

Peripheral Endothelial Function and Cardiovascular Events in High-Risk Patients

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Background—Endothelial dysfunction is a key component of vascular vulnerability. Reactive hyperemia index (RHI), as assessed by the peripheral arterial tonometry, can noninvasively evaluate endothelial function. This study was designed to determine the additional prognostic value of endothelial function to the Synergy Between PCI With Taxus and Cardiac Surgery Score (SYNTAXsc) and the Framingham Risk Score (FRS) in predicting cardiovascular events in high-risk patients.

Methods and Results—We undertook a two-center prospective study in 528 stable patients at high-risk for cardiovascular events from the years 2006–2011. The RHI was measured before coronary angiography and coronary complexity was assessed by SYNTAXsc. After optimal therapies including coronary revascularization, there was follow-up with patients until August 2012. Cardiovascular events consist of cardiovascular death, myocardial infarction, unstable angina, ischemic stroke, coronary revascularization, heart failure-induced hospitalization, aortic disease, and peripheral arterial disease. During 1468 person-years of follow-up, 105 patients developed cardiovascular events. Multivariate Cox proportional hazards analysis identified B-type natriuretic peptide (BNP), SYNTAXsc, and RHI as independent cardiovascular event predictors (hazard ratio [95% confidence interval]: natural logarithm of BNP per 0.1: 1.019 [1.002 to 1.037]; $P=0.023$, SYNTAXsc per tertile: 2.426 [1.825 to 3.225]; $P<0.0001$, RHI per 0.1: 0.761 [0.673 to 0.859]; $P<0.0001$). When RHI was added to the FRS, BNP, and SYNTAXsc, net reclassification index was significantly improved (27.5%; $P<0.0001$), with a significant increase in the C-statistic (from 0.728 [0.679 to 0.778] to 0.766 [0.726 to 0.806]; $P=0.031$).

Conclusions—Advanced endothelial dysfunction significantly correlated with near future cardiovascular events in high-risk patients. This physiological vascular measurement improved risk discrimination when added to the FRS, BNP, and SYNTAXsc.

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An integrated approach for identifying patients who are at a high risk for near-future cardiovascular events is desirable for developing proper therapeutic strategies in cardiovascular medicine.¹ Cardiovascular risk assessment using established risk factors such as the Framingham Risk Score (FRS) does not exhaustively predict cardiovascular disease development. Endothelial dysfunction is associated with atherosclerotic progression and can often predict future cardiovascular events.^{2–6}

Coronary plaques and morphological findings using coronary angiography (CAG) reportedly improve cardiovascular event prediction. Synergy Between PCI With Taxus and Cardiac Surgery Score (SYNTAXsc) is an angiographic scoring system used to evaluate coronary atherosclerosis and anatomy complexity.⁷ The SYNTAXsc is reportedly an effective tool for predicting a major cardiovascular event risk in stable

patients with multivessel or left main coronary artery disease (CAD) and in patients with acute coronary syndromes,^{8,9} but not in stable patients with high-risk condition.

Reactive hyperemia-peripheral arterial tonometry (RH-PAT), which is used to measure the digital hyperemic response, is a noninvasive, automatic, and less operator-dependent test that is clinically used to evaluate endothelial function.^{10,11} Recently, we reported that the RH-PAT index (RHI) was useful for identifying female patients who were at high-risk for

ischemic heart disease,¹² and Rubinshtein et al reported that the RHI predicted adverse cardiovascular events in patients without known CAD.¹³

We hypothesized that adding RHI as an assessment of physiological endothelial function to the coronary complexity morphological assessment and classical risk score would provide substantial cardiovascular event prognostic information in high-risk patients, including stable patients with established CAD.

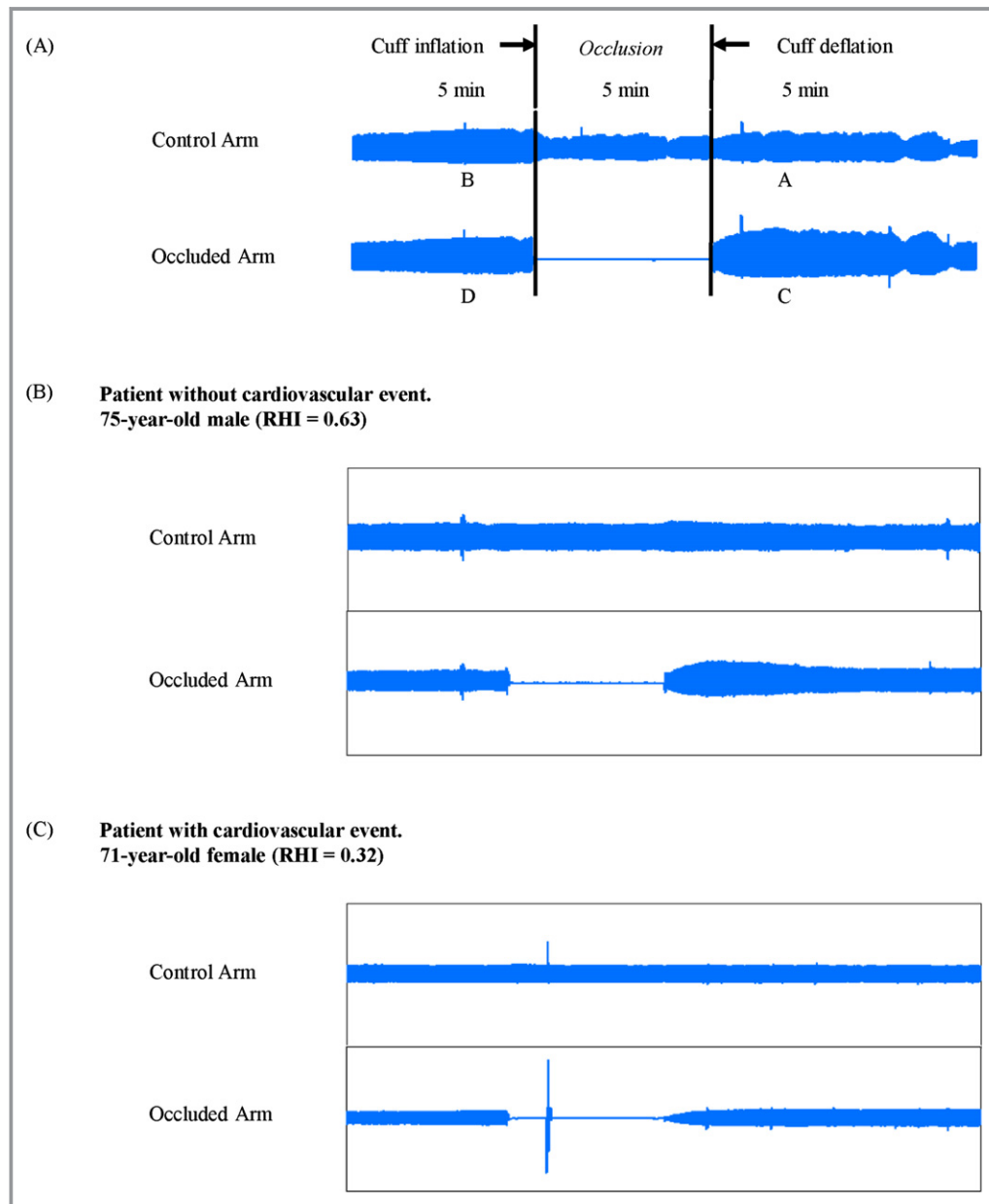


Figure 1. Representative RH-PAT signals. A, RH-PAT ratio was calculated with the following equation: $\text{RH-PAT ratio} = (C/D)/(A/B)$. The representative results of RH-PAT of the patient without cardiovascular event (B) and the patient with cardiovascular event (C). RHI indicates reactive hyperemia-peripheral arterial tonometry index; RH-PAT, reactive hyperemia-peripheral arterial tonometry.

Methods

Study Design and Population

This is a prospective observational study and we recruited 577 consecutive, stable, high-risk patients with diabetes mellitus or >2 conventional coronary risk factors without heart failure (left ventricular ejection fraction <50%, cardiomyopathy, and severe valvular heart disease). These patients had been referred to Kumamoto University Hospital and Yokohama City University Medical Center for CAG because of angina-like chest symptoms and abnormality in electrocardiogram with high-risk background for CAD between August 2006 and December 2011. RH-PAT examination and the subsequent CAG were performed. Exclusion criteria were prespecified as a clinical status that could evidently affect their noncardiovascular prognosis and endothelial function, including systemic illness (advanced endocrine disease, hepatic disease, end-stage renal disease, active inflammatory

disease, and cancer), and cerebrovascular disease with residual hemiplegia.

The present study was approved by the Kumamoto University Institutional Review Board and Yokohama City University Institutional Review Board and was conducted in accordance with the guidelines of our institutional ethics committees and the Declaration of Helsinki. Written informed consent was obtained from each patient before participation.

Angiographic Analysis

Based on quantitative CAG analysis, we defined CAD as $\geq 50\%$ narrowing of coronary artery diameter in at least 1 coronary artery. The SYNTAXsc for each angiogram was independently evaluated by 2 experienced cardiologists who were blinded to the RH-PAT results. In case of disagreement, consensus was achieved by consulting 2 cardiologists. Briefly, each lesion with $\geq 50\%$ luminal narrowing in ≥ 1.5 mm vessels was defined

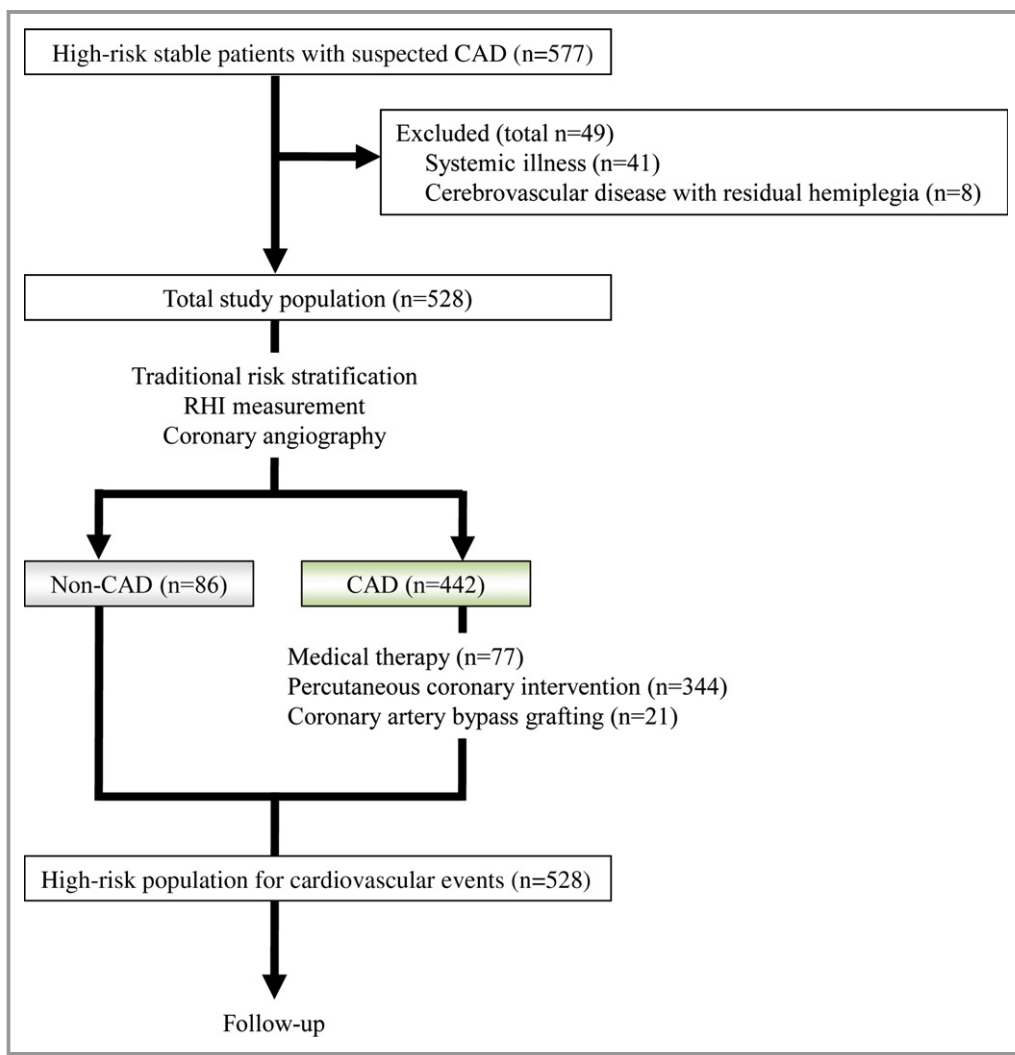


Figure 2. Study protocol flow chart. CAD indicates coronary artery disease; RHI, reactive hyperemia-peripheral arterial tonometry index.

based on the modified American Heart Association coronary tree segment classification and separately scored regarding bifurcations or trifurcations or aortic ostial localization, chronic occlusion, vessel tortuosity, length, calcification, and thrombus formation. Finally, each lesion score was added to obtain the patient's raw SYNTAXsc. Angiographers who calculated SYNTAXsc were blinded to clinical data and RH-

PAT data, and patients with occluded infarct-related arteries were scored as occlusions of unknown duration. A high SYNTAXsc is indicative of complex coronary disease.⁷ We assessed reproducibility of the SYNTAXsc in a random sample of 30 patients. The kappa values for SYNTAXsc (tertile partitioning) intra- and interobserver agreement were 0.80 and 0.69, respectively.

Table 1. Baseline Characteristics of 528 High-Risk Patients

	All High-risk Patients (n=528)	RHI		P Value
		High RHI 0.531< (n=267)	Low RHI ≤0.531 (n=261)	
Age, mean (SD), y	67.2 (10.7)	66.2 (11.3)	68.2 (9.9)	0.027
Male sex, no. (%)	365 (69.1)	168 (62.9)	197 (75.5)	0.0019
Body mass index, mean (SD), kg/m ²	24.2 (3.5)	24.0 (3.5)	24.3 (3.4)	0.297
Hypertension, no. (%)	429 (81.3)	210 (78.7)	219 (83.9)	0.147
Diabetes, no. (%)	248 (47.0)	128 (47.9)	120 (46.0)	0.664
Dyslipidemia, no. (%)	438 (83.0)	222 (83.1)	216 (82.8)	0.908
Current smoking, no. (%)	112 (21.2)	47 (17.6)	65 (24.9)	0.043
Family history of CAD, no. (%)	130 (24.6)	75 (28.1)	55 (21.1)	0.069
Systolic BP, mean (SD), mm Hg	129.3 (18.4)	129.1 (18.6)	129.4 (18.2)	0.890
Diastolic BP, mean (SD), mm Hg	72.7 (12.1)	71.9 (12.3)	73.5 (11.8)	0.149
Hemoglobin A1c, mean (SD), %	6.4 (1.0)	6.4 (1.0)	6.4 (1.1)	0.912
Total/HDL cholesterol ratio, mean (SD)	3.7 (1.1)	3.6 (1.1)	3.7 (1.1)	0.049
LDL cholesterol, mean (SD), mg/dL	105.2 (31.6)	106.1 (32.2)	104.3 (30.9)	0.525
HDL cholesterol, mean (SD), mg/dL	51.9 (14.5)	53.7 (14.6)	50.1 (14.1)	0.0042
Triglycerides, median (IQR), mg/dL	115 (83 to 154)	115 (80 to 151)	116 (85 to 160)	0.561
LVEF, mean (SD), %	63.6 (6.9)	64.0 (7.2)	63.2 (6.8)	0.220
BNP, median (IQR), pg/mL	28.6 (13.3 to 62.3)	28.0 (11.5 to 54.4)	29.4 (15.0 to 67.9)	0.019
hsCRP, median (IQR), mg/L	0.76 (0.30 to 1.80)	0.70 (0.30 to 1.76)	0.90 (0.37 to 1.90)	0.192
eGFR, mean (SD), mL/min per 1.73 m ²	67.3 (18.1)	68.8 (17.1)	65.8 (19.1)	0.057
Aspirin, no. (%)	434 (82.2)	202 (75.7)	232 (88.9)	0.0001
HMG-CoA RIs, no. (%)	373 (70.6)	178 (66.7)	195 (74.7)	0.045
CCB, no. (%)	305 (57.8)	142 (53.2)	163 (62.5)	0.035
ACE-I or ARB, no. (%)	296 (56.1)	142 (53.2)	154 (59.0)	0.189
β-blockers, no. (%)	228 (43.2)	105 (39.3)	123 (47.1)	0.079
Anti-diabetic drugs, no. (%)	175 (33.1)	90 (33.7)	85 (32.6)	0.782
Coronary artery disease, no. (%)	442 (83.7)	190 (71.2)	252 (96.6)	<0.0001
FRS, median (IQR), %	8.0 (4.0 to 11.0)	7.0 (2.0 to 11.0)	9.0 (7.0 to 13.0)	<0.0001
SYNTAXsc, median (IQR)	13.0 (5.0 to 20.0)	9.0 (0.0 to 18.0)	16.0 (7.8 to 22.5)	<0.0001
SYNTAXsc ≥23, no. (%)	91 (17.2)	34 (12.7)	57 (21.8)	0.0058
RHI, mean (SD)	0.566 (0.210)	0.725 (0.168)	0.402 (0.088)	<0.0001
Cardiovascular events, no. (%)	105 (19.9)	22 (8.2)	83 (31.8)	<0.0001

Data are the mean (SD), median values (25th to 75th percentile range), or no. (%). Significance was assessed by an unpaired *t* test, the Mann-Whitney U test or Fisher's exact test. ACE-I indicates angiotensin-converting enzyme-inhibitors; ARB, angiotensin II receptor blockers; BNP, B-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blockers; eGFR, estimated glomerular filtration rate; FRS, Framingham Risk Score; HDL, high-density lipoprotein; HMG-CoA RIs, hydroxymethylglutaryl-CoA reductase inhibitors; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia-peripheral arterial tonometry index; SD, standard deviation; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

RH-PAT Examination

The RH-PAT studies in all of the patients were uniformly performed early in the morning in the fasted state before medication intake and prior to CAG, but within 7 days of CAG. The PAT method has been previously described.¹⁴

RH-PAT measurements were analyzed with a computerized, automated algorithm to reduce intra- and interobserver variability (Endo-PAT2000 software, version 3.0.4, Itamar Medical Ltd). The RH-PAT ratio was calculated using the ratio of the average PAT signal amplitude over a 1-minute time interval, starting 1.5 minutes after cuff deflation (where

Table 2. Baseline Characteristics of 528 High-risk Patients Divided by Cardiovascular Events

	All High-risk Patients (n=528)	Cardiovascular Events During Follow-up		P Value
		No (n=423)	Yes (n=105)	
Age, mean (SD), y	67.2 (10.7)	66.3 (10.8)	70.7 (9.7)	0.0002
Male sex, no. (%)	365 (69.1)	289 (68.3)	76 (72.4)	0.479
Body mass index, mean (SD), kg/m ²	24.2 (3.5)	24.4 (3.6)	23.4 (2.9)	0.012
Hypertension, no. (%)	429 (81.3)	339 (80.1)	90 (85.7)	0.211
Diabetes, no. (%)	248 (47.0)	197 (46.6)	51 (48.6)	0.744
Dyslipidemia, no. (%)	438 (83.0)	353 (83.5)	85 (81.0)	0.563
Current smoking, no. (%)	112 (21.2)	88 (20.8)	24 (22.9)	0.689
Family history of CAD, no. (%)	130 (24.6)	106 (25.1)	24 (22.9)	0.705
Systolic BP, mean (SD), mm Hg	129.3 (18.4)	129.4 (18.3)	128.9 (18.7)	0.804
Diastolic BP, mean (SD), mm Hg	72.7 (12.1)	73.0 (12.3)	71.4 (10.9)	0.235
Hemoglobin A1c, mean (SD), %	6.4 (1.0)	6.4 (1.0)	6.5 (1.3)	0.108
Total/HDL cholesterol ratio, mean (SD)	3.7 (1.1)	3.7 (1.1)	3.6 (1.1)	0.713
LDL cholesterol, mean (SD), mg/dL	105.2 (31.6)	106.2 (32.0)	101.5 (29.7)	0.180
HDL cholesterol, mean (SD), mg/dL	51.9 (14.5)	52.1 (14.1)	51.1 (15.8)	0.556
Triglycerides, median (IQR), mg/dL	115 (83 to 154)	117 (85 to 153)	107 (75 to 157)	0.234
LVEF, mean (SD), %	63.6 (6.9)	63.8 (6.8)	62.7 (7.4)	0.150
BNP, median (IQR), pg/mL	28.6 (13.3 to 62.3)	26.1 (12.4 to 54.4)	45.3 (20.8 to 100.2)	<0.0001
hsCRP, median (IQR), mg/L	0.76 (0.30 to 1.80)	0.70 (0.30 to 1.70)	0.90 (0.50 to 2.40)	0.033
eGFR, mean (SD), mL/min per 1.73 m ²	67.3 (18.1)	68.4 (17.8)	62.8 (18.8)	0.0048
Aspirin, no. (%)	434 (82.2)	342 (80.9)	92 (87.6)	0.118
HMG-CoA RIs, no. (%)	373 (70.6)	297 (70.2)	76 (72.4)	0.720
CCB, no. (%)	305 (57.8)	242 (57.2)	63 (60.0)	0.659
ACE-I or ARB, no. (%)	296 (56.1)	233 (55.1)	63 (60.0)	0.381
β-blockers, no. (%)	228 (43.2)	174 (41.1)	54 (51.4)	0.062
Anti-diabetic drugs, no. (%)	175 (33.1)	132 (31.2)	43 (41.0)	0.064
Coronary artery disease, no. (%)	442 (83.7)	340 (80.4)	102 (97.1)	<0.0001
FRS, median (IQR), %	8.0 (4.0 to 11.0)	8.0 (3.0 to 11.0)	9.0 (7.0 to 13.0)	0.0022
SYNTAXsc, median (IQR)	13.0 (5.0 to 20.0)	10.0 (3.0 to 18.0)	19.0 (15.0 to 25.8)	<0.0001
SYNTAXsc ≥23, no. (%)	91 (17.2)	57 (13.5)	34 (32.4)	<0.0001
Baseline pulse amplitude, mean (SD)	899 (413)	910 (418)	856 (388)	0.235
Ln_RH-PAT ratio, mean (SD)	0.292 (0.260)	0.322 (0.267)	0.171 (0.185)	<0.0001
RHI, mean (SD)	0.566 (0.210)	0.595 (0.211)	0.449 (0.163)	<0.0001

Data are the mean (SD), median values (25th to 75th percentile range), or no. (%). Significance was assessed by an unpaired *t* test, the Mann-Whitney U test or Fisher's exact test. ACE-I indicates angiotensin-converting enzyme-inhibitors; ARB, angiotensin II receptor blockers; BNP, B-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blockers; eGFR, estimated glomerular filtration rate; FRS, Framingham Risk Score; HDL, high-density lipoprotein; HMG-CoA RIs, hydroxymethylglutaryl-CoA reductase inhibitors; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia-peripheral arterial tonometry index; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SD, standard deviation; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

control arm=A and the occluded arm=C), divided by the average PAT signal amplitude 2.5 minutes before cuff inflation (baseline) (where the control arm=B and the occluded arm=D), and the RH-PAT ratio=(C/D)/(A/B) (Figure 1A). Because RH-PAT ratio results have a skewed distribution, we used the Ln_RH-PAT ratio and the RHI for analyses. The RHI was derived from the following equation: $RHI = \text{Ln}\{[\text{RH-PAT ratio}] \times [0.226 \times \text{Ln}(\text{baseline}) - 0.2]\}$.^{13,15} Ln_RH-PAT ratio and baseline pulse amplitude were retrospectively analyzed using Endo-PAT2000 software (version 3.4.4); however, reanalysis was impossible in 2 patients for unknown reasons. Peripheral endothelial function as assessed by the RHI was validated by

coronary artery response to acetylcholine, which is the gold standard coronary endothelial functional measurement.^{12,16} Previous studies demonstrated that RH-PAT technology has excellent reproducibility.^{14,17–19}

Coronary Risk Factors

Coronary risk factors were defined as current smoking (within 1 year), hypertension (>140/90 mm Hg or antihypertensive medication), dyslipidemia (high-density lipoprotein [HDL] cholesterol <40 mg/dL, low-density lipoprotein [LDL] cholesterol \geq 140 mg/dL, triglycerides \geq 150 mg/dL or medications

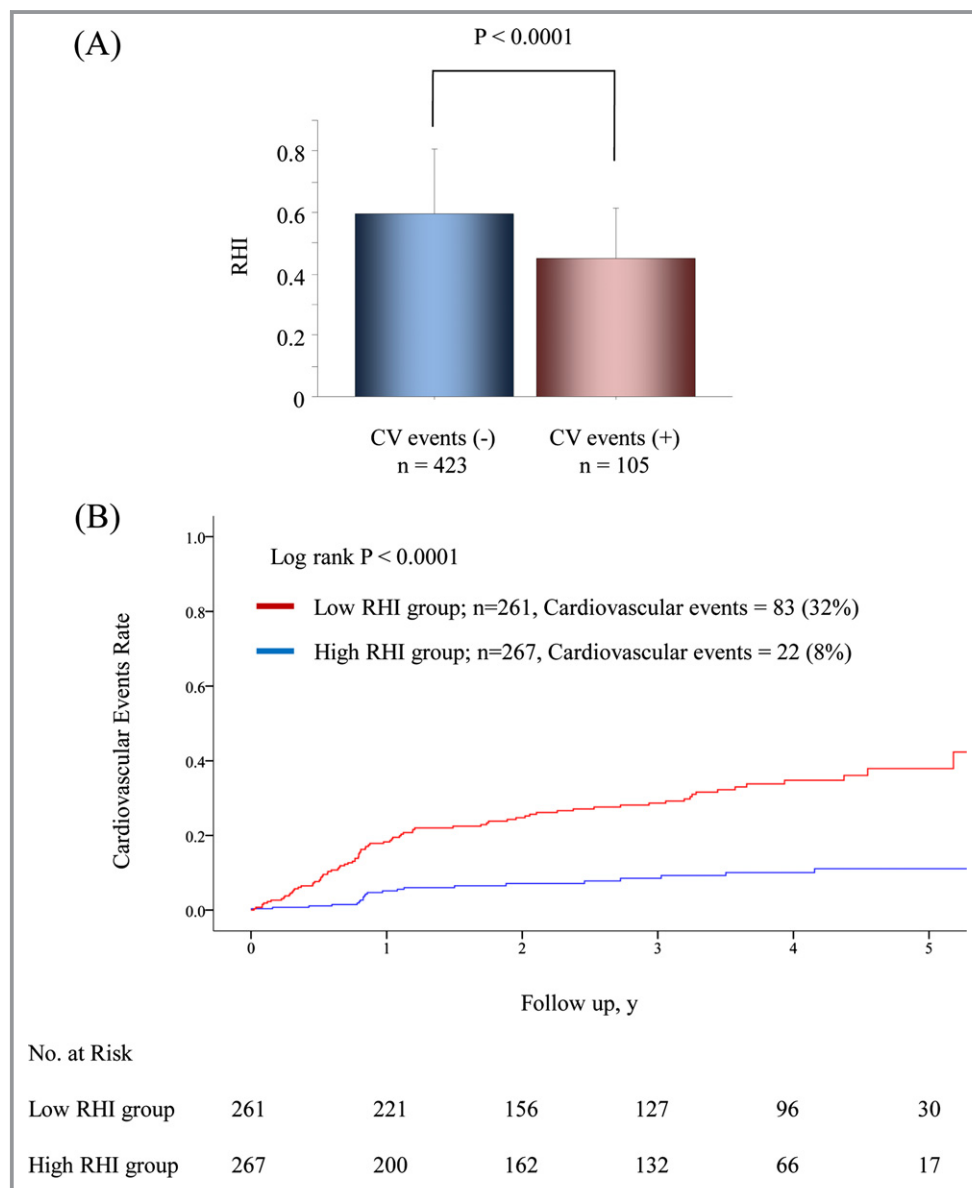


Figure 3. RHI and cardiovascular events. A, These bars represent RHI averages for each group. T-bars indicate standard deviation. B, Kaplan-Meier analysis for cardiovascular event probability in high-risk patients based on a median RHI value of 0.531. CV indicates cardiovascular; RHI, reactive hyperemia-peripheral arterial tonometry index.

for dyslipidemia) and, diabetes mellitus (diabetes symptoms and casual plasma glucose concentration ≥ 200 mg/dL, fasting plasma glucose concentration ≥ 126 mg/dL, 2-hour plasma glucose concentration ≥ 200 mg/dL during 75 g oral glucose tolerance test or hypoglycemic medication), and the presence of a family history of CAD.

Blood Tests and Risk Assessment by Framingham Risk Score (FRS)

Venous blood samples were obtained early in the morning after a 12-hour fast on the day after admission to measure fasting blood glucose, hemoglobin A1c, total cholesterol, HDL

Table 3. Baseline Characteristics of 526 High-risk Patients According to Ln_RH-PAT Ratio

	Ln_RH-PAT Ratio		P Value
	High Ln_RH-PAT Ratio 0.246 < (n=263)	Low Ln_RH-PAT Ratio ≤ 0.246 (n=263)	
Age, mean (SD), y	66.3 (11.3)	68.0 (10.0)	0.068
Male sex, no. (%)	174 (66.2)	190 (72.2)	0.156
Body mass index, mean (SD), kg/m ²	23.8 (3.5)	24.5 (3.4)	0.024
Hypertension, no. (%)	204 (77.6)	223 (84.8)	0.044
Diabetes, no. (%)	125 (47.5)	121 (46.0)	0.793
Dyslipidemia, no. (%)	214 (81.4)	223 (84.8)	0.352
Current smoking, no. (%)	48 (18.3)	64 (24.3)	0.110
Family history of CAD, no. (%)	75 (28.5)	55 (20.9)	0.055
Systolic BP, mean (SD), mm Hg	128.5 (18.6)	129.9 (18.0)	0.377
Diastolic BP, mean (SD), mm Hg	72.3 (12.4)	72.9 (11.6)	0.547
Hemoglobin A1c, mean (SD), %	6.4 (1.0)	6.4 (1.1)	0.794
Total/HDL cholesterol ratio, mean (SD)	3.5 (1.0)	3.8 (1.1)	0.008
LDL cholesterol, mean (SD), mg/dL	104.5 (31.5)	106.0 (31.7)	0.595
HDL cholesterol, mean (SD), mg/dL	53.4 (14.2)	50.4 (14.6)	0.016
Triglycerides, median (IQR), mg/dL	114 (78 to 150)	119 (87 to 161)	0.100
LVEF, mean (SD), %	63.8 (7.3)	63.5 (6.5)	0.647
BNP, median (IQR), pg/mL	28.0 (11.2 to 56.8)	28.0 (14.7 to 63.2)	0.183
hsCRP, median (IQR), mg/L	0.70 (0.30 to 1.90)	0.80 (0.38 to 1.70)	0.479
eGFR, mean (SD), mL/min per 1.73 m ²	68.7 (17.6)	65.9 (18.6)	0.076
Aspirin, no. (%)	202 (76.8)	231 (87.8)	0.001
HMG-CoA RIs, no. (%)	176 (66.9)	196 (74.5)	0.068
CCB, no. (%)	151 (57.4)	154 (58.6)	0.792
ACE-I or ARB, no. (%)	140 (53.2)	155 (58.9)	0.219
β -blockers, no. (%)	103 (39.2)	124 (47.1)	0.078
Anti-diabetic drugs, no. (%)	86 (32.7)	87 (33.1)	>0.99
Coronary artery disease, no. (%)	191 (72.6)	249 (94.7)	<0.0001
FRS, median (IQR), %	7.0 (2.0 to 11.0)	9.0 (6.0 to 11.0)	<0.0001
SYNTAXsc, median (IQR)	11.0 (0.0 to 19.0)	15.0 (7.0 to 22.0)	<0.0001
SYNTAXsc ≥ 23 , no. (%)	39 (14.8)	52 (19.8)	0.166
Ln_RH-PAT ratio, mean (SD)	0.490 (0.213)	0.094 (0.105)	<0.0001
Cardiovascular events, no. (%)	29 (11.0)	75 (28.5)	<0.0001

Data are the mean (SD), median values (25th to 75th percentile range), or no. (%). Significance was assessed by an unpaired *t* test, the Mann-Whitney U test or Fisher's exact test. ACE-I indicates angiotensin-converting enzyme-inhibitors; ARB, angiotensin II receptor blockers; BNP, B-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blockers; eGFR, estimated glomerular filtration rate; FRS, Framingham Risk Score; HDL, high-density lipoprotein; HMG-CoA RIs, hydroxymethylglutaryl-CoA reductase inhibitors; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SD, standard deviation; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

cholesterol, LDL cholesterol, triglycerides, insulin, creatinine, B-type natriuretic peptide (BNP), and high-sensitivity C-reactive protein (hsCRP) levels. Estimated glomerular filtration rates (eGFRs) were determined using the prediction equation proposed by the Japanese Society of Nephrology and based on the equation described in the Modification of Diet in Renal

Disease Study.²⁰ D'Agostino et al reported the FRS for primary and secondary prevention of the composite cardiovascular events.²¹ In their study, cardiovascular events occurred in 24.7% of patients with coronary heart disease or stroke during 4-year follow-up period. We adopted this risk model in our study, and stratified study patients into 1 of 3 risk categories

Table 4. Baseline Characteristics of 526 High-risk Patients According to Baseline Pulse Amplitude

	Mean Baseline		P Value
	High Mean Baseline 857< (n=263)	Low Mean Baseline ≤857 (n=263)	
Age, mean (SD), y	67.6 (10.3)	66.7 (11.0)	0.324
Male sex, no. (%)	187 (71.1)	177 (67.3)	0.395
Body mass index, mean (SD), kg/m ²	24.4 (3.2)	24.0 (3.7)	0.199
Hypertension, no. (%)	214 (81.4)	213 (81.0)	>0.99
Diabetes, no. (%)	127 (48.3)	119 (45.2)	0.541
Dyslipidemia, no. (%)	218 (82.9)	219 (83.3)	>0.99
Current smoking, no. (%)	55 (20.9)	57 (21.7)	0.915
Family history of CAD, no. (%)	69 (26.2)	61 (23.2)	0.479
Systolic BP, mean (SD), mm Hg	130.6 (18.2)	127.7 (18.4)	0.076
Diastolic BP, mean (SD), mm Hg	72.8 (11.5)	72.5 (12.5)	0.783
Hemoglobin A1c, mean (SD), %	6.4 (1.0)	6.4 (1.0)	0.788
Total/HDL cholesterol ratio, mean (SD)	3.7 (1.1)	3.7 (1.1)	0.974
LDL cholesterol, mean (SD), mg/dL	104.4 (30.8)	106.0 (32.4)	0.576
HDL cholesterol, mean (SD), mg/dL	51.7 (14.7)	52.0 (14.6)	0.814
Triglycerides, median (IQR), mg/dL	118 (86 to 154)	113 (80 to 156)	0.713
LVEF, mean (SD), %	63.8 (6.6)	63.5 (7.2)	0.646
BNP, median (IQR), pg/mL	28.3 (14.8 to 57.6)	27.9 (11.7 to 63.4)	0.482
hsCRP, median (IQR), mg/L	0.70 (0.30 to 1.80)	0.78 (0.33 to 1.76)	0.711
eGFR, mean (SD), mL/min per 1.73 m ²	66.8 (17.6)	67.8 (18.8)	0.565
Aspirin, no. (%)	222 (84.4)	211 (80.2)	0.253
HMG-CoA RIs, no. (%)	189 (71.9)	183 (69.6)	0.632
CCB, no. (%)	145 (55.1)	160 (60.8)	0.216
ACE-I or ARB, no. (%)	147 (55.9)	148 (56.3)	>0.99
β-blockers, no. (%)	121 (46.0)	106 (40.3)	0.218
Anti-diabetic drugs, no. (%)	91 (34.6)	82 (31.2)	0.458
Coronary artery disease, no. (%)	227 (86.3)	213 (81.0)	0.125
FRS, median (IQR), %	9.0 (5.0 to 11.0)	8.0 (3.0 to 11.0)	0.059
SYNTAXsc, median (IQR)	14.0 (5.0 to 20.0)	12.0 (5.0 to 19.0)	0.156
SYNTAXsc ≥23, no. (%)	51 (19.4)	40 (15.2)	0.249
Baseline Pulse Amplitude, mean (SD)	1226 (304)	573 (187)	<0.0001
Cardiovascular events, no. (%)	50 (19.0)	54 (20.5)	0.663

Data are the mean (SD), median values (25th to 75th percentile range), or no. (%). Significance was assessed by an unpaired *t* test, the Mann-Whitney U test or Fisher's exact test. ACE-I indicates angiotensin-converting enzyme-inhibitors; ARB, angiotensin II receptor blockers; BNP, B-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blockers; eGFR, estimated glomerular filtration rate; FRS, Framingham Risk Score; HDL, high-density lipoprotein; HMG-CoA RIs, hydroxymethylglutaryl-CoA reductase inhibitors; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; SD, standard deviation; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

Table 5. Cardiovascular Events in Patients With Low or High RHI

All High-Risk Patients	High RHI Group (n=267)	Low RHI Group (n=261)	P Value
	No. (% [95% CI])	No. (% [95% CI])	
Total cardiovascular events	22 (8.2 [4.9 to 11.5])	83 (31.8 [26.2 to 37.5])	<0.0001
Cardiovascular death	0 (0 [0 to 0])	1 (0.4 [−0.4 to 1.1])	0.296
AMI or unstable angina	1 (0.4 [−0.4 to 1.1])	28 (10.7 [7.0 to 14.5])	<0.0001
Heart failure	2 (0.7 [−0.3 to 1.8])	13 (5.0 [2.3 to 7.6])	0.0036
Coronary revascularization	14 (5.2 [2.6 to 7.9])	29 (11.1 [7.3 to 14.9])	0.0089
Aortic disease or PAD	4 (1.5 [0.0 to 3.0])	6 (2.3 [0.5 to 4.1])	0.384
Stroke	1 (0.4 [−0.4 to 1.1])	6 (2.3 [0.5 to 4.1])	0.048

Significance was assessed by the log rank test. AMI indicates acute myocardial infarction; CI, confidence interval; PAD, peripheral arterial disease; RHI, reactive hyperemia-peripheral arterial tonometry index.

(calculated as a 2-year score): low-intermediate risk (<12%), high risk (12% to 25%), or very high risk (>25%).

Follow-up and Cardiovascular Events

After optimal therapies including coronary revascularization, patients were followed until August 2012. Cardiovascular events consisted of cardiovascular death, nonfatal myocardial infarction, unstable angina, ischemic stroke, coronary revascularization, heart failure-induced hospitalization, nonfatal aortic disease, and peripheral arterial disease. Cardiovascular events were documented by phone calls to patients or their families and direct consultation with their physicians. In order to verify diagnosis of the cardiovascular events, 3 independent physicians comprising events committee reviewed all medical records (electrocardiograms, ultrasound echocardiograms, cardiac enzyme data, radiographic images, and death certificates) and validated cardiovascular events. If the reviewing physicians disagreed on the event classification, they adjudicated differences. Cardiovascular death was defined as death because of myocardial infarction (within 28 days), congestive heart failure, or documented sudden death without apparent noncardiovascular causes. Myocardial infarction was diagnosed by a rise or fall in cardiac biomarkers (plasma creatine kinase-MB or cardiac troponin) above the 99th percentile of the normal range upper limit together with evidence of myocardial ischemia and at least 1 of the following symptoms: electrocardiogram changes (new ST-T changes, left bundle branch block, or pathological Q wave) or imaging evidence of new viable myocardium loss, or a new regional wall motion abnormality. A diagnosis of unstable angina pectoris was made by new or accelerating myocardial ischemia symptoms accompanied by new ischemic ST-T-wave changes. Ischemic stroke diagnosis was based on the documented focal neurologic deficit with radiological evidence of brain infarction excluding intracranial hemor-

rhage. Hospitalization for heart failure decompensation was made if the patient was admitted with typical heart failure symptoms and had objective signs of worsening heart failure that required intravenous drug administration. A diagnosis of aortic and peripheral arterial disease was based on radiological imaging evidence and surgery requirement or percutaneous intervention.

Statistical Analysis

The continuous variable distributions were tested for normality using the Kolmogorov-Smirnov test. Continuous variable data with normal distribution were expressed as the mean (standard deviation), and the data with skewed distributions were expressed as medians [interquartile range]. Differences between normally distributed continuous variables were analyzed by an unpaired *t* test or one-way analysis of variance (ANOVA) with the post-hoc Bonferroni test. We used the Mann-Whitney U test for continuous variables with a skewed distribution. We compared groups using the chi-squared test. We calculated the cumulative cardiovascular event incidence with the Kaplan-Meier method and compared cardiovascular event incidence with the log-rank test. We used the median value RHI of 0.531 to divide patients into 2 groups (high and low RHI). To account for the confounding variables, propensity score was calculated in each patient using a logistic regression model in which the dependent variable was high RHI (>median), high Ln_RH-PAT ratio (>median), or high baseline pulse amplitude (>median), respectively. Independent variables included in the propensity score model were age, gender, body mass index, hypertension, diabetes, current smoking, family history of CAD, systolic blood pressure, diastolic blood pressure, hemoglobin A1c, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, left ventricular ejection fraction, BNP, hsCRP, eGFR, treatment with aspirin, hydroxymethylglutaryl-CoA reductase inhibitors, calcium

Table 6. Cox Proportional Hazards Analysis for Cardiovascular Events

Variable	Univariate			Multivariate Model 1			Multivariate Model 2			Multivariate Model 3		
	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value	HR	95% CI	P Value
Male sex	1.211	0.789 to 1.859	0.381		Not selected		1.178	0.730 to 1.901	0.502	—	—	—
Age, per year	1.043	1.022 to 1.065	<0.0001	1.024	1.000 to 1.048	0.046	1.029	1.002 to 1.056	0.038	—	—	—
Body mass index, per kg/m ²	0.925	0.872 to 0.982	0.011	0.950	0.894 to 1.010	0.103	0.947	0.885 to 1.013	0.113	—	—	—
Current smoking	1.162	0.737 to 1.833	0.519		Not selected		1.284	0.780 to 2.113	0.325	—	—	—
Hypertension	1.426	0.826 to 2.465	0.203		Not selected		1.306	0.731 to 2.335	0.367	—	—	—
Diabetes mellitus	1.135	0.774 to 1.665	0.517		Not selected		1.214	0.811 to 1.819	0.346	—	—	—
HDL cholesterol, per mg/dL	0.994	0.980 to 1.007	0.376		Not selected		1.009	0.994 to 1.024	0.233	—	—	—
LDL cholesterol, per mg/dL	0.994	0.988 to 1.001	0.081		Not selected		0.999	0.992 to 1.006	0.775	—	—	—
Ln BNP, per 0.1	1.036	1.019 to 1.052	<0.0001	1.019	1.002 to 1.037	0.023	1.018	1.000 to 1.037	0.050	1.026	1.009 to 1.044	0.003
Ln hsCRP, per 0.1	1.008	0.998 to 1.019	0.125		Not selected		1.004	0.993 to 1.015	0.514	1.001	0.991 to 1.012	0.813
LVEF, per %	0.977	0.952 to 1.003	0.081		Not selected		0.997	0.970 to 1.026	0.859	1.004	0.977 to 1.032	0.778
eGFR, per mL/min per 1.73 m ²	0.985	0.975 to 0.996	0.0067		Not selected		1.000	0.989 to 1.012	0.951	0.997	0.986 to 1.008	0.550
Coronary artery disease	8.587	2.720 to 27.11	0.0003		Not selected		1.110	0.310 to 3.980	0.873	1.577	0.433 to 5.747	0.490
SYNTAXsc, per tertile increment	2.854	2.167 to 3.759	<0.0001	2.426	1.825 to 3.225	<0.0001	2.447	1.781 to 3.361	<0.0001	2.360	1.736 to 3.208	<0.0001
FRS, per tertile increment	1.445	1.126 to 1.854	0.0038	—	—	—	—	—	—	0.976	0.740 to 1.288	0.863
RHI, per 0.1	0.693	0.614 to 0.781	<0.0001	0.761	0.673 to 0.859	<0.0001	0.757	0.666 to 0.860	<0.0001	0.771	0.681 to 0.873	<0.0001

Multivariate analysis Model 1 was performed using a backward algorithm with a 0.20 significance level. Model 2 and 3 was performed using a forced entry algorithm. Model 2; gender, age, body mass index, smoking, hypertension, diabetes mellitus, HDL cholesterol, LDL cholesterol, BNP, hsCRP, LVEF, eGFR, coronary artery disease, SYNTAXsc, and RHI. Model 3; FRS, BNP, hsCRP, LVEF, eGFR, coronary artery disease, SYNTAXsc, and RHI. BNP indicates B-type natriuretic peptide; CI, confidence interval; eGFR, estimated glomerular filtration rate; FRS, Framingham Risk Score; HDL, high-density lipoprotein; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

channel blockers, angiotensin-converting enzyme-inhibitors or angiotensin II receptor blockers, β blocker, and anti-diabetic drugs, CAD, FRS, and SYNTAXsc. We used Cox proportional hazard models to estimate cardiovascular event hazard ratios (HR) and their 95% confidence intervals (CI) in high-risk patients by univariate analysis and multivariate analysis with a

backward algorithm and forced inclusion models. Multicollinearity between covariates was examined by calculating the mean and individual covariate variance inflation factors. None of the individual covariate variance inflation factors were >2 , and the mean variance inflation factor for all covariates included in the Cox hazard model was 1.26.

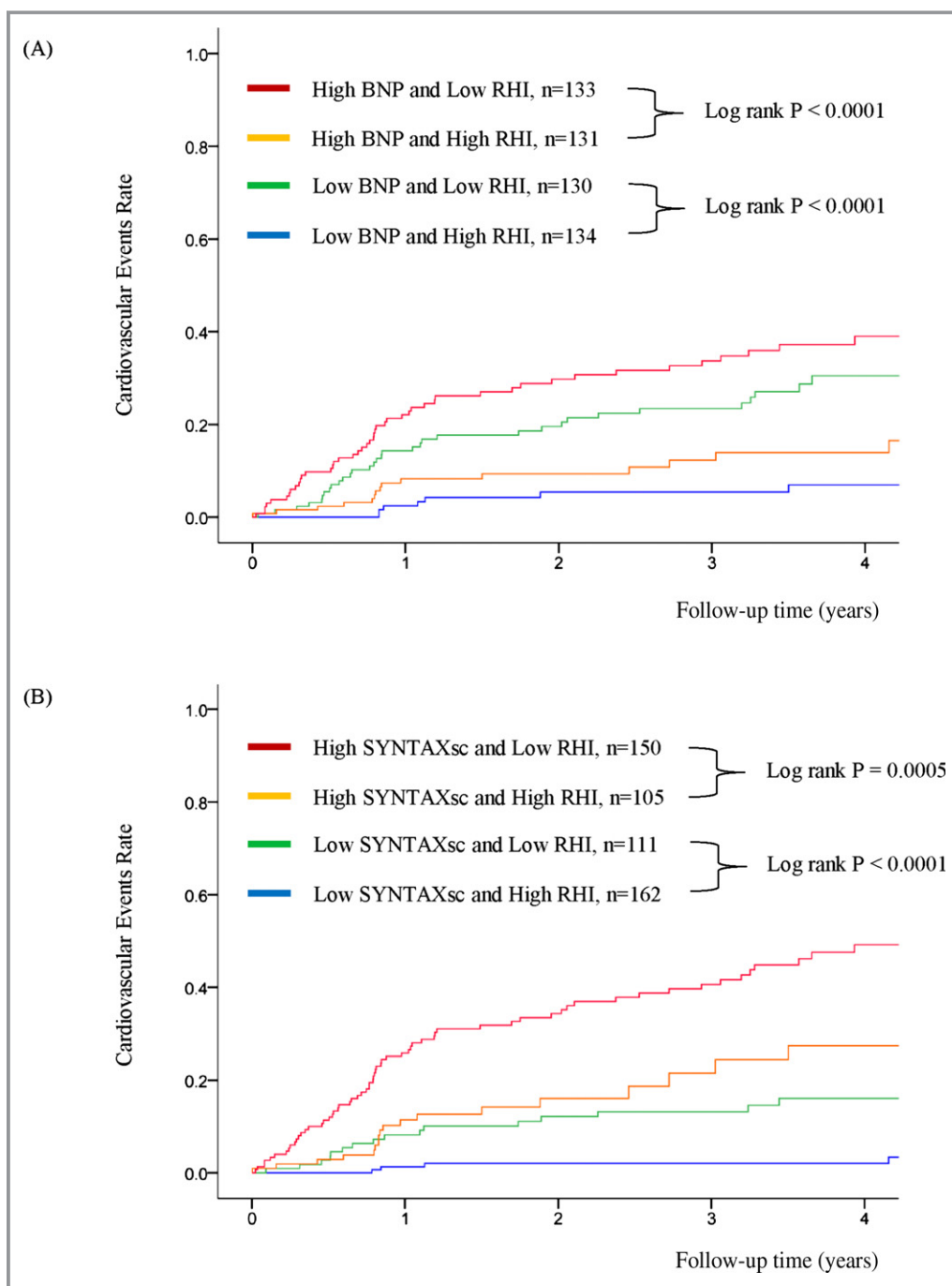


Figure 4. Kaplan-Meier analysis for cardiovascular event probability in high-risk patients based on BNP, SYNTAXsc, and RHI. (n=528). A, Analysis in subgroups stratified by RHI and BNP. B, Analysis in subgroups stratified by RHI and SYNTAXsc. Based on each cut-off point (median value) of RHI, BNP and SYNTAX Score. RHI 0.531, BNP 28.6 pg/mL, and SYNTAX Score 13.0. BNP indicates B-type natriuretic peptide; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery Score.

We confirmed the proportional hazards assumption using Schoenfeld's test. Estimates of C-statistics for the Cox proportional hazards regression models were calculated.²² The C-statistics were compared after the addition of BNP levels, the SYNTAXsc, and the RHI to the FRS.²¹ We also examined whether various combinations of these parameters improved the model's discriminatory power.

We performed likelihood ratio tests to evaluate whether the global model fit improved after RHI addition. We also evaluated whether adding the RHI to the FRS, BNP levels, and SYNTAXscs had an incremental effect in predicting cardiovascular events using the net reclassification index.²³ To assess reclassification improvement, we defined 3 risk categories on the basis of the FRS (primary and secondary prevention for 2-year risk for cardiovascular events)²¹: low-intermediate risk; <12%, high risk; 12% to 25%, or very high risk; >25%.

When we performed power analysis, we used the past report from Japan.²⁴ When we fixed parameters as follows, the estimated required patient number was 463: event-free rate 85%, hazard ratio 2.1, power 0.9, and alpha error 0.05. The number that we enrolled in the present study (n=528) was appropriate when compared with the number that was estimated by the power analysis (n=463). Statistical significance was defined as $P<0.05$ and all of the tests were 2 tailed. All analyses were performed using PASW 18 for Windows (SPSS Inc), STATA version 11.2 (StataCorp LP), and the SAS 9.2 program for Windows (SAS Institute Inc).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patient Enrollment

Figure 2 shows the study flow chart. At baseline, we initially included 577 stable, high-risk patients with suspected CAD without heart failure. We excluded a total of 49 patients from the analysis on the basis of advanced endocrine disease (n=6), hepatic disease (n=9), renal disease (n=11), active inflammatory disease (n=8), cancer (n=7), and cerebrovascular disease with residual hemiplegia (n=8). The study did not include patients with acute coronary syndromes. After the baseline evaluation that included the RHI, CAG was performed in all of the patients. Four hundred and forty-two patients had $\geq 50\%$ coronary artery diameter narrowing and were diagnosed as having stable CAD. Depending on their coronary anatomy, patients with CAD were then treated with percutaneous coronary intervention (PCI) (n=344, 77.8%), coronary artery bypass graft surgery (n=21, 4.8%), or medical therapy alone

(n=77, 17.4%). In patients undergoing PCI, a bare metal stent (n=64, 18.6%) or a drug-eluting stent (n=269, 78.2%) was used per operator discretion. At discharge, 98.4%, 93.4%, 63.6%, and 75.1% of the CAD patients were taking aspirin, hydroxymethylglutaryl-CoA reductase inhibitors, β -blockers, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers for achieving the optimal medical therapy, respectively.

Baseline Clinical Characteristics

Study patient baseline characteristics are shown in Tables 1 through 4. The mean patient age was 67.2 ± 10.7 years, and coronary risk factor prevalence for hypertension, diabetes, dyslipidemia, current smoking, and family histories of CAD was 81.3%, 47.0%, 83.0%, 21.2%, and 24.6%, respectively. Representative records of RH-PAT signals in patients with or without cardiovascular events are shown in Figure 1B and 1C.

Table 7. Reclassification by RHI Addition to FRS Alone and FRS, BNP and SYNTAXsc (n=528)

	Low-Intermediate Risk	High Risk	Very High Risk
Risk Category Using FRS Alone	New Risk Category Using FRS+RHI		
Patients Without Cardiovascular Events			
Low-intermediate risk	2	0	0
High risk	135	159	93
Very high risk	6	14	14
Patients With Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	9	34	48
Very high risk	0	3	11
Risk Category Using FRS+BNP+SYNTAXsc	New Risk Category by FRS+BNP+SYNTAXsc+RHI		
Patients Without Cardiovascular Events			
Low-intermediate risk	136	27	1
High risk	63	78	22
Very high risk	15	16	65
Patients With Cardiovascular Events			
Low-intermediate risk	4	6	0
High risk	4	15	20
Very high risk	1	3	52

According to the Framingham Risk Score that was calculated for a 2-year cardiovascular event risk, low-intermediate risk was <12%, high risk was 12% to 25%, and very high risk was >25%. The overall net reclassification index was 48.9%, $P<0.0001$ when RHI was used in conjunction with FRS alone, and 27.5%, $P<0.0001$ when used in conjunction with FRS+BNP+SYNTAXsc. BNP indicates B-type natriuretic peptide; FRS, Framingham Risk Score; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

Cardiovascular Events

During a mean follow-up period of 2.8 years with a 1.7-year SD, which corresponds to 1468 person-years, 105 patients developed cardiovascular events. Those patients who developed cardiovascular events during the follow-up period were significantly older and had a lower body mass index, higher BNP levels, higher hsCRP levels, reduced eGFR, increased CAD prevalence, higher FRS, higher SYNTAXsc, and a lower RHI than patients without cardiovascular events (Table 2 and Figure 3A). Patients in the low-RHI group developed significantly more cardiovascular events ($n=83$; 31.8% [95% CI, 26.2 to 37.5]) than patients in the high-RHI group ($n=22$; 8.2% [95% CI, 4.9 to 11.5]) during the follow-up period, ($P<0.0001$) (Table 5). Specifically, acute coronary syndrome, ischemic stroke, coronary revascularization, and heart failure were significantly higher in the low-RHI group than in the high-RHI group (Table 5).

Cox Proportional Hazard Analysis for Cardiovascular Events

The multivariate analysis with backward algorithms revealed that age, BNP levels, SYNTAXsc, and RHI were independent

predictors of cardiovascular events (age HR per 1 year was 1.024 with a 95% CI of 1.000 to 1.048; $P=0.046$, natural logarithm of BNP level HR per 0.1 was 1.019 with a 95% CI of 1.002 to 1.037; $P=0.023$, the SYNTAXsc HR per tertile increase was 2.426 with a 95% CI of 1.825 to 3.225; $P<0.0001$, the RHI HR per 0.1 was 0.761 with a 95% CI of 0.673 to 0.859; $P<0.0001$) (Table 6, Model 1). RHI was also significantly associated with cardiovascular events in the model with propensity score adjustment (the RHI HR per 0.1 was 0.739 with a 95% CI of 0.649 to 0.842; $P<0.0001$), and in the forced inclusion model with traditional risk factors, BNP, hsCRP, left ventricular ejection fraction, eGFR, CAD, SYNTAXsc, and RHI (Table 6, Model 2). Furthermore, the FRS was a significant predictor of cardiovascular events (HR for FRS per tertile increase was 1.445, 95% CI: 1.126 to 1.854; $P=0.0038$), and the forced inclusion model with FRS, BNP, hsCRP, left ventricular ejection fraction, eGFR, CAD, SYNTAXsc, and RHI demonstrated that RHI was independently associated with future cardiovascular events (the RHI HR per 0.1 was 0.771 with a 95% CI of 0.681 to 0.873; $P<0.0001$) (Table 6, Model 3). Limiting to the coronary heart disease events ($n=73$), RHI was independently associated with the occurrence of future coronary events by forced-entry multivariate Cox analysis with FRS, BNP, eGFR, and SYNTAXsc

Table 8. C-Statistics for Cox Proportional Hazards Models to Predict Cardiovascular Events

	C-Statistics (95% CI)	Increment in C-Statistics (95% CI)	P Value
All High-Risk Patients (n=528)			
FRS	0.596 (0.540 to 0.653)		0.0002
FRS+RHI	0.699 (0.655 to 0.743)	0.103 (0.048 to 0.158)	
FRS+BNP	0.640 (0.588 to 0.692)		0.0055
FRS+BNP+RHI	0.706 (0.664 to 0.747)	0.066 (0.019 to 0.112)	
FRS+SYNTAXsc	0.733 (0.683 to 0.782)		0.017
FRS+SYNTAXsc+RHI	0.774 (0.736 to 0.812)	0.041 (0.008 to 0.075)	
FRS+BNP+SYNTAXsc	0.728 (0.679 to 0.778)		0.031
FRS+BNP+SYNTAXsc+RHI	0.766 (0.726 to 0.806)	0.038 (0.004 to 0.072)	
CAD Patients (n=442)			
FRS	0.510 (0.448 to 0.572)		<0.0001
FRS+RHI	0.654 (0.603 to 0.704)	0.144 (0.078 to 0.209)	
FRS+BNP	0.610 (0.553 to 0.667)		0.0094
FRS+BNP+RHI	0.676 (0.631 to 0.720)	0.066 (0.016 to 0.115)	
FRS+SYNTAXsc	0.682 (0.624 to 0.740)		0.0070
FRS+SYNTAXsc+RHI	0.733 (0.689 to 0.776)	0.051 (0.014 to 0.088)	
FRS+BNP+SYNTAXsc	0.694 (0.638 to 0.751)		0.046
FRS+BNP+SYNTAXsc+RHI	0.735 (0.692 to 0.779)	0.042 (0.001 to 0.083)	

BNP, SYNTAXsc, and RHI were incorporated as continuous variables. We used natural logarithmic transformations of BNP and SYNTAXsc because of skewed distributions. BNP indicates B-type natriuretic peptide; CAD, coronary artery disease; CI, confidence interval; FRS, Framingham Risk Score; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

(RHI; per 0.1, HR 0.742, 95% CI; 0.643 to 0.856, $P<0.0001$). Kaplan-Meier analysis based on high and low RHI values demonstrated that there was a significantly higher cardiovascular event probability in the low RHI group during the follow-up period (log-rank test $P<0.0001$) (Figure 3B). Kaplan-Meier estimates for patients in each category by BNP levels and SYNTAXsc confirmed that low RHI values significantly improved cardiovascular events prediction (Figure 4A and 4B).

Net Reclassification Index and C-statistics for Cox Proportional Hazard Models to Predict Cardiovascular Events

We treated BNP levels, SYNTAXsc, and RHI values as continuous and reclassified risk scores for the study patients. Significance was achieved in the resultant net reclassification index by adding RHI to the FRS alone (net reclassification index 48.9%; $P<0.0001$) or to FRS+BNP+SYNTAXsc (net reclassification index 27.5%; $P<0.0001$) (Table 7).

We estimated the C-statistic of the FRS alone. Individual BNP level, SYNTAXsc, and RHI value incorporation into the FRS increased the C-statistic for cardiovascular event prediction. Adding RHI values to the FRS, BNP level, and SYNTAXsc produced a significant increase in the C-statistic, from 0.728 to 0.766 ($P=0.031$, Table 8). We confirmed appropriate proportional hazards assumptions using Schoenfeld's test ($P=0.476$). RHI addition to the model with FRS, BNP levels, and SYNTAXsc demonstrated a better global fit compared with the model without RHI, as evaluated by the likelihood ratio test ($P=0.017$). We examined the interaction among all of the variables for effect modification and found that only SYNTAXsc had an interaction with RHI ($P=0.005$).

We conducted an analysis of a prespecified subgroup of established CAD patients ($n=442$). CAD patients with cardiovascular events had significantly lower RHI values than those without cardiovascular events (Figure 5A). Kaplan-Meier estimates for CAD patients in each category by RHI values, BNP levels, and SYNTAXsc revealed that the low RHI values significantly improved cardiovascular event prediction (Figures 5B, 6A, and 6B). Among CAD patients, the net reclassification index and C-statistics were also significantly improved by adding RHI to the FRS, BNP levels, and SYNTAXsc (net reclassification index was 29.2%; $P<0.0001$; C-statistic changed from 0.694 to 0.735; $P=0.046$) (Tables 8 and 9).

Ln_{RH}-PAT Ratio and Baseline Pulse Amplitude

We also evaluated the prognostic value of baseline pulse amplitude and Ln_{RH}-PAT ratio, which is uncorrected for baseline pulse amplitude. The relation of baseline pulse

Table 9. Reclassification by RHI Addition to the FRS Alone and the FRS, BNP and SYNTAXsc in CAD Patients ($n=442$)

	Low-Intermediate Risk	High Risk	Very High Risk
Risk Category by FRS Alone		New Risk Category Using FRS+RHI	
Patients Without Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	65	117	88
Very high risk	4	33	33
Patients With Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	3	28	41
Very high risk	2	5	23
Risk Category Using FRS+BNP+SYNTAXsc		New Risk Category Using FRS+BNP+SYNTAXsc+RHI	
Patients Without Cardiovascular Events			
Low-intermediate risk	54	19	0
High risk	59	80	22
Very high risk	15	23	68
Patients With Cardiovascular Events			
Low-intermediate risk	2	4	0
High risk	4	16	18
Very high risk	1	4	53

According to the Framingham Risk Score, which was calculated for a 2-year cardiovascular event risk, low-intermediate risk was <12%, high risk was 12% to 25%, and very high risk was more than 25%. The overall net reclassification index was 34.5%, $P<0.0001$ when used in combination with FRS alone, and 29.2%, $P<0.0001$ when used in combination with FRS+BNP+SYNTAXsc. BNP indicates B-type natriuretic peptide; CAD, coronary artery disease; FRS, Framingham Risk Score; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

amplitude and Ln_{RH}-PAT ratio with cardiovascular risk factors are shown in Table 10. Cox proportional hazard analysis revealed that Ln_{RH}-PAT ratio was significantly associated with cardiovascular events in the single model and the model with propensity score adjustment (single analysis, the Ln_{RH}-PAT ratio HR per 0.1 was 0.796 with a 95% CI of 0.727 to 0.873; $P<0.0001$) (the model with propensity score adjustment, the Ln_{RH}-PAT ratio HR per 0.1 was 0.816 with a 95% CI of 0.740 to 0.899; $P<0.0001$). Baseline pulse amplitude did not have significant relation to cardiovascular events in the single and the adjustment model (single analysis, the baseline pulse amplitude HR per 1 was 1.000 with a 95% CI of 0.999 to 1.000; $P=0.349$) (the model with propensity score adjustment, the baseline pulse amplitude HR per 1 was 1.000 with a 95% CI of 0.999 to 1.000; $P=0.233$). The addition of Ln_{RH}-PAT ratio to the FRS, BNP levels, and SYNTAXsc increased the C-statistics (Table 11), and the net reclassification indices were significant with the

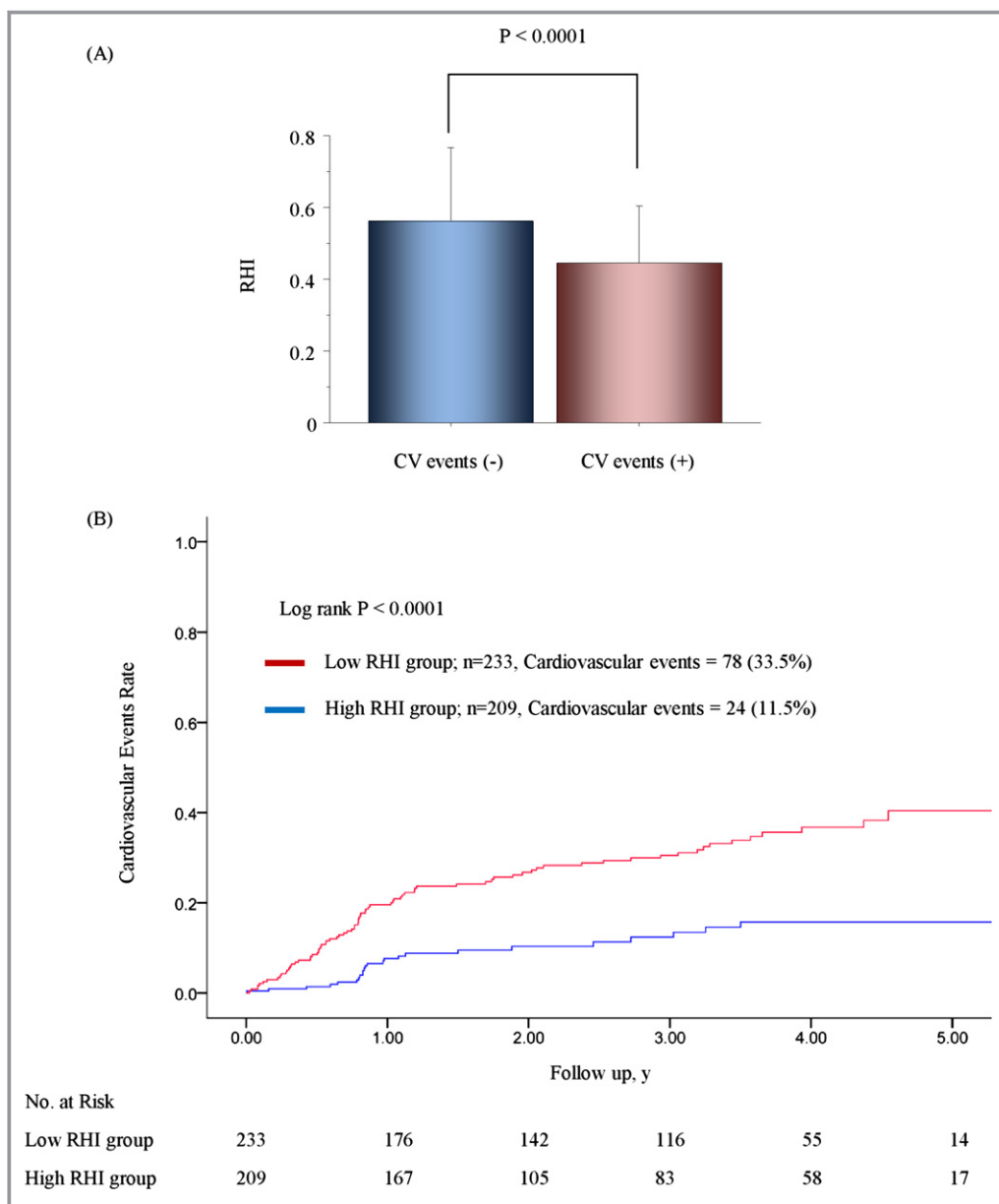


Figure 5. RHI and cardiovascular events in 442 CAD patients. A, These bars represent averages of the RHI in each group (CV events [–] [n=340], CV events [+] [n=102]). T-bars indicate standard deviation. B, Kaplan-Meier analysis for the probability of cardiovascular events in CAD patients based on median value of RHI (0.501). CAD indicates coronary artery disease; CV, cardiovascular; RHI, reactive hyperemia-peripheral arterial tonometry index.

inclusion of Ln_RH-PAT ratio in the whole study population and in CAD patients (Tables 12 and 13).

Discussion

Among patients who were high risk for a cardiovascular event, RHI values successfully and incrementally predicted future cardiovascular events. We demonstrated that adding the RHI value to the Framingham risk model, SYNTAXsc anatomical assessment, and BNP levels improved risk

classification, as evidenced by a net reclassification index and a significant increase in the C-statistics. These findings indicated that an additional physiological assessment of endothelial function could be clinically valuable to identify vulnerable patients who may develop near-future cardiovascular events.

Atherosclerotic lesions with a high probability of acute thrombotic complications because of plaque rupture or superficial endothelial erosion should be considered “vulnerable plaques.” Currently, cardiovascular risk stratification with

Table 10. The Relation of Baseline Pulse Amplitude, Ln_RH-PAT Ratio, and RHI With Cardiovascular Risk Factors

Variables	Baseline Pulse Amplitude			Ln_RH-PAT Ratio			RHI		
	B (SE)	Partial R ²	P Value	B (SE)	Partial R ²	P Value	B (SE)	Partial R ²	P Value
Age	0.104 (0.044)	0.011	0.018	-0.110 (0.044)	0.012	0.012	-0.125 (0.043)	0.016	0.004
Male	0.203 (0.095)	0.009	0.033	-0.280 (0.094)	0.017	0.003	-0.294 (0.094)	0.018	0.002
Currently smoking	0.046 (0.113)	<0.001	0.68	-0.121 (0.112)	0.002	0.28	-0.090 (0.112)	0.001	0.42
Diabetes	0.131 (0.087)	0.004	0.13	-0.044 (0.087)	<0.001	0.61	-0.009 (0.086)	<0.001	0.92
Hypertension	-0.032 (0.113)	<0.001	0.78	-0.114 (0.112)	0.002	0.31	-0.133 (0.112)	0.003	0.24
Body mass index	0.174 (0.044)	0.017	0.003	-0.113 (0.044)	0.013	0.011	-0.072 (0.044)	0.005	0.11
Systolic blood pressure	0.104 (0.043)	0.011	0.017	-0.004 (0.043)	<0.001	0.93	0.003 (0.043)	<0.001	0.94
Diastolic blood pressure	-0.013 (0.044)	<0.001	0.76	-0.052 (0.044)	0.003	0.24	-0.102 (0.043)	0.010	0.020
Fasting blood glucose	0.049 (0.044)	0.002	0.27	-0.016 (0.044)	<0.001	0.72	-0.007 (0.043)	<0.001	0.87
Hemoglobin A1c	0.001 (0.044)	<0.001	0.98	-0.020 (0.044)	<0.001	0.64	-0.030 (0.043)	<0.001	0.49
Total/HDL cholesterol ratio	0.030 (0.044)	<0.001	0.50	-0.114 (0.044)	0.013	0.009	-0.112 (0.043)	0.013	0.010
Triglycerides	0.040 (0.044)	0.002	0.37	-0.031 (0.044)	<0.001	0.48	-0.028 (0.044)	<0.001	0.53
High-sensitivity CRP	-0.063 (0.043)	0.004	0.15	0.036 (0.043)	0.001	0.41	0.034 (0.043)	0.001	0.44
Anti-hypertensive drugs	-0.038 (0.119)	<0.001	0.75	-0.333 (0.118)	0.016	0.005	-0.380 (0.117)	0.021	0.001
HMG-CoA RIs	0.102 (0.096)	0.002	0.29	-0.158 (0.095)	0.005	0.097	-0.123 (0.095)	0.003	0.20
Coronary artery disease	0.132 (0.123)	0.002	0.29	-0.836 (0.117)	0.095	<0.001	-0.838 (0.117)	0.096	<0.001
SYNTAXsc	-0.003 (0.045)	<0.001	0.95	-0.181 (0.044)	0.033	<0.001	-0.186 (0.044)	0.035	<0.001

The first 2 rows present models for age and sex separately, with no adjustment for the other variable. Age and sex were forced into all other models. Continuous variables were standardized to mean of 0 and SD of 1, and all categorical variables were coded 1=presence and 0=absence of factor. CRP indicates C-reactive protein; HDL, high-density lipoprotein; HMG-CoA RIs, hydroxymethylglutaryl-CoA reductase inhibitors; RHI, reactive hyperemia-peripheral arterial tonometry index; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SE, standard error; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

established coronary risk factors cannot fully predict the development of acute cardiovascular complications, especially the near-future cardiovascular events.^{1,25} The cardiovascular events frequently occurred in the highest-risk population with history of CAD and those with the established evidence of athero-thrombotic diseases. Thus, these populations should be the most clinically important patients to prevent the occurrence of cardiovascular events, however the practical risk stratification strategy for these patients has not been proposed.¹ Most plaque disruption occurs in moderately stenotic plaques that contain a soft, lipid-rich core covered by a thin, inflamed fibrous cap, which often accompanies endothelial dysfunction.²⁶ Severe endothelial dysfunction predisposes an individual to vulnerable endothelium, which could lead to plaque disruption and thrombosis. In the present study, we showed that using the RHI value in conjunction with the FRS improved future cardiovascular event prediction. Based on these findings, we propose an additional physiological biomarker assessing endothelial dysfunction, which could be an integrated measurement of all atherogenic and atheroprotective factors. A combined approach with an FRS-based risk classification, noninvasive physiological RHI, anatomical coronary plaque complexity,

and BNP levels might be clinically valuable and could be an integrative strategy for cardiovascular risk assessment in high-risk patients. Complementing unknown risk conditions assessed by RH-PAT would open a new era of patient risk stratification in cardiovascular medicine.

Although endothelial function testing is expectedly desired in clinical practice, endothelial function as determined by brachial artery flow-dependent vasodilation (FMD) has not been successfully incorporated into the current integrative risk stratification system because of its operator dependency and technical problems.^{18,27} The additional benefits of FMD to traditional risk factors in the cardiovascular risk reclassification have not been established.^{4,6} Practically with clinical utility, digital RHI is a reproducible and less operator-dependent technique for peripheral endothelial function assessment^{10,12,14,15,18} that noninvasively reflects coronary endothelial function.^{12,16} FMD attenuates markedly with advancing age. In contrast, digital vascular function (RHI) well reflects metabolic risk factors including obesity, high cholesterol, diabetes, and smoking.²⁸ Rubinshtein et al demonstrated a significant association between RHI and future cardiovascular events during a 7-year follow-up period among patients without CAD.¹³ In the high-risk

Table 11. C-Statistics for Cox Proportional Hazards Models to Predict Cardiovascular Events

	C-Statistics (95% CI)	Increment in C-Statistics (95% CI)	P Value
All High-Risk Patients (n=526)			
FRS	0.596 (0.539 to 0.659)		0.022
FRS+Ln_RH-PAT ratio	0.659 (0.608 to 0.709)	0.062 (0.009 to 0.116)	
FRS+BNP	0.652 (0.570 to 0.733)		0.080
FRS+BNP+Ln_RH-PAT ratio	0.688 (0.623 to 0.753)	0.036 (−0.004 to 0.076)	
FRS+SYNTAXsc	0.733 (0.690 to 0.776)		0.119
FRS+SYNTAXsc+Ln_RH-PAT ratio	0.753 (0.709 to 0.797)	0.020 (−0.005 to 0.045)	
FRS+BNP+SYNTAXsc	0.740 (0.679 to 0.801)		0.072
FRS+BNP+SYNTAXsc+Ln_RH-PAT ratio	0.760 (0.704 to 0.816)	0.020 (−0.002 to 0.041)	
CAD Patients (n=440)			
FRS	0.510 (0.440 to 0.580)		0.0030
FRS+Ln_RH-PAT ratio	0.603 (0.545 to 0.661)	0.093 (0.032 to 0.154)	
FRS+BNP	0.610 (0.511 to 0.709)		0.0998
FRS+BNP+Ln_RH-PAT ratio	0.645 (0.570 to 0.719)	0.035 (−0.007 to 0.076)	
FRS+SYNTAXsc	0.682 (0.630 to 0.733)		0.0596
FRS+SYNTAXsc+Ln_RH-PAT ratio	0.709 (0.657 to 0.761)	0.028 (−0.001 to 0.056)	
FRS+BNP+SYNTAXsc	0.694 (0.622 to 0.766)		0.0693
FRS+BNP+SYNTAXsc+Ln_RH-PAT ratio	0.717 (0.650 to 0.784)	0.023 (−0.002 to 0.047)	

BNP, SYNTAXsc, and RHI were incorporated as continuous variables. We used natural logarithmic transformations of BNP and SYNTAXsc because of skewed distributions. BNP indicates B-type natriuretic peptide; CAD, coronary artery disease; CI, confidence interval; FRS, Framingham Risk Score; RHI, reactive hyperemia-peripheral arterial tonometry index; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

population and in CAD patients, accurate classification of the near-future event risk is clinically imperative.¹ We demonstrated that low RHI values were significantly associated with the near-future cardiovascular events independent from FRS and coronary plaque complexity, as assessed by SYNTAXsc in high-risk patients, which indicated the usefulness of the RH-PAT test in the practical medicine. Compared with medical therapy for atherosclerosis risk factors, prompt coronary revascularization did not effectively reduce cardiovascular event risk.²⁹ Although invasive coronary revascularization can anatomically treat local coronary stenotic plaques, it cannot treat physiological vascular disorders, such as endothelial dysfunction in the systemic vasculature. Thus, effective identification of vulnerable patients with severe endothelial dysfunction is important to investigate potential treatments and improve prognosis. Use of the RHI as a noninvasive assessment of endothelial function could represent an important advance in comprehensive clinical cardiovascular risk evaluation, even after invasive coronary revascularization with optimal medical treatments. Recently, Matsubara et al reported that the new treatment strategy for diabetes improved endothelial function in CAD patients with lower RHI and uncontrolled diabetes.³⁰ We can introduce and evaluate the clinical

efficacy of new approaches to achieve optimal therapies with improving endothelial function.

RH-PAT reflects changes in flow and digital microvessel dilation.¹⁸ Validation studies have shown that impairment in peripheral finger endothelial function measured with RH-PAT is correlated with coronary microvascular function.¹⁶ In this study, we clarified the new aspect that peripheral microvascular endothelial function as assessed by RHI can predict cardiovascular events in conductance vessels. The fact that endothelial dysfunction is a systemic condition may explain why peripheral microvascular endothelial function correlates with endothelial function in the coronary arteries (conductance vessels).^{13,31–33} Taken together, we suggest that peripheral microvascular endothelial dysfunction could associate with the burden of cardiovascular risk and could be considered a barometer of the total risk burden (the risk of the risk factors).

Endothelial dysfunction, as assessed by RHI, could be modestly explained by clinically available risk factors,³⁴ which suggests that the majority of contributing pathogenic factors to endothelial dysfunction have not yet been fully elucidated. The medical community really needs to recognize that a clinical assessment of endothelial function could be an integrated parameter reflecting unknown atherogenic factors,

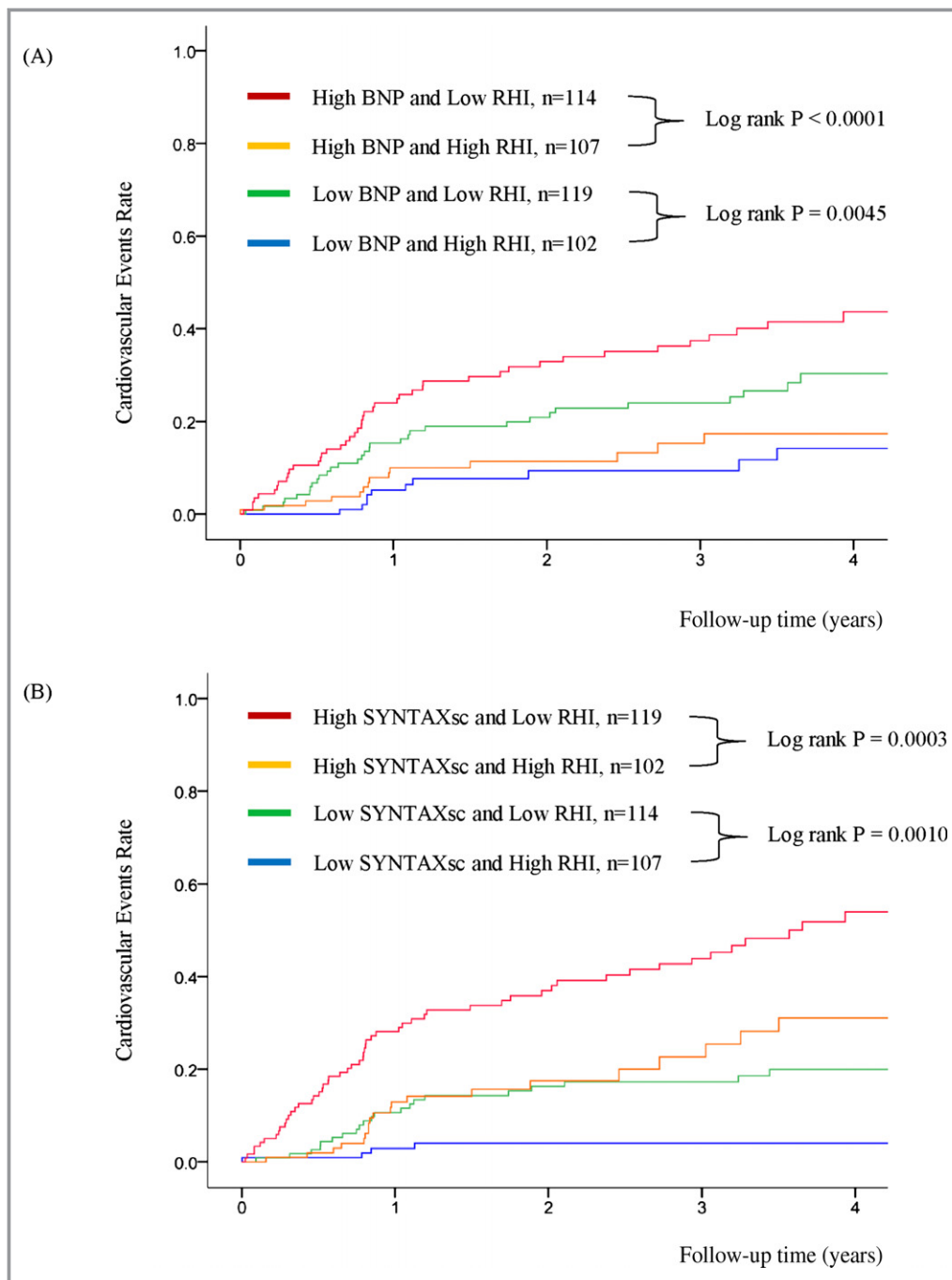


Figure 6. Kaplan-Meier analysis for cardiovascular event probability in CAD patients based on BNP, SYNTAXsc, and RHI. (n=442). A, Analysis in subgroups stratified by RHI and BNP. B, Analysis in subgroups stratified by RHI and SYNTAX score. Based on each cut-off point (median value) of RHI, BNP, and SYNTAX Score. RHI 0.501, BNP 31.7 pg/mL, and SYNTAX Score 15.8. BNP indicates B-type natriuretic peptide; CAD, coronary artery disease; RHI, reactive hyperemia-peripheral arterial tonometry index; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery Score.

including mental stress, environmental, and genetic background. The RH-PAT technique is less operator-dependent and has good reproducibility.³⁵ We would potentially introduce the noninvasive endothelial function tests in the future practical medicine.¹⁸

Past studies, including the Framingham study, demonstrated that baseline pulse amplitude was positively related to most cardiovascular risk factors and Ln_{RH-PAT} ratio was negatively associated with most cardiovascular disease risk factors.^{15,28} Whereas in this study, significant positive relation

Table 12. Reclassification by Ln_RH-PAT Ratio Addition to FRS Alone and FRS, BNP, and SYNTAXsc (n=526)

	Low-Intermediate Risk	High Risk	Very High Risk
Risk Category Using FRS Alone	New Risk Category Using FRS+Ln_RH-PAT Ratio		
Patients Without Cardiovascular Events			
Low-intermediate risk	2	0	0
High risk	128	175	93
Very high risk	6	13	15
Patients With Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	8	40	43
Very high risk	0	5	8
Risk Category Using FRS+BNP+SYNTAXsc	New Risk Category by FRS+BNP+SYNTAXsc+Ln_RH-PAT Ratio		
Patients Without Cardiovascular Events			
Low-intermediate risk	134	30	0
High risk	45	92	25
Very high risk	7	26	63
Patients With Cardiovascular Events			
Low-intermediate risk	6	4	0
High risk	2	19	18
Very high risk	1	3	51

According to the Framingham Risk Score that was calculated for a 2-year cardiovascular event risk, low-intermediate risk was <12%, high risk was 12% to 25%, and very high risk was >25%. The overall net reclassification index was 41.3%, $P<0.0001$ when Ln_RH-PAT ratio was used in conjunction with FRS alone, and 20.8%, $P=0.0003$ when used in conjunction with FRS+BNP+SYNTAXsc. BNP indicates B-type natriuretic peptide; FRS, Framingham Risk Score; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

to baseline pulse amplitude was observed only in age, male sex, body mass index, and systolic blood pressure. Age, male sex, body mass index, total/HDL cholesterol ratio, use of antihypertensive drugs, CAD, and SYNTAXsc were negatively correlated with Ln_RH-PAT ratio. There are several plausible explanations for the discrepancies with prior reports. The high-aged study patients, the small sample size, and the high prevalence of cardiovascular risk factors (hypertension, dyslipidemia, diabetes, and male) and CAD could cause these discrepancies. These high-risk profiles could also be one reason for the lower Ln_RH-PAT ratio in this study compared to previous studies.^{15,28}

The present trial was limited because it was a 2-center design with a small patient population. Further multicenter studies will be required to confirm our results in a larger patient population.

In conclusion, advanced endothelial dysfunction was significantly associated with adverse cardiovascular events

Table 13. Reclassification by Ln_RH-PAT Ratio Addition to the FRS Alone and the FRS, BNP, and SYNTAXsc in CAD Patients (n=440)

	Low-Intermediate Risk	High Risk	Very High Risk
Risk Category by FRS Alone	New Risk Category Using FRS+Ln_RH-PAT Ratio		
Patients Without Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	46	132	91
Very high risk	2	28	40
Patients With Cardiovascular Events			
Low-intermediate risk	0	0	0
High risk	2	29	41
Very high risk	1	10	18
Risk Category Using FRS+BNP+SYNTAXsc	New Risk Category Using FRS+BNP+Syntaxsc+Ln_RH-PAT Ratio		
Patients Without Cardiovascular Events			
Low-intermediate risk	54	19	0
High risk	41	95	24
Very high risk	5	36	65
Patients With Cardiovascular Events			
Low-intermediate risk	3	3	0
High risk	1	21	16
Very high risk	1	4	52

According to the Framingham Risk Score, which was calculated for a 2-year cardiovascular event risk, low-intermediate risk was <12%, high risk was 12% to 25%, and very high risk was more than 25%. The overall net reclassification index was 23.3%, $P=0.0045$ when used in combination with FRS alone, and 24.4%, $P<0.0001$ when used in combination with FRS+BNP+SYNTAXsc. BNP indicates B-type natriuretic peptide; CAD, coronary artery disease; FRS, Framingham Risk Score; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SYNTAXsc, Synergy Between PCI With Taxus and Cardiac Surgery score.

in high-risk patients. Patients with advanced endothelial dysfunction as identified by the lower RHI might have vulnerable vasculature and endothelium. Clinical evaluation of endothelial function with RHI could provide useful and complementary prognostic information to improve risk assessment for the near-future cardiovascular events in high-risk patients.

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Disclosures

None.

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