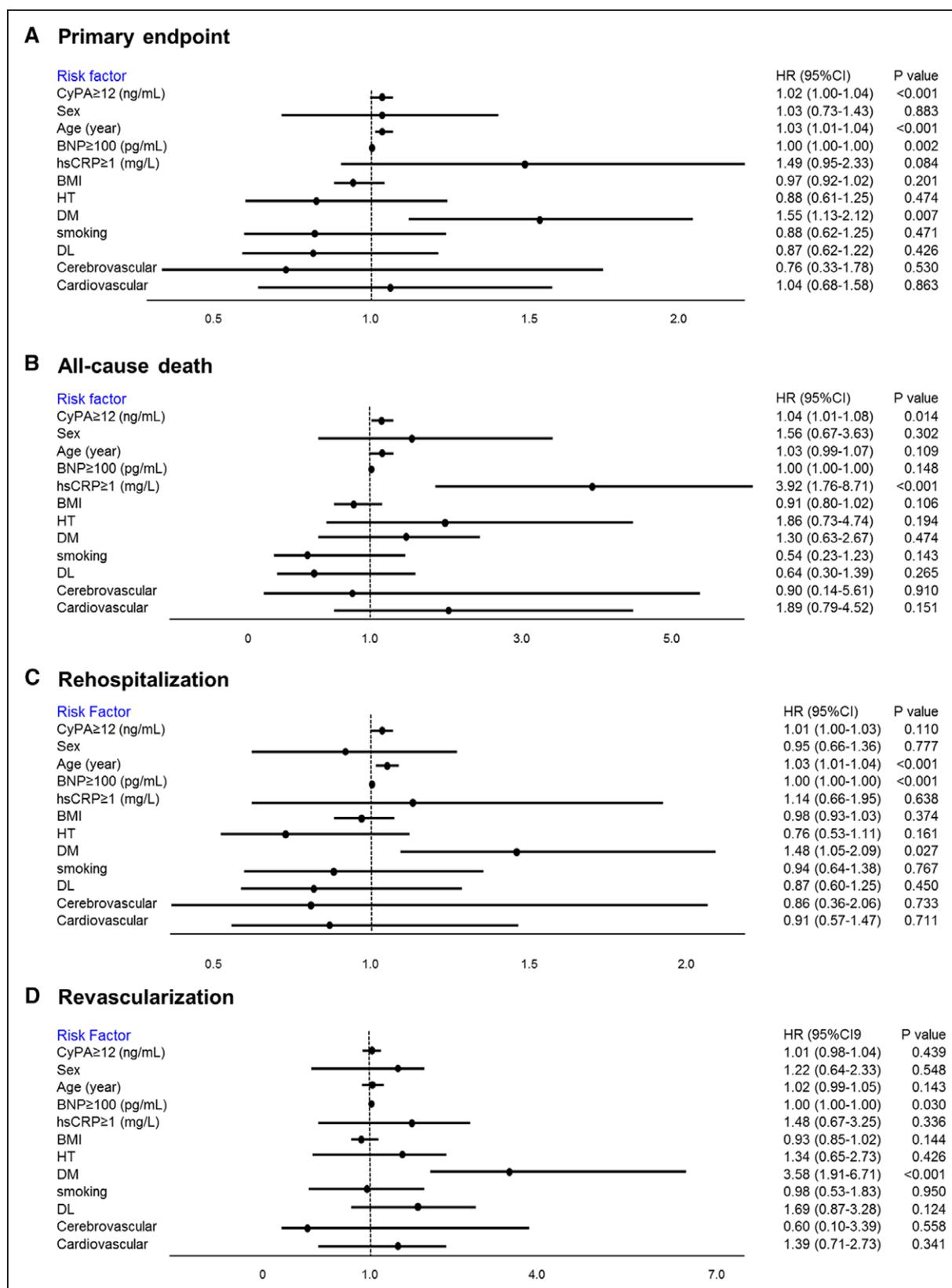
Study Limitations

Several limitations should be mentioned for this study. First, the number of patients in this study is relatively small. However, despite this small sample size, plasma CyPA levels had a prognostic impact to predict AMI. In addition, this limitation did not affect the validity of the main result of this study. Second, it has been reported that plasma CyPA levels were elevated in several disease status, such as infectious diseases,[47,48] cancer,[49,50] collagen diseases,[49,51] asthma,[49,52] and diabetes mellitus[49,53] in addition to arteriosclerosis. Third, age and hypertension were not associated with all-cause

death. Because most of the patients with hypertension enrolled in this study had already been controlled by medication, we consider that one of the reasons can be explained by the medical controls. In addition, we also consider the limited number of patients in this study may have affected these results. Further analysis with increased number of patients may show the prognostic impacts of plasma levels of CyPA in CAD. Third, CyPA is mainly secreted from VSMCs in response to oxidative stress. VSMCs are mainly present in the heart, kidney, lungs, aorta, and peripheral arteries. This suggests that plasma CyPA levels are elevated not only in CAD but also in other vascular diseases, such as aortic aneurysm, renal artery stenosis, and peripheral artery disease. Thus, it is important to measure plasma CyPA levels to evaluate its roles in other vascular diseases. Future prospective analysis in a large cohort will further elucidate the importance of plasma CyPA levels.

Conclusions

This study demonstrates, for the first time, that higher plasma CyPA levels in patients with CAD can predict all-cause death, rehospitalization, and revascularization. Combining CyPA levels with other biomarkers (BNP or hsCRP levels) would further enhance the prognostic impacts for patients with CAD. Thus, plasma CyPA levels are a novel biomarker to predict mortality, rehospitalization, and revascularization in patients with CAD.

A Primary endpoint

Risk factor	HR (95%CI)	P value
CyPA≥12 (ng/mL)	1.02 (1.00-1.04)	<0.001
Sex	1.03 (0.73-1.43)	0.883
Age (year)	1.03 (1.01-1.04)	<0.001
BNP≥100 (pg/mL)	1.00 (1.00-1.00)	0.002
hsCRP≥1 (mg/L)	1.49 (0.95-2.33)	0.084
BMI	0.97 (0.92-1.02)	0.201
HT	0.88 (0.61-1.25)	0.474
DM	1.55 (1.13-2.12)	0.007
smoking	0.88 (0.62-1.25)	0.471
DL	0.87 (0.62-1.22)	0.426
Cerebrovascular	0.76 (0.33-1.78)	0.530
Cardiovascular	1.04 (0.68-1.58)	0.863

B All-cause death

Risk factor	HR (95%CI)	P value
CyPA≥12 (ng/mL)	1.04 (1.01-1.08)	0.014
Sex	1.56 (0.67-3.63)	0.302
Age (year)	1.03 (0.99-1.07)	0.109
BNP≥100 (pg/mL)	1.00 (1.00-1.00)	0.148
hsCRP≥1 (mg/L)	3.92 (1.76-8.71)	<0.001
BMI	0.91 (0.80-1.02)	0.106
HT	1.86 (0.73-4.74)	0.194
DM	1.30 (0.63-2.67)	0.474
smoking	0.54 (0.23-1.23)	0.143
DL	0.64 (0.30-1.39)	0.265
Cerebrovascular	0.90 (0.14-5.61)	0.910
Cardiovascular	1.89 (0.79-4.52)	0.151

C Rehospitalization

Risk Factor	HR (95%CI)	P value
CyPA≥12 (ng/mL)	1.01 (1.00-1.03)	0.110
Sex	0.95 (0.66-1.36)	0.777
Age (year)	1.03 (1.01-1.04)	<0.001
BNP≥100 (pg/mL)	1.00 (1.00-1.00)	<0.001
hsCRP≥1 (mg/L)	1.14 (0.66-1.95)	0.638
BMI	0.98 (0.93-1.03)	0.374
HT	0.76 (0.53-1.11)	0.161
DM	1.48 (1.05-2.09)	0.027
smoking	0.94 (0.64-1.38)	0.767
DL	0.87 (0.60-1.25)	0.450
Cerebrovascular	0.86 (0.36-2.06)	0.733
Cardiovascular	0.91 (0.57-1.47)	0.711

D Revascularization

Risk Factor	HR (95%CI9	P value
CyPA≥12 (ng/mL)	1.01 (0.98-1.04)	0.439
Sex	1.22 (0.64-2.33)	0.548
Age (year)	1.02 (0.99-1.05)	0.143
BNP≥100 (pg/mL)	1.00 (1.00-1.00)	0.030
hsCRP≥1 (mg/L)	1.48 (0.67-3.25)	0.336
BMI	0.93 (0.85-1.02)	0.144
HT	1.34 (0.65-2.73)	0.426
DM	3.58 (1.91-6.71)	<0.001
smoking	0.98 (0.53-1.83)	0.950
DL	1.69 (0.87-3.28)	0.124
Cerebrovascular	0.60 (0.10-3.39)	0.558
Cardiovascular	1.39 (0.71-2.73)	0.341

Figure 4. Plasma levels of CyPA and cardiovascular risk factors. Cox's proportional hazards model was performed for multivariate analysis including age, sex, body mass index (BMI), presence of hypertension, dyslipidemia, diabetes mellitus, past cardiovascular events, past cerebrovascular events, smoking status, brain natriuretic peptide (BNP) level, high-sensitivity C-reactive protein (hsCRP) level, and plasma CyPA level. CyPA≥12 ng/mL remained associated with the primary end point (**A**) and all-cause death (**B**), but not with rehospitalization (**C**) or revascularization (**D**). CI indicates confidence interval; and HR, hazard ratio.

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Disclosures

None.

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Highlights

- Plasma CyPA levels have prognostic impacts to predict all-cause death, rehospitalization, and revascularization.
- Plasma CyPA levels have a significantly high specificity in predicting the primary end point.
- Plasma CyPA levels have enhanced prognostic impacts in patients with CAD, especially when combined with the levels of BNP or hsCRP.

Arteriosclerosis, Thrombosis, and Vascular Biology

Prognostic Impacts of Plasma Levels of Cyclophilin A in Patients With Coronary Artery Disease

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Supplementary Figure I. Plasma Levels of CyPA and Cardiovascular Risk Factors

Distribution of plasma CyPA levels based on the advanced age (>66 years), hypertension, smoking, diabetes mellitus, and dyslipidemia, and sex. Results are expressed by box-plots analyses.
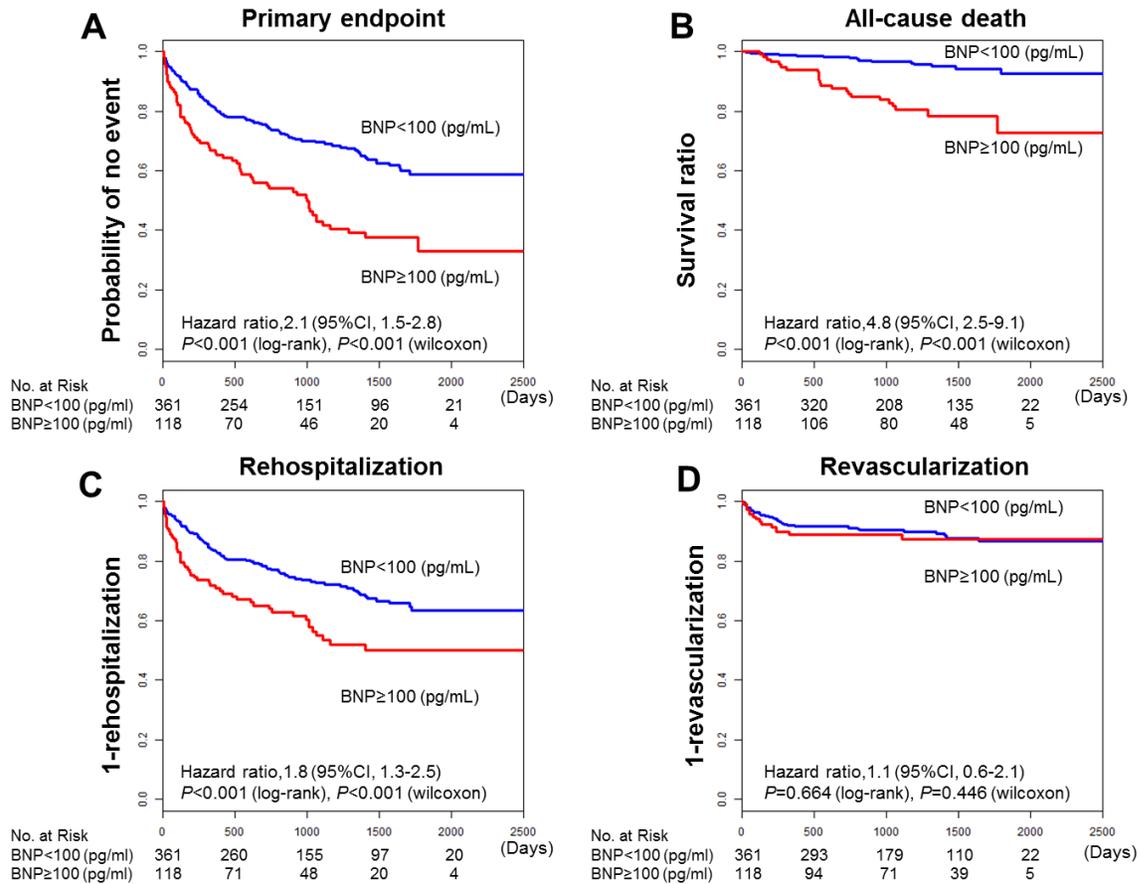
Supplementary Figure II

A Cardiovascular death

B Composite cardiovascular death
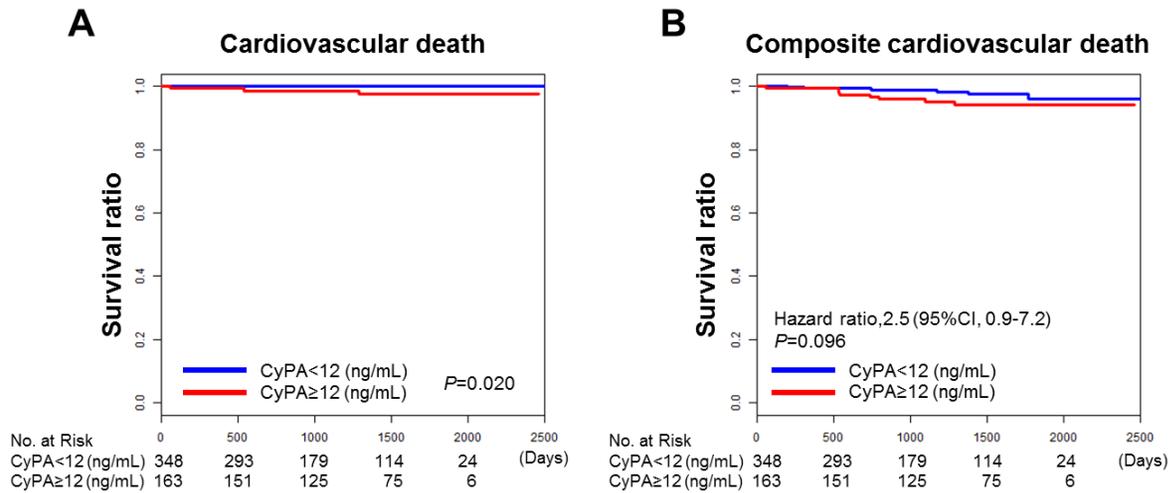
Supplementary Figure II. Kaplan-Meier Curve based on Plasma CyPA Levels

Plasma CyPA levels were associated with cardiovascular death (**A**), but not with composite cardiovascular death (**B**). Cardiovascular death was considered if the death was caused by acute myocardial infraction or heart failure. Composite cardiovascular death was defined if the death was caused by acute myocardial infraction, heart failure, stroke, or other cardiovascular causes.
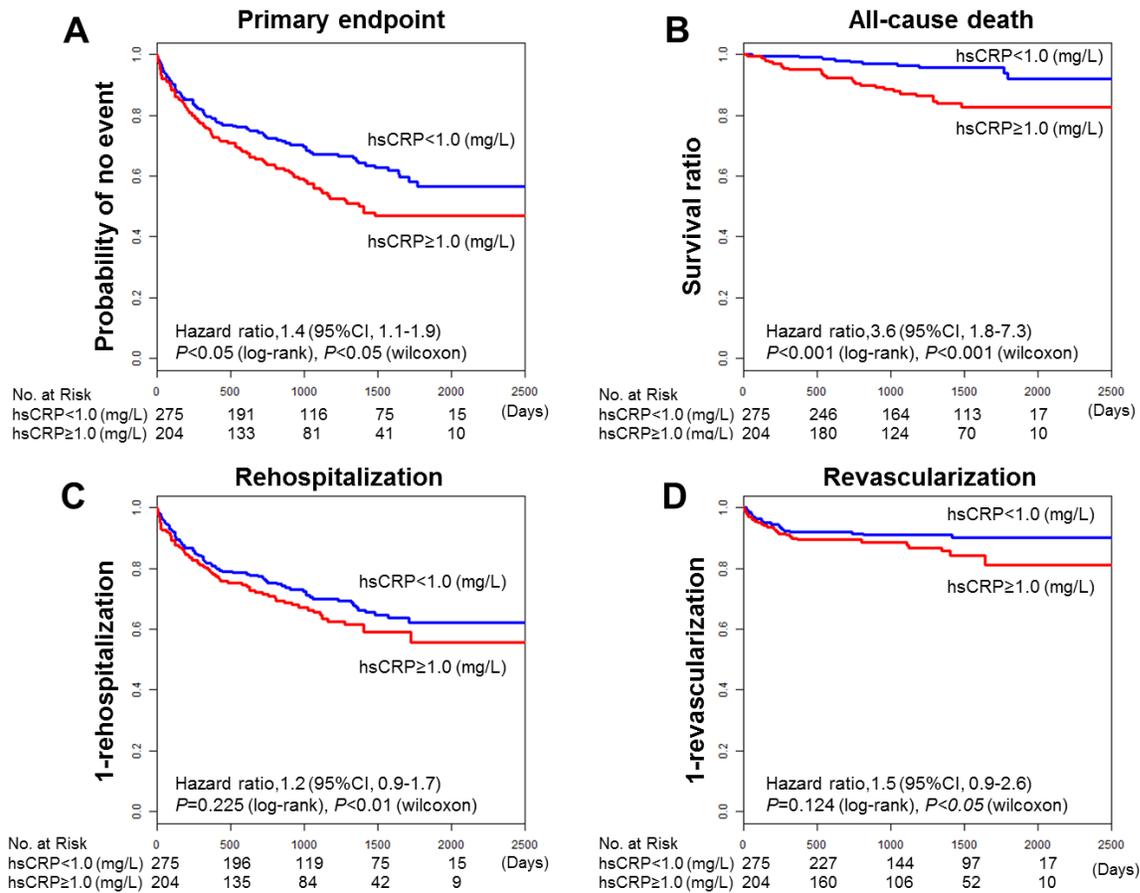
A Primary endpoint

B All-cause death

C Rehospitalization

D Revascularization

Supplementary Figure III. Kaplan-Meier Curve Based on Plasma BNP Levels

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.
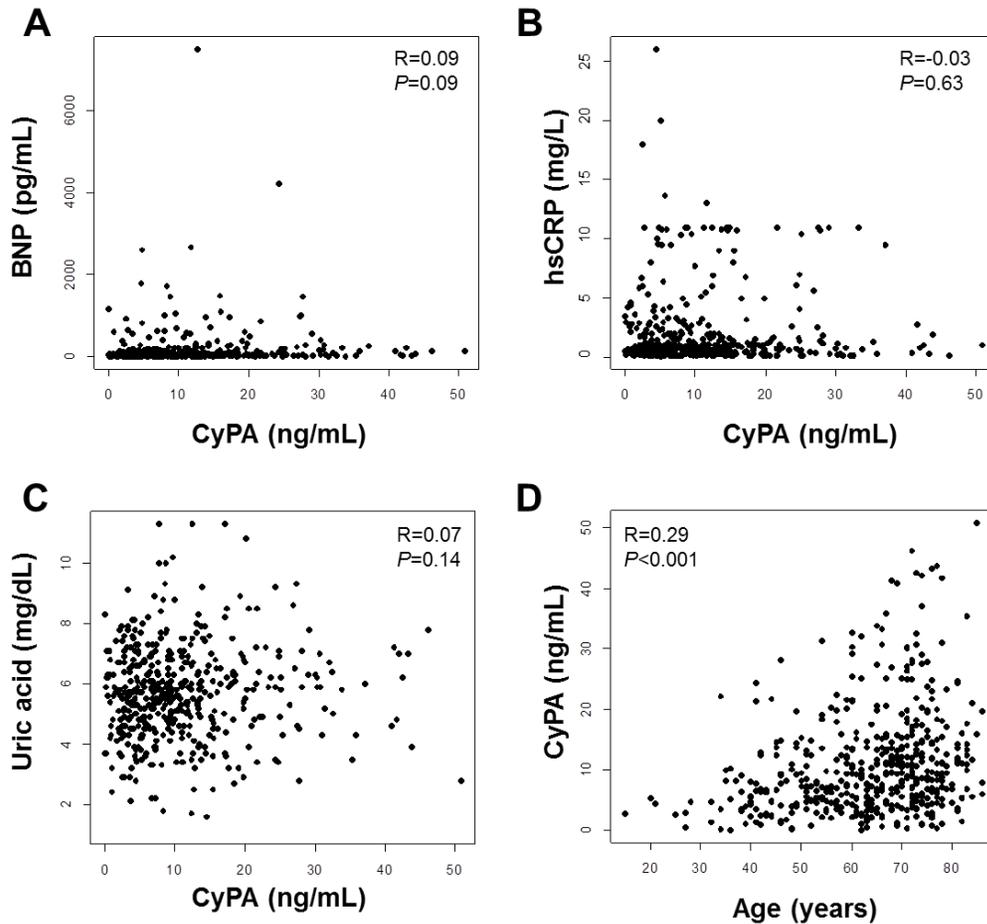
Supplementary Figure IV

Supplementary Figure IV. Kaplan-Meier Curve Based on Plasma hsCRP Levels

Higher hsCRP levels were significantly associated with (**A**) primary endpoint and (**B**) all-cause death, but not with (**C**) rehospitalization or (**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.
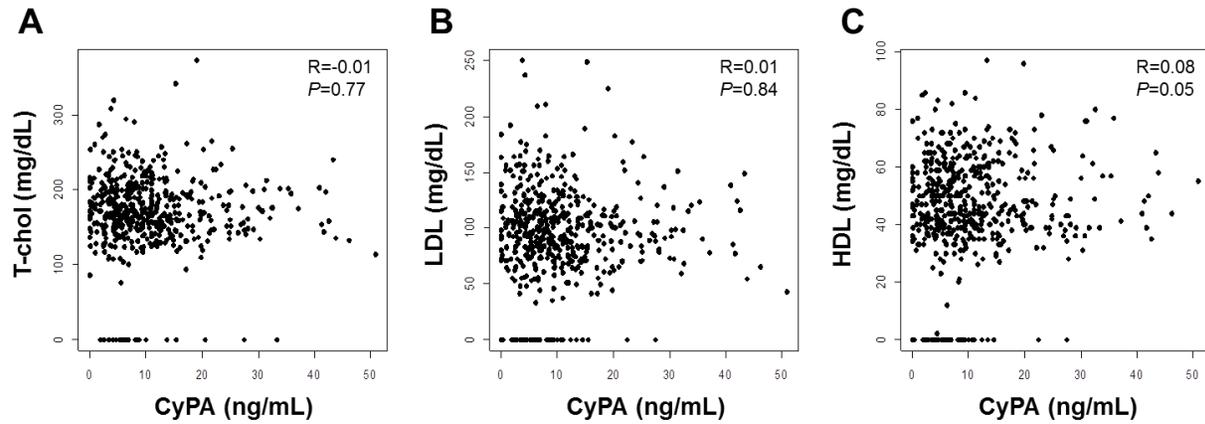
Supplementary Figure V

Supplementary Figure V. Scatter Plots Showing the Relation Between Plasma CyPA Levels and Other Variables

Scatter plots showed that plasma levels of CyPA were not correlated with plasma levels of (**A**) BNP, (**B**) hsCRP, or (**C**) uric acid.　In contrast, plasma CyPA levels were positively correlated with (**D**) advanced age.
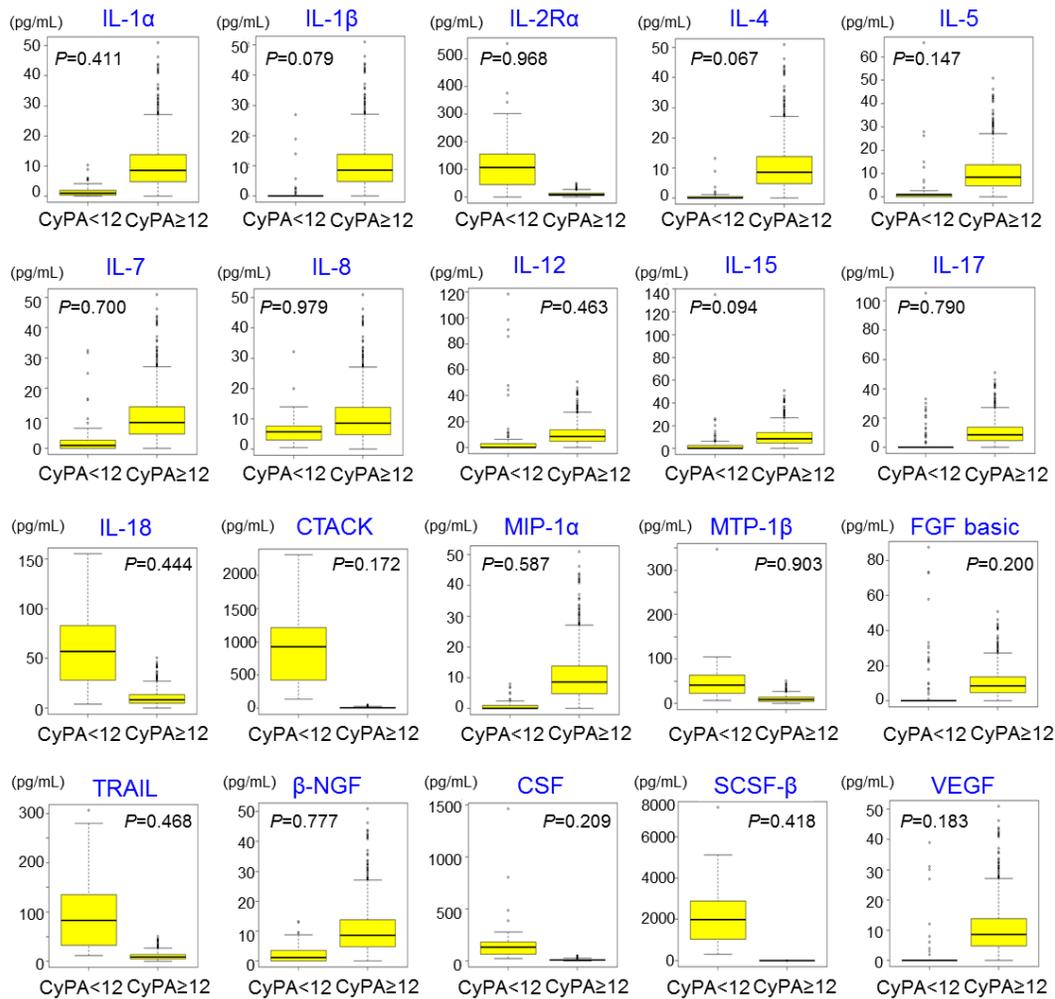
Supplementary Figure VI

Supplementary Figure VI. Scatter Plots Showing the Plasma CyPA Levels and Serum Cholesterol Levels.   Scatter plots showed that plasma levels of CyPA were not correlated with serum levels of (**A**) T-chol, (**B**) LDL, or (**C**) HDL.   Plasma CyPA levels were positively correlated with (**D**) advanced age.   Tchol, Total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; R, correlation coefficient.
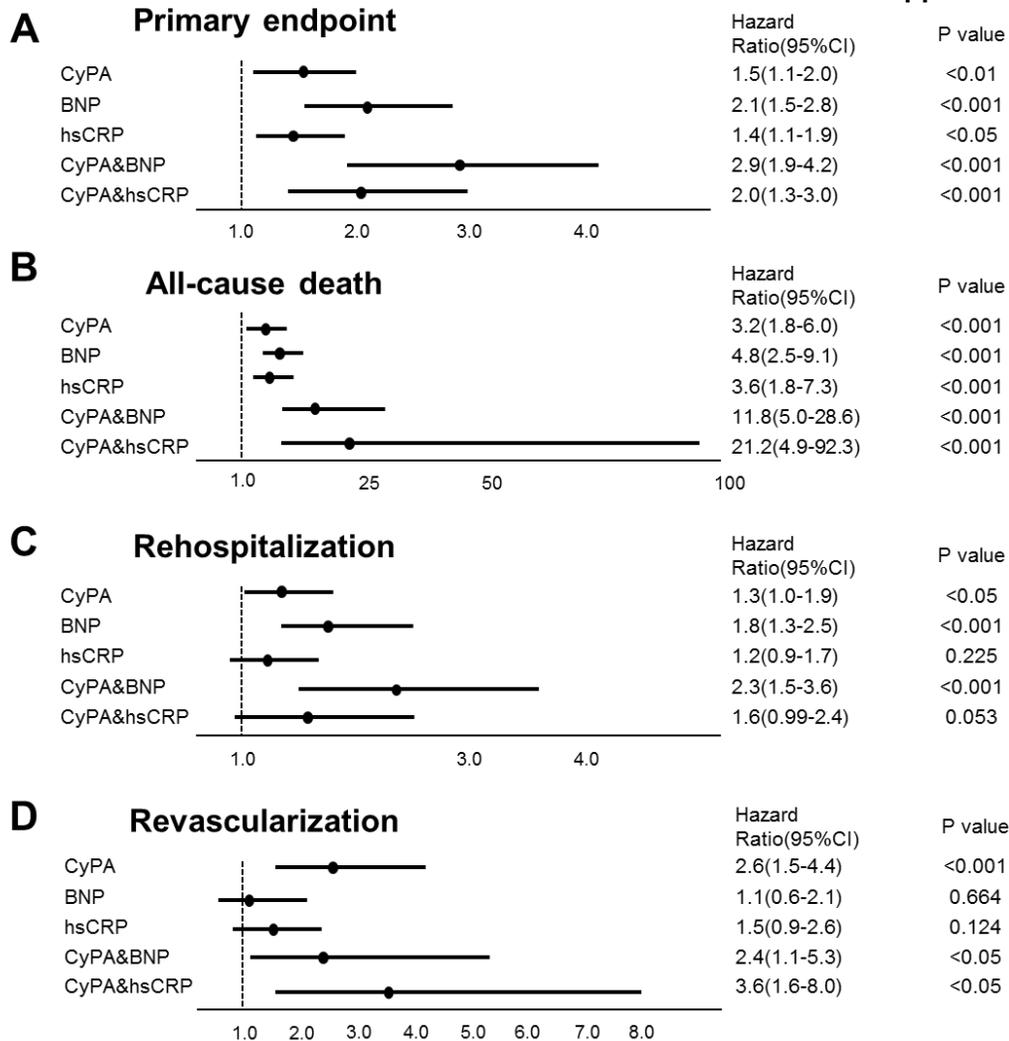
Supplementary Figure VII.   Serum Levels of Cytokines/chemokines and Growth Factors in CAD Patients

Serum levels of cytokines/chemokines and growth factors in CAD patients (*n*=101).   Each experiment was performed in duplicate.   Results are expressed by box plots analyses.

Supplementary Figure VIII

Supplementary Figure VIII. Hazard Ratios of Plasma Levels of CyPA and Other Biomarkers

Only plasma CyPA levels had a significant prognostic impact for (**A**) primary endpoint, (**B**) all-cause death, (**C**) rehospitalization, and (**D**) revascularization.   In addition, combining with other biomarkers (BNP, hsCRP levels) further enhanced the prognostic impacts of the CyPA levels.

SUPPLEMENTAL MATERIAL

Prognostic Impacts of Plasma Levels of Cyclophilin A in Patients with Coronary Artery Disease

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Online Material and Methods
Supplementary Figure I-VIII
Supplementary Figure Legend I-VIII

Material and Methods

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 2009 to May 2014, a total of 531 patients were referred for diagnostic catheterization for evaluation of chest pain and/or ECG abnormalities at the Tohoku University Hospital.   The enrolled patients had evidence of ischemia on exercise ECG or myocardial radionuclide imaging.   Patients with unstable angina or myocardial infarction were excluded from the present study.   Twenty of the enrolled patients were lost to follow-up.

Evaluation of Coronary Artery Stenosis

Two experienced cardiologists, who were blinded to the patients' data, including plasma levels of CyPA, evaluated the coronary angiograms.   The severity of coronary stenosis was assessed according to the American Heart Association standards.[1]   A narrowing of the lumen by more than 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, diabetes, and smoking status.   Patients with hypertension were regarded as being at risk if their blood pressure was ≥140/90 mmHg or if they had a history of antihypertensive drug use.   Patients with diabetes mellitus 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 LDL cholesterol level was ≥140 mg/dL or their HDL cholesterol level was ≤40 mg/dL, or if they were taking a lipid-lowering drug.   Fasting blood samples were collected for measurement of CyPA concentration immediately before CAG from the antecubital vein in the supine position.   Plasma samples were collected using EDTA and were centrifuged for 10 min at 2,500 g within 30 min of blood collection, and aliquots were stored at -80°C.   Plasma CyPA levels were measured using an immunoassay based on the sandwich technique according to the manufacturer's protocol (Human Cyclophilin A ELISA Kit, CSB-E09920h, Cusabio).   Plasma levels of hsCRP were measured using the sandwich technique (Roche Diagnostics).   Values of other laboratory parameters were obtained with an auto analyzer at the Tohoku University Hospital.

Measurement of Cytokines/Chemokines and Growth Factors by Bioplex System

Serum levels of cytokines/chemokines and growth factors were measured with a Bioplex system (Bio-Rad, Tokyo, Japan) 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, #MF0-005KMII). Each experiment was performed in duplicate (**Supplementary Figure VII**).

Statistical Analysis

Information on death, rehospitalization, and revascularization was collected annually using follow-up questionnaires, telephone interviews, and medical records. The primary endpoint was the composite of all-cause death, rehospitalization, and revascularization, while the secondary endpoints consisted of all-cause death, rehospitalization, and revascularization. The primary and secondary endpoints were analyzed based on the time to the first occurrence. Cardiovascular death was defined as death caused by AMI or heart failure. 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 causes after enrollment in the present study. Revascularization was defined as undergoing percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) after enrollment in the study.

Categorical variables were presented as numerals and percentages. Continuous variables were presented as means±standard deviations. Correlations between plasma CyPA levels and age or plasma levels of BNP, hsCRP, and uric acid were analyzed using the Spearman rank correlation coefficient. Differences in plasma CyPA levels depending on sex, smoking status, and presence of hypertension, diabetes mellitus or dyslipidemia were analyzed by Mann-Whitney U test. In order to determine the most appropriate cut-off points for high and low plasma CyPA levels, we performed classification and regression tree (CART) analysis.[2] CART analysis is an empirical, statistical technique based on recursive partitioning of the data space to predict the response. 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 two 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.[2] We also performed the CART analysis to determine the most appropriate cut-off point for plasma levels

of BNP and hsCRP. Kaplan-Meier curves were plotted based on the cut-off points of all-cause death for each biomarker. Kaplan-Meier curves were plotted for the primary end point, all-cause death, rehospitalization, and revascularization relative to the cut-off points. The log rank test and generalized Wilcoxon test were applied to compare event-free survival between groups. Cox's proportional hazards model was used for univariate analysis and multivariate analysis. All P values were two-tailed, and a P <0.05 was considered to be statistical significant. All analyses were performed with R, version 3.1.3 (R Foundation for Statistical Computing, Vienna. http://www.R-project.org/).[3]

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