Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 60, No. 18, 2012
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.07.036

Incremental Prognostic Significance of Peripheral Endothelial Dysfunction in Patients With Heart Failure With Normal Left Ventricular Ejection Fraction

Eiichi Akiyama, MD,*† Seigo Sugiyama, MD, PHD,* Yasushi Matsuzawa, MD,*† Masaaki Konishi, MD, PHD,*† Hiroyuki Suzuki, MD,† Toshimitsu Nozaki, MD, PHD,* Keisuke Ohba, MD,* Junichi Matsubara, MD, PHD,* Hirofumi Maeda, MD,* Yoko Horibata, MD,* Kenji Sakamoto, MD, PHD,* Koichi Sugamura, MD, PHD,* Megumi Yamamuro, MD, PHD,* Hitoshi Sumida, MD, PHD,‡ Koichi Kaikita, MD, PHD,* Satomi Iwashita, MT,* Kunihiko Matsui, MD, MPH,§ Kazuo Kimura, MD, PHD,† Satoshi Umemura, MD, PHD,‖ Hisao Ogawa, MD, PHD*

Kumamoto, Yokohama, and Ube, Japan

Objectives The purpose of this study was to investigate whether peripheral endothelial dysfunction could predict the occurrence of cardiovascular events in patients with heart failure (HF) with normal left ventricular ejection fraction (HFNEF).

Background Endothelial dysfunction plays an important role in HF, but the relation between peripheral endothelial dysfunction and prognosis in HFNEF remains unknown.

Methods We conducted a prospective cohort study of 321 patients with HFNEF. We evaluated cardiac function by echocardiography measuring the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity (E/e'), noninvasively assessed peripheral endothelial function by reactive hyperemia-peripheral arterial tonometry (RH-PAT) as the RH-PAT index (RHI), and followed cardiovascular events.

Results A total of 59 patients had a cardiovascular event. Kaplan-Meier analysis demonstrated a significantly higher probability of cardiovascular events in the low RHI group than in the high RHI group (mean follow-up: 20 months; log-rank test: $p < 0.001$). Multivariate Cox hazard analysis identified RHI (per 0.1) (hazard ratio [HR]: 0.80; 95% confidence interval [CI]: 0.67 to 0.94; $p = 0.007$), E/e' (Ln[E/e'] [per 0.1]) (HR: 1.15; 95% CI: 1.04 to 1.26; $p = 0.006$), and B-type natriuretic peptide (BNP) (Ln[BNP] [per picogram/milliliter]) (HR: 1.81; 95% CI: 1.44 to 2.28; $p < 0.001$) as independent predictors of cardiovascular events. The C-statistics for cardiovascular events substantially increased when the RHI was added to the HFNEF prognostic 5 factors (PF5)—age, diabetes, New York Heart Association classification, HF hospitalization history, and left ventricular ejection fraction—which were identified in the I-PRESERVE (Irbesartan in Heart Failure with Preserved Ejection Fraction Study) (PF5 alone: 0.671; PF5 + RHI: 0.712). The net reclassification index was significant after addition of the RHI (19.0%, $p = 0.01$).

Conclusions Peripheral endothelial dysfunction independently correlated with future cardiovascular events, adding incremental clinical significance for risk stratification in patients with HFNEF. (Endothelial Dysfunction Assessed by Reactive Hyperemia Peripheral Arterial Tonometry and Heart Failure with Preserved Left Ventricular Ejection Fraction; UMIN000002640)
(J Am Coll Cardiol 2012;60:1778–86) © 2012 by the American College of Cardiology Foundation

Approximately 50% of patients with heart failure (HF) have normal or preserved left ventricular ejection fraction (LVEF) (1,2). The prognosis of patients with HF with normal LVEF (HFNEF) is similar to that of patients with HF with

From the *Department of Cardiovascular Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan; †Division of Cardiology, Yokohama City University Medical Center, Yokohama, Japan; ‡Interventional Cardiology, Kumamoto University Hospital, Kumamoto, Japan; §Department of General Internal Medicine, Yamaguchi University Hospital, Ube, Japan; and the ‖Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan. Funding: This study was supported in part by a grant-in-aid for Scientific Research (No. C22590786 for S. Sugiyama) from the Ministry of Education, Science, and Culture in Japan. All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received March 20, 2012; revised manuscript received July 4, 2012, accepted July 23, 2012.

reduced LVEF (HFREF) (1,3). Effective treatments for patients with HFNEF have not been established because the precise mechanism underlying HFNEF remains unclear. Left ventricular diastolic dysfunction (LVDD) plays an important role in patients with HFNEF (4), and HFNEF could be due to structural and molecular abnormalities of the cardiovascular system. These abnormalities include myocardial ischemia, cardiomyocyte hypertrophy, cardiac inflammation (5), and ventricular-vascular stiffening, in part due to the reduced effects of nitric oxide and impaired endothelial function (6). Borlaug et al. (7) recently demonstrated that global cardiovascular reserve functions, including endothelial function, are impaired in subjects with HFNEF who have hypertension.

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Impairment of coronary and peripheral circulation with endothelial dysfunction has been shown to be involved in the pathogenesis of HF, mainly HFREF. Coronary endothelial function is impaired in HFREF (8). Several studies have reported that peripheral endothelial dysfunction is associated with the severity of HF symptoms and clinical outcome in patients with HFREF (9,10). Vascular dysfunction leading to increased vascular stiffness and resistance with elevated blood pressure has been proposed as a potential and important noncardiac factor in the pathogenesis of acute HF syndrome (AHFS) as the clinical manifestation in many patients with HFNEF (11).

In contrast to patients with HFREF, the factors associated with clinical outcome remain to be fully understood in patients with HFNEF. The I-PRESERVE (Irbesartan in Heart Failure with Preserved Ejection Fraction Study) demonstrated that age, presence of diabetes mellitus (DM), presence of a previous hospitalization for HF, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and LVEF were associated with adverse outcomes in patients with HFNEF (12), although the relation between peripheral endothelial dysfunction and prognosis in HFNEF remains unknown.

The main hypothesis of this study was that peripheral endothelial dysfunction could be a prognostic factor for future cardiovascular events in patients with HFNEF in the prospective cohort study.

Methods

Study population. The study included patients who presented with signs or symptoms of HF and were referred for diagnosis or treatment of HF at Kumamoto University Hospital and Yokohama City University Medical Center between August 2006 and August 2011 (n = 762). All of the patients were free of noncardiac causes of HF-like symptoms, such as chronic obstructive pulmonary disease and end-stage renal disease with hemodialysis. The exclusion criteria included reduced LVEF (≤50, n = 280), acute coronary syndromes (n = 56), severe valvular heart disease (n = 29), hypertrophic obstructive cardiomyopathy (n = 16), significant inflammatory disease (n = 3), and neoplasms (n = 4).

We applied the diagnostic criteria of the European Working Group to HFNEF (13). We excluded 53 patients with HFNEF who did not meet these diagnostic criteria. Finally, 321 patients with HFNEF were enrolled in the present study (Fig. 1).

During the same study period, age-, gender-, rate of hypertension, and DM-matched patients with normal LVEF who did not present with HF symptoms and had never been diagnosed or treated for HF were also enrolled as patients without HF (n = 173).

The study complied with the Declaration of the Helsinki regarding investigation in humans, was approved by each institutional review committee, and was conducted in accordance with the guidelines of the ethics committee at each institution. Written informed consent was obtained from all patients. This study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000002640).

Assessment of endothelial function by reactive hyperemia-peripheral arterial tonometry. Peripheral endothelial function was assessed by reactive hyperemia-peripheral arterial tonometry (RH-PAT) using the EndoPAT2000 system (Itamar Medical, Caesarea, Israel), as described previously (14). RH-PAT measurement is largely operator independent, and a computerized algorithm with an online system automatically calculates the RH-PAT index (RHI); thus, there is minimal interoperator and intraoperator variability. RH-PAT studies were performed when patients were in stable, compensated condition after implementing medical therapies for HF and in the fasting state in the early morning before taking any medications. The RH-PAT value that reflected the extent of RH was calculated as the ratio of the average pulse amplitude of PAT signal over a 1-minute time interval starting 1.5 minutes after cuff deflation (control arm, A; study arm, C) to the average pulse amplitude of PAT signal of the

Abbreviations and Acronyms

AHFS = acute heart failure syndrome
BMI = body mass index
BNP = B-type natriuretic peptide
CI = confidence interval
DM = diabetes mellitus
E/e' = ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity
eGFR = estimated glomerular filtration rate
HF = heart failure
HFNEF = heart failure with normal left ventricular ejection fraction
HFREF = heart failure with reduced left ventricular ejection fraction
HR = hazard ratio
LV = left ventricular
LVDD = left ventricular diastolic dysfunction
LVEF = left ventricular ejection fraction
NRI = net reclassification index
NT-proBNP = N-terminal pro-B-type natriuretic peptide
NYHA = New York Heart Association
OR = odds ratio
PF5 = HFNEF prognostic 5 factors
RHI = reactive hyperemia-peripheral arterial tonometry index
RH-PAT = reactive hyperemia-peripheral arterial tonometry

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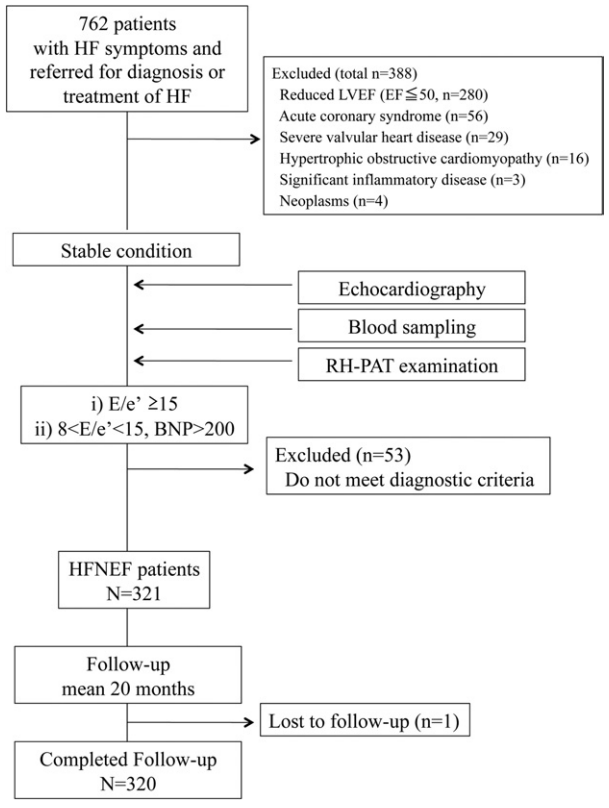

Figure 1 **Flow Chart Showing the Protocol Used for Enrollment of Patients With HFNEF**

BNP = B-type natriuretic peptide; E/e' = the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity; HF = heart failure; HFNEF = heart failure with normal left ventricular ejection fraction; LVEF = left ventricular ejection fraction; RH-PAT = reactive hyperemia peripheral arterial tonometry.

2.5-minute time period before cuff inflation (baseline) (control arm, B; study arm, D) (Fig. 2). The RH-PAT value was calculated by the following equation: RH-PAT value = (C/D)/(A/B). We used a natural logarithmic transformation of the RH-PAT value to calculate the RHI: RHI = Ln{[RH-PAT ratio] · [0.226 · Ln(baseline) − 0.2]} (14,15). Previous studies have demonstrated that RH-PAT technology has excellent reproducibility (16,17) (Online Fig. 1).

Follow-up. After the assessment of endothelial function by RH-PAT, patients with HFNEF were followed prospectively every month at the outpatient clinics until September 2011 or an endpoint occurred. The endpoint was a composite of cardiovascular death, nonfatal myocardial infarction, unstable angina pectoris, nonfatal ischemic stroke, hospitalization for HF decompensation, or coronary revascularization (definition in the Online Appendix). Cardiovascular events were ascertained from a review of medical records and confirmed by direct contact with the patients, their families, and physicians. For subjects who had more than 2 cardiovascular events, only the first event was considered in the analysis. We used the median value of the RHI to divide patients with HFNEF into low and high RHI groups.

Statistical analysis. Continuous variables with normal distribution were expressed as the mean ± SD. Continuous variables with skewed distribution were summarized as the median (interquartile range). Estimates of the C-statistic for Cox proportional hazards regression models were calculated (18–20). The comparison of C-statistics was estimated after the addition of RHI, B-type natriuretic peptide (BNP), and the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular velocity (E/e') to the HFNEF prognostic 5 factors (PF5), which were identified in the I-PRESERVE (12), including age, presence of DM, presence of a previous hospitalization for HF, New York Heart Association (NYHA) classification, and LVEF. We also examined whether the addition of RHI, BNP, and E/e' improved the discriminatory power of the model. The proportional hazards assumption was confirmed by Schoenfeld's global test. The calibration of Cox regression models also was assessed by the Grønnesby and Borgan calibration test (21).

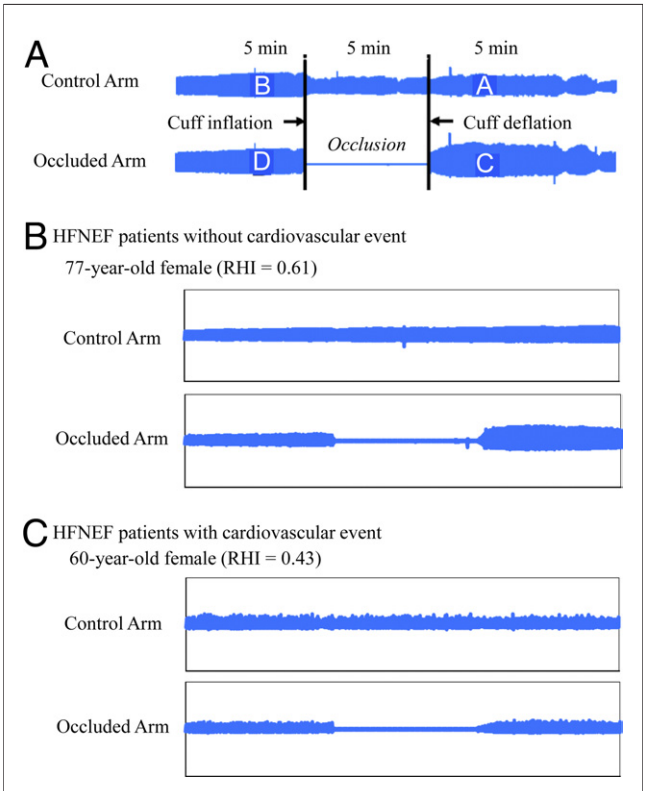

Figure 2 **Representative RH-PAT Signals**

(A) RH-PAT index (RHI) was calculated by the following equation: RHI = Ln{[(C/D)/(A/B)] · [0.226 · Ln(baseline) − 0.2]}. The representative results of RH-PAT examination of the patient with heart failure with normal left ventricular ejection fraction (HFNEF) without **(B)** and with **(C)** a cardiovascular event. RH-PAT examination was performed at the study entry point in patients in a stable and compensated condition. HFNEF = heart failure with normal left ventricular ejection fraction; RH-PAT = reactive hyperemia-peripheral arterial tonometry; RHI = RH-PAT index.

The incremental effect of adding the RHI to the PF5 for predicting future cardiovascular events was evaluated using the net reclassification index (NRI) as previously described (22). A p value <0.05 was considered statistically significant. An expanded Methods section is available in the Online Appendix.

Results

Study population. A total of 321 patients with HFNEF and 173 patients without HF were enrolled in this study. Patients with HFNEF had a higher body mass index (BMI) and heart rate than patients without HF (Table 1). Patients with HFNEF had lower levels of estimated glomerular filtration rate (eGFR) and higher levels of BNP and high-sensitivity C-reactive protein compared with patients without HF. The E/e' ratio was higher, left atrial diameter was larger, and left ventricular (LV) mass index was higher in patients with HFNEF compared with patients without HF (Table 1). Of 321 patients with HFNEF, patients in the low RHI group (below median, cutoff value: 0.49) had higher NYHA classes than those in the high RHI group (above median) (Table 1).

Endothelial function in patients with HFNEF. The RHI was significantly lower in patients with HFNEF compared with patients without HF (0.53 ± 0.20 vs. 0.64 ± 0.20; p < 0.001) (Fig. 3). Stepwise backward multivariate logistic regression analysis among various clinical factors demonstrated that BMI (odds ratio [OR]: 1.08; 95% confidence interval [CI]: 1.02 to 1.15; p = 0.01), eGFR (OR: 0.98; 95% CI: 0.97 to 0.99; p = 0.002), heart rate (OR: 1.01; 95% CI: 1.00 to 1.03; p = 0.04), and RHI (per 0.1) (OR: 0.77; 95% CI: 0.70 to 0.85; p < 0.001) were independently correlated with the presence of HFNEF among the age-, gender-, and rate of hypertension or DM-matched subjects with normal LVEF and patients with HFNEF (Hosmer-Lemeshow chi-square = 14.8 and

Table 1 Clinical and Echocardiographic Characteristics of the Study Population

	Patients Without HF (n = 173)	Patients With HFNEF (n = 321)	p Value	Low RHI Group (n = 160)	High RHI Group (n = 161)	p Value
Age, yrs	71 ± 9	72 ± 10	0.27	72 ± 10	71 ± 9	0.82
Female	83 (48)	161 (50)	0.64	77 (48)	84 (52)	0.50
BMI, kg/m^2	23.4 ± 3.1	24.3 ± 3.6	0.006	24.2 ± 3.3	24.4 ± 3.9	0.56
Waist circumference, cm	87 ± 9	89 ± 10	0.05	89 ± 10	89 ± 10	0.95
NYHA functional class II/III, IV	—	246/75	—	107/53	139/22	<0.001
CAD	85 (49)	145 (45)	0.40	77 (48)	68 (42)	0.29
Atrial fibrillation	—	102 (32)	—	58 (36)	44 (27)	0.09
Hypertension	143 (83)	268 (83)	0.81	129 (81)	139 (86)	0.18
Hypercholesterolemia	100 (58)	168 (52)	0.25	91 (57)	77 (48)	0.09
DM	67 (39)	140 (44)	0.29	71 (44)	69 (43)	0.82
Metabolic syndrome	79 (46)	139 (43)	0.61	73 (46)	66 (41)	0.40
Current smoker	20 (12)	32 (10)	0.58	19 (12)	13 (8)	0.26
Heart rate, beats/min	69 ± 11	72 ± 18	0.03	73 ± 18	71 ± 18	0.40
BNP, pg/ml	25 (14–50)	121 (33–302)	<0.001	166 (43–330)	106 (30–275)	0.07
Hemoglobin, g/dl	13.0 ± 1.5	12.8 ± 1.8	0.12	12.9 ± 1.7	12.6 ± 1.9	0.16
eGFR, ml/min/1.73 m^2	68 ± 18	60 ± 20	<0.001	61 ± 21	60 ± 18	0.71
Severe renal dysfunction (eGFR < 30 ml/min/1.73 m^2)	1 (0.6)	20 (6)	0.003	11 (7)	9 (6)	0.63
hsCRP (mg/dl)	0.05 (0.03–0.15)	0.09 (0.04–0.22)	0.001	0.10 (0.05–0.24)	0.08 (0.04–0.18)	0.17
LVEF	65 ± 5	63 ± 6	<0.001	62 ± 7	63 ± 6	0.35
E/e'	9.9 (8.4–11.6)	16.2 (15.0–18.8)	<0.001	16.3 (15.0–19.1)	16.2 (15.1–18.6)	0.71
Left atrial diameter, mm	36.3 ± 5.7	40.8 ± 7.1	<0.001	40.9 ± 6.8	40.8 ± 7.4	0.96
LV mass index, g/m^2	107 (93–133)	130 (105–157)	<0.001	125 (105–154)	136 (110–161)	0.14
Medications						
Beta-blocker	55 (32)	166 (52)	<0.001	87 (54)	79 (49)	0.34
ACEIs or ARBs	85 (49)	210 (65)	<0.001	104 (65)	106 (66)	0.88
Calcium-channel blockers	89 (51)	171 (53)	0.70	83 (52)	88 (55)	0.62
Nitrates	27 (16)	57 (18)	0.54	29 (18)	28 (17)	0.86
Diuretics	14 (8)	110 (34)	<0.001	64 (40)	46 (29)	0.03
Spironolactone	3 (2)	50 (16)	<0.001	28 (18)	22 (14)	0.34
Statins	109 (63)	181 (56)	0.17	92 (58)	89 (55)	0.69
Aspirin	111 (64)	193 (60)	0.38	99 (62)	94 (58)	0.52

Values are mean ± SD, median (25th to 75th percentile range), or n (%). The p values represent comparisons of patients with HFNEF versus patients without HF or low versus high RHI group.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; CAD = coronary artery disease; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; HFNEF = heart failure with normal ejection fraction; hsCRP = high-sensitivity C-reactive protein; LV = left ventricular; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

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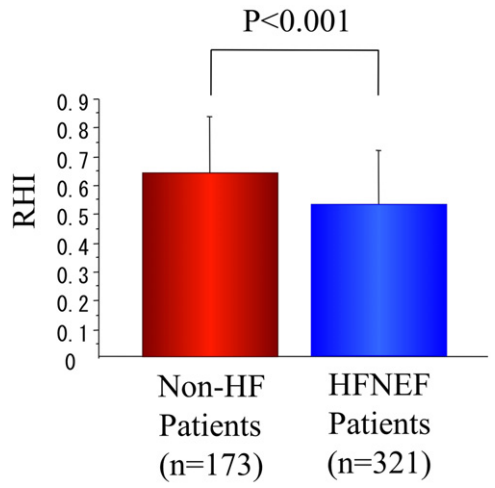

Figure 3 RHI in Patients With HFNEF and Patients Without HF

Bars represent averages of the RH-PAT index (RHI) in each group. T-bars indicate SD. HF = heart failure; HFNEF = heart failure with normal left ventricular ejection fraction; RHI = RH-PAT index.

p = 0.06). Even after the forced adjustment for BMI, eGFR, and the presence of hypertension and DM, the RHI was significantly correlated with the presence of HFNEF (OR: 0.77; 95% CI: 0.70 to 0.85; p < 0.001; Hosmer-Lemeshow chi-square = 12.6 and p = 0.13). All baseline medications were not significantly associated with endothelial dysfunction (low RHI group) in patients with HFNEF according to a simple logistic regression analysis (Online Table 1).

Follow-up. The data of 320 patients with HFNEF were available for analyzing cardiovascular events. One patient was lost to follow-up. The follow-up period was 1 to 57 months (mean: 20 months). Overall, 59 cardiovascular events were recorded in patients with HFNEF during the follow-up period. Details of the cardiovascular events are as follows: cardiovascular death (n = 6), nonfatal myocardial infarction (n = 1), ischemic stroke (n = 4), hospitalization for HF decompensation (n = 32), unstable angina pectoris (n = 10), and coronary revascularization (n = 6). The frequency of cardiovascular events was significantly higher in the low RHI group (below median, cutoff value: 0.49) compared with the high RHI group (above median) (n = 45 vs. n = 14, p < 0.001). Kaplan-Meier analysis demonstrated a significantly higher probability of cardiovascular events in the low RHI group compared with the high RHI group (log-rank test: p = 0.001) (Table 2, Fig. 4A).

Cox proportional hazards analysis and C-statistics for cardiovascular events. The results of the univariate and multivariate Cox proportional hazards analyses for cardiovascular events are summarized in Table 3. Stepwise multivariate Cox proportional hazard analysis identified RHI (per 0.1) (hazard ratio [HR]: 0.80; 95% CI: 0.67 to 0.94; p = 0.007), E/e' (Ln[E/e'] [per 0.1]) (HR: 1.15; 95% CI: 1.04 to 1.26; p = 0.006), and BNP (Ln[BNP] [per picogram/milliliter]) (HR: 1.81; 95% CI: 1.44 to 2.28; p < 0.001) as independent predictors of future cardiovascular events (Table 3). By using the 5 forced inclusion models with various clinical parameters in the multivariate Cox hazard analysis, the RHI still significantly predicted cardiovascular events (Table 3). As shown in Figure 4B, the combination of RHI and BNP identified subgroups with a significantly different probability of cardiovascular events (log-rank test: p < 0.001). In both the low and high BNP groups, the low RHI group had a significantly higher probability of cardiovascular events compared with the high RHI group (log-rank test: p = 0.01 and p = 0.02, respectively). Even in patients with mild HF symptoms (NYHA class II), the low RHI group was associated with worse outcomes than the high RHI group (log-rank test p = 0.006) (Fig. 4C).

We estimated the C-statistic of PF5 alone. Separate incorporation of RHI, E/e', and BNP into the PF5 increased the C-statistic for prediction of future cardiovascular events (C-statistics: PF5 alone 0.671, PF5 + RHI 0.712, PF5 + E/e' 0.700, and PF5 + BNP 0.732) (Table 4). Moreover, we examined the additive usefulness of RHI to PF5 and E/e', BNP, or both. The RHI increased the C-statistics in each model (C-statistics: PF5 + E/e' 0.700, PF5 + E/e' + RHI 0.737, PH5 + BNP 0.732, PF5 + BNP + RHI 0.753, PF5 + E/e' + BNP 0.742, PF5 + E/e' + BNP + RHI 0.761) (Table 4). The addition of RHI, E/e', and BNP to PF5 resulted in a significant increase in the C-statistics from 0.671 to 0.761 (p = 0.02).

The Schoenfeld's tests indicated that the proportional hazards assumptions were appropriate (p = 0.95). We also confirmed good calibration for the analysis by using Grønnesby and Borgan (21) statistics (p = 0.30).

Net reclassification index. We reclassified the risk of PF5 for the patients with HFNEF. The NRI was significant with the inclusion of the RHI (8.8% for patients without cardiovascular events, 10.2% for those with cardiovascular events, and 19.0% overall, p = 0.01) (Table 5).

Table 2 Cardiovascular Events in Patients With HFNEF With Low or High RHI

	Low RHI Group (n = 160)	High RHI Group (n = 160)	p Value
Total cardiovascular events	45 (28.1)	14 (8.8)	0.001
Cardiovascular death	5 (3.1)	1 (0.6)	0.11
Nonfatal myocardial infarction	0 (0)	1 (0.6)	0.32
Unstable angina	8 (5.0)	2 (1.3)	0.15
Ischemic stroke	2 (1.3)	2 (1.3)	0.99
Hospitalization for HF decompensation	26 (16.3)	6 (3.8)	0.002
Coronary revascularization	4 (2.5)	2 (1.3)	0.48

Values are n (%). Significance was assessed by the log-rank test.

HF = heart failure; HFNEF = heart failure with normal left ventricular ejection fraction; RHI = reactive hyperemia-peripheral arterial tonometry index.

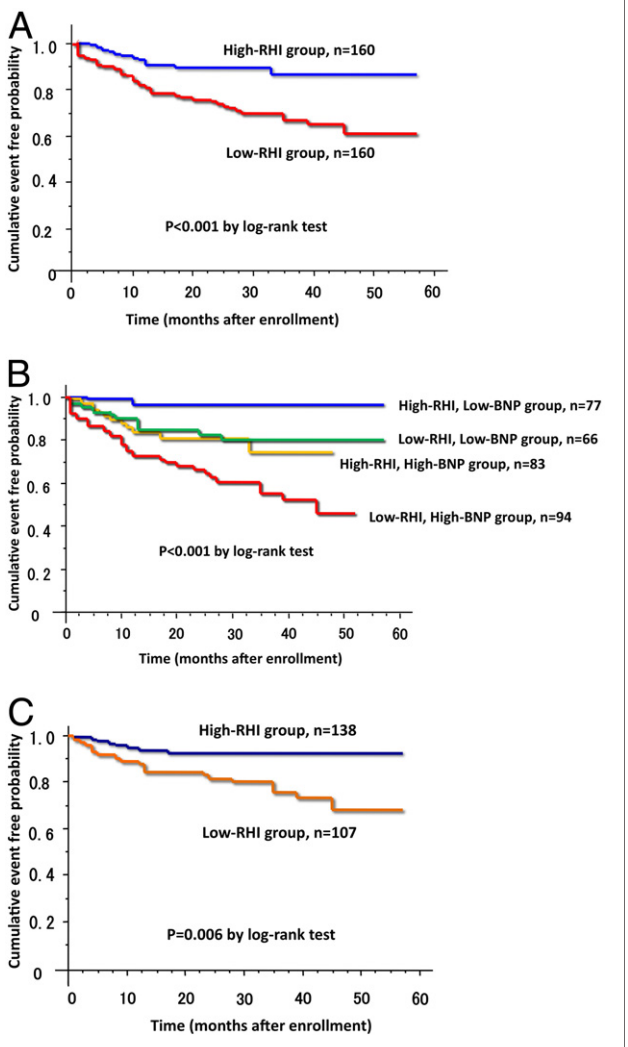

Figure 4 **Kaplan-Meier Analysis**

Kaplan-Meier analysis for the probability of cardiovascular events in patients with high or low RHI **(A)**, in subgroups of patients with high or low RHI and B-type natriuretic peptide (BNP) **(B)**, and in subgroups of New York Heart Association (NYHA) class II patients with high or low RHI **(C)**. On the basis of a cut-off point: RHI 0.49 (median), BNP 100 pg/ml. BNP = B-type natriuretic peptide; NYHA = New York Heart Association; RHI = RH-PAT index.

Discussion

This is the first report to reveal a significant association between peripheral endothelial dysfunction and adverse cardiovascular outcomes in patients with HFNEF. Significant endothelial dysfunction was demonstrated in patients with HFNEF, and the RHI was significantly correlated with the presence of HFNEF, independently of various comorbid factors and diseases. Endothelial dysfunction, BNP, and E/e' were identified as independent predictors of future cardiovascular events in patients with HFNEF. The addition of endothelial function as assessed by the RHI to the previously described HFNEF prognostic factors with BNP and E/e' improved risk stratification in patients with HFNEF, as indicated by a substantial increase in the C-statistics and a significant NRI.

Patients with HFNEF represent a heterogeneous population, and the precise mechanism underlying HFNEF is not fully understood. The I-PRESERVE demonstrated that age, presence of DM, presence of a previous hospitalization for HF, NT-proBNP, and LVEF were associated with adverse clinical outcome in patients with HFNEF (12); however, the determinants of prognosis in patients with HFNEF remain largely unexplored.

Endothelial dysfunction has been shown to be involved in the pathogenesis of HF, mainly HFREF. Several studies have reported that peripheral endothelial dysfunction is associated with clinical outcome in patients with HFREF (10). Borlaug et al. (23) recently reported that subjects with HFNEF had limited arterial vasodilatory response to exercise, which might impair cardiac output reserve under stress conditions and demonstrated that global cardiovascular reserve functions, including peripheral endothelial function, are impaired in subjects with HFNEF (7). However, no previous report has discussed the association between peripheral endothelial function and adverse clinical outcome in patients with HFNEF. In the present study, we first reported that peripheral endothelial function assessed by RH-PAT significantly correlates with future cardiovascular events in patients with HFNEF. Peripheral endothelial function is still an independent predictor after adjusting various clinical parameters. The NRI was significant when the RHI was added to the PF5. The present study also demonstrated that peripheral endothelial function, BNP, and E/e' were independent predictors of future cardiovascular events in patients with HFNEF. The combination of these parameters adding to PF5 significantly increased the C-statistics and may be useful to improve risk stratification in patients with HFNEF.

The present study also demonstrated that BNP independently correlated with future cardiovascular events in patients with HFNEF. This result was consistent with the result of a recent report of NT-proBNP from the I-PRESERVE (12). BNP was still an independent predictor of adverse outcomes in patients with HFNEF or HFREF.

Moreover, the present study identified E/e' as an independent predictor of future cardiovascular events in patients with HFNEF. E/e' has been used as an index of LV filling pressure and abnormal LV relaxation (24). LVDD plays an important role in patients with HFNEF (4). Prior studies have demonstrated that elevated LV filling pressure or LVDD was associated with adverse outcomes in patients with HFREF (25–27) or community subjects with normal LVEF and without HF (2); however, few previous reports showed the relationship between LVDD and adverse clinical outcomes in patients with HFNEF. Ren et al. (28) reported that the presence of asymptomatic LVDD could predict subsequent hospitalization for HF events in patients with coronary heart disease without a

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Table 3 Cox Proportional Hazards Analysis for Future Cardiovascular Events in Patients With HFNEF

	Univariate Regression			Stepwise Backward Multivariate Regression			Multivariate Regression Using Forced Inclusion Model 1		
Variable	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
Age (per year)	1.00	0.98–1.03	0.81		Not selected		1.00	0.97–1.02	0.87
Female	0.98	0.59–1.63	0.94		Not selected			—	
Body mass index (per kg/m^2)	1.00	0.93–1.08	0.99		Not selected			—	
Waist circumferences (per cm)	1.00	0.97–1.02	0.77		Not selected			—	
NYHA functional class III or IV vs. II	4.18	2.50–6.99	<0.001		Not selected		2.41	1.18–4.94	0.02
Previous history of decompensated HF hospitalization (yes)	2.92	1.74–4.89	<0.001		Not selected		1.20	0.61–2.35	0.60
CAD (yes)	1.13	0.68–1.89	0.63		Not selected			—	
Atrial fibrillation (yes)	2.25	1.35–3.76	0.002		Not selected			—	
Diabetes mellitus (yes)	1.18	0.71–1.96	0.53		Not selected		1.05	0.62–1.78	0.85
Metabolic syndrome (yes)	0.93	0.56–1.56	0.79		Not selected			—	
Current smoker (yes)	1.00	0.43–2.32	0.99		Not selected			—	
Heart rate (per beats/min)	1.02	1.00–1.03	0.02		Not selected			—	
Systolic blood pressure (per mm Hg)	0.99	0.98–1.00	0.08		Not selected			—	
Diastolic blood pressure (per mm Hg)	0.98	0.96–1.00	0.07		Not selected			—	
LDL cholesterol (per mg/dl)	1.00	0.99–1.00	0.25		Not selected			—	
HDL cholesterol (per mg/dl)	0.99	0.97–1.00	0.13		Not selected			—	
Ln (Triglycerides) (per mg/dl)	0.80	0.46–1.41	0.44		Not selected			—	
Ln (BNP) (per pg/ml)	1.89	1.52–2.36	<0.001	1.81	1.44–2.28	<0.001		—	
Hemoglobin (per g/dl)	0.96	0.84–1.11	0.60		Not selected			—	
eGFR (per ml/min/1.73 m^2)	0.99	0.98–1.01	0.38		Not selected			—	
hsCRP (mg/dl)	1.37	0.94–2.01	0.10		Not selected			—	
LVEF (per %)	0.93	0.89–0.97	<0.001		Not selected		0.96	0.92–1.00	0.07
Ln (E/e') (per 0.1)	1.16	1.04–1.29	0.009	1.15	1.04–1.26	0.006		—	
RHI (per 0.1)	0.72	0.61–0.85	<0.001	0.80	0.67–0.94	0.007	0.82	0.69–0.97	0.02

	Multivariate Regression Using Forced Inclusion											
	Model 2			Model 3			Model 4			Model 5		
Variable	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
NYHA functional class III or IV vs. II	1.76	0.86–3.64	0.12	1.97	0.94–4.14	0.07	1.76	0.85–3.66	0.13	1.68	0.81–3.51	0.17
Previous history of decompensated HF hospitalization (yes)	0.76	0.38–1.53	0.44	0.68	0.33–1.39	0.28	0.72	0.35–1.48	0.37	0.73	0.36–1.48	0.38
Atrial fibrillation (yes)		—		1.54	0.88–2.68	0.13		—			—	
Heart rate (per beats/min)		—			—		1.01	1.00–1.02	0.15		—	
Ln (BNP) (per pg/ml)	1.68	1.30–2.19	<0.001	1.63	1.25–2.13	<0.001	1.67	1.29–2.17	<0.001	1.64	1.26–2.14	<0.001
LVEF (per %)		—			—			—		0.97	0.93–1.02	0.24
Ln (E/e') (per 0.1)	1.11	1.02–1.21	0.02	1.13	1.03–1.24	0.01	1.11	1.02–1.22	0.02	1.11	1.02–1.21	0.02
RHI (per 0.1)	0.80	0.67–0.95	0.01	0.82	0.69–0.97	0.02	0.80	0.68–0.94	0.008	0.80	0.68–0.95	0.01

Not selected indicates the backward algorithm selection did not reach the 0.10 significance level, when each independent variable was once included into the stepwise regression model.

CI = confidence interval; HR = hazard ratio; RHI = reactive hyperemia peripheral arterial tonometry index; other abbreviations as in Table 1.

history of HF symptoms. A small study (29) recently showed that elevated E/e' after optimized medical therapy predicts future HF events in patients with HFNEF. Our data confirmed this result in a relative larger population.

Effective treatments for patients with HFNEF have not been established because the precise mechanisms underlying HFNEF remain unclear. HFNEF often has been shown to cause AHFS, and the high-risk population with AHFS after discharge has not been clarified. To achieve the better postdischarge outcomes in patients hospitalized for AHFS, optimizing treatments in the early postdischarge period will require the improvement of risk stratification and patient triage during the compensated condition of HF symptoms (30). HFNEF could be due to structural and molecular abnormalities of the cardiovascular system, including cardiac and noncardiac factors such as vascular functions (7). The prognostic impact of the RHI in patients with HFNEF suggests that endothelial dysfunction may not be a passive finding, but rather that endothelial function may play an active and important pathophysiologic role in HFNEF. This in turn supports the concept that endothelial dysfunction may be a novel therapeutic target in HFNEF. To determine the molecular mechanisms of endothelial dysfunction in patients with HFNEF, further clinical and experimental studies are required, and we need to develop the therapeutic strategy for improving endothelial dysfunction in HFNEF.

Study limitations. First, endothelial function in HFNEF may be related to a metabolic risk profile, but the results of a case-control observational study cannot prove causality. Impairment of endothelial function in HFNEF may be a result of progression to HF. Measurement of plasma neurohormones or exercise oxygen uptake would help to clarify the pathophysiological link between endothelial function and HFNEF. Further study is needed. Second, patients with HFNEF were referred to 2 tertiary centers, and RHI measurements were performed in a stable compensated state after treatment. These results might have referral bias and were applicable to such stable patients with HFNEF. The pathophysiological and prognostic importance of endothelial dysfunction in AHFS should be discussed in a further study. Third, the sample size was small, and the design was a 2-center study in Japan, resulting in the more predominantly male population and the better prognosis compared with that found in Western studies. Therefore, a large multiracial and multicenter study is required to confirm our results. Fourth, to select patients with HFNEF, we applied the European Society of Cardiology consensus statement for HFNEF, which contained some limited data. Because the diagnosis of HFNEF is practically important, we continuously need to discuss and evaluate the criteria for HFNEF to establish the definitive diagnosis. Fifth, endothelial function assessed by RHI measurement might be affected by various factors in the clinical practice, which may be a limitation to the applicability of the present findings. The present study clearly demonstrated the independent prognostic impact of endothelial dysfunction assessed by RH-PAT on future cardiovascular events in patients with HFNEF; however, the immediate clinical applicability of RH-PAT as a prognostic tool in patients with HFNEF might be hampered by the lack of validation in a broader sample and lack of standardization of methodology. Further continuous clinical studies are necessary.

Table 4 C-Statistics for Cox Proportional Hazards Analysis to Predict Future Cardiovascular Events in Patients With HFNEF

Variable	C-statistic	95% CI	Increment in C-statistic
PF5	0.671	0.576–0.766	
PF5 + RHI	0.712	0.636–0.789	0.041
PF5 + E/e'	0.700	0.613–0.786	
PF5 + E/e' + RHI	0.737	0.663–0.811	0.037
PF5 + BNP	0.732	0.649–0.814	
PF5 + BNP + RHI	0.753	0.680–0.826	0.021
PF5 + E/e' + BNP	0.742	0.662–0.821	
PF5 + E/e' + BNP + RHI	0.761	0.691–0.832	0.019

BNP = B-type natriuretic peptide; CI = confidence interval; E/e' = the ratio of early transmitral flow velocity to tissue Doppler early diastolic mitral annular (medial) velocity; PF5 = HFNEF prognostic 5 factors that were identified in the I-PRESERVE, including age, presence of DM, presence of a previous hospitalization for HF, NYHA classification, and LVEF; RHI = reactive hyperemia-peripheral arterial tonometry index; other abbreviations as in Table 1.

Table 5 Reclassification of Risk of PF5 for Cardiovascular Events After Addition of RHI

Original Risk Category	Reclassification: PF5 + RHI Low Risk	PF5 + RHI Intermediate Risk	PF5 + RHI High Risk
Patients without cardiovascular events			
PF5 low risk	59	13	1
PF5 intermediate risk	44	80	14
PF5 high risk	0	7	43
Patients with cardiovascular events			
PF5 low risk	3	8	0
PF5 intermediate risk	5	10	3
PF5 high risk	0	0	30

According to the risk of PF5 for cardiovascular events during the follow-up period (20 months), low risk indicates a risk <10%, intermediate risk indicates a risk of 10% to 20%, and high risk indicates a risk of >20%. The NRI was 8.8% (23 of 261 patients) for patients without cardiovascular events, 10.2% (6 of 59 patients) for those with cardiovascular events, and 19.0% overall.
Abbreviations as in Tables 1, 2 and 4.

Conclusions

Peripheral endothelial dysfunction assessed by RH-PAT independently correlated with future cardiovascular events in patients with HFNEF. In combination with BNP and E/e', the clinical assessment of endothelial function can provide incremental prognostic significance, leading to improved risk stratification in HFNEF.

Reprint requests and correspondence: Dr. Seigo Sugiyama, Department of Cardiovascular Medicine, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku Kumamoto City 860-8556, Japan. E-mail: ssugiyam@kumamoto-u.ac.jp.

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Key Words: endothelial function ■ heart failure with normal left ventricular ejection fraction ■ reactive hyperemia-peripheral arterial tonometry.

APPENDIX

For supplementary material, table, and figure, please see the online version of this article.