Table 2. Changes in blood pressure during the study period

Therapy	CCB-based		Telmisartan-based		p (between therapies)
	baseline	12 months	baseline	12 months	
Clinical SBP	155 ± 19	134 ± 12*	156 ± 14	136 ± 13*	0.935
Clinical DBP	95 ± 11	85 ± 10*	101 ± 10	87 ± 11*	0.232
24-hour SBP	138 ± 18	130 ± 13	144 ± 11	128 ± 11*	0.053
24-hour DBP	88 ± 9	80 ± 8*	91 ± 9	81 ± 7*	0.045
Daytime SBP	142 ± 20	135 ± 14	148 ± 10	132 ± 11*	0.045
Daytime DBP	88 ± 12	83 ± 9*	94 ± 7	84 ± 8*	0.050
Nighttime SBP	125 ± 20	116 ± 12	131 ± 17	113 ± 13*	0.124
Nighttime DBP	76 ± 11	71 ± 9	83 ± 11	72 ± 9*	0.076
Nocturnal decrease	18 ± 16	19 ± 12	17 ± 13	18 ± 12	0.994
Morning surge	44 ± 12	47 ± 20	47 ± 20	43 ± 17	0.317
SBP variability	20 ± 4	20 ± 5	19 ± 5	19 ± 5	0.763

Units are mm Hg. Data are the means ± SD. SBP = Systolic blood pressure; DBP = diastolic blood pressure.
* $p < 0.05$ vs. the baseline value.

creased significantly after the telmisartan-based therapy, although no significant difference was observed between the two groups. The clinical diastolic BP decreased significantly after the CCB-based therapy, and also declined significantly after the telmisartan-based therapy, although no significant difference was observed between the two groups. The 24-hour ambulatory systolic BP decreased significantly after the telmisartan-based therapy, whereas it did not change significantly after the CCB-based therapy. The 24-hour ambulatory diastolic BP decreased significantly after both the CCB-based therapy and the telmisartan-based therapy. The reduction in the 24-hour ambulatory diastolic BP after the telmisartan-based therapy was significantly greater than that after the CCB-based therapy. The daytime systolic BP decreased significantly after the telmisartan-based therapy, whereas it did not change significantly after the CCB-based therapy. The reduction in the daytime systolic BP after the telmisartan-based therapy was significantly greater than that after the CCB-based therapy. The daytime diastolic BP decreased significantly after the CCB-based therapy and also decreased significantly after the telmisartan-based therapy, although no significant difference was observed between the two groups. The nighttime systolic BP decreased significantly after the telmisartan-based therapy, but did not change significantly after the CCB-based therapy. The nighttime diastolic BP decreased significantly after the telmisartan-based therapy, but did not change significantly after the CCB-based therapy. The reduction in the nighttime diastolic pressure after the telmisartan-based therapy was significantly greater than that after the CCB-based therapy. No significant changes in the nocturnal decrease in systolic BP, the morning systolic BP surge or the systolic BP variability were seen during the 12-month observation period in either group.

Figure 1 shows the change in the BNP, UAE and arterial stiffness as assessed by CAVI, AI and MAX-IMT in both groups. The log UAE level decreased significantly from 1.03 ± 0.66 to 0.57 ± 0.52 after the telmisartan-based therapy, whereas it did not change significantly after the CCB-based therapy. CAVI decreased significantly from 8.2 ± 1.0 to 7.7 ± 0.9 after the telmisartan-based therapy, whereas it did not change significantly after the CCB-based therapy. CAVI also decreased significantly from 100 to 93.7 ± 9.8% after the telmisartan-based therapy when the baseline values of CAVI were adjusted, but did not change significantly after the CCB-based therapy. No significant changes in the levels of BNP, AI or MAX-IMT were observed in either group.

Figure 2 shows the changes in the metabolic parameters in both groups. During the 12-month observation period, the serum LDL-C level decreased significantly from 130 ± 34 to 119 ± 32 mg/dl after the CCB-based treatment and also decreased significantly from 121 ± 24 to 115 ± 29 mg/dl after the telmisartan-based therapy. However, no significant difference was observed between the two groups. The plasma ARC increased significantly from 9.6 ± 8.7 to 46.7 ± 84.7 pg/ml after the telmisartan-based therapy, whereas no significant change was seen af-

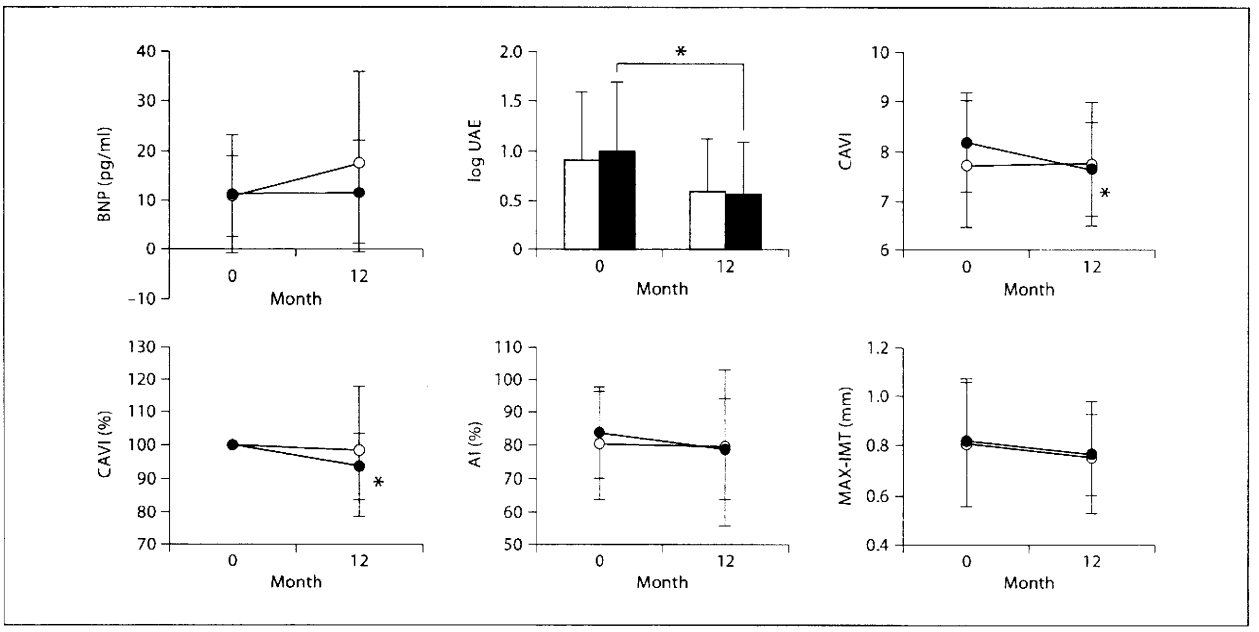

Fig. 1. Serum BNP, log UAE, AI, CAVI, the baseline adjusted CAVI and MAX-IMT at baseline and after 12 months of treatment in the CCB-based therapy (open circles) and the telmisartan-based therapy (closed circles) groups. * $p < 0.05$ vs. the baseline value.

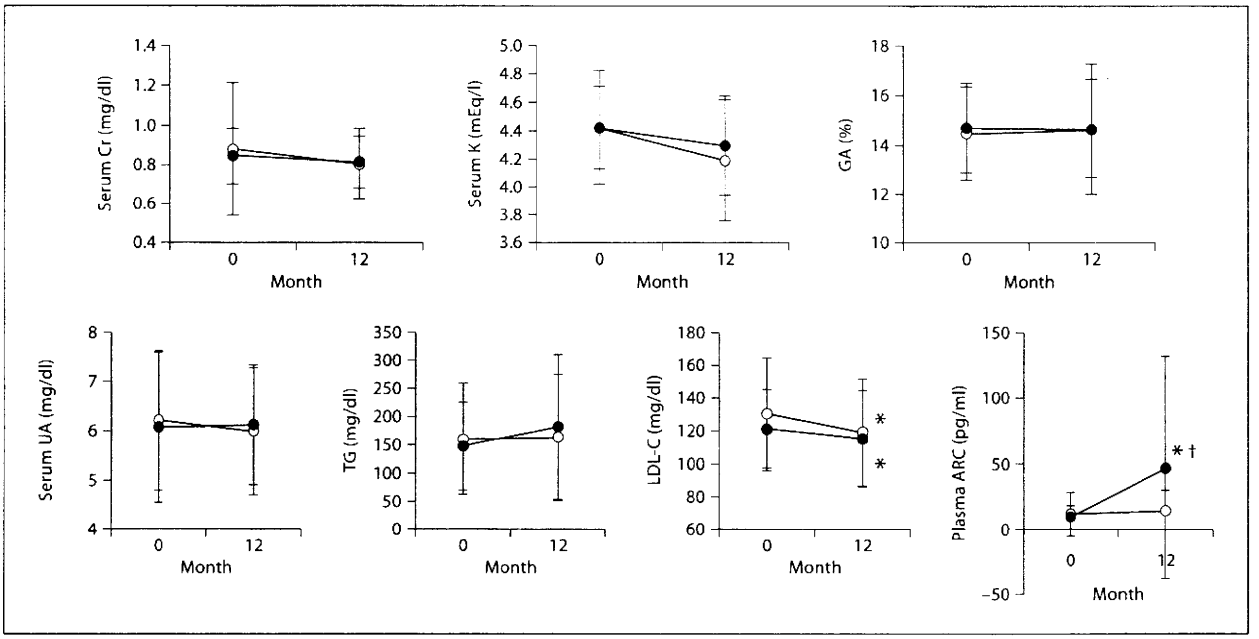

Fig. 2. Serum creatinine (Cr), serum K, glycoalbumin (GA), serum uric acid (UA), serum triglyceride (TG), serum LDL-C and plasma ARC at baseline and after 12 months of treatment in the CCB-based therapy (open circles) and the telmisartan-based therapy (closed circles) groups. * $p < 0.05$ vs. the baseline value; † $p < 0.05$ vs. the group treated with the CCB-based therapy.

Table 3. Effects of percent changes in LDL, ARC, log UAE, clinical BP, 24-hour BP, daytime BP and nighttime BP on percent changes in CAVI after telmisartan-based therapy

ANCOVA	Coefficient	SE	t test	p
Intercept	−0.058	0.016	−3.640	0.0008
ΔLDL	0.004	0.088	0.046	0.9638
Intercept	−0.044	0.019	−2.281	0.0298
ΔARC	−0.005	0.002	−2.382	0.0238
Intercept	−0.039	0.024	−1.623	0.1151
Δlog UAE	0.064	0.051	1.263	0.2165
Intercept	−0.038	0.024	−1.570	0.1241
Δclinical SBP	0.182	0.157	1.154	0.2552
Intercept	−0.039	0.024	−1.637	0.1093
Δclinical DBP	0.167	0.142	1.181	0.2446
Intercept	−0.027	0.028	−0.963	0.3427
Δ24-hour SBP	0.290	0.204	1.423	0.1645
Intercept	−0.001	0.028	−0.042	0.9671
Δ24-hour DBP	0.449	0.199	2.515	0.0171
Intercept	−0.033	0.028	−1.190	0.2429
Δdaytime SBP	0.246	0.205	1.201	0.2386
Intercept	−0.015	0.027	−0.570	0.5729
Δdaytime DBP	0.397	0.193	2.054	0.0483
Intercept	−0.019	0.024	−0.782	0.4398
Δnighttime SBP	0.320	0.136	2.350	0.0251
Intercept	−0.024	0.021	−1.114	0.2734
Δnighttime DBP	0.305	0.120	2.531	0.0165

SBP = Systolic blood pressure; DBP = diastolic blood pressure.

Table 4. Effects of percent changes in LDL, ARC, CAVI, clinical BP, 24-hour BP, daytime BP and nighttime BP on percent changes in log UAE after telmisartan-based therapy

ANCOVA	Coefficient	SE	t test	p
Intercept	−0.301	0.065	−4.641	<0.0001
ΔLDL	0.041	0.408	0.101	0.9201
Intercept	−0.265	0.085	−3.130	0.0044
ΔARC	−0.013	0.009	−1.438	0.1629
Intercept	−0.265	0.074	−3.571	0.0012
ΔCAVI	0.787	0.624	1.263	0.2165
Intercept	−0.271	0.113	−2.403	0.0227
Δclinical SBP	0.265	0.773	0.342	0.7347
Intercept	−0.282	0.112	−2.522	0.0172
Δclinical DBP	0.150	0.683	0.220	0.8276
Intercept	−0.222	0.121	−1.830	0.0783
Δ24-hour SBP	0.946	0.841	1.125	0.2705
Intercept	−0.224	0.127	−1.761	0.0896
Δ24-hour DBP	0.890	0.870	1.023	0.3152
Intercept	−0.230	0.128	−1.793	0.0842
Δdaytime SBP	0.858	0.898	0.956	0.3475
Intercept	−0.274	0.123	−2.232	0.0341
Δdaytime DBP	0.497	0.843	0.589	0.5606
Intercept	−0.215	0.094	−2.287	0.0302
Δnighttime SBP	0.950	0.525	1.810	0.0815
Intercept	−0.262	0.093	−2.812	0.0091
Δnighttime DBP	0.581	0.506	1.150	0.2602

SBP = Systolic blood pressure; DBP = diastolic blood pressure.

ter the CCB-based therapy. The increase in the plasma ARC after the telmisartan-based therapy was significantly greater than that after the CCB-based therapy. No significant changes in the serum levels of creatinine, potassium, glycoalbumin, uric acid or triglyceride were seen during the 12-month observation period in either group.

In the present study, serum LDL-C, ARC, log UAE, clinical BP, 24-hour BP, daytime BP and nighttime BP changed significantly after the telmisartan-based therapy. As table 3 shows, we performed ANCOVA (analysis of covariance) to examine whether these variables affected the change in CAVI during telmisartan-based therapy. The changes in the plasma ARC, 24-hour diastolic BP, daytime diastolic BP and nighttime systolic and diastolic BP contributed significantly to the decrease in CAVI after the telmisartan-based therapy.

As table 4 shows, ANCOVA was also performed to examine whether the changes in the serum LDL-C, ARC, CAVI, clinical BP, 24-hour BP, daytime BP and nighttime BP affected the change in log UAE during the telmisartan-based therapy. However, none of the parameter changes contributed to the decrease in log UAE after the telmisartan-based therapy.

Discussion

This study demonstrated for the first time that telmisartan significantly improves CAVI, which reflects arterial stiffness in a manner that is less dependent on BP, compared with PWV in hypertensive patients [5]. The average coefficient of variation of CAVI is less than 5%,

which is small enough for clinical usage and indicates that CAVI has good reproducibility and validation of the measurement of arterial stiffness [13]. In addition, CAVI is a new marker for arterial stiffness since the previous study has reported that CAVI reflects the fibrosis of arterial walls histologically [5]. In the present study, telmisartan also significantly improved CAVI by 6.3% when the baseline values were adjusted, which was larger than the average coefficient of variation of the CAVI. The results of this study agree with those of previous studies, which showed improvements in vascular compliance after treatment with other ARBs [12]. In fact, previous studies have revealed that the long-term administration of telmisartan improved the PWV in spite of a BP-lowering efficacy comparable to those of other antihypertensive medications [9, 14]. Since angiotensin II stiffens the vascular wall by constricting the vascular smooth muscle and promoting vascular wall remodeling, ARBs might improve arterial stiffness, at least in part, through BP-independent effects [7]. In the present study, ANCOVA showed that the changes in plasma ARC contributed significantly to the decrease in CAVI after the telmisartan-based therapy, although the changes in the 24-hour diastolic BP, daytime diastolic BP and nighttime systolic and diastolic BP also contributed significantly to the decrease in CAVI after the telmisartan-based therapy. Thus, the improvement of CAVI after telmisartan-based therapy might result from RAS blockade as well as antihypertensive effects. In addition, previous studies showed that telmisartan has the unique activity of enhancing PPAR-γ, which plays critical roles in the vasculature [8]. In vascular endothelial cells, PPAR-γ activation inhibits endothelial inflammation by suppressing inflammatory gene expression, leading to endothelial dysfunction [15]. In vascular smooth muscle cells, PPAR-γ activation inhibits proliferation and migration, and promotes apoptosis [15]. Taken together, the properties of telmisartan, such as RAS blockade and PPAR-γ activation, are thought to be involved in the improvement of CAVI. Since arterial stiffness is a powerful and independent risk factor for mortality in cardiovascular events [16], telmisartan could be a first-line antihypertensive drug with additional cardiovascular protective properties.

AI is another marker of arterial stiffness; this marker is associated with the plasma BNP because both markers reflect the central aortic pressure [13, 17]. Vascular stiffening causes an increase in the amplitude and early return of the reflected wave during systole, with augmentation of the central systolic BP and a resultant increase in AI [18]. Furthermore, the carotid IMT represents structural changes like wall thickening and the formation of atherosclerotic plaques, which are correlated with a greater incidence of atherosclerosis in the coronary circulation and other large arteries [19]. Telmisartan has been shown to cause an insignificant reduction in AI [18] and significant decreases in both BNP [20] and IMT [21]. In the present study, telmisartan-based therapy also tended to decrease the AI and to regress the IMT, consistent with the above-mentioned studies, whereas telmisartan-based therapy did not decrease the BNP. While a previous study demonstrated a significant reduction in BNP after treatment with telmisartan when the initial baseline level was 27.8 ± 21 pg/ml [20], the baseline level of BNP in the present study was 11.2 ± 11.9 pg/ml. Therefore, the plasma BNP level in the subjects in the present study might have been too low to detect any notable changes.

Albuminuria is an important predictor of cardiovascular events and of progression to end-stage renal disease in hypertensive patients [22, 23]. In the present study, telmisartan-based therapy significantly reduced microalbuminuria in hypertensive patients. This result was consistent with those of previous studies showing that ARB treatment inhibits the progression to macroalbuminuria and overt proteinuria in diabetic patients with microalbuminuria [24, 25] and that telmisartan treatment reduces microalbuminuria in hypertensive patients [14]. The renoprotective effects exerted by telmisartan might be partly mediated by BP-independent mechanisms, since ANCOVA showed that the BP changes did not contribute to the decrease in log UAE after the telmisartan-based therapy. Telmisartan, a dual ARB/PPAR-γ agonist, might have a great antiproteinuric effect [26] since treatment with telmisartan enabled a greater reduction in microalbuminuria than treatment with an ARB without any PPAR-γ agonistic action [27]. The present study demonstrated a significant reduction in the log UAE value, even though ACE inhibitors or ARBs other than telmisartan were prescribed for 40% of the patients treated with CCB-based therapy. Thus, ARBs with properties that enhance PPAR-γ might be useful for reducing microalbuminuria in hypertensive patients.

Telmisartan has been shown to be characterized by a balanced antihypertensive efficacy that fully covers a 24-hour period, thereby antagonizing the adverse effects of early morning BP elevations on cardiovascular risk [19]. The beneficial effects of telmisartan on the 24-hour BP values consist not only of lowering the daytime and nighttime BP, but also effectively controlling BP variability [28, 29], which has been shown to be closely correlated with PWV [11], target-organ damage [30] and the incidence of

cardiovascular events and mortality independent of the absolute BP load [31]. Previous studies have shown that the inhibitory effects of telmisartan on BP variability were associated with a reduction in sympathetic nerve activity in hypertensive patients with overt nephropathy [28]. Thus, telmisartan could be beneficial in hypertensive patients with a greater BP variability and/or autonomic dysfunction, such as elderly subjects and diabetic patients. Although reductions in morning surge and BP fluctuations were not observed in the present study, a sustained antihypertensive effect was observed throughout the 24-hour observation period among the subjects treated with telmisartan-based therapy.

ARB has also been shown to have a favorable lipid profile [32]. In the present study, telmisartan significantly decreased the serum level of LDL-C, which was in line with previous studies [33]. However, the reduction in the serum LDL-C levels after telmisartan-based therapy was similar to that observed after CCB-based therapy, probably because of the relatively large proportion of patients with prescriptions for ACE inhibitors or ARBs other than telmisartan among the patients treated with the CCB-based therapy. In the present study, the telmisartan-based therapy did not increase the serum levels of creatinine or potassium, which is consistent with the ONTARGET study (which demonstrated a similar number of patients whose serum creatinine levels and potassium levels increased after telmisartan or ramipril therapy) [34]. Thus, the present study also confirmed the metabolic safety of telmisartan.

In the present study, ARBs and CCBs were added in the group treated with CCBs and telmisartan, respectively, unless the target BP was achieved. The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial revealed that BP reduction is important for the cardiovascular protection at an early stage, while the contribution of RAS inhibition to the reduction of the cardiovascular events is apparent in a delayed fashion [35]. Based on the CASE-J trial, one would expect that the early initiation of ARBs will be beneficial for the inhibition of the arterial stiffness. In fact, the current study clearly provided evidence that the arterial stiffness assessed by CAVI significantly improved in the group treated with telmisartan from the start of the study, whereas the improvement of the arterial stiffness in the CCB-based therapy was not as much as that in the telmisartan-based therapy, even after the addition of ARB. Therefore, telmisartan should be the first drug to be initiated in view of the protection from the arterial stiffness.

Some limitations in the interpretation of the results of the present study exist. First, the trial population was comparatively small and the observation period was relatively short. A longer observation with a larger number of subjects might elucidate the beneficial and adverse effects of telmisartan more clearly. In addition, prognostic events were not examined in the present study. Further studies are needed to confirm the benefits and safety of telmisartan-based therapy.

In conclusion, telmisartan-based therapy exerted beneficial effects on arterial stiffness, as assessed using CAVI, albuminuria, 24-hour BP and metabolism compared with CCB-based therapy. Since these markers are known to influence the future risk for cardiovascular events in hypertensive patients, telmisartan could well be a first-line antihypertensive drug for the treatment of hypertensive patients.

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Significance of a Multiple Biomarkers Strategy Including Endothelial Dysfunction to Improve Risk Stratification for Cardiovascular Events in Patients at High Risk for Coronary Heart Disease

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Objectives We investigated whether a multiple biomarkers strategy that includes plasma levels of endothelium-derived microparticles (EMP), reflecting endothelial dysfunction, can improve prediction of future cardiovascular events in patients at high risk for coronary heart disease (CHD).

Background Detailed risk stratification using multiple biomarkers can provide clinical benefits in high-risk patients. Endothelial dysfunction has been described as a predictor of cardiovascular complications.

Methods We measured 3 biomarkers in 488 consecutive patients with various CHD risks: B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (hsCRP), and EMP. We followed 387 stable patients at high risk for CHD and examined future cardiovascular events.

Results During a mean follow-up of 36 months, 55 patients developed cardiovascular events. Multivariate Cox proportional hazards analysis adjusted for established risk factors identified age, BNP, hsCRP, and EMP as significant and independent predictors of future cardiovascular events (age: hazard ratio [HR]: 1.042, 95% confidence interval [CI]: 1.007 to 1.080, p = 0.02; BNP: HR: 1.242, 95% CI: 1.004 to 1.536, p = 0.046; hsCRP: HR: 1.468, 95% CI: 1.150 to 1.875, p = 0.002; EMP: HR: 1.345, 95% CI: 1.094 to 1.652, p = 0.005). The C statistics for cardiovascular events increased when each biomarker or combinations of biomarkers were added to the Framingham risk model (C statistics: Framingham risk model alone 0.636, Framingham risk + BNP 0.695, Framingham risk + hsCRP 0.696, Framingham risk + EMP 0.682, and Framingham risk + BNP + hsCRP + EMP 0.763).

Conclusions The assessment of endothelial dysfunction by plasma levels of EMP can independently predict future cardiovascular events in patients at high risk for CHD. A multiple biomarkers strategy that includes endothelial dysfunction assessed by EMP can identify patients vulnerable to cardiovascular disease. (University Hospital Medical Information Network number: UMIN000000876) (J Am Coll Cardiol 2009;54:601–8) © 2009 by the American College of Cardiology Foundation

The present cardiovascular risk stratification with established coronary risk factors cannot fully predict the development of cardiovascular events (1). Several biomarkers including B-type natriuretic peptide (BNP) and high-sensitivity C-reactive protein (hsCRP) have been reported to be useful for identifying the high-risk patients, independent of the established risk factors, and the multiple biomarkers strategy has been demonstrated to improve the risk stratification for cardiovascular events beyond the risk assessment based on established risk factors alone (2,3). Biomarkers reflecting different disease pathways may have the potential advantage of improving predictive power utility, and improvement of the assessment of cardiovascular risk with new biomarkers is desirable. It has been demonstrated that endothelial dysfunction is involved in the

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Abbreviations and Acronyms

ACS = acute coronary syndromes

BNP = B-type natriuretic peptide

CAD = coronary artery disease

CHD = coronary heart disease

CI = confidence interval

DM = diabetes mellitus

eGFR = estimated glomerular filtration rate

EMP = endothelium-derived microparticle(s)

HDL = high-density lipoprotein

HR = hazard ratio

hsCRP = high-sensitivity C-reactive protein

LDL = low-density lipoprotein

development of atherothrombogenic complications (4) and associated with future cardiovascular events in high-risk patients (5–7); however, it has not been incorporated into the previous multiple biomarkers strategy. Endothelial dysfunction can be clinically detected by measuring impairment of endothelium-dependent vasodilatation in response to acetylcholine during coronary angiography or by brachial artery flow-mediated vasodilation (5,8). These physiological tests are complex, operator dependent, and provide limited quantitative data (9,10).

Endothelium-derived microparticles (EMP) are small membrane-shed vesicles generated from endothelial cell surfaces in response to cellular activation or injury/apoptosis, and can potentially reflect endothelial dysfunction (11,12). Recently, we reported that CD144-EMP is derived selectively from human endothelial cells (13) and that circulating plasma CD144-EMP levels correlate significantly with coronary endothelial dysfunction and are significantly elevated in patients with type 2 diabetes and atherosclerosis (13). Although EMP are still only used for research purpose and in specialized laboratories because of their elusive nature and difficult assessment due to very small size (14), these findings underscore the potential application of CD144-EMP as a quantitative biomarker of endothelial dysfunction.

We hypothesized that the addition of a quantitative measure of endothelial dysfunction to a multiple biomarkers strategy could improve the prediction of future cardiovascular events. The hypothesis was tested by investigating the utility of plasma CD144-EMP levels for prediction of future cardiovascular events in stable patients at high risk for coronary heart disease (CHD), and examined the usefulness of the modified multiple biomarkers strategy, including endothelial dysfunction assessed by EMP, to predict cardiovascular complications.

Methods

Study patients. In this prospective study, we screened 519 consecutive Japanese patients between May 2003 and August 2007 at Kumamoto University Hospital. Patients with severe valvular heart disease requiring surgical intervention within 1 month, scheduled for coronary revascularization, active infection, or malignant disease were excluded from the study (n = 31). The 488 patients who fulfilled the study criteria were divided into the following 4 groups: low-risk patients who had no or 1 CHD risk factor, patients with multiple risk factors without documented coronary artery disease (CAD), patients with documented CAD at stable condition (stable-CAD), and patients with acute coronary syndromes (ACS) (Fig. 1). Stable-CAD represented patients with angiographically documented organic coronary

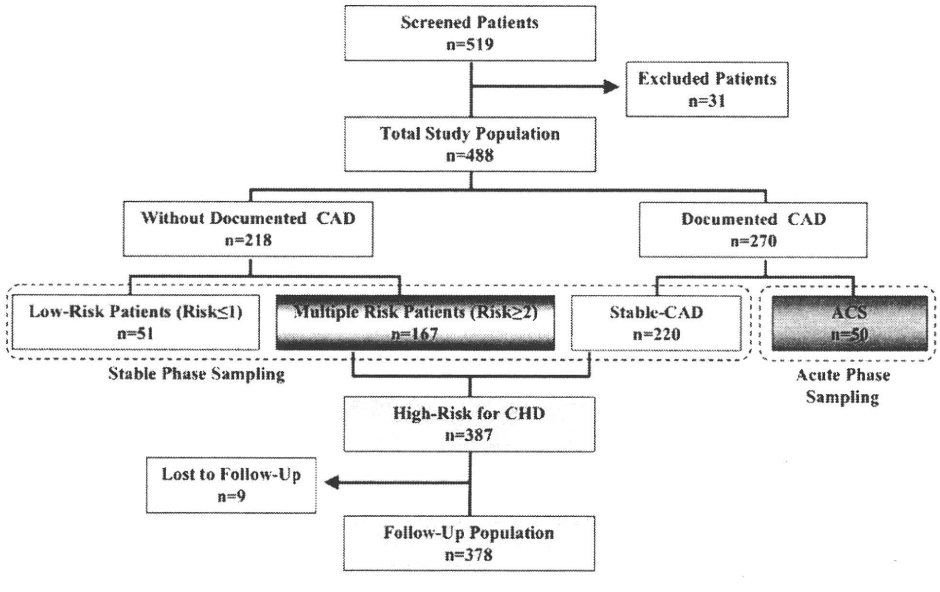

Figure 1 Flow Diagram of Subject Recruitment

Thirty-one patients were excluded for the following reasons: malignant diseases (n = 20), unstable conditions (n = 6), systemic inflammatory disease (n = 3), and active infections (n = 2). ACS = acute coronary syndromes; CAD = coronary artery disease; CHD = coronary heart disease.

stenosis of >50% by quantitative coronary angiography in major coronary arteries. Risk factors for CHD were defined as age ≥65 years (15); current smoking; family history of ischemic heart disease; hypertension (>140/90 mm Hg or taking antihypertensive medication) (16); dyslipidemia (high-density lipoprotein [HDL] cholesterol <40 mg/dl, low-density lipoprotein [LDL] cholesterol ≥140 mg/dl, triglycerides ≥150 mg/dl, or receiving lipid-lowering treatment); diabetes mellitus (DM) (17); body mass index ≥25.0 kg/m^2 (16); hsCRP ≥2.0 mg/l; or chronic kidney disease (estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m^2). The glomerular filtration rate was estimated using the modified formula of Modification of Diet in Renal Disease study equation, which was proposed by the Japanese Society of Nephrology (18). This study protocol was conducted in accordance with guidelines approved by the ethics committee at our institution.

Measurement of plasma levels of CD144-EMP and blood parameters. Blood samples were withdrawn by venipuncture into vacutainer tubes containing sodium citrate after a 12-h overnight fast for stable patients and on admission to the emergency room for ACS patients, before any mechanical intervention. Fresh plasma was assayed immediately for CD144-EMP by flow cytometry using the method described previously (13,14). We verified plasma levels of CD144-EMP with standard plasma for each sample. Standard plasma were subdivided into 1-use volume and stocked at −80°C. One thawing of stock plasma did not affect CD144-EMP levels. We measured hsCRP by a nephelometry with BN II (Siemens, Berlin, Germany) and BNP by a fluorescence enzyme immunoassay with AIA-21 (Tosoh Bioscience, Tokyo, Japan). Total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, and creatinine concentrations were determined by routine laboratory methods.

Study protocol. First, we compared plasma levels of CD144-EMP among low-risk patients (CHD risk factor ≤1), multiple risk patients (CHD risk factors ≥2), stable-CAD, and ACS patients. Second, patients with multiple risk factors or stable-CAD were categorized as high-risk patients for CHD and followed up every month at the outpatient department until July 2008 or at end point (Fig. 1). The end point was cardiovascular death, nonfatal myocardial infarction, unstable angina, ischemic stroke, or coronary revascularization to new lesions. Cardiovascular events were documented by phone calls to the patients or their families, followed by a review of medical records, electrocardiogram, ultrasound echocardiogram, and cardiac enzyme data. Cardiovascular death was defined as death due to myocardial infarction, congestive heart failure, or documented sudden cardiac death. Diagnosis of ischemic stroke was made if the patient had clinical and radiological evidence of stroke without intracranial hemorrhage. For subjects experiencing more than 2 acute events, only the first event was considered in the analysis. Revascularization therapy based only on angiographic data, including percutaneous coronary intervention-mediated restenosis, was not counted as a cardiovascular event. We used the previously reported cutoff values of 52.6 pg/ml (19) and 2.0 mg/l (20), and the median levels for BNP, hsCRP, and CD144-EMP, respectively, to divide our follow-up population into 2 groups: the high-level group and low-level group for the particular parameter.

Statistical analysis. Results were expressed as mean ± SD or as frequencies (percentages), while BNP, hsCRP, and CD144-EMP levels were expressed as median and interquartile range. The frequencies of risk factors and medications were compared between 2 groups by using chi-square analysis. Continuous variables were compared between 2 groups by the unpaired *t* test or Mann-Whitney *U* test, as appropriate. Data of the 4 groups were compared by 1-way analysis of variance, Kruskal-Wallis test, and chi-square analysis. Survival analysis was performed using the Kaplan-Meier method and assessed with the log-rank test.

The predictive value for cardiovascular events was assessed by Cox proportional hazards regression. The following variables were incorporated first into the univariate model: age, sex, current smoking, hypertension, DM, body mass index, HDL cholesterol, LDL cholesterol, eGFR, BNP, hsCRP, and CD144-EMP. Variables with p values <0.20 were then entered into a forward stepwise multivariate Cox proportional hazards analysis. In this model, we evaluated the effect of the biomarkers, BNP, hsCRP, and CD144-EMP, according to quintile increment in biomarkers levels.

Proportional hazards assumption was confirmed by Schoenfeld's test. Estimates of the C statistic for Cox proportional hazards regression models were calculated (21). The comparison of C statistics after the addition of the biomarkers to the model with Framingham risk was estimated (22). We also examined whether the addition of various combinations of biomarkers improved the discriminatory power of the model.

We assessed the calibration of Cox regression models by the Grønnesby and Borgan (23) calibration test, which compares the number of events that are expected based on estimation from 5 risk score groups. To evaluate whether the global model fit improved after the addition of the biomarkers, we performed likelihood ratio tests.

The statistical analyses were carried out using SPSS version 15.0J for Windows (SPSS Inc., Chicago, Illinois), STATA version 10.0 (StataCorp LP, College Station, Texas), and SAS version 9.1.3 (SAS Institute Inc., Cary, North Carolina). Statistical significance was defined as a value of $p < 0.05$ from 2-sided tests.

Results

Enrollment, classification, and follow-up of patients. We screened 519 patients, but 31 patients were excluded (Fig. 1). Data of the remaining 488 patients were subjected to analysis. In this study population, 387 patients at high

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risk for CHD were followed up, and the data of 378 patients (multiple risk factors, n = 167; stable-CAD, n = 220) were available for analysis of cardiovascular events while 9 patients were lost to follow-up (Fig. 1). The follow-up period was 1 to 62 months (mean 36 months).

Comparison of CD144-EMP levels. All clinical factors except the frequency of current smoking were significantly different among patients with various CHD risk. The plasma levels of CD144-EMP increased significantly with increased coronary risk factors and with complicated clinical manifestations (patients at low-risk: n = 51, median [interquartile range], 0.303 [0.142 to 0.367] × 10^6; multiple risk factors: n = 167, 0.508 [0.387 to 0.681] × 10^6; stable-CAD: n = 220, 0.604 [0.449 to 0.795] × 10^6; ACS: n = 50, 0.983 [0.718 to 1.150] × 10^6/ml, p < 0.001) (Fig. 2). LDL cholesterol, eGFR, and hsCRP were higher in ACS than stable-CAD (ACS vs. stable-CAD: LDL cholesterol: 121.2 ± 30.0 mg/dl vs. 110.8 ± 32.7 mg/dl, eGFR: 65.7 ± 20.8 ml/min/1.73 m^2vs. 58.9 ± 21.4 ml/min/1.73 m^2, and hsCRP: 2.2 [0.7 to 7.8] mg/l vs. 1.2 [0.5 to 3.6] mg/l). Moreover, CD144-EMP levels were significantly higher in ACS patients than in stable-CAD patients (Fig. 2).

Baseline clinical features of patients at high risk for CHD. Table 1 summarizes the baseline clinical features of patients at high risk for CHD (multiple risk factors or stable-CAD; follow-up population). The mean age was 66.9 years and 61.4% were men. Plasma levels of CD144-EMP correlated weakly with hsCRP (r = 0.16, p = 0.002) and did not correlate with BNP (r = 0.08, p = 0.14). Multivariate logistic regression analysis identified male sex and DM as significant risk factors of high EMP levels (above median) (men: hazard ratio [HR]: 1.685, 95% confidence interval [CI]: 1.076 to 2.639, p = 0.02; DM: HR: 1.551, 95% CI: 1.009 to 2.386, p = 0.046).

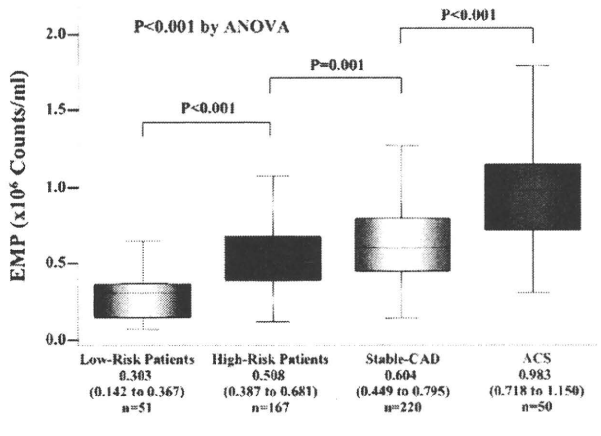

Figure 2 **Plasma Levels of CD144-EMP in Patients With Various Cardiovascular Risks**

The **line within the box** represents the median value; the **top and bottom lines of the box** represent the 25th and 75th percentiles, respectively; and the **top and bottom vertical lines outside the boxes** represent the 90th and 10th percentiles, respectively. ANOVA = analysis of variance; EMP = endothelium-derived microparticle; other abbreviations as in Figure 1.

Table 1 **Baseline Clinical Characteristics of 378 Follow-Up Patients at High Risk for CHD**

	All Subjects (n = 378)
Age, yrs	66.9 ± 9.8
Sex, male/female (%/%)	232/146 (61.4/38.6)
Current smoking	68 (18.0)
Hypertension	279 (73.8)
Diabetes mellitus	157 (41.5)
Body mass index, kg/m^2	23.7 ± 3.4
HDL cholesterol, mg/dl	52.2 ± 16.4
LDL cholesterol, mg/dl	114.4 ± 31.4
eGFR, ml/min/1.73 m^2	63.0 ± 20.9
BNP, pg/ml	57.0 (22.7–156.3)
High-sensitivity CRP, mg/l	0.9 (0.4–2.4)
EMP, × 10^6/ml	0.569 (0.427–0.761)
Medications	
Antihypertensive drugs	338 (89.4)
Statins	174 (46.0)

Data are mean ± SD, n (%), or median (interquartile range).
BNP = B-type natriuretic peptide; CHD = coronary heart disease; CRP = C-reactive protein; eGFR = estimated glomerular filtration rate; EMP = endothelium-derived microparticle; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Cardiovascular events and biomarker levels. We recorded 55 cardiovascular events in patients at high risk for CHD during the follow-up period. Patients of the high EMP group developed significantly more cardiovascular events than the low EMP group during the follow-up (Table 2). Specifically, the incidences of cardiovascular death and ACS were significantly higher in the high-EMP group than in the low-EMP group (Table 2). Kaplan-Meier analysis based on high and low levels of biomarkers showed a significantly higher probability of cardiovascular events in the presence of high levels of BNP, hsCRP, and EMP during the follow-up (log-rank test: BNP p < 0.001, hsCRP p < 0.001, and EMP p < 0.001) (Figs. 3A to 3C).

Cox proportional hazard analysis and C statistics for cardiovascular events. Univariate and multivariate Cox proportional hazards analysis for cardiovascular events showed that age, BNP, hsCRP, and CD144-EMP were independent predictors of future cardiovascular events in

Table 2 **Cardiovascular Events in Patients With High or Low EMP Levels**

	High EMP Group (n = 189)	Low EMP Group (n = 189)	p Value
Total cardiovascular events	41	14	<0.001
Cardiovascular death	14	3	0.01
Acute coronary syndromes	12	3	0.03
Nonfatal myocardial infarction	4	0	0.12
Unstable angina	8	3	0.22
Ischemic stroke	5	5	1.00
Coronary revascularization to new lesions	10	3	0.09

Data are number of patients.
EMP = endothelium-derived microparticle.

patients at high risk for CHD (age: HR: 1.042, 95% CI: 1.007 to 1.080, p = 0.02; BNP: HR: 1.242, 95% CI: 1.004 to 1.536, p = 0.046; hsCRP: HR: 1.468, 95% CI: 1.150 to 1.875, p = 0.002; EMP: HR: 1.345, 95% CI: 1.094 to 1.652, p = 0.005) (Table 3). Framingham risk was not incorporated into multivariate analysis because it was constructed by the same variables in univariate analysis. Framingham risk was confirmed to be a significant factor by univariate analysis in the present study (HR: 1.043, 95% CI: 1.011 to 1.076, p = 0.008). We then estimated the C statistic of Framingham risk alone. Separate incorporation of each biomarker into the Framingham risk model showed that all biomarkers increased the C statistic for prediction of cardiovascular events (C statistics: Framingham risk alone 0.636, Framingham risk + BNP 0.695, Framingham risk + hsCRP 0.696, and Framingham risk + EMP 0.682) (Table 4). Moreover, we examined the additive usefulness of EMP in multiple biomarkers strategy based on Framingham risk and BNP, hsCRP, or both. EMP increased the C statistics in multiple biomarkers strategy (C statistics: Framingham risk + BNP 0.695, Framingham risk + BNP + EMP 0.741; Framingham risk + hsCRP 0.696, Framingham risk + hsCRP + EMP 0.734; and Framingham risk + BNP + hsCRP 0.732, Framingham risk + BNP + hsCRP + EMP 0.763) (Table 4). The p value for the Schoenfeld's tests indicated that proportional hazards assumptions were appropriate (p = 0.70). We also confirmed good calibration for the model in patients at high risk for CHD by Grønnesby and Borgan (23) statistics (p = 0.34). Furthermore, models that included all biomarkers had better global fit than models with only Framingham risk, as evaluated by the likelihood ratio test (p = 0.02).

We examined the effect modification of interaction among all biomarkers and found that there was an interaction term between EMP and hsCRP (p = 0.03).

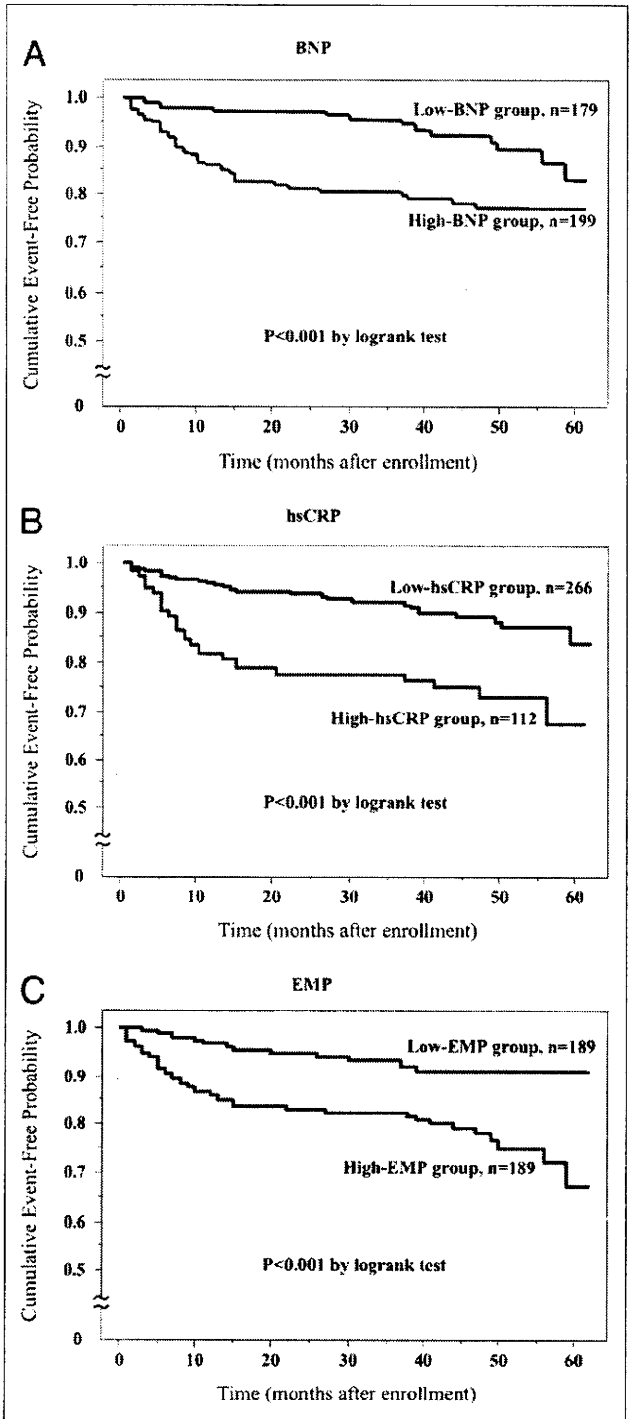

Figure 3 **Kaplan-Meier Analysis for the Probability of Cardiovascular Events**

(A to C) Based on each cutoff point of B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (hsCRP), and CD144 endothelium-derived microparticles (EMP). BNP 52.6 pg/ml, hsCRP 2.0 mg/l, and CD144-EMP 0.569 × 10^6/ml.

Discussion

We demonstrated that circulating plasma levels of CD144-EMP in patients at high risk for CHD were independent predictors of future cardiovascular events. We also found that the addition of multiple biomarkers, including endothelial dysfunction, as assessed by CD144-EMP, to the Framingham risk model improved classification of risk, as evidenced by a substantial increase in the C statistics. Thus, quantitative evaluation of cardiovascular risk leading to atherothrombogenic complications from multiple aspects that include endothelial dysfunction can be clinically useful and valuable in patients at high risk for CHD.

Although the mean age of the study population and combination of biomarkers were issues of concern in the study design, the multiple biomarkers strategy, which is based on adding several biomarkers to the prediction model, including the established risk factors, is useful for risk stratification of cardiovascular events (2,3). It has already been demonstrated that BNP and hsCRP are independent predictors in healthy subjects (24,25) and CHD patients (26,27), and are significant biomarkers that improve C statistics for death and cardiovascular events (2,3). Endo-

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Table 3 Univariate and Multivariate Cox Proportional Hazards Analysis for Cardiovascular Events in Follow-Up Patients

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age, per yr	1.045 (1.010–1.080)	0.01	1.042 (1.007–1.080)	0.02
Sex (male)	1.498 (0.845–2.655)	0.17	Not selected	—
Current smoking	0.886 (0.433–1.810)	0.74	Not selected	—
Hypertension	0.808 (0.451–1.447)	0.47	0.616 (0.341–1.112)	0.11
Diabetes mellitus	1.967 (1.150–3.364)	0.01	1.597 (0.922–2.766)	0.10
Body mass index, per kg/m^2	0.975 (0.904–1.052)	0.52	Not selected	—
HDL cholesterol, per mg/dl	0.988 (0.971–1.006)	0.19	Not selected	—
LDL cholesterol, per mg/dl	0.997 (0.989–1.006)	0.53	Not selected	—
eGFR, per ml/min/1.73 m^2	0.982 (0.969–0.995)	0.006	Not selected	—
BNP, quintile increment	1.461 (1.190–1.792)	<0.001	1.242 (1.004–1.536)	0.046
High-sensitivity CRP, quintile increment	1.693 (1.335–2.146)	<0.001	1.468 (1.150–1.875)	0.002
EMP, quintile increment	1.469 (1.203–1.792)	<0.001	1.345 (1.094–1.652)	0.005

CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

thelial dysfunction has also been recognized as an independent predictor of future cardiovascular events (5–7). Despite the pathophysiological significance of endothelial dysfunction in cardiovascular medicine, one cannot clinically assess coronary endothelial dysfunction because the available method is complex and invasive. It is probably for this reason that endothelial dysfunction was not incorporated into the multiple biomarkers strategy. In addition to the use of coronary reactivity to acetylcholine or brachial artery flow-mediated vasodilation, endothelial dysfunction can be assessed by measuring circulating levels of intercellular adhesion molecule 1, E-selectin (28), and von Willebrand factor (29). Soluble biomarkers offer the advantage of convenience and quantitative assessment; however, there is little evidence at present that such markers can accurately predict future cardiovascular events. Because the aforementioned molecules can be produced from cells other than endothelial cells such as leukocytes (28) and platelets, we need to identify a highly specific soluble biomarker that reflects endothelial dysfunction and can predict the prognosis of CHD patients.

Microparticles are released from various circulating blood cells and have many pathophysiological properties, as procoagulants and messengers (11). Microparticles detected by CD144 antigens (vascular endothelial cadherin), which are endothelial cell-type specific transmembrane adhesion molecules located only on the endothelium, exist in human plasma and are derived selectively from human endothelial cells, and their plasma levels can be a clinically specific marker for endothelial dysfunction (13,30). In the present study, we used the CD144-EMP assay to quantitate endothelial dysfunction. Although the clinical significance of measurement of microparticles has not been established yet, as stated in the preceding text, the method used for measurement of CD144-EMP is more specific, safe, simple, and rapid. Moreover, the fact that plasma levels of CD144-EMP independently predicted future cardiovascular events in the present study indicates that measurement of plasma CD144-EMP levels could be potentially useful for risk assessment of endothelial dysfunction with potential cardiovascular complications.

Table 4 C Statistics for Cox Proportional Hazards Model to Predict Cardiovascular Events in Follow-Up Patients

Risk Factors and Biomarkers	C Statistic	Increment in C Statistic
Framingham risk	0.636	0.046
Framingham risk + EMP	0.682	
Framingham risk + BNP	0.695	0.046
Framingham risk + BNP + EMP	0.741	
Framingham risk + hsCRP	0.696	0.038
Framingham risk + hsCRP + EMP	0.734	
Framingham risk + BNP + hsCRP	0.732	0.031
Framingham risk + BNP + hsCRP + EMP	0.763	

Biomarkers were incorporated as variables of 5 ingredients that were divided by quintiles.
hsCRP = high-sensitivity C-reactive protein; other abbreviations as in Table 1.

Endothelial dysfunction is one component of vulnerable plaques and closely associated with the occurrence of ACS (31). Vulnerable plaques are characterized by a thin fibrous cap with a large lipid core and superficial erosion of the luminal endothelium. Severe endothelial dysfunction may predispose to vulnerable endothelium, and the main feature of endothelial vulnerability is probably endothelial erosion. A vulnerable endothelium can promote atherothrombogenic complications through endothelial erosion, but there are no reliable methods for evaluating the risk of endothelial vulnerability, including endothelial erosion (31,32). Therefore, cardiovascular risk stratification that includes evaluation of endothelial dysfunction is a sound approach. Analysis of the risk in different disease pathways is important, and we propose that evaluation of endothelial dysfunction could be an important and clinically useful strategy. Based on the concept of vascular protection, a specific and quantifiable marker that can monitor endothelial dysfunction is neces-

sary, as is the need to design intensive treatment to improve endothelial dysfunction.

A weak correlation between EMP and hsCRP resulted in statistical modification of interaction between EMP and hsCRP. EMP levels correlated to some extent with various inflammatory markers, because inflammatory cytokines can induce the release of EMP, and the latter, in turn, promote endothelial injury, leading to endothelial dysfunction (11).

Study limitations. One limitation of the present study is the relatively small number of patients in a single center. However, this should result in underestimation, stressing the need for further multicenter studies in a larger population to confirm the present results. There is no consensus about measurement of EMP for assessment of endothelial damage and prothrombotic state at this stage, and microparticles are still used only for research purposes. There is a need to standardize the EMP assay for the development and establishment of routine clinical tests, because measurement of CD144-EMP could be potentially useful for the evaluation of endothelial dysfunction. The number of this study population was not estimated by power calculation. It is effective and necessary to have a plan for the number of patients required for a prospective study.

Conclusions

Endothelial dysfunction leading to cardiovascular complications can be assessed quantitatively by measurement of plasma levels of CD144-EMP. Moreover, a multiple biomarkers strategy that includes endothelial dysfunction assessed by CD144-EMP can provide better risk stratification of cardiovascular events and, hence, more thorough clinical assessment of patients who might benefit from more aggressive treatment strategies that improve prognosis.

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Key Words: biomarkers ▪ endothelium ▪ microparticles ▪ follow-up studies ▪ coronary heart disease.

Lack of Effect of Oral Beta-Blocker Therapy at Discharge on Long-Term Clinical Outcomes of ST-Segment Elevation Acute Myocardial Infarction After Primary Percutaneous Coronary Intervention

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Beta-blocker therapy is recommended after ST-segment elevation acute myocardial infarction (STEMI) in current guidelines, although its efficacy in those patients who have undergone primary percutaneous coronary intervention (PCI) has not been adequately evaluated. Of 12,824 consecutive patients who underwent sirolimus-eluting stent implantation in the J-Cypher registry, we identified 910 patients who underwent PCI within 24 hours from onset of STEMI. Three-year outcomes were evaluated according to use of β blockers at hospital discharge (349 patients in β-blocker group and 561 patients in no–β-blocker group). Patients in the β-blocker group more frequently had hypertension, low left ventricular ejection fraction (LVEF), a left anterior descending artery infarct, and statin use than those in the no-β-blocker group. No difference was observed between the β-blocker and no–β-blocker groups in mortality (6.6% vs 6.6%, p = 0.85; propensity score adjusted hazard ratio 1.10, 95% confidence interval 0.64 to 1.90, p = 0.70) or in incidence of major adverse cardiac events (all-cause death, recurrent myocardial infarction, and heart failure hospitalization, 13.5% vs 12.1%, p = 0.91; hazard ratio 1.13, 95% confidence interval 0.76 to 1.66, p = 0.53). Better outcomes were observed in the β-blocker group than in the no–β-blocker group in a subgroup of patients with LVEF ≤40% (n = 125, death 6.4% vs 17.4%, p = 0.04; major adverse cardiac events 14.5% vs 31.8%, p = 0.009). In conclusion, β-blocker therapy was not associated with better 3-year clinical outcomes in patients with STEMI who underwent primary PCI and had preserved LVEF. © 2010 Elsevier Inc. All rights reserved. (Am J Cardiol 2010;106:1225–1233)

Randomized controlled trials and meta-analyses have demonstrated beneficial effects of β blockers on survival in patients with ST-segment elevation acute myocardial infarction (STEMI).[1–6] Based on the results of these studies, current American College of Cardiology/American Heart Association guidelines for treatment of STEMI recommend daily oral administration of β blockers to hemodynamically stable patients who have no contraindications to β blockers.[7,8] However, it is less clear whether β-blocker therapy improves long-term clinical outcomes in patients who have undergone primary percutaneous coronary intervention (PCI) after STEMI. In this study, the long-term effect of β-blocker use was investigated in consecutive patients who underwent primary PCI after STEMI in patients enrolled in the j-Cypher Registry.

Methods

The study design and patient enrollment for the j-Cypher Registry have been described in detail elsewhere.[9] In brief, the j-Cypher Registry is a physician-directed prospective multicenter registry in Japan enrolling consecutive patients undergoing sirolimus-eluting stent implantation without any exclusion criteria. Although data entry was basically left to the individual sites, clinical research coordinators in the data management center (Department of Cardiology, Kyoto University Hospital, Kyoto, Japan) supported data entry when necessary. Logical inconsistencies were resolved by inquiries to the site investigators and/or by audits against the

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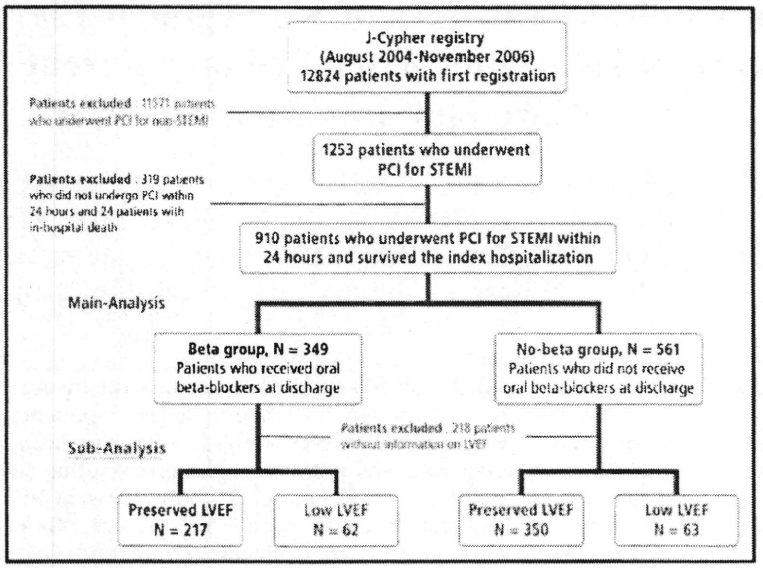


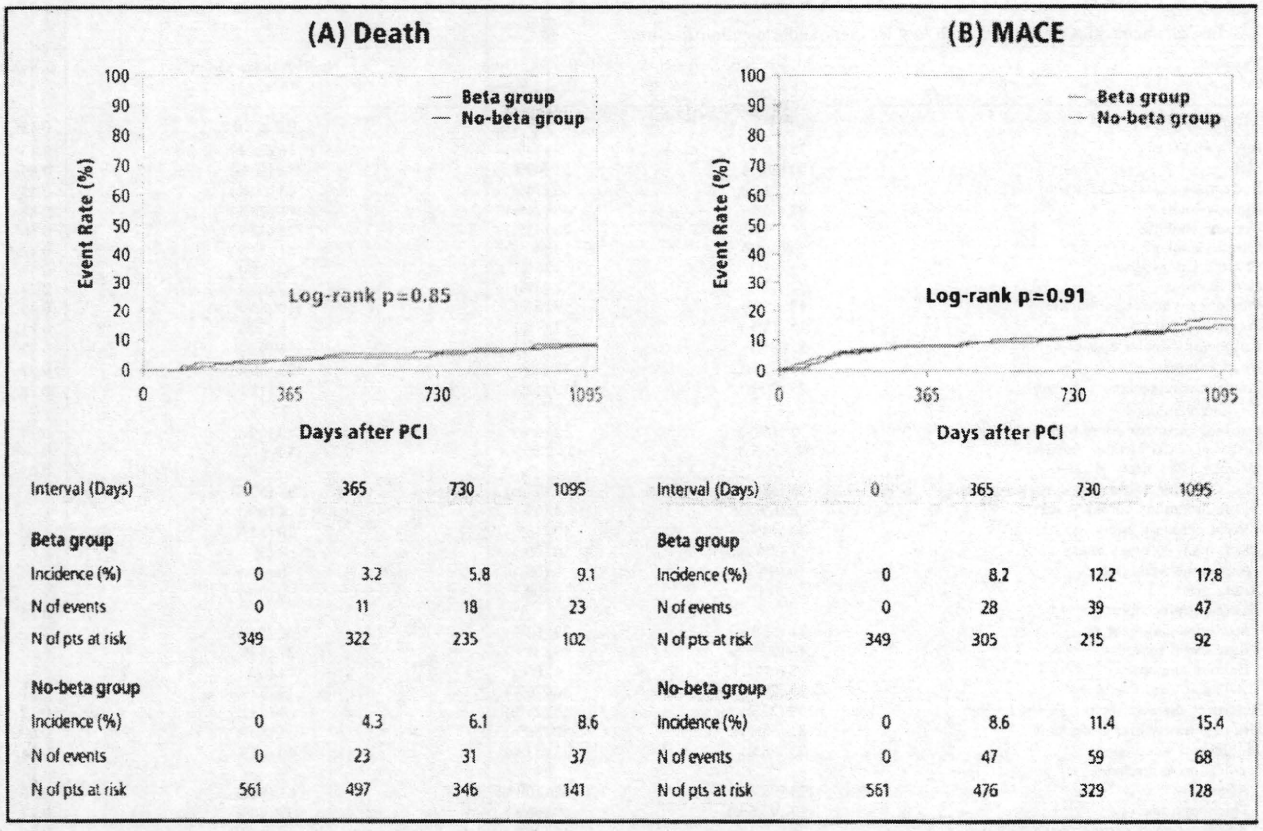
Figure 1. Study flow chart.

Table 1
Baseline characteristics

Variable	All Patients (n = 910)	β-Blocker Group (n = 349)	No-β-Blocker Group (n = 561)	p Value
Age (years)	67.4 ± 11.6	66.4 ± 11.5	68.0 ± 11.6	0.05
Age ≥80 years	146 (16%)	51 (14%)	95 (16%)	0.35
Men	694 (76%)	264 (75%)	430 (76%)	0.73
Body mass index <25.0 kg/m^2	615 (67%)	232 (66%)	383 (68%)	0.52
Hypertension	622 (68%)	256 (73%)	366 (65%)	0.01
Diabetes mellitus	349 (38%)	144 (41%)	205 (36%)	0.16
Current smoker	346 (38%)	148 (42%)	198 (35%)	0.03
Chronic kidney disease	46 (5%)	22 (6%)	24 (4%)	0.18
Hemodialysis	16 (1%)	9 (2%)	7 (1%)	0.14
Previous myocardial infarction	80 (8%)	28 (8%)	52 (9%)	0.52
Previous stroke	79 (8%)	27 (7%)	52 (9%)	0.42
Peripheral vascular disease	51 (5%)	18 (5%)	33 (5%)	0.64
Heart failure	158 (17%)	63 (18%)	95 (16%)	0.67
Previous percutaneous coronary intervention	120 (13%)	41 (11%)	79 (14%)	0.31
Previous coronary artery bypass surgery	13 (1%)	6 (1%)	7 (1%)	0.56
Left ventricular ejection fraction	52.3 ± 12.1	51.0 ± 11.9	53.2 ± 12.2	0.02
Left ventricular ejection fraction ≤40%	125 (18%)	62 (22%)	63 (15%)	0.02
Infarct-related artery location				0.007
Left anterior descending coronary artery	415 (46%)	186 (53%)	229 (41%)	
Left circumflex coronary artery	117 (13%)	42 (12%)	75 (13%)	
Right coronary artery	361 (40%)	114 (33%)	247 (44%)	
Left main coronary artery	11 (1%)	5 (1%)	6 (1%)	
Saphenous vein graft	5 (0.5%)	2 (0.6%)	3 (0.5%)	
Artery graft	1 (0.1%)	0 (0%)	1 (0.2%)	
Treatment procedures				0.16
Sirolimus-eluting stent	511 (56%)	201 (57%)	310 (55%)	
Bare metal stent	352 (39%)	125 (36%)	227 (40%)	
Balloon angioplasty	47 (5%)	23 (7%)	24 (4%)	
Total stent length >28 mm	188 (21%)	78 (23%)	110 (20%)	0.39
Reference diameter before PCI <2.5 mm	300 (34%)	110 (32%)	190 (35%)	0.49
Use of intravascular ultrasound	457 (51%)	208 (60%)	249 (45%)	0.0001
Multivessel stenting	550 (60%)	203 (58%)	347 (61%)	0.27
Medication at discharge				
Aspirin	902 (99%)	347 (99%)	555 (98%)	0.72
Thienopyridine	865 (95%)	331 (94%)	534 (95%)	0.82
Angiotensin-converting enzyme inhibitors	228 (25%)	93 (26%)	135 (24%)	0.38
Angiotensin receptor blockers	465 (51%)	185 (53%)	280 (49%)	0.36
Statins	497 (54%)	223 (63%)	274 (48%)	0.0001

original data sources. Follow-up data were obtained from hospital charts or by contacting patients and/or referring physicians at 30 days, 6 months, 1 year, and yearly thereafter. When death, MI, and stent thrombosis (ST) were reported, the events were adjudicated using the original source documents by a clinical events committee.

The present post hoc subanalysis of the j-Cypher Registry was intended to evaluate the efficacy of oral β



Interval (Days)	0	365	730	1095
Beta group				
Incidence (%)	0	3.2	5.8	9.1
N of events	0	11	18	23
N of pts at risk	349	322	235	102
No-beta group				
Incidence (%)	0	4.3	6.1	8.6
N of events	0	23	31	37
N of pts at risk	561	497	346	141

Interval (Days)	0	365	730	1095
Beta group				
Incidence (%)	0	8.2	12.2	17.8
N of events	0	28	39	47
N of pts at risk	349	305	215	92
No-beta group				
Incidence (%)	0	8.6	11.4	15.4
N of events	0	47	59	68
N of pts at risk	561	476	329	128

Figure 2. Kaplan-Meier curves depict cumulative incidences of death *(A)* and MACEs *(B)* in patients treated with or without β blockers. MACEs include all-cause death, recurrent MI, and heart failure hospitalization.

Table 2
Frequency of events during three-year clinical follow-up in all patients

Event	All Patients (n = 910)	β-Blocker Group (n = 349)	No–β-Blocker Group (n = 561)	Log-Rank p Value
Death	60 (6.6%)	23 (6.6%)	37 (6.6%)	0.85
Cardiac death	27 (3.0%)	9 (2.6%)	18 (3.2%)	0.53
Sudden death	8 (0.9%)	3 (0.9%)	5 (0.9%)	0.92
Reinfarction	24 (2.6%)	9 (2.6%)	15 (2.7%)	0.85
Stent thrombosis (definite)	16 (1.8%)	7 (2.0%)	9 (1.6%)	0.70
Stroke	32 (3.5%)	13 (3.7%)	19 (3.4%)	0.88
Heart failure hospitalization	61 (6.7%)	26 (7.5%)	35 (6.2%)	0.56
Target lesion revascularization	133 (14.6%)	52 (14.9%)	81 (14.4%)	0.93
Any revascularization	247 (27.1%)	93 (26.6%)	154 (27.4%)	0.61
Major adverse cardiac events	115 (12.6%)	47 (13.5%)	68 (12.1%)	0.71

Values are numbers of events (incidences).

blockers in patients with STEMI who have undergone primary PCI in real-world clinical practice. Of 12,824 patients enrolled in the j-Cypher Registry from August 2004 to November 2006, 1,253 patients had an admission diagnosis of STEMI. PCI was performed within 24 hours from onset in 934 patients (74.5%), from 24 hours to 7 days in 169 patients (13.5%), and after ≥7 days in 150 patients (12.0%). Patients who did not undergo PCI within 24 hours from onset and 24 patients with in-hospital death were excluded from the present analysis. Therefore, the present study population consisted of 910 patients who underwent primary PCI within 24 hours after onset of STEMI and were discharged alive from the index hospitalization (Figure 1). We classified patients into the β-blocker group (those who received β blockers at discharge, n = 349, 38.4%) and the no–β-blocker group (those who did not receive β blockers at discharge, n = 561, 61.6%). Left ventricular ejection fraction (LVEF) measured by echocardiography was reported in 692 patients (76.0%). Subgroup analysis was also conducted in these 692 patients with known LVEF.

The relevant review boards in all 37 participating centers approved the study protocol. Written informed consent was obtained from all patients enrolled. The study sponsor was not involved in the study design; in the collection, analysis, and interpretation of data; writing of the report; or the decision to submit the report for publication.

The primary outcome measurement for the present analysis was all-cause death. The secondary outcome measure-

Table 3
Baseline characteristics of patients with low left ventricular ejection fraction

Variable	All Patients (n = 125)	β-Blocker Group (n = 62)	No–β-Blocker Group (n = 63)	p Value
Age (years)	68.7 ± 11.1	67.3 ± 11.5	70.2 ± 10.7	0.15
Age ≥80 years	25 (20%)	9 (14%)	16 (25%)	0.13
Men	100 (80%)	51 (49%)	49 (50%)	0.53
Body mass index <25.0 kg/m^2	89 (71%)	46 (74%)	43 (69%)	0.55
Hypertension	92 (73%)	46 (74%)	46 (73%)	0.88
Diabetes mellitus	54 (43%)	28 (45%)	26 (42%)	0.66
Current smoker	50 (40%)	29 (46%)	21 (33%)	0.12
Chronic kidney disease	6 (4%)	4 (6%)	2 (3%)	0.44
Hemodialysis	2 (1%)	2 (3%)	0 (0%)	0.24
Previous myocardial infarction	17 (13%)	7 (8%)	10 (8%)	0.45
Previous stroke	15 (12%)	7 (7%)	8 (7%)	0.81
Peripheral vascular disease	11 (8%)	5 (5%)	6 (9%)	0.77
Heart failure	41 (32%)	18 (20%)	23 (36%)	0.37
Previous percutaneous coronary intervention	25 (20%)	10 (12%)	15 (12%)	0.28
Previous coronary artery bypass surgery	2 (1%)	2 (3%)	0 (0%)	0.24
Left ventricular ejection fraction	34.8 ± 5.3	33.9 ± 5.8	35.8 ± 4.6	0.04
Infarct-related artery location				0.05
Left anterior descending coronary artery	85 (68%)	48 (77%)	37 (59%)	
Left circumflex coronary artery	8 (6%)	4 (6%)	4 (6%)	
Right coronary artery	30 (24%)	10 (16%)	20 (32%)	
Left main coronary artery	2 (2%)	0 (0%)	2 (3%)	
Saphenous vein graft	0 (0%)	0 (0%)	0 (0%)	
Artery graft	0 (0%)	0 (0%)	0 (0%)	
Treatment procedures				0.35
Sirolimus-eluting stent	71 (57%)	39 (63%)	32 (51%)	
Bare metal stent	46 (37%)	19 (31%)	27 (43%)	
Balloon angioplasty	8 (6%)	4 (6%)	4 (6%)	
Total stent length >28 mm	35 (29%)	16 (26%)	19 (32%)	0.51
Reference diameter before PCI <2.5 mm	39 (32%)	15 (25%)	24 (39%)	0.12
Use of intravascular ultrasound	81 (65%)	50 (80%)	31 (50%)	0.0003
Multivessel stenting	73 (58%)	33 (53%)	40 (63%)	0.24
Medication at discharge				
Aspirin	123 (98.4%)	62 (100%)	61 (96%)	0.50
Thienopyridine	117 (93.6%)	60 (96%)	57 (90%)	0.27
Angiotensin-converting enzyme inhibitors	24 (19.2%)	16 (25%)	8 (12%)	0.06
Angiotensin receptor blockers	69 (55.2%)	32 (51%)	37 (58%)	0.42
Statins	62 (49.6%)	39 (62%)	23 (36%)	0.003

ment was major adverse cardiac events (MACEs) defined as a composite of all-cause death, recurrent MI, and heart failure hospitalization. Other end points assessed included cardiac death, sudden death, recurrent MI, ST, stroke, heart failure hospitalization, target lesion revascularization, and any coronary revascularization.

During follow-up, death was regarded as cardiac in origin unless obvious noncardiac causes could be identified. MI was adjudicated according to the definition in the Arterial Revascularization Therapy Study.[10] Heart failure hospitalization was defined as hospitalization due to exacerbation of heart failure adjudicated by local investigators. ST was defined according to the Academic Research Consortium definition.[11] Definite ST assessed on an individual patient basis was used as the end point for ST. Stroke was defined as symptomatic cerebral infarction or intracranial bleeding necessitating hospitalization. Low LVEF was defined as ≤40% and preserved LVEF was defined as >40%. Presence of chronic kidney disease was defined by an estimated glomerular filtration rate <30 ml/min/1.73 m^2 according to the Modification of Diet in Renal Disease study equation modified for Japanese patients.[12]

Continuous variables are presented as mean ± SD, and categorical variables are expressed as number and percentages. Categorical variables were compared with chi-square test when appropriate; otherwise, Fisher's exact test was used. Continuous variables were compared with *t* test or Wilcoxon rank-sum test based on distribution. Cumulative incidences of clinical event rates were estimated by the Kaplan-Meier method and differences were assessed with log-rank test. Because use of β blockers was decided by physicians, we developed a propensity score for use of β blockers based on variables that were associated with their use. Potential variables assessed for associations using β blockers by univariate analysis were as follows: age ≥80 years, male gender, body mass index <25.0 kg/m^2, hypertension, diabetes mellitus, current smoking, chronic kidney disease, previous MI, previous stroke, peripheral vascular disease, heart failure, previous PCI, previous coronary artery bypass graft, infarct-related coronary artery location, total stent length >28 mm, reference diameter before PCI <2.5 mm, use of intravascular ultrasound, multivessel stenting, and use of statins. We also evaluated the association of LVEF with use of β blockers, but we replaced this with heart failure if the association was significant because we wanted to avoid the loss of samples due to missing data for LVEF. We developed a propensity score from the multivariable logistic model with variables with significant associations for use of β blockers by univariate analysis. We then developed a Cox proportional hazard model with variables of use of β blockers and the decile propensity scores. Results of multivariable analysis are expressed as adjusted hazard ratios and their 95% confidence intervals of use of β blockers for the primary and secondary outcome measure-

Table 4
Baseline characteristics of patients with preserved left ventricular ejection fraction

Variable	All Patients (n = 567)	β-Blocker Group (n = 217)	No–β-Blocker Group (n = 350)	p Value
Age (years)	67.0 ± 11.8	66.7 ± 11.7	67.2 ± 11.8	0.59
Age ≥80 years	87 (15%)	34 (15%)	53 (15%)	0.87
Men	426 (75%)	161 (74%)	265 (75%)	0.68
Body mass index <25.0 kg/m^2	376 (66%)	142 (65%)	234 (67%)	0.69
Hypertension	381 (67%)	156 (71%)	225 (64%)	0.06
Diabetes mellitus	200 (35%)	87 (40%)	113 (32%)	0.06
Current smoker	212 (37%)	89 (41%)	123 (35%)	0.16
Chronic kidney disease	28 (4%)	11 (5%)	17 (4%)	0.91
Hemodialysis	9 (1%)	4 (1%)	5 (1%)	0.73
Previous myocardial infarction	50 (8%)	17 (7%)	33 (9%)	0.51
Previous stroke	47 (8%)	16 (7%)	31 (8%)	0.53
Peripheral vascular disease	29 (5%)	11 (5%)	18 (5%)	0.97
Heart failure	80 (14%)	32 (14%)	48 (13%)	0.73
Previous percutaneous coronary intervention	68 (12%)	22 (10%)	46 (13%)	0.28
Previous coronary artery bypass surgery	8 (1%)	3 (1%)	5 (1%)	0.35
Left ventricular ejection fraction	56.2 ± 9.5	55.4 ± 9.5	56.7 ± 9.5	0.12
Infarct-related coronary artery location				0.19
Left anterior descending	240 (42%)	106 (49%)	134 (38%)	
Left circumflex	78 (14%)	27 (12%)	51 (15%)	
Right	242 (43%)	82 (38%)	160 (46%)	
Left main	4 (0.7%)	1 (0.5%)	3 (1%)	
Saphenous vein graft	2 (0.4%)	1 (0.5%)	1 (0.3%)	
Artery graft	1 (0.2%)	0 (0%)	1 (0.3%)	
Treatment procedures				0.12
Sirolimus-eluting stent	323 (57%)	124 (57%)	199 (57%)	
Bare metal stent	213 (38%)	76 (35%)	137 (39%)	
Balloon angioplasty	31 (5%)	17 (8%)	14 (4%)	
Total stent length >28 mm	106 (19%)	46 (22%)	60 (18%)	0.24
Reference diameter before PCI <2.5 mm	179 (32%)	70 (33%)	109 (32%)	0.76
Use of intravascular ultrasound	338 (60%)	142 (66%)	196 (57%)	0.04
Multivessel stenting	334 (58%)	123 (56%)	211 (60%)	0.40
Medication at discharge				
Aspirin	561 (98%)	215 (99%)	346 (98%)	0.99
Thienopyridine	536 (94%)	202 (93%)	334 (95%)	0.24
Angiotensin-converting enzyme inhibitors	122 (21%)	55 (25%)	67 (19%)	0.08
Angiotensin receptor blockers	303 (53%)	118 (54%)	185 (52%)	0.72
Statins	329 (58%)	140 (64%)	189 (54%)	0.01

ments. Subgroup analyses were also performed in patients with preserved LVEF and low LVEF. All analyses were conducted by physicians (NO and TK) and a statistician (TM) using JMP 7 and SAS 9.2 (SAS Institute, Cary, North Carolina). All reported p values were 2-sided and p values <0.05 were regarded as statistically significant.

Results

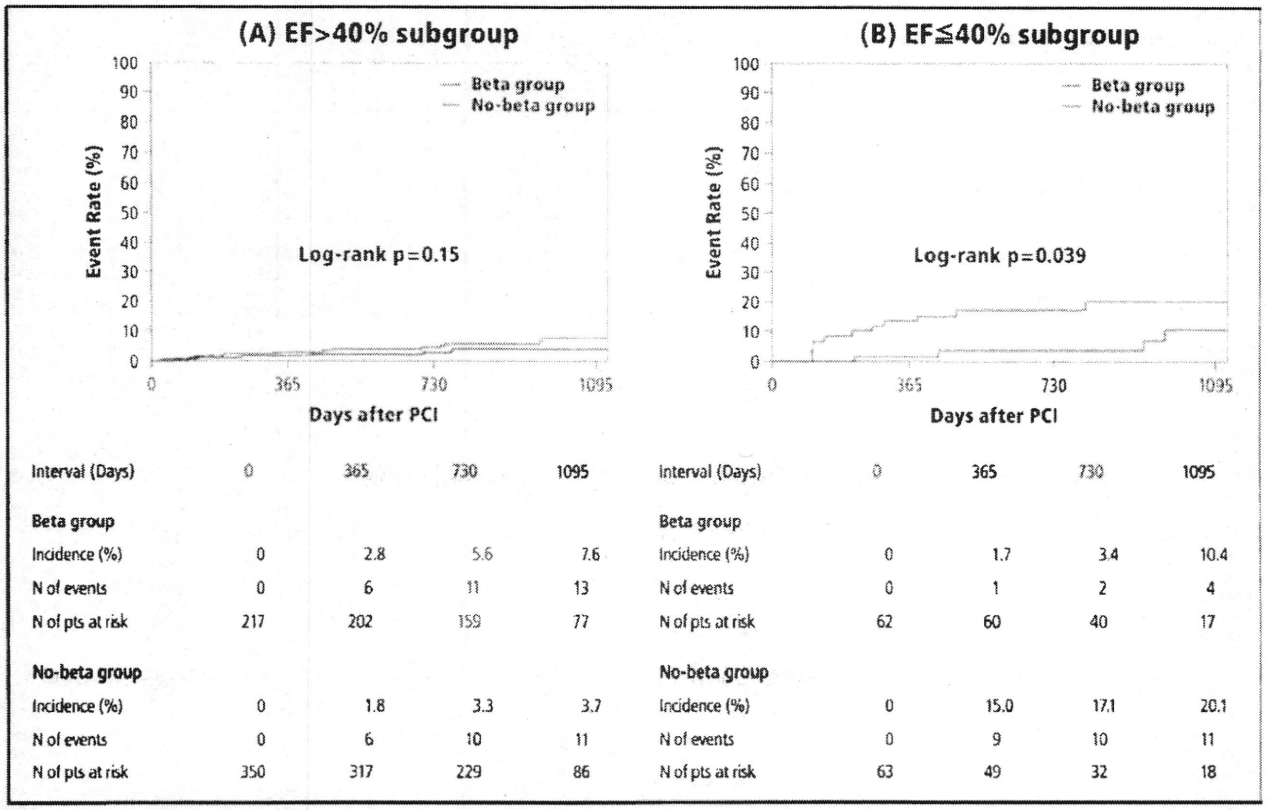
The proportion of patients for whom β blockers were prescribed at hospital discharge after primary PCI within 24 hours from onset of STEMI was 38.3% and varied widely according to institutions (range 0% to 100%, median 42.5%). Patients in the β-blocker group more frequently had hypertension, low LVEF, a left anterior descending coronary artery infarct, statin use, and intravascular ultrasound use than those in the no–β-blocker group. Current smokers were more prevalent in the β-blocker group. Prevalences of diabetes mellitus, previous stroke, and previous MI were not different between the 2 groups (Table 1). There were 6 factors that were associated with β-blocker use, namely hypertension, current smoking, low LVEF, left anterior descending coronary artery-related infarction, use of statins, and use of intravascular ultrasound. Because there were missing data for LVEF, we substituted it with heart failure, and used these 6 factors to develop the propensity score thereafter.

Cumulative incidence of death at 3 years was 6.6% for the entire study population. No difference was observed in 3-year mortality between patients in the β-blocker group and those in the no–β-blocker group (Figure 2, Table 2). Adjusted hazard ratios of use of β blockers for 3-year mortality was 1.10 (95% confidence interval 0.64 to 1.90, p = 0.70). Cumulative incidence of MACEs at 3 years was 12.6% for the entire study population. No difference was observed in 3-year MACE between patients in the β-blocker group and those in the no–β-blocker group (Figure 2, Table 2). Adjusted hazard ratios of use of β blockers for 3-year MACEs was 1.13 (95% confidence interval 0.76 to 1.66, p = 0.53).

Baseline characteristics in the low and preserved LVEF subgroups are presented in Tables 3 and 4, respectively. In 125 patients with low LVEF, mortality and MACEs in the β-blocker group were significantly lower than those in the no–β-blocker group (Figures 3 and 4, Table 5). However, in 567 patients with preserved LVEF, there were no differences in mortality and MACE between the 2 groups (Figures 3 and 4, Table 6).

Discussion

In the present study, oral β-blocker therapy was not associated with better 3-year clinical outcomes in patients with STEMI who underwent PCI within 24 hours from



Interval (Days)	0	365	730	1095
Beta group				
Incidence (%)	0	2.8	5.6	7.6
N of events	0	6	11	13
N of pts at risk	217	202	159	77
No-beta group				
Incidence (%)	0	1.8	3.3	3.7
N of events	0	6	10	11
N of pts at risk	350	317	229	86

Interval (Days)	0	365	730	1095
Beta group				
Incidence (%)	0	1.7	3.4	10.4
N of events	0	1	2	4
N of pts at risk	62	60	40	17
No-beta group				
Incidence (%)	0	15.0	17.1	20.1
N of events	0	9	10	11
N of pts at risk	63	49	32	18

Figure 3. Cumulative incidences of death in patients with preserved LVEF *(A)* and in patients with low LVEF *(B)* were compared according to use of β blockers.

onset. It was also noted that β-blocker therapy was associated with lower 3-year mortality and MACEs in the low LVEF group; in contrast, no difference was observed in patients with preserved LVEF.

The main mechanism of the beneficial effect of β-blocker therapy in patients with STEMI is probably attenuation of the myocardial oxygen demand by decreases in heart rate, systemic arterial pressure, and myocardial contractility. Prolongation of diastole caused by a decrease in heart rate may augment perfusion to ischemic myocardium, particularly in the subendocardium, limiting the infarct size.[13] In the prefibrinolytic period, intravenous β-blocker administration followed by oral β-blocker therapy was associated with lower mortality or recurrent MI in patients with STEMI.[1–6] The beneficial effects of β blockers, however, were less pronounced in patients with STEMI who received antiplatelets and fibrinolytic therapy, probably because of the decrease in residual myocardial ischemia.[14,15]

Current American College of Cardiology/American Heart Association guidelines for treatment of STEMI recommend daily oral β-blocker therapy in survivors of STEMI undergoing coronary revascularization unless contraindicated.[7,8] However, the evidence suggesting benefits of β-blocker therapy in patients undergoing primary PCI is not sufficient. The recommendation is based on findings of the large database Cooperative Cardiovascular Project, in which most patients who underwent percutaneous transluminal coronary angioplasty or coronary artery bypass grafting did not undergo early reperfusion.[16]

Management of patients with STEMI has changed dramatically compared to a decade ago, including widespread use of primary PCI, efforts to shorten door-to-balloon time, technical and technologic improvements of PCI, and widespread use of evidence-based medications such as statins, newer antiplatelet agents, and drugs that inhibit the renin-angiotensin-aldosterone system. Based on this progress in treatment of patients with STEMI, the mortality and morbidity after STEMI have been markedly improved.[7,17] Our findings suggesting better prognosis with β blockers in patients with low LVEF are consistent with previous studies.[18–21] However, the present analysis suggested that use of β blockers was not associated with better clinical outcomes in patients with STEMI who underwent primary PCI and had preserved LVEF. There are potential adverse effects with oral β blockers, i.e., mild to severe hypotension, bradycardia, dizziness, depression, metabolic disorders, and drug allergy.[5] In the Japanese Beta-Blockers and Calcium Antagonist Myocardial Infarction Study, the incidence of coronary spasm was significantly higher in the β-blocker group than in the calcium antagonist group, without any difference in the incidence of cardiovascular death.[22] In addition, the increased daily administration of pills could worsen patient compliance, increase errors in medical treatment, and increase health care costs.[23] It is therefore important