The strength of this study is that it is a nationwide survey with a relatively large sample size, but several study limitations need to be discussed. First, the gender differences we observed might be accounted for by a selection bias. Overconfident residents may have been preferentially selected among male peers relative to their female peers. However, this scenario is unlikely because the study is a nationwide resident survey with a random sampling method. According to the Ministry,[27] 5,019 (66%) men and 2,549 (34%) women passed the National Board Examination for a doctor's license in 2005 and started residency. Although the number of responses by gender was not available in our dataset, the gender ratio in 2005 was comparable with that of our study subjects. Second, our study showed that only 13% of women residents reported they were more work- than life-oriented; a significant proportion of women residents chose "family" as "the most important thing in life." These findings might have been due to generational differences. The Women Physicians Health Study[7] reported that older women physicians in the United States attained greater job satisfaction and had only vague recall of training's rigor, showed "pioneer pride," or belonged to a cohort of "survivors." By contrast, our study participants were limited to young residents, a fact that requires careful interpretation. Third, although one of our hypotheses included the possibility that lower confidence levels may have a negative impact on career development, the findings indicated that perspectives on life and work were not significantly associated with confidence levels. In this regard, it is suggested that a more precise and direct measure to assess the negative impacts of lower confidence levels needs to be developed. Finally, because of the cross-sectional nature of this study, a causal relationship is difficult to determine. Our results might not truly demonstrate definite relationships but, rather, reflect surrogate indicators of unknown factors.

In spite of these limitations, our study demonstrated that women residents in Japan are less likely to be confident about some basic skills and knowledge than are men, even adjusting for the number of clinical experiences. Previous studies suggested that lower confidence levels may have negative impacts on career satisfaction and even on decision making about continuing in the profession. The gender difference in clinical confidence may thus indicate an additional barrier women face in academic career development. Given that the number of women entering medicine is increasing, to attract and retain more women into the physician workforce, studies in this area require careful monitoring of self-confidence and further assessments. In this regard, quantitative research is useful for investigating the impact of lower confidence levels among women on their professional development, whereas qualitative research is useful for unveiling factors that influence the underestimation of self-confidence. In addition, an education program incorporated into the residency program that addresses gender difference is also important for helping young women physicians overcome barriers to career development in their future.

Acknowledgments: The authors thank the reviewers and editor of *Academic Medicine* for their helpful comments, and Dr. Shunsaku Mizushima, Dr. Makoto Aoki, Dr. Hiroyoshi Endo, and Dr. Osamu Takahashi for their support of collecting data.

Funding/Support: This study was supported in part by grant H17-Iryo-015 from the Ministry of Health, Labor, and Welfare of Japan.

Other disclosures: None.

Ethical approval: This study was approved by the institutional review board of the Japanese Ministry of Health, Labor, and Welfare.

Disclaimer: The opinions expressed in this article are those of the authors alone and do not reflect the views of the Japanese Ministry of Health, Labor, and Welfare.

References

1 Kaneto C, Toyokawa S, Inoue K, Kobayashi Y. Gender difference in physician workforce participation in Japan. Health Policy. 2009; 89:115–123.
2 McMurray JE, Cohen M, Angus G, et al. Women in medicine: A four-nation comparison. J Am Med Womens Assoc. 2002; 57:185–190.
3 Ness RB, Ukoli F, Hunt S, et al. Salary equity among male and female internists in Pennsylvania. Ann Intern Med. 2000;133: 104–110.
4 Levinson W, Kaufman K, Bickel J. Part-time faculty in academic medicine: Present status and future challenges. Ann Intern Med. 1993; 119:220–225.
5 Heiliger PJ, Hingstman L. Career preferences and the work–family balance in medicine: Gender differences among medical specialists. Soc Sci Med. 2000;50:1235–1246.
6 McMurray JE, Linzer M, Konrad TR, Douglas J, Shugerman R, Nelson K. The work lives of women physicians results from the physician work life study. The SGIM Career Satisfaction Study Group. J Gen Intern Med. 2000;15: 372–380.
7 Frank E, McMurray JE, Linzer M, Elon L. Career satisfaction of US women physicians: Results from the Women Physicians' Health Study. Society of General Internal Medicine Career Satisfaction Study Group. Arch Intern Med. 1999;159:1417–1426.
8 Carr PL, Friedman RH, Moskowitz MA, Kazis LE. Comparing the status of women and men in academic medicine. Ann Intern Med. 1993;119:908–913.
9 Shollen SL, Bland CJ, Finstad DA, Taylor AL. Organizational climate and family life: How these factors affect the status of women faculty at one medical school. Acad Med. 2009;84:87–94.
10 McManus IC, Sproston KA. Women in hospital medicine in the United Kingdom: Glass ceiling, preference, prejudice or cohort effect? J Epidemiol Community Health. 2000; 54:10–16.
11 Wright AL, Schwindt LA, Bassford TL, et al. Gender differences in academic advancement: Patterns, causes, and potential solutions in one US college of medicine. Acad Med. 2003; 78:500–508.
12 Nonnemaker L. Women physicians in academic medicine: New insights from cohort studies. N Engl J Med. 2000;342:399–405.
13 Ministry of Health, Labour and Welfare. New postgraduate medical education system [in Japanese]. Available at: http://www.mhlw.go.jp/topics/bukyoku/isei/rinsyo. Accessed August 27, 2009.
14 Nomura K, Yano E, Aoki M, Kawaminami K, Endo H, Fukui T. Improvement of residents of clinical competency after the introduction of new postgraduate medical education program in Japan. Med Teach. 2008;30:e161–e169.
15 Nomura K, Yano E, Mizushima S, et al. The shift of residents from university to non-university hospitals in Japan: A survey study. J Gen Intern Med. 2008;23:1105–1109.
16 Lind DS, Rekkas S, Bui V, Lam T, Beierle E, Copeland EM 3rd. Competency-based student self-assessment on a surgery rotation. J Surg Res. 2002;105:31–34.
17 Minter RM, Gruppen LD, Napolitano KS, Gauger PG. Gender differences in the self-assessment of surgical residents. Am J Surg. 2005;189:647–650.
18 Bakken LL, Sheridan J, Carnes M. Gender differences among physician–scientists in self-assessed abilities to perform clinical research. Acad Med. 2003;78:1281–1286.
19 Moffat KJ, McConnachie A, Ross S, Morrison JM. First year medical student stress and coping in a problem-based learning medical curriculum. Med Educ. 2004;38:482–491.
20 Dahlin ME, Runeson B. Burnout and psychiatric morbidity among medical students entering clinical training: A three

year prospective questionnaire and interview-based study. BMC Med Educ. 2007;7:6.
21 Gude T, Vaglum P, Tyssen R, et al. Identification with the role of doctor at the end of medical school: A nationwide longitudinal study. Med Educ. 2005;39:66–74.
22 Sambunjak D, Straus SE, Marusic A. Mentoring in academic medicine: A systematic review. JAMA. 2006;296:1103–1115.
23 Tesch BJ, Wood HM, Helwig AL, Nattinger AB. Promotion of women physicians in academic medicine. Glass ceiling or sticky floor? JAMA. 1995;273:1022–1025.
24 Roter DL, Hall JA. Why physician gender matters in shaping the physician–patient relationship. J Womens Health. 1998;7:1093–1097.
25 Bylund CL, Makoul G. Empathic communication and gender in the physician–patient encounter. Patient Educ Couns. 2002;48:207–216.
26 Hall JA, Roter DL. Medical communication and gender: A summary of research. J Gend Specif Med. 1998;1:39–42.
27 The Ministry of Health, Labour and Welfare. National Board Examination for Doctor's License [in Japanese]. Available at: http://www.mhlw.go.jp/general/sikaku/index.html. Accessed August 18, 2009.

Circulation
Cardiovascular Interventions
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association
Learn and Live

Coronary Spasm Preferentially Occurs at Branch Points: An Angiographic Comparison With Atherosclerotic Plaque

Hitoshi Nakagawa, Yoshinobu Morikawa, Yuji Mizuno, Eisaku Harada, Teruhiko Ito, Kunihiko Matsui, Yoshihiko Saito and Hirofumi Yasue

Circ Cardiovasc Interv 2009;2;97-104; originally published online Feb 20, 2009;
DOI: 10.1161/CIRCINTERVENTIONS.108.803767
Circulation: Cardiovascular Interventions is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 1941-7640. Online ISSN: 1941-7632

Coronary Spasm Preferentially Occurs at Branch Points
An Angiographic Comparison With Atherosclerotic Plaque

Hitoshi Nakagawa, MD; Yoshinobu Morikawa, MD; Yuji Mizuno, MD; Eisaku Harada, MD; Teruhiko Ito, MD; Kunihiko Matsui, MD, MPH; Yoshihiko Saito, MD; Hirofumi Yasue, MD

Background—Coronary spasm plays an important role in the pathogenesis of ischemic heart disease. However, similarities and differences between coronary spasm and atherosclerosis are not known. We examined the angiographic characteristics of coronary spasm in comparison with those of atherosclerosis.

Methods and Results—Thirty-two left anterior descending arteries, 11 left circumflex arteries, and 23 right coronary arteries with spasm and atherosclerotic plaque were analyzed for the localization of spasm in comparison with that of plaque in 47 patients (38 men and 9 women, mean age 66.8±10.3 yrs). Spasm predominantly occurred at the branch point as compared with plaque in each of the 3 arteries (76.7% versus 23.3%, $P<0.0001$; 72.7% versus 9.1%, $P<0.039$; and 60.0% versus 10.0%, $P=0.002$, in the left anterior descending, left circumflex, and right coronary arteries, respectively). Spasm involved the proximal segment less frequently as compared with plaque in each of the 3 arteries (56.7% versus 93.3%, $P<0.0001$; 18.2% versus 81.8%, $P=0.016$; and 15.0% versus 75.0%, $P<0.0001$ in the left anterior descending, left circumflex, and right coronary arteries, respectively). Most spasms occurred at the nonplaque site in each of the 3 arteries (73.3%, $P=0.018$; 100%, $P<0.0001$; and 75.0%, $P=0.041$ in the left anterior descending, left circumflex, and right coronary arteries, respectively).

Conclusion—Coronary spasm preferentially occurred at branch points and nonplaque sites, whereas the atherosclerotic lesion was predominantly localized at the nonbranch points of the curved proximal segments. Coronary spasm may thus be a manifestation of a distinct type of arteriosclerosis different from the lipid-laden coronary atherosclerosis. (***Circ Cardiovasc Intervent.* 2009;2:97-104.**)

Key Words: atherosclerosis ■ coronary spasm ■ endothelium ■ nitric oxide ■ vasoconstriction

Coronary spasm is not only the cause of variant angina but also participates in the pathogenesis of unstable angina, acute myocardial infarction, and sudden death, particularly in Japan.[1–3] However, precise mechanisms by which coronary spasm occurs are not fully understood. We have shown that endothelial nitric oxide (NO) activity is deficient and endothelial function is impaired in the coronary arteries involved in spasm.[4] Endothelial NO enhances vascular functions, including vessel relaxation, survival of vascular endothelial cells, inhibition of platelet aggregation, and attenuation of leukocyte infiltration.[5,6] Impaired NO activity has been suggested as the earliest pathophysiological events contributing to atherosclerosis.[7,8]

Clinical Perspective see p 104

Flow-generated shear stress is an important physiological stimulus that enhances the production of NO and high shear stress augments the bioavailability of NO, whereas disturbed shear stress reduces it.[5,9,10] Although the entire vasculature is exposed to the atherogenic effect of systemic risk factors, atherosclerotic lesions form at specific arterial regions such as curvatures or branch sites where flow is disturbed.[9,10] Thus, local hemodynamic factors play a major role in the regional localization of atherosclerosis. It is, therefore, possible that coronary spasm also may preferentially occur at the sites of coronary arterial tree where flow is disturbed. However, no previous studies have examined this possibility and the relationship between coronary spasm and atherosclerosis is not clear. This study was designed to examine whether there are predilection sites for spasm in the coronary arteries and, if there are, whether these sites are similar to those of atherosclerosis.

Methods

Patients

Ninety-eight (67 men and 31 women, with a mean age of 65.5±10.1 years ranging from 35 to 86) Japanese patients in whom coronary

Received October 1, 2008; accepted February 11, 2009.

From the Division of Cardiovascular Medicine (H.N., Y. Morikawa, Y. Mizuno, E.H., T.I., H.Y.), Kumamoto Kinoh Hospital, Kumamoto Aging Research Institute; Clinical Education Center (K.M.), Kumamoto University Hospital, Kumamoto City; and First Department of Internal Medicine (Y.S.), Nara Medical University, Kashihara City, Japan.

The online-only Data Supplement is available at http://circinterventions.ahajournals.org/cgi/content/full/10.1161/CIRCINTERVENTIONS.108.803767/DC1.

Correspondence to Hirofumi Yasue, MD, Kumamoto Aging Research Institute, 6-8-1, Yamamuro, Kumamoto City 860-8518, Japan. E-mail yasue@juryo.or.jp

Circ Cardiovasc Intervent **is available at http://circinterventions.ahajournals.org** **DOI: 10.1161/CIRCINTERVENTIONS.108.803767**

Table 1. Clinical Characteristics of the Study Subjects

Variables	Normal Angiogram Group (n=51)	Atherosclerosis Group (n=47)
Age, yr	64.2±9.5	66.8±10.3
Gender (male/female)	29/22	38/9
Body mass index, kg/m^2	24.3±3.8	24.2±3.4
Hypertension	19/51 (37%)	25/47 (53%)
Diabetes mellitus	8/51 (16%)	17/47 (36%)
History of smoking	32/51 (63%)	28/47 (60%)
Leukocyte, per μL	6,160±1,668	6925±1944
Hemoglobin, g/dL	13.6±1.7	14.0±2.1
Platelet, ×10^4/μ	24.1±8.0	24.8±7.5
CRP, mg/L*	0.99 (0.32–2.81)	2.00 (0.48–3.52)
Total protein, g/dL	6.6±0.4	6.8±0.6
Albumin, g/dL	3.9±0.3	3.9±0.4
Fast plasma glucose level, mmol/L	5.86±1.51	5.69±1.01
AST, u/L	25.2±11.0	26.1±8.6
ALT, u/L	22.6±14.5	23.8±11.8
CK, u/L	105.4±71.8	106.7±71.3
Total cholesterol, mmol/L	5.11±0.81	5.35±1.00
LDL cholesterol, mmol/L	2.98±0.74	3.32±0.84
HDL cholesterol, mmol/L	1.54±0.40	1.32±0.38
Triglyceride, mmol/L	1.52±0.70	1.71±0.65

*Median (25th and 75th percentile). ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

spasm was induced by intracoronary injection of acetylcholine (ACh; Daiichi-Sankyo Co, Tokyo, Japan) were the subjects of this study. They had been admitted to our hospital because of chest pain or ECG abnormalities suspected of ischemic heart disease between January 2003 and January 2009. The study consisted of the 2 parts: the first part of the 51 patients with normal or almost normal coronary angiogram (<25% stenosis of luminal diameter) in whom the confounding effect of organic stenosis on coronary flow could be excluded (normal angiogram group) and the second part of the 47 patients with organic stenosis (25% to 90% stenosis of luminal diameter) (atherosclerosis group) based on the consensus of 3 to 4 investigators. We defined coronary spasm as a total or subtotal occlusion or severe diffuse constriction of an angiographically demonstrable coronary artery associated with transient ischemic ST segment changes on ECG. In each spasm artery, we defined the site of spasm as that of total or subtotal occlusion or as that of the most severe and proximal constriction in the case of segmental diffuse or multifocal spasm and the site of atherosclerotic lesion (plaque) as the most narrowed based on the consensus of 3 to 4 investigators. Patients with recent myocardial infarction, acute coronary syndrome, left main trunk disease, severe organic stenosis of >90%, multivessel coronary disease with >75% organic stenosis, heart failure, liver disease, creatinine level >1.5 mg/dL, acute inflammation, malignant diseases, and cholesterol lowering medication within a month were excluded from the study. None of the study patients were on statins or other lipid-lowering drugs. The clinical characteristics of the study patients are presented in Table 1. Hypertension was defined as >140/90 mm Hg and diabetes mellitus as fasting plasma glucose level >7 mmol/L (126 mg/dL) or 2-hour postload glucose level >11.1 mmol/L (200 mg/dL).

The protocol of this study was approved by the institutional review board and each patient provided written informed consent.

Induction of Coronary Spasm

Ca-channel blockers and other vasodilators, if they had been administered, were stopped for at least 5 days. Coronary spasm was induced by intracoronary injection of ACh (Daiichi-Sankyo Co) after diagnostic catheterization in the morning. The details of the method were previously reported.[11] Briefly, ACh was injected in incremental doses of 20, 50, and 100 μg into the left coronary artery and then 20 and 50 μg into the right coronary artery (RCA) in 20 seconds under continuous monitoring of ECG and blood pressure. Coronary spasm induced by this method usually disappeared spontaneously within 1 to 2 minutes and both the left coronary artery and RCA could be examined separately unless severe spasm occurred in the left coronary artery and necessitated the prompt injection of isosorbide dinitrate (ISDN) into the arteries. After the end of the test, ISDN (0.1 mg) was injected into the coronary artery and angiography was again performed. The specificity of this test for variant or resting angina was 99%.[12] The test did not induce coronary spasm in any of the patients with normal coronary angiogram and without ischemic heart disease.[12,13] The specificity of this test for spasm arteries was also confirmed in the vitro study.[14]

Assessment of Coronary Artery Diameter and Length

We quantitatively measured the diameter of the coronary arteries and the distance from the branch point. An end-diastolic frame was digitized and the diameter of the index vessel was measured by CAAS II software (PIE Medical). We defined the branch point segment as that within 5-mm distal from the apex of the flow divider because the median distance between each adjacent branch was 14.3 mm (interquartile range was 9.1 to 21.1 mm). We divided the left anterior descending (LAD) artery, the left circumflex (LCx) artery, and the RCA into the proximal segments (segments 6, 7, and 9 in the LAD; segments 11 to 13 in the LCx; and segments 1 to 2 in the RCA) and the distal segments (segments 8 and 10 in the LAD; segments 14 and 15 in the LCx; and segments 3 and 4 in the RCA) according to the AHA coronary segment reporting system[15] and compared the incidence of spasm between the proximal and distal segments. The coronary diameter was expressed as percent narrowing in luminal diameter after ISDN injection. Total or subtotal obstruction or severe coronary spasm with a lumen diameter <0.4 mm could not be accurately quantified because of technical limitations of the computer-assisted quantitative coronary angiography.[16]

Laboratory Methods

Fasting blood samples were drawn by venipuncture 1 to 2 days before coronary angiography and the hematologic and biochemical analyses were done using standard laboratory procedures.

Statistical Analysis

Each of the 3 coronary arteries (LAD, LCx, and RCA) was separately analyzed. Discrete variables were expressed as counts and percentages and were compared using McNemar or binomial exact test between the paired data of the same artery. Probability value of <0.05 was considered to be statistically significant. Continuous data were expressed as mean±SD. However, when the variable was significantly skewed, the median (25th to 75th percentile) was reported. Statistical analysis was performed by using commercially available software (SPSS STATISTICS 17.0 BASE WIN, SPSS Japan Inc, Tokyo, Japan). The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Table 2 shows the coronary angiographic findings of the 2 groups. In the normal angiogram group, spasm was induced in 106 (45, 28, and 33 in the LAD, LCx, and RCA, respectively) arteries. Of these, 9 (8.5%) were total occlusion, 18 (17.0%) subtotal occlusion, 50 (47.2%) segmental diffuse

Table 2. Coronary Angiographic Findings

	Normal Angiogram Group Spasm Site (n=106)	Atherosclerosis Group Spasm Site (n=66)	*P*	Plaque Site (n=66)
Entire artery spasm				
LAD, n	12	2		2
LCX, n	10	0		0
RCA, n	12	3		3
Total, n	34	5		5
LAD, n	33	30		30
Proximal segment, n (%)	23 (69.7)	17 (56.7)	<0.0001	28 (93.3)
LCX, n	18	11		11
Proximal segment, n (%)	12 (66.7)	2 (18.2)	0.016	9 (81.8)
RCA, N	21	20		20
Proximal segment, n (%)	4 (19.0)	3 (15.0)	<0.0001	15 (75.0)
Total, n	72	61		61

LAD indicates left anterior descending artery; LCA, left coronary artery; LCX, left circumflex artery; RCA, right coronary artery.

spasm involving the branch site and 29 (27.4%) diffuse and extensive spasm involving the entire arterial tree affecting the proximal and distal epicardial vessels and their branches. Five shifted from entire artery spasm into total occlusion. Accordingly, 34 (32.1%) of the 106 spasms involved the entire arterial tree in this group. Spasm of 1 vessel, 2 vessels, and 3 vessels was demonstrated in 16, 15, and 20 patients, respectively. Of the 44 patients in whom ACh was injected into both the left coronary artery and RCA, 15 had 1-vessel, 9 had 2-vessel, and 20 had 3-vessel spasm, and thus most (65.9%) patients had multivessel coronary spasm demonstrated. For the analysis of the localization of spasm at branch or nonbranch point, 34 (12 LAD, 10 LCx, and 12 RCA) entire artery diffuse spasms were excluded and the remaining 72 arteries (33 LAD, 18 LCx, and 21 RCA) were analyzed. Spasm occurred at the branch point in 27 (81.8%) of 33 LAD, 17 (94.4%) of 18 LCx, and 17 (81.0%) of 21 RCA. Coronary spasm thus preferentially occurred at the branch point in all of the 3 arteries (Figure 1). Spasm involved the proximal segment in 23 (69.7%) of the 33 LAD, 12 (66.7%) of the 18 LCx, and 4 (19.0%) of the 21 RCA. Thus, spasm occurred more frequently at the proximal than the distal segments in the LAD, whereas it occurred more frequently at the distal than the proximal segments in the RCA (Figure 2). This is probably related to the fact that branch site is more numerous at the proximal segment in the LAD, whereas it is more numerous at the distal segment in the RCA[15] and confirms the close relation of the branch point to spasm. In the atherosclerosis group, the organic stenosis was identified in the 66 (32 LAD, 11 LCx, and 23 RCA) arteries as shown in Table 2. Of these, 52 (22 LAD, 8 LCx, and 22 RCA) arteries had 25% to 75% and 14 (10 LAD, 3 LCx, and 1 RCA) arteries had 75% to 90% luminal diameter narrowing. Thus, most (78.8%) patients had mild to moderate organic stenosis in the atherosclerosis group. Spasm was induced in 66 (32 LAD, 11 LCx, and 23 RCA) arteries. Of these, 6 (9.1%) were total occlusion, 9 (13.6%) were subtotal occlusion, 48 (77.2%) were segmental diffuse spasm, and 3 (4.5%) were entire artery diffuse spasm. Two shifted from entire artery diffuse spasm into total occlusion. Accordingly, the entire artery diffuse spasm occurred in 5 (7.6%) of the 66 spasms in the atherosclerosis group (Table 2). For the analysis of the localization of spasm at the branch or nonbranch points, 5 entire artery diffuse spasms were excluded and the remaining 61 (30 LAD, 11

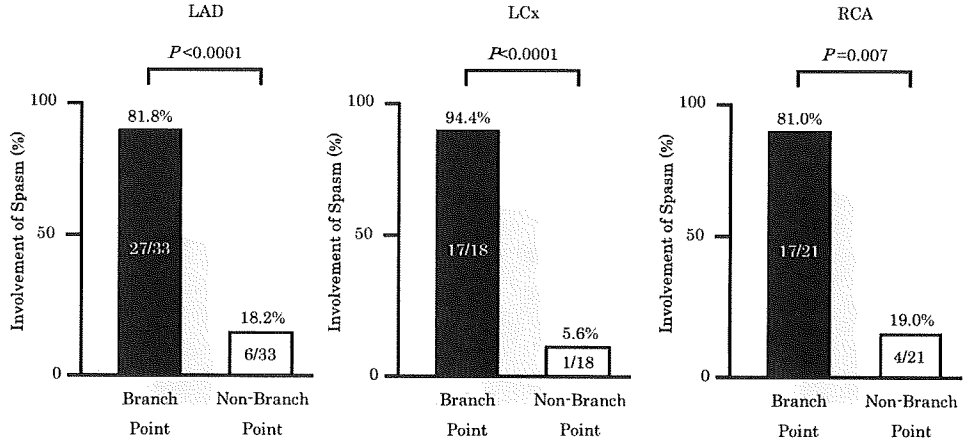

Figure 1. Comparison of the involvement of coronary spasm between the branch and nonbranch points in the normal angiogram group. LAD, left anterior descending coronary artery; LCx; left circumflex artery; RCA, right coronary artery.

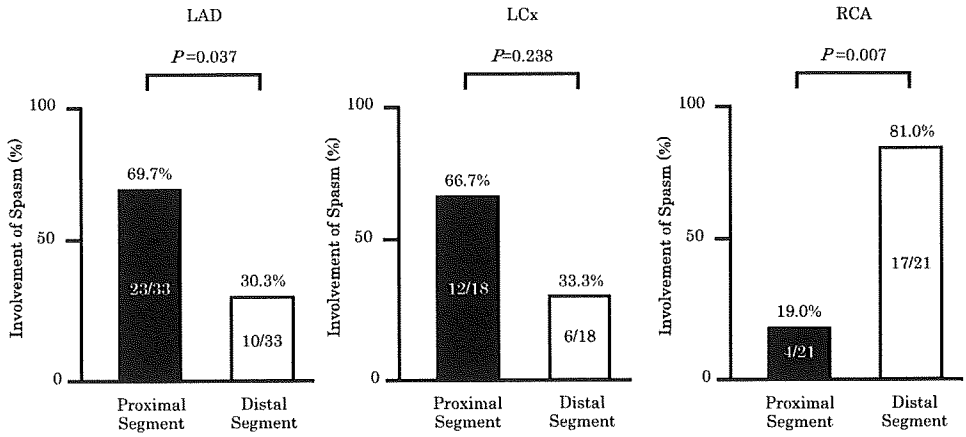

Figure 2. Comparison of the involvement of coronary spasm between the proximal and distal segments in the normal angiogram group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

LCx, and 20 RCA) were analyzed for comparison of localization between spasm and plaque in the atherosclerosis group. Spasm occurred at the branch site in 23 (76.7%) of the 30 LAD, 8 (72.7%) of the 11 LCx, and 12 (60.0%) of the 20 RCA. These results are in agreement with those in the normal angiogram group. On the other hand, plaque was localized at the branch point in only 7 (23.3%) of the 30 LAD, 1 (9.1%) of the 11 LCx, and 2 (10.0%) of the 20 RCA. Thus, there was a significant difference in the involvement of the branch point between spasm and plaque in each of the 3 arteries ($P<0.0001$ in LAD, $P=0.039$ in LCx, and $P=0.002$ in RCA, respectively) (Figure 3). Spasm involved the proximal segment in 17 (56.7%) of the 30 LAD, 2 (18.2%) of the 11 LCx, and 3 (15.0%) of the 20 RCA, whereas plaque was localized at the proximal segment in 28 (93.3%) of the 30 LAD, 9 (81.8%) of the 11 LCx, and 15 (75.0%) of the 20 RCA. Thus, there was a significant difference in the involvement of the proximal segment between spasm and plaque in each of the 3 arteries ($P<0.0001$ in LAD, $P=0.016$ in LCx, and $P<0.0001$ in RCA, respectively) (Figure 4). In accordance with these results, most spasms occurred at the nonplaque site in each of the 3 arteries ($P=0.018$ in LAD, $P<0.0001$ in LCx, and $P=0.041$ in RCA, respectively) (Figure 5). Spasm thus preferentially occurred at branch points and nonplaque sites, whereas the plaque preferentially occurred at nonbranch point sites of the proximal segment in each of the 3 coronary arteries. Paired data using 2×2 tables for Figures 1 to 5 are shown in Online Data supplements.

Nineteen (17.9%) of the 106 spasms in the normal angiogram group and 11 (16.7%) of the 66 in the atherosclerosis group were associated with ST-segment elevation and the 87 (82.1%) and 55 (83.3%) with ST-segment depression on the ECG, respectively, indicating that coronary spasm with ST-segment depression is more numerous than that with ST-segment elevation in both groups ($P<0.0001$, respectively). Of the 11 spasms with ST-segment elevation, 8 (72.7%) involved the organic stenosis and 8 were total or subtotal occlusion in the atherosclerosis group.

Figures 6 and 7 show the representative angiograms of spasm in the normal angiogram group and those of atherosclerosis group, respectively.

Discussion

This study showed that most spasms were diffused and extensive, and the substantial number of these involved the entire arterial tree affecting the proximal and distal epicardial vessels and their intramural branches in the normal angiogram group. These findings are in agreement with the results of our previous angiographic study[17] and also with our intravascular ultrasound study, which revealed the existence

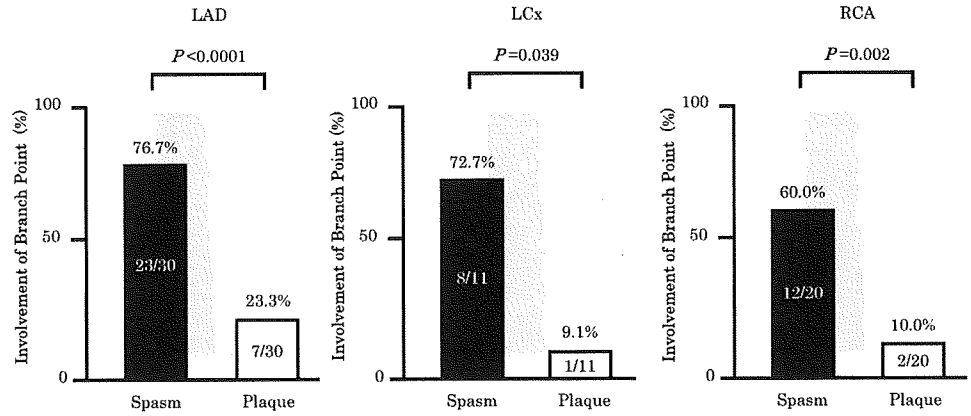

Figure 3. Comparison of the involvement of the branch point between spasm and plaque in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

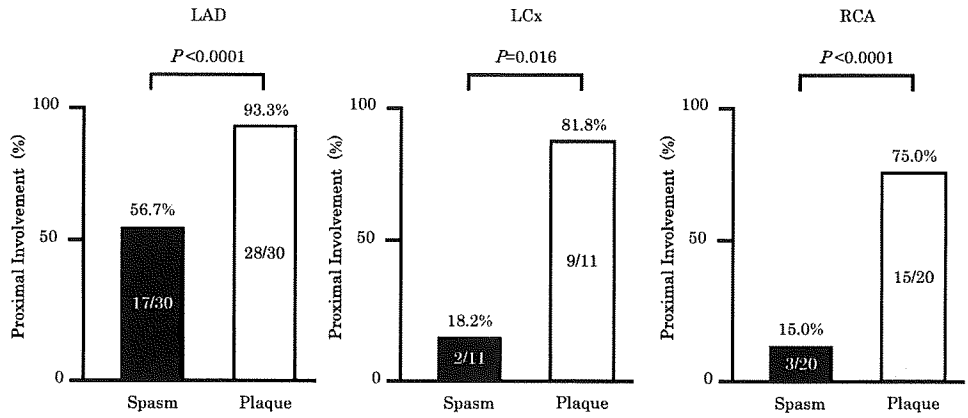

Figure 4. Comparison of the involvement of the proximal segment between spasm and plaque in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

of diffuse intimal thickening in an entire coronary artery in patients with coronary spasm and normal angiograms.[18] Accordingly, the results strongly suggest that systemic factors play an important role in the pathogenesis of coronary spasm.[18–21] On the other hand, the atherosclerotic plaque lesion was focal and largely localized to the proximal segments in agreement with previous studies.[7,8,22–24] This suggests that local factors are more important in the pathogenesis of atherosclerosis as compared with those of coronary spasm.

We have shown that endothelial NO activity is deficient and endothelial function impaired in the spasm arteries.[4] NO not only modulates vasomotor tone, but also inhibits inflammation, production of reactive oxygen species, vascular smooth muscle proliferation, and platelet aggregation,[5,6] and reduced endothelial NO activity represents the early steps in the development of atherosclerosis.[7–9] The endothelium is exposed to shear stress and unidirectional laminar shear stress in straight parts of the arterial tree potently stimulates NO production, whereas disturbed flows at curvatures or branches have the opposite effect.[5,9,10]

Studies of human coronary arteries provide evidence that regions prone to the development of atherosclerosis occur at sites of intimal thickening, which is mainly composed of smooth muscle cells (SMCs), suggesting that SMCs in intimal thickening play a pathogenic role in the initiation and development of atherosclerosis.[22–24] Low shear stress occurs at the curvature or upstream of stenosis, whereas oscillatory shear stress occurs downstream of stenosis or branch points.[9,10] Recent studies revealed that low–shear stress lesions contained fewer SMCs and more lipids and were larger and more progressive and vulnerable,[10,25,26] whereas oscillatory–shear stress lesions contained more SMCs and fewer lipids and are more stable.[25]

This study further showed that spasm preferentially occurred at the branch point or downstream of the flow divider where shear stress is presumed to be oscillatory[9,10] both in the normal angiogram and atherosclerosis groups. This is in agreement with the result of Selwyn's group, which showed that branch point constricted more intensely than nonbranch sites in response to ACh infusion.[27] On the other hand, the atherosclerotic stenosis was localized predominantly at the nonbranch point of the curved proximal segment where shear stress is presumed to be low.[9,10] Thus, there was a difference in the predilection site between the spasm and atherosclerotic plaque and most spasms occurred at different sites from those of the plaque. These results thus suggest that atherosclerosis does not contribute to the occurrence of spasm or rather tends

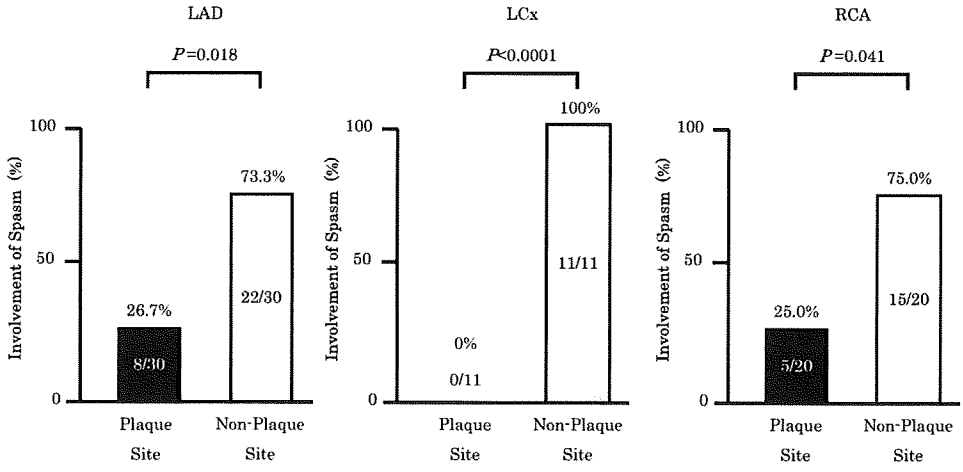

Figure 5. Comparison of the involvement of spasm between the plaque and nonplaque sites in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

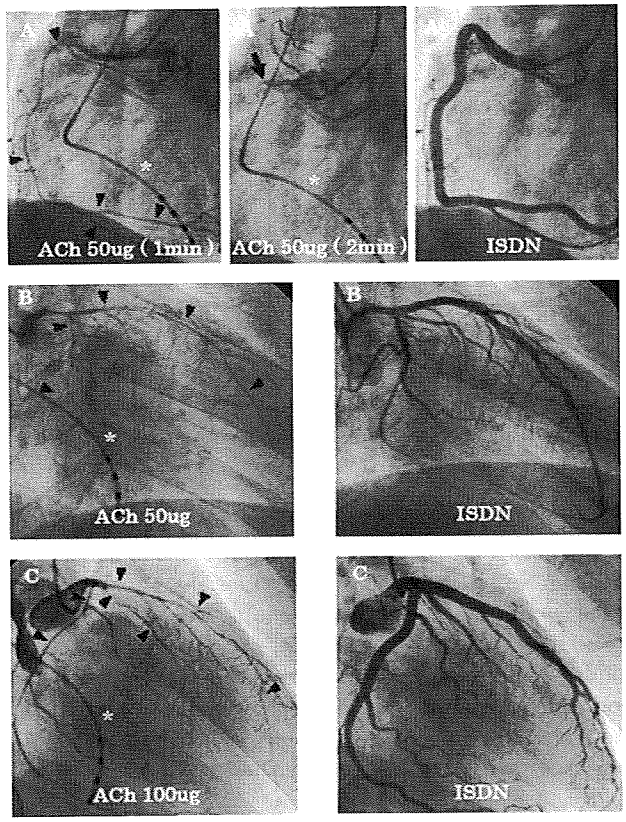

Figure 6. Coronary angiograms during spasm induced by ACh injection and after ISDN in the normal angiogram group. A, Severe diffuse spasm involving the entire RCA appeared after ACh (left) and converted into a total occlusion at the origin of the artery 2 minutes later (center, arrow). After ISDN, the artery was marked dilated and normal (right). B, Severe diffuse spasm involving the entire left coronary artery including intramural branches appeared after ACh (left, arrow heads) and disappeared after ISDN (right). C, Severe diffuse spasm involving the entire arterial tree of both the LAD and LCx appeared after ACh injection (left, arrow heads) and disappeared after ISDN (right). ACh indicates acetylcholine; ISDN, isosorbide dinitrate; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; *, a pacing catheter.

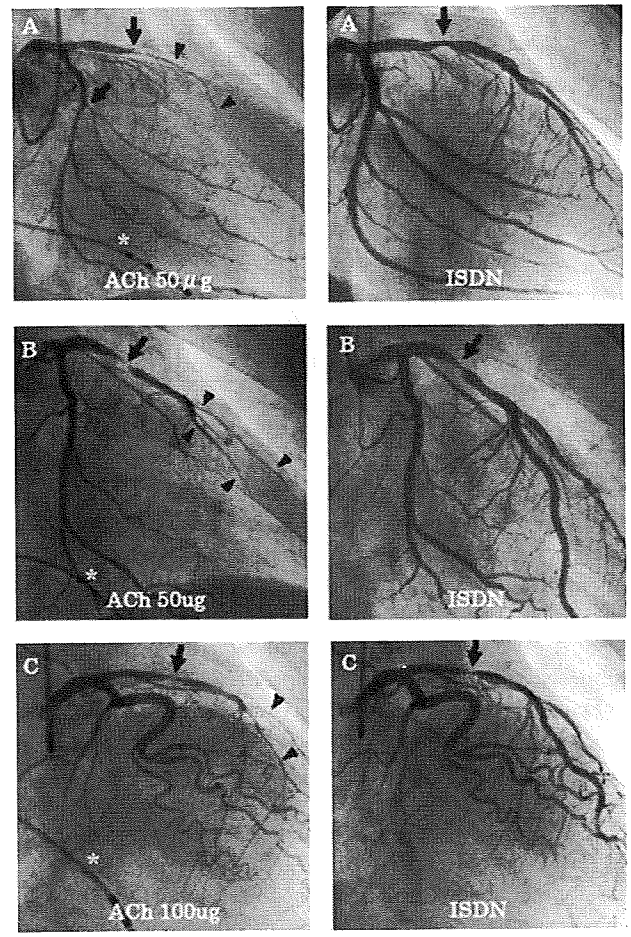

Figure 7. Coronary angiograms during spasm induced by ACh injection and after ISDN in the atherosclerosis group. A, A subtotal occlusion spasm with diffuse vasoconstriction involving the curved proximal segment of the LAD and a focal spasm at the branch site of the LCx appeared after ACh (left, arrows and arrow heads) and disappeared after ISDN (right). A significant organic stenotic lesion was revealed at the curved proximal segment (right, arrow). Spasm was superimposed on the lesion (left, arrow). B, Subtotal occlusion at the proximal segment and diffuse spasm at its distal branch site of the LAD appeared after ACh (left, arrow and arrow heads) and disappeared after ISDN, revealing a severe organic stenosis at the nonbranch site of the curved proximal segment (right, arrow). Despite severe stenosis, total occlusion did not occur at this site during spasm. C, Diffuse spasm occurred at the distal branches, not at the site of severe organic stenosis at the curved proximal segment of the LAD after ACh (left, arrow heads and arrow). A severe organic stenosis lesion was revealed at the nonbranch site of the curved proximal segment after ISDN (right). ACh indicates acetylcholine; ISDN, isosorbide dinitrate; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; *, a pacing catheter.

to suppress it and are in agreement with those of Maseri's group.[1,28] However, MacAlpin[29] reported on the basis of the literature that most spasms were localized at the site of an organic lesion. The discrepancy between his results and ours may probably be explained by the difference of the study subjects. He reported on the patients with "variant angina," ie, angina associated with ST elevation on ECG. On the other hand, most spasms were associated with ST depression and mild to moderate organic stenosis in the atherosclerosis group of this study.

Coronary spasm has risk factors, such as smoking and aging,[18–21] and is associated with endothelial dysfunction,[2,4] inflammation,[20,30] and intimal thickening.[18] It thus shares the common risk and pathogenetic factors with atherosclerosis.[7,8] However, atherosclerosis is characterized by subendothelial retention of atherogenic lipoproteins,[7,8,22–26] develops early from infants,[31] and is usually associated with hyperlipidemia,[7,8,32] whereas coronary spasm does not occur in the young but in the old patients (mean age of 65.5±10.1 in this study), and hyperlipidemia is not a risk factor for coronary spasm.[18–21] Indeed, Morikawa et al have recently reported by using intravascular optical coherence tomography that the spasm arteries with normal angiogram had a diffuse intimal thickening and contained almost no lipid deposits, whereas the no-spasm arteries with normal angiogram had either intimal thickening containing lipid deposits or had no intimal thickening.[33]

Coronary spasm is caused by abnormal contraction of vascular SMCs and therefore contractile and not synthetic phenotype SMCs are likely to play a crucial role in the

pathogenesis of coronary spasm. We, therefore, propose that coronary spasm may be a manifestation of coronary arteriosclerosis distinctly different from coronary atherosclerosis, which is characterized by lipid accumulation and SMCs of synthetic phenotype.[7,8,22–24,32] Recent studies showed that oxidized lipids suppress SMCs marker genes[34] and that lipid lowering promotes accumulation of mature SMCs.[35] To be noted in this connection is the fact that the patients with coronary spasm with angiographically normal or almost normal coronary arteries are less prone to develop acute myocardial infarction as compared with those with other types of unstable angina.[36,37] Intriguingly, the incidence of coronary spasm, particularly variant angina, has decreased recently,[38,39] whereas that of hyperlipidemia has risen in Japan.[40,41]

This study further demonstrates that most coronary spasms were associated with ST-segment depression rather than ST segment elevation on ECG and thereby confirms the concept that variant angina is only one aspect of the spectrum of coronary spastic myocardial ischemia.[42]

Study Limitations

In this study, we defined the site of spasm as that of total or subtotal obstruction or as that of the most severe and proximal constriction in the case of multifocal or segmental diffuse spasm and the site of atherosclerotic lesion as that of the most narrowed in each artery for the purpose of analysis. However, spasm is often, diffuse and or multifocal, or even migrates from site to site and thus the actual images of spasm may be more complex and dynamic than described in this study.[2] Atherosclerotic lesions also are often multifocal. In this study, however, most atherosclerotic lesions were mild and mostly monofocal, because we excluded the patients with multivessel or sever organic stenosis disease from the study. Thus, the results of this study may not necessarily be applicable to advanced atherosclerotic lesions with multiple plaques. Moreover, angiogram is not sensitive enough to detect atherosclerosis because it is highly likely that vascular remodeling may have occurred, and thus, the patients in the normal angiogram group in this study might not have been free from atherosclerosis.[10] In this study, we did not perform the intravascular ultrasound examination concurrently with angiography and thus could not present the data on shear stress and constituents of vessels walls. However, we have previously shown that the intimal thickening involved the entire arterial tree in the patients with coronary spasm and normal angiogram using intravascular ultrasound.[18]

Conclusions

Diffuse spasm involving the entire arterial tree occurred in a substantial number of angiographically normal or almost normal coronary arteries in the patients with chest pain. Spasm preferentially occurred involving branch points, whereas atherosclerosis was predominantly focal and localized at the nonbranch points of the curved proximal segments. Most spasms occurred at the sites different from those of the atherosclerotic plaque. These results strongly suggest that coronary spasm may be a manifestation of a distinct type of arteriosclerosis different from the lipid-laden coronary atherosclerosis.

Sources of Funding

This study was supported in part from Japan Heart Foundation, Tokyo; and Japan Vascular Disease Research Foundation, Kyoto, Japan.

Disclosures

None.

References

1. Maseri A, Davies G, Hackett D, Kaski JC. Coronary artery spasm and vasoconstriction. The case for a distinction. *Circulation*. 1990;81:1983–1991.
2. Yasue H, Kugiyama K. Coronary spasm: clinical features and pathogenesis. *Intern Med*. 1997;36:760–765.
3. Pristipino C, Beltrame JF, Finocchiaro ML, Hattori R, Fujita M, Mongiardo R, Cianflone D, Sanna T, Sasayama S, Maseri A. Major racial differences in coronary constrictor response between Japanese and Caucasians with recent myocardial infarction. *Circulation*. 2001;101:1102–1108.
4. Kugiyama K, Yasue H, Okumura K, Ogawa H, Fujimoto K, Nakao K, Yoshimura M, Motoyama T, Inobe Y, Kawano H. Nitric oxide activity is deficient in spasm arteries of patients with coronary spastic angina. *Circulation*. 1996;94:266–271.
5. Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med*. 2006;259:351–363.
6. Moncada S, Higgs EA. Nitric oxide and the vascular endothelium. *Handb Exp Pharmacol*. 2006;(176 Pt 1):213–254.
7. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111:3481–3488.
8. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685–1695.
9. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardeña G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci*. 2000;902:230–239.
10. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*. 2007;49:2379–2393.
11. Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, Kugiyama K, Obata K, Morikami Y, Kimura T. Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation*. 1986;74:955–963.
12. Okumura K, Yasue H, Matsuyama K, Goto K, Miyagi H, Ogawa H, Matsuyama K. Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J Am Coll Cardiol*. 1988;12:883–888.
13. Yasue H, Matsuyama K, Matsuyama K, Okumura K, Morikami Y, Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment. Possible role of early coronary atherosclerosis. *Circulation*. 1990;81:482–490.
14. Kugiyama K, Murohara T, Yasue H, Kimura T, Sakaino N, Ohgushi M, Sugiyama S, Okumura K. Increased constrictor response to acetylcholine of the isolated coronary arteries from patients with variant angina. *Int J Cardiol*. 1995;52:223–233.
15. AHA Committee Report. A reporting system on patients evaluated for coronary artery disease. *Circulation*. 1975;51:7–37.
16. Waters D, Lespérance J, Craven TE, Hudon G, Gillam LD. Advantages and limitations of serial coronary arteriography for the assessment of progression and regression of coronary atherosclerosis. Implications for clinical trials. *Circulation*. 1993;87(3 Suppl):II38–II47.
17. Okumura K, Yasue H, Matsuyama K, Ogawa H, Kugiyama K, Ishizaka H, Sumida H, Fujii H, Matsunaga T, Tsunoda R. Diffuse disorder of coronary artery vasomotility in patients with coronary spastic angina. Hyperreactivity to the constrictor effects of acetylcholine and the dilator effects of nitroglycerin. *J Am Coll Cardiol*. 1996;27:45–52.
18. Miyao Y, Kugiyama K, Kawano H, Motoyama T, Ogawa H, Yoshimura M, Sakamoto T, Yasue H. Diffuse intimal thickening of coronary arteries

in patients with coronary spastic angina. *J Am Coll Cardiol.* 2000;36: 432–437.
19. Takaoka K, Yoshimura M, Ogawa H, Kugiyama K, Nakayama M, Shimasaki Y, Mizuno Y, Sakamoto T, Yasue H. Comparison of the risk factors for coronary artery spasm with those for organic stenosis in a Japanese population: role of cigarette smoking. *Int J Cardiol.* 2000;72: 121–126.
20. Itoh T, Mizuno Y, Harada E, Yoshimura M, Ogawa H, Yasue H. Coronary spasm is associated with chronic low-grade inflammation. *Circ J.* 2007;71:1074–1078.
21. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, Motoyama T, Saito Y, Ogawa Y, Miyamoto Y, Nakao K. T-786–>C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation.* 1999;99:2864–2870.
22. Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation.* 1992;85: 391–405.
23. Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arterioscler Thromb Vasc Biol.* 2007;27:1159–1165.
24. Doran AC, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2008;28:812–819.
25. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation.* 2006;113:2744–2753.
26. Chatzizisis YS, Jonas M, Coskun AU, Beigel R, Stone BV, Maynard C, Gerrity RG, Daley W, Rogers C, Edelman ER, Feldman CL, Stone PH. Prediction of the localization of high-risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: an intravascular ultrasound and histopathology natural history study. *Circulation.* 2008; 117:993–1002.
27. McLenachan JM, Vita J, Fish DR, Treasure CB, Cox DA, Ganz P, Selwyn AP. Early evidence of endothelial vasodilator dysfunction at coronary branch points. *Circulation.* 1990;82:1169–1173.
28. Newman CM, Maseri A, Hackett DR, el-Tamimi HM, Davies GJ. Response of angiographically normal and atherosclerotic left anterior descending coronary arteries to acetylcholine. *Am J Cardiol.* 1990;66: 1070–1076.
29. MacAlpin RN. Relation of coronary arterial spasm to sites of organic stenosis. *Am J Cardiol.* 1980;46:143–153.
30. Soejima H, Irie A, Miyamoto S, Kajiwara I, Kojima S, Hokamaki J, Sakamoto T, Tanaka T, Yoshimura M, Nishimura Y, Ogawa H. Preference toward a T-helper type 1 response in patients with coronary spastic angina. *Circulation.* 2003;107:2196–2200.
31. Tuzcu EM, Kapadia SR, Tutar E, Ziada KM, Hobbs RE, McCarthy PM, Young JB, Nissen SE. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: evidence from intravascular ultrasound. *Circulation.* 2001;103:2705–2710.
32. Tabas I, Williams KJ, Borén J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation.* 2007;116:1832–1844.
33. Morikawa Y, Uemura S, Ishigami K, Soeda T, Okayama S, Takemoto Y, Onoue K, Somekawa S, Nishida T, Takeda Y, Kawata H, Horii M, Nakajima T, Saito Y. Coronary spasm occurs in coronary arteries with diffuse intimal thickening without lipid deposition and/or calcification: evaluation with intravascular optical coherence tomography [abstract]. *Circulation* 2008;118(Suppl 2):S734.
34. Pidkovka NA, Cherepanova OA, Yoshida T, Alexander MR, Deaton RA, Thomas JA, Leitinger N, Owens GK. Oxidized phospholipids induce phenotypic switching of vascular smooth muscle cells in vivo and in vitro. *Circ Res.* 2007;101:792–801.
35. Aikawa M, Rabkin E, Voglic SJ, Shing H, Nagai R, Schoen FJ, Libby P. Lipid lowering promotes accumulation of mature smooth muscle cells expressing smooth muscle myosin heavy chain isoforms in rabbit atheroma. *Circ Res.* 1998;83:1015–1026.
36. Walling A, Waters DD, Miller DD, Roy D, Pelletier GB, Théroux P. Long-term prognosis of patients with variant angina. *Circulation.* 1987; 76:990–997.
37. Yasue H, Takizawa A, Nagao M, Nishida S, Horie M, Kubota J, Omote S, Takaoka K, Okumura K. Long-term prognosis for patients with variant angina and influential factors. *Circulation.* 1988;78:1–9.
38. Sueda S, Kohno H, Fukuda H, Uraoka T. Did the widespread use of long-acting calcium antagonists decrease the occurrence of variant angina? *Chest.* 2003;124:2074–2078.
39. Japanese beta-Blockers and Calcium Antagonists Myocardial Infarction (JBCMI) Investigators. Comparison of the effects of beta blockers and calcium antagonists on cardiovascular events after acute myocardial infarction in Japanese subjects. *Am J Cardiol.* 2004;93:969–973.
40. Okamura T, Tanaka H, Miyamatsu N, Hayakawa T, Kadowaki T, Kita Y, Nakamura Y, Okayama A, Ueshima H; NIPPON DATA80 Research Group. The relationship between serum total cholesterol and all-cause or cause-specific mortality in a 17.3-year study of a Japanese cohort. *Atherosclerosis.* 2007;190:216–223.
41. Kitamura A, Sato S, Kiyama M, Imano H, Iso H, Okada T, Ohira T, Tanigawa T, Yamagishi K, Nakamura M, Konishi M, Shimamoto T, Iida M, Komachi Y. Trends in the incidence of coronary heart disease and stroke and their risk factors in Japan, 1964 to 2003: the Akita-Osaka study. *J Am Coll Cardiol.* 2008;52:71–79.
42. Maseri A, Severi S, Nes MD, L'Abbate A, Chierchia S, Marzilli M, Ballestra AM, Parodi O, Biagini A, Distante A. "Variant" angina: one aspect of a continuous spectrum of vasospastic myocardial ischemia. Pathogenetic mechanisms, estimated incidence and clinical and coronary arteriographic findings in 138 patients. *Am J Cardiol.* 1978;42:1019–1035.

CLINICAL PERSPECTIVE

Coronary spasm plays an important role in the pathogenesis of ischemic heart disease. However, similarities and differences between coronary spasm and atherosclerosis are not known. This study examined the angiographic characteristics of coronary spasm in comparison with those of atherosclerotic plaque, first in the angiographically normal or almost normal coronary arteries and then in those with atherosclerotic plaque. The results showed that diffuse spasm involving the entire artery appeared in the substantial number of the angiographically normal arteries and that spasm preferentially occurred at branch points in both the angiographically normal arteries and those with plaque. On the other hand, plaque was predominantly localized at nonbranch point sites of the curved proximal segments. Most spasms did not occur at the sites of plaque. These results suggest that coronary spasm may be a manifestation of a distinct type of arteriosclerosis different from the lipid-laden coronary atherosclerosis. This study, thus, may provide a new insight into the pathogenesis not only of coronary spasm but also of atherosclerosis and may explain at least partially the decline of the number of coronary spasm with the increase of hyperlipidemia among Japanese in recent years.

SUPPLEMENTAL MATERIAL

Coronary Spasm Preferentially Occurs at Branch Points

-an angiographic comparison with atherosclerotic plaque -

Hitoshi Nakagawa, MD, Yoshinobu Morikawa, Yuji Mizuno, MD,
Eisaku Harada, MD, MD, Teruhiko Ito, MD, Kunihiko Matsui, MD,
MPH*, Yoshihiko Saito, MD#,
Hirofumi Yasue, MD.

Figure 1
LAD

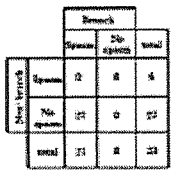

Figure 1
LCx

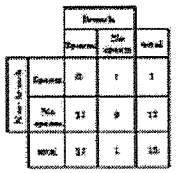

Figure 1
RCA

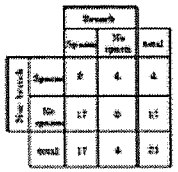

Figure 2
LAD

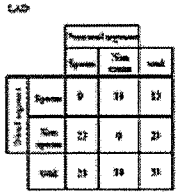

Figure 2
LCx

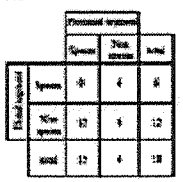

Figure 2
RCA

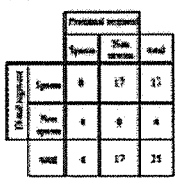

Figure 3
LAD

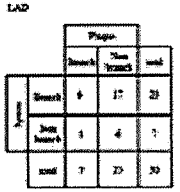

Figure 3
LCx

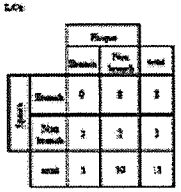

Figure 3
RCA

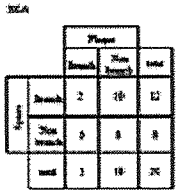

Figure 4
LAD

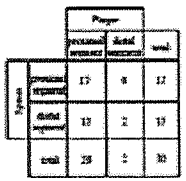

Figure 4
LCx

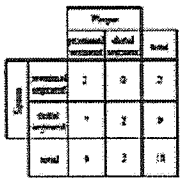

Figure 4
RCA

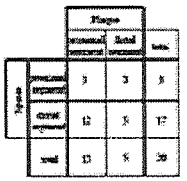

Figure 5
LAD

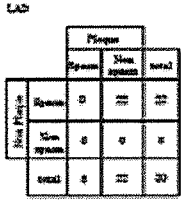

Figure 5
LCx

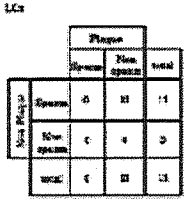

Figure 5
RCA

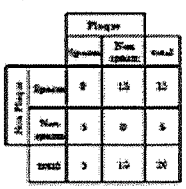

Figure legends

Figure 1. Comparison of the involvement of coronary spasm between the branch and non-branch points in the normal angiogram group. Branch indicates the branch point; Non-branch, the non-branch point; LAD, left anterior descending coronary artery; LCx; left circumflex artery; RCA, right coronary artery.

Figure 2. Comparison of the involvement of coronary spasm between the proximal and distal segments in the normal angiogram group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

Figure 3. Comparison of the involvement of the branch point between spasm and plaque in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

Figure 4. Comparison of the involvement of the proximal segment between spasm and plaque in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

Figure 5. Comparison of the involvement of spasm between the plaque and non-plaque sites in the atherosclerosis group. LAD indicates left anterior descending coronary artery; LCx; left circumflex artery; RCA, right coronary artery.

Journal of the American College of Cardiology
© 2009 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 54, No. 7, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.05.022

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

Toshimitsu Nozaki, MD,* Seigo Sugiyama, MD, PhD,* Hidenobu Koga, MD, PhD,* Koichi Sugamura, MD,* Keisuke Ohba, MD,* Yasushi Matsuzawa, MD,* Hitoshi Sumida, MD, PhD,† Kunihiko Matsui, MD, PhD,‡ Hideaki Jinnouchi, MD, PhD,§ Hisao Ogawa, MD, PhD*

Kumamoto, Japan

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

From the Departments of *Cardiovascular Medicine, †Interventional Cardiology, and ‡General Medicine, Graduate School of Medical Science, Kumamoto University, Kumamoto, Japan; and the §Jinnouchi Hospital, Kumamoto, Japan. This study was supported, in part, by grants-in-aid for Scientific Research (#C19590869 to Dr. Sugiyama) from the Ministry of Education, Science, and Culture, Japan; Advanced Education Program for Integrated Clinical, Basic and Social Medicine, Graduate School of Medical Sciences, Kumamoto University in Kumamoto, Japan (Support Program for Improvement of Graduate School Education, MEXT, Japan); and Kimura Memorial Heart Foundation Bayer Grant for Clinical Vascular Function 2008, Kurume, Japan.

Manuscript received January 5, 2009; revised manuscript received May 21, 2009, accepted May 25, 2009.

JACC Vol. 54, No. 7, 2009
August 11, 2009:601–8

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

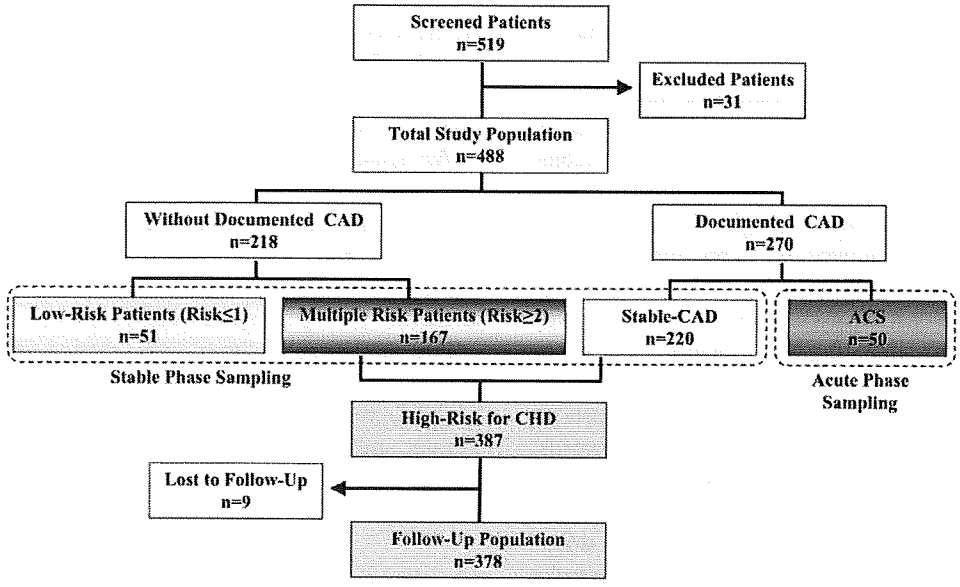

Figure 1 Flow Diagram of Subject Recruitment

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

JACC Vol. 54, No. 7, 2009
August 11, 2009:601–8

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).

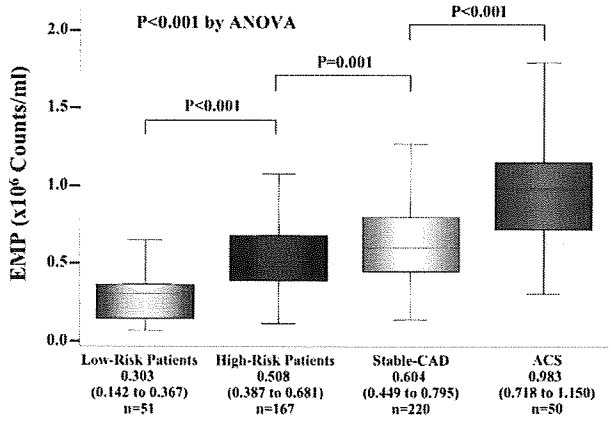

Figure 2 Plasma Levels of CD144-EMP in Patients With Various Cardiovascular Risks

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).

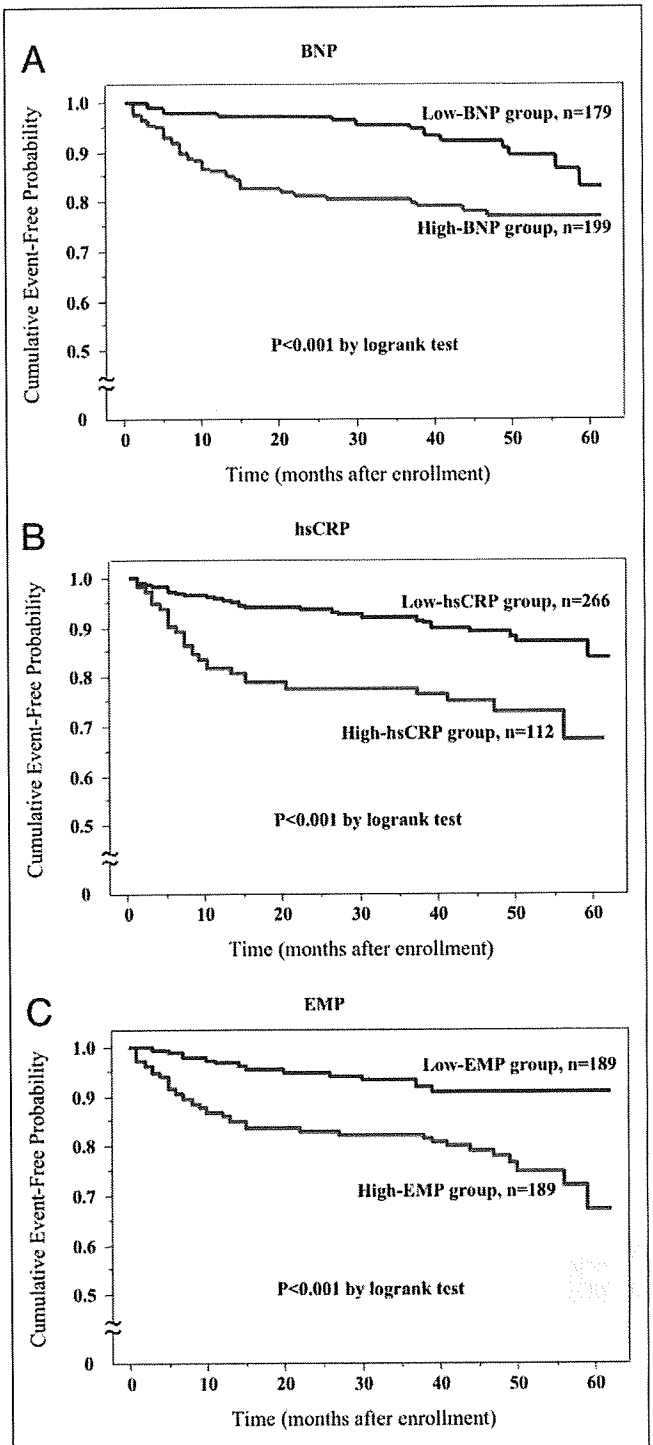

Figure 3 Kaplan-Meier Analysis for the Probability of Cardiovascular Events

(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-

JACC Vol. 54, No. 7, 2009
August 11, 2009:601–8

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-