17 Siegrist J, Matschinger H, Motz W. Untreated hypertensives and their quality of life. *J Hypertens Suppl* 1987; **5**: S15–S20.
18 Kjellgren KI, Ahlner J, Dahlöf B, Gill H, Hedner T, Säljö R. Perceived symptoms amongst hypertensive patients in routine clinical practice -a population-based study. *J Intern Med* 1998; **244**: 325–332.
19 Muller A, Montoya P, Schandry R, Hartl L. Changes in physical symptoms, blood pressure and quality of life over 30 days. *Behav Res Ther* 1994; **32**: 593–603.
20 Guyatt GH, Feeny DH, Patrick DL. Measuring health-related quality of life. *Ann Intern Med* 1993; **118**: 622–629.
21 Fitzpatrick R, Fletcher A, Gore S, Jones D, Spiegelhalter D, Cox D. Quality of life measures in health care. I. Applications and issues in assessment. *BMJ* 1992; **305**: 1074–1077.
22 Weinberger M, Kirkman MS, Samsa GP, Cowper PA, Shortliffe EA, Simel DL, Feussner JR. The relationship between glycemic control and health-related quality of life in patients with non-insulin-dependent diabetes mellitus. *Med Care* 1994; **32**: 1173–1181.
23 Beto JA, Bansal VK. Quality of life in treatment of hypertension. A metaanalysis of clinical trials. *Am J Hypertens* 1992; **5**: 125–133.
24 Fiorentini A, Valente R, Perciaccante A, Tubani L. Sleep's quality disorders in patients with hyperlension and type 2 diabetes mellitus. *Int J Cardiol* 2007; **114**: e50–e52.

nature publishing group

Relationships Among Hyperuricemia, Metabolic Syndrome, and Endothelial Function

Hirofumi Tomiyama[1], Yukihito Higashi[2], Bonpei Takase[3], Kohichi Node[4], Masataka Sata[5], Teruo Inoue[6], Yutaka Ishibashi[7], Shinichiro Ueda[8], Kenei Shimada[9] and Akira Yamashina[1]

BACKGROUND

We evaluated the relationship of the severity of hyperuricemia and the flow-mediated vasodilatation of the brachial artery (FMD) in patients with and without the metabolic syndrome (MetS).

METHODS

In a cross-sectional study, FMD was obtained in 2,732 Japanese healthy men (49 ± 8 years) who had no cardiovascular (CV) disease and were not on any medication for CV risk factors. MetS was defined according to Japanese criteria, and serum uric acid (UA) levels in the upper half of the fifth (highest) quintile range were defined as severe hyperuricemia, whereas those in the lower half of this quintile range were defined as mild hyperuricemia.

RESULTS

Overall, the adjusted values of FMD were lower in the subjects with MetS (5.6 ± 0.1%; $n = 413$) than in those without MetS (6.2 ± 0.1%; $n = 2,319$) ($P < 0.01$). Among the subjects without MetS, the adjusted values of FMD were lower in both the subgroups with mild hyperuricemia and severe hyperuricemia than in the subgroup without hyperuricemia. On the contrary, among the subjects with MetS, the adjusted value of FMD was lower only in the subgroup with severe hyperuricemia (4.8 ± 0.3%) as compared with that in the group without hyperuricemia (5.7 ± 0.2%) ($P < 0.05$).

CONCLUSIONS

In middle-aged healthy Japanese men without MetS, not only severe, but also mild hyperuricemia may be a significant independent risk factor for endothelial dysfunction in subjects without MetS, whereas only severe hyperuricemia (but not mild hyperuricemia) appeared to exacerbate endothelial dysfunction in similar subjects with MetS.

Keywords: blood pressure; endothelial function; hypertension; hyperuricemia; metabolic syndrome; risk factors; uric acid

American Journal of Hypertension, advance online publication 14 April 2011; doi:10.1038/ajh.2011.55

Recent studies have suggested that hyperuricemia is an independent risk factor for cardiovascular (CV) disease.[1–4] The endothelium is a direct, sensitive target for the injurious effects of CV risk factors,[5] and endothelial dysfunction, assessed by flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia (FMD), is a marker of the early stages of atherosclerotic vascular damage.[6,7] Some studies have indicated that the FMD is impaired in subjects with hyperuricemia.[8,9] However, it still remains to be clarified whether not only severe hyperuricemia, but also mild hyperuricemia might impair FMD. On the other hand, the metabolic syndrome (MetS), represents a clustering of risk factors of metabolic origin, is associated with an elevated risk of CV diseases,[10,11] and FMD is also impaired in subjects with MetS.[12] Although hyperuricemia is common in subjects with MetS,[13] it still remains to be clarified whether MetS and hyperuricemia might independently impair endothelial dysfunction.

The present cross-sectional study was conducted in a large cohort of middle-aged Japanese men who had no CV disease and were not on any medication for CV risk factors, including hyperuricemia, to evaluate the relationship between the severity of hyperuricemia and FMD in patients with and without MetS.

METHODS

Design and subjects. This cross-sectional study was conducted with the participation of three health care centers of companies and three health care clinics. All the participants were informed about the measurement of the FMD to examine its potential relationship with CV events. After providing written informed consent, the subjects underwent FMD measurement in addition to the routine annual health checkup, which included evaluation of atherosclerotic risk factors, between May and December 2008. A part of the data was reported

[1]Second Department of Internal Medicine, Tokyo Medical University, Tokyo, Japan; [2]Department of Cardiovascular Physiology and Medicine, Hiroshima University Graduate School of Biomedical Science, Hiroshima, Japan; [3]Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan; [4]Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga, Japan; [5]Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; [6]Department of Cardiovascular Medicine, Dokkyo Medical University, Tochigi, Japan; [7]Division of Cardiovascular Medicine, Department of Medicine, Shimane University Faculty of Medicine, Izumo, Japan; [8]Department of Clinical Pharmacology and Therapeutics, University of the Ryukyus School of Medicine, Okinawa, Japan; [9]Department of Medicine, Cardiovascular Division, Osaka Ekisaikai Hospital, Osaka, Japan. Correspondence: Hirofumi Tomiyama (tomiyama@tokyo-med.ac.jp)

Received 5 October 2010; first decision 27 November 2010; accepted 20 January 2011.

elsewhere.[7] The study protocol conformed to the principles of the Declaration of Helsinki (1964), and the study was conducted with the approval of the ethical guidelines committee of each of the participating institutions.

Assessment of FMD. The subjects were instructed to fast for at least 4 h, and to abstain from alcohol, smoking, caffeine and antioxidant vitamins for at least 12 h prior to the measurements. They were asked to rest in the sitting position in a quiet, dark, air-conditioned room (22–25 °C) for 5 min, followed by blood pressure measurement by the oscillometric method (UA 767; A&D Co. Ltd, Saitama, Japan). Then, after the subjects had rested again for at least 15 min in the supine position in the same room, the FMD measurement was conducted. We performed ultrasound measurements according to the guidelines for ultrasound assessment of FMD.[14]

Using high-resolution ultrasound with a 10-MHz linear array transducer, longitudinal images of the right brachial artery were recorded at the baseline and then continuously from 30 s before to 2 min or more after the cuff deflation following suprasystolic compression (50 mm Hg over the systolic blood pressure) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter (Unex Co. Ltd, Nagoya, Japan). In brief, continuous recordings of the two-dimensional gray-scale images and A-mode waves of the brachial artery in the longitudinal plane were conducted with a novel stereotactic probe-holding device. A segment with clear anterior (media-adventitia) and posterior (intima-media) interfaces was manually determined. These border interfaces were identified automatically on the A-mode waves as a signal of the intima-media complex, and the diastolic diameter of the brachial artery beat was synchronized with the electrocardiographic R-waves and tracked automatically. Changes in the diastolic diameter were continuously recorded. Then, FMD was estimated as the percent change of the diameter of the brachial artery over the baseline value at maximal dilatation during reactive hyperemia.

The reproducibility of the FMD measurements at each institute was determined from the measurements conducted at the three company health care centers and the health care clinic supervised by the Tokyo Medical University (Tokyo, Japan) (Pearson's correlation coefficient of the FMD between visits 1 and 2 was 0.86, $P < 0.01$, and the coefficient of variation was 11.2%; $n = 39$); it was also confirmed in the health care clinic supervised by the Hiroshima University (Hiroshima, Japan) (Pearson's correlation coefficient of the FMD between visit 1 and visit 2 was 0.89, $P < 0.01$, where the coefficient of variation was determined to be 10.1%; $N = 20$).

Laboratory measurements. We collected blood samples from fasting subjects after an interval of at least 1 h following assessment of the arterial endothelial function. The serum triglyceride, high-density lipoprotein cholesterol, total cholesterol, creatinine and uric acid (UA) levels, and the fasting blood glucose were measured using enzymatic methods.

Definition of metabolic syndrome, mild hyperuricemia and severe hyperuricemia. We adopted the criteria of the Japanese Expert Committee on the Diagnosis and Classification of Metabolic Syndrome for the clinical diagnosis of MetS;[15] namely, central obesity (waist circumference ≥85 cm for men), plus at least two of the following three criteria: dyslipidemia (hypertriglyceridemia (triglyceride ≥1.70 mmol/l) and/or low high-density lipoprotein cholesterol (high-density lipoprotein cholesterol <1.03 mmol/l), elevated blood pressure (blood pressure ≥130/85 mm Hg), and elevated plasma glucose (fasting blood glucose ≥6.11 mmol/l).

Serum UA levels in the upper half of the fifth (highest) quintile range were defined as severe hyperuricemia, whereas those in the lower half of this quintile range were defined as mild hyperuricemia.

Statistical analysis. The normality of the distribution of the variables was assessed by the Kolmogorov–Smirnov test. The Kolmogorov–Smirnov test demonstrated that none of the continuous variables showed normal distribution. The linearity between the serum UA levels and FMD was assessed by the calculation of Pearson's correlation coefficient, and the relationship between the two variables was found to be weak, although significant ($r = 0.07$, $P < 0.01$). Therefore, linear model analyses were not applied for assessment of the relationship between the two variables. The continuous variables are represented as median values (25th–75th percentile). The Mann–Whitney test was applied to evaluate the differences in FMD between subjects with and without MetS. For assessment of the differences in the status of each variable among the groups, the Kruskal–Wallis test for continuous variables and Pearson's χ^2 test for categorical variables were applied. Furthermore, for assessment of the differences in the adjusted values of FMD among the groups, a general linear model analysis-*post hoc* pairwise comparison model with adjustments by the simple contrast method was applied. In the assessment of the differences in FMD between the subjects with and without MetS, the covariates used for the adjustments included the age, smoking status, total cholesterol, and creatinine. In the assessment of the differences in FMD among the three subgroups of subjects classified based on the presence/absence of mild/severe hyperuricemia, the covariates used for the adjustments included the age, waist circumference, smoking status, mean blood pressure, total cholesterol, high-density lipoprotein cholesterol, triglyceride, fasting blood glucose, and creatinine. All the analyses were conducted using the SPSS software for Windows, version 11.0J (SPSS, Chicago, IL); P values <0.05 were considered to denote statistical significance.

RESULTS

In total, 4,447 subjects were successfully enrolled for the FMD measurement in the aforementioned six institutes. Among them, the following subjects were excluded from the study: 324 who failed to provide blood samples for measurement of the conventional CV risk factors or serum UA levels, 80 who were under medication for CV diseases, 513 who were under

Table 1 | Clinical characteristics of all the study subjects

Variable	
Number	2,732
Age (years)	49 (43–54)
WC (cm)	83 (78–88)
BMI	23.2 (21.5–25.1)
Smoking (%)	1038 (38.0)
SBP (mm Hg)	125 (115–136)
DBP (mm Hg)	78 (70–86)
HR (beats/min)	62 (56–68)
TC (mg/dl)	206 (185–228)
HDL(mg/dl)	57 (48–67)
TG (mg/dl)	116 (83–162)
FBS (mg/dl)	97 (91–104)
UA (mg/dl)	6.1 (5.4–6.9)
Crnn (mg/dl)	0.86 (0.79–0.93)
mildHUA (%)	305 (11.2)
sevHUA (%)	291 (10.7)
MetS (%)	413 (15.1)
FMD (%)	5.9 (4.1–7.8)

BMI, body mass index; Crnn, serum creatinine; DBP, diastolic blood pressure; FBS, fasting blood sugar; FMD, flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia; HDL, serum high-density lipoprotein cholesterol; HR, heart rate; MetS, number of subjects with metabolic syndrome alone; mildHUA, number of subjects with mild hyperuricemia (UA levels ≥425–<461 μmol/l); sevHUA, number of subjects with severe hyperuricemia (tserum UA levels ≥461 μmol/l); Smoking, number of current smokers; SBP, systolic blood pressure; TC, serum total cholesterol; TG, serum triglycerides; UA, serum uric acid; WC, waist circumference.

medication for CV risk factors, including hyperuricemia, 75 who were age <30, and 723 who were women (i.e., a relatively small percentage of the study subjects). Finally, the data of 2,732 subjects (age 49 ± 8 years) were included for the analyses.

Table 1 shows the clinical characteristics of the entire study population. In the entire study population, 413 subjects were diagnosed as having MetS. In the serum UA levels in the entire study population (150–712 μmol/l), serum UA levels >425 μmol/l but <461 μmol/l (in the lower half of the fifth (highest) quintile range) were defined as mild hyperuricemia, and serum UA levels >461 μmol/l (in the upper half of the fifth (highest) quintile range) were defined as severe hyperuricemia. Then, 305 of the subjects were classified as having mild hyperuricemia and 291 as having severe hyperuricemia. The prevalence of mild hyperuricemia (with MetS (number of subjects = 70/413: 16.9%) vs. without MetS (number of subjects = 235/2319: 10.1%)) and that of severe hyperuricemia (with MetS (number of subjects = 86/413: 20.8%) vs. without MetS (number of subjects = 205/2319: 8.8%)) were significantly higher in the subjects with MetS than in those without MetS ($P < 0.01$).

In the study population as a whole ($n = 2,732$), the Mann–Whitney test demonstrated that the crude value of FMD was different between the subjects with and without MetS. The general linear model analysis demonstrated that the adjusted values of FMD were lower in the subjects with MetS (5.6 ± 0.1%; $n = 413$) than in those without MetS (6.2 ± 0.1%; $n = 2,319$). Then, the differences of the FMD among three subgroups of subjects classified based on the presence/absence of

Table 2 | Clinical characteristics of the study subjects with and without metabolic syndrome

Variable	Non	mildHUA	sevHUA	*P* value
In subjects without MetS (n = 2,319)				
Number	1,879	235	205	
Age (years)	49 (43–54)	49 (42–52)	49 (43–53)	0.17
WC (cm)	82 (77–86)	83 (80–87)	84 (80–89)	<0.01
BMI	22.6 (21.0–24.3)	23.4 (22.0–25.0)	23.7 (22.1–25.8)	<0.01
Smoking (%)	699 (37.2)	76 (32.3)	85 (41.5)	0.14
SBP (mm Hg)	122 (113–133)	124 (116–133)	125 (115–136)	<0.05
DBP (mm Hg)	76 (68–84)	77 (72–85)	77 (71–87)	<0.01
HR (beats/min)	61 (55–67)	62 (57–68)	62 (56–68)	0.06
TC (mg/dl)	203 (182–225)	209 (189–231)	210 (191–230)	<0.01
HDL (mg/dl)	59 (49–69)	56 (48–67)	57 (49–66)	0.07
TG (mg/dl)	106 (77–139)	116 (87–165)	126 (93–168)	<0.01
FBS (mg/dl)	96 (90–101)	97 (91–102)	97 (92–104)	<0.01
UA (mg/dl)	5.8 (5.2–6.4)	7.3 (7.2–7.4)	8.1 (7.9–8.6)	<0.01
Crnn (mg/dl)	0.85 (0.78–0.91)	0.90 (0.81–0.99)	0.92 (0.83–1.00)	<0.01
FMD (%)	6.2 (4.3–8.0)	5.7 (3.9–7.4)	5.6 (4.0–7.7)	<0.05
In subjects with MetS (n = 413)				
Number	257	70	86	
Age (years)	50 (47–56)	50 (49–58)	49 (41–54)	<0.05
WC (cm)	90 (87–93)	89 (86–92)	91 (87–95)	<0.05
BMI	25.5 (24.1–27.3)	25.8 (24.1–27.0)	26.4 (25.0–28.3)	<0.05
Smoking (%)	111 (43.1)	33 (47.1)	34 (39.5)	0.63
SBP (mm Hg)	136 (129–142)	137 (130–145)	136 (130–145)	0.56
DBP (mm Hg)	85 (79–91)	87 (80–93)	87 (82–94)	0.16
HR (beats/min)	65 (59–73)	65 (60–72)	65 (59–74)	0.83
TC (mg/dl)	214 (190–239)	226 (203–245)	227 (213–246)	<0.01
HDL (mg/dl)	49 (42–58)	51 (44–57)	49 (41–57)	0.71
TG (mg/dl)	182 (145–242)	183 (155–237)	216 (172–270)	<0.01
FBS (mg/dl)	110 (99–119)	105 (96–115)	105 (96–116)	<0.05
UA (mg/dl)	6.0 (5.4–6.5)	7.3 (7.2–7.5)	8.1(7.9–8.7)	<0.01
Crnn (mg/dl)	0.83 (0.76–0.91)	0.87 (0.80–0.93)	0.90 (0.82–1.00)	<0.01
FMD (%)	5.4 (3.6–7.4)	5.2 (3.6–7.2)	4.9 (3.2–7.0)	<0.05

BMI, body mass index; Crnn, serum creatinine; DBP, diastolic blood pressure; FBS, fasting blood sugar; FMD, flow-mediated vasodilatation of the brachial artery caused by reactive hyperemia; HDL, serum high-density lipoprotein cholesterol; HR, heart rate; MetS, metabolic syndrome; mildHUA, subjects with mild hyperuricemia (serum UA levels ≥425 μmol/l but <461 μmol/l); non, subjects without hyperuricemia; SBP, systolic blood pressure; sevHUA, subjects with severe hyperuricemia (serum UA levels ≥461 μmol/l); Smoking, number of current smokers; TC, serum total cholesterol; TG, serum triglycerides; UA, serum uric acid; WC, waist circumference.
P value was assessed by Kruskal–Wallis test for continuous variables and Pearson's χ^2 test for categorical variables.

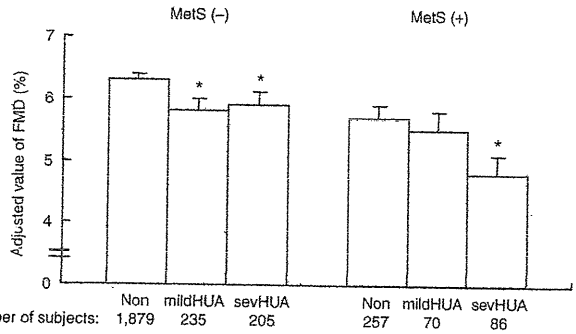

Figure 1 | The adjusted values of flow-mediated vasodilatation of the brachial artery (FMD) in the three groups of subjects classified based on the presence/absence of mild hyperuricemia/severe hyperuricemia in subjects with and without metabolic syndrome. MetS (+), with metabolic syndrome; MetS (–), without metabolic syndrome; mildHUA, subjects with mild hyperuricemia; non, subjects with neither mild hyperuricemia nor severe hyperuricemia; sevHUA, subjects with severe hyperuricemia. *$P < 0.05$ vs. the subjects with neither mild hyperuricemia nor severe hyperuricemia.

mild/severe hyperuricemia were examined separately in the subjects with and without MetS.

Table 2 shows the clinical characteristics of the three subgroups of subjects with/without MetS classified based on the presence/absence of mild/severe hyperuricemia. In both subjects with and without MetS, in addition to the FMD value, the waist circumference, total cholesterol, triglyceride, fasting blood glucose and creatinine also differed significantly among the three subgroups of subjects classified based on the presence/absence of mild/severe hyperuricemia.

The Kruskal–Wallis test demonstrated that the crude value of FMD was different among the subgroups with mild hyperuricemia, severe hyperuricemia and without hyperuricemia, not only in the subject population without MetS but also in that with MetS. As shown in Figure 1, the general linear model analysis with the simple contrast method demonstrated that in subjects without MetS ($n = 2,319$), the adjusted values of FMD in both the subgroups with mild hyperuricemia and severe hyperuricemia were lower than those in the subgroup without hyperuricemia. On the contrary, in the subjects with MetS ($n = 413$), the adjusted value of FMD was lower only in the subgroup with severe hyperuricemia (and not in the group with mild hyperuricemia) as compared with that in the subgroup without hyperuricemia.

DISCUSSION

Several studies have indicated that the conventional risk factors for CV disease are predictive of impaired FMD.[6,7] Several studies have suggested that hyperuricemia is a causal factor for vascular damage.[16,17] An experimental study demonstrated that UA impairs endothelial function via reduction of nitric oxide synthase.[18] Although allopurinol has been shown to improve the FMD, it has not been clarified whether this effect is mediated by the reduction of the serum UA levels by the drug or reduction of xanthine oxidase activity by the drug.[19,20] In addition, only studies with a relatively small number of subjects have reported the existence of a significant relationship between the serum UA levels and the FMD.[8,9] The present study, conducted on a large number of middle-aged healthy Japanese men, confirmed that not only severe hyperuricemia (serum UA levels ≥ 461 μmol/l) but also mild hyperuricemia (serum UA levels ≥ 425 μmol/l but <461 μmol/l) impairs FMD, independent of the risk factors for CV disease, including the components of MetS.

Existence of a significant relationship between MetS and hyperuricemia has been reported.[2,4] In this connection, the hyperinsulinemia associated with MetS has been shown to increase UA reabsorption from the proximal renal tubules, and the microvascular damage associated with elevated blood pressure has been shown to enhance UA generation.[2,4] The present study demonstrated a higher prevalence of mild/severe hyperuricemia in the subjects with MetS than in those without MetS. Thus, clarification of the effects of hyperuricemia on the atherogenic abnormalities associated with MetS is crucial. It is noteworthy that the clustering of CV risk factors in MetS is associated with a greater risk for CV diseases than that reflected by the sum of the risks associated with the individual risk factors.[10,11] The Framingham Offspring study demonstrated that the clustering of the CV risk factors in MetS is associated with progressive impairment of the FMD.[12] In the present study, different from the subjects without MetS, severe hyperuricemia, but not mild hyperuricemia, significantly impaired FMD. Thus, severe hyperuricemia may make a significant additional contribution to the progressive impairment of endothelial function associated with the clustering of CV risk factors in subjects with MetS.

Some prospective studies have demonstrated that hyperuricemia is a risk factor for CV events independent of the presence of MetS.[4,13] On the other hand, hyperuricemia has also been reported to be a risk factor for increased carotid intima-media thickness, a morphological marker of atherosclerosis, as assessed by intravascular ultrasound examination.[21] However, in two recent studies, hyperuricemia was not demonstrated as a potent risk factor for increased carotid intima-media thickness in subjects with MetS.[22,23] As compared to the carotid intima-media thickness, impaired FMD is considered to be an earlier marker of atherosclerosis, preceding the appearance of ultrasonic evidence of atherosclerosis.24 Several factors, such as conventional risk factors for CV disease, inflammation, oxidative stress, nitric oxide and so on, contribute to the initiation/progression of atherosclerosis.[24–26] FMD is a marker related to endothelial nitric oxide bioavailability,[25,26] and therefore, hyperuricemia may affect atherogenic abnormalities related to nitric oxide bioavailability in MetS.

This study had some limitations: (i) The linearity between the serum UA levels and the FMD was weak, although significant and therefore, the significance of the interaction of the effects of hyperuricemia and MetS on FMD was not assessed. (ii) The present study had some technical limitations related to the measurement of FMD;[27] (ii-A) Although FMD shows diurnal variations,[27] it was measured in the morning in some of the study subjects, but around noontime in others; (ii-B) Furthermore, the results of FMD measurement are operator

dependent. The reproducibility of FMD measurement at the participating study institutions is described in the Methods section, and for the present study, the FMD measurements were conducted using the same protocol and the same instrument. Even so, the consistency of FMD measurements among institutions was not examined in the present study. (iii) In the present study, abnormal glucose metabolism, a well-known risk factor for endothelial dysfunction, was not significantly related to the impairment of FMD. A plausible mechanisms to explain this unexpected result are that the status of abnormal glucose metabolism was not severe enough to impair FMD. (iv) The present study could not clarify the mechanisms underlying the impairment of endothelial function induced by hyperuricemia. (v) Gender-related differences in serum UA levels are widely recognized,[1,10,13] and some studies have suggested that the relationship between hyperuricemia and MetS is relatively weak in males.[22,23] Thus, any gender-related differences in the relationships among hyperuricemia, endothelial function, and MetS should be clarified in the future study. (vi) Although endothelium-independent vasodilatation is also a marker of vascular damage,[24] we did not evaluate the effect of hyperuricemia on endothelium-independent vasodilatation in the present study. (vii) In this multicenter study, although the FMD was assessed using the same protocol and the same instrument, the biochemical assays were not calibrated. Notwithstanding, however, the serum uric acid levels were similar among the clinics (data not shown). (viii) In the present study, the lipid profile was entered as a covariate for the analyses, and the National Cholesterol Education Program has recommended a fasting period of at least 8-h duration prior to its assessment.[28] However, in the present study, the lipid profile was determined after 5-h fasting in the subjects.

In middle-aged healthy Japanese men without MetS, not only severe, but also mild hyperuricemia may be a significant independent risk factor for endothelial dysfunction, whereas only severe hyperuricemia (but not mild hyperuricemia) appeared to exacerbate endothelial dysfunction in similar subjects with MetS.

Acknowledgment: This study was partially supported by grant in aid of Japanese Arteriosclerosis Prevention Fund.

Disclosure: The authors declared no conflict of interest.

1. Gagliardi AC, Miname MH, Santos RD. Uric acid: A marker of increased cardiovascular risk. *Atherosclerosis* 2009; 202:11–17.
2. Kim SY, Guevara JP, Kim KM, Choi HK, Heitjan DF, Albert DA. Hyperuricemia and risk of stroke: a systematic review and meta-analysis. *Arthritis Rheum* 2009; 61:885–892.
3. Ioachimescu AG, Brennan DM, Hoar BM, Hazen SL, Hoogwerf BJ. Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study. *Arthritis Rheum* 2008; 58:623–630.
4. Chien KL, Hsu HC, Sung FC, Su TC, Chen MF, Lee YT. Hyperuricemia as a risk factor on cardiovascular events in Taiwan: The Chin-Shan Community Cardiovascular Cohort Study. *Atherosclerosis* 2005; 183:147–155.
5. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; 115:1285–1295.
6. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, Lehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004; 109:613–619.
7. Tomiyama H, Matsumoto C, Yamada J, Teramoto T, Abe K, Ohta H, Kiso Y, Kawauchi T, Yamashina A. The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. *Hypertens Res* 2008; 31:2019–2025.
8. Erdogan D, Gullu H, Caliskan M, Yildirim E, Bilgi M, Ulus T, Sezgin N, Muderrisoglu H. Relationship of serum uric acid to measures of endothelial function and atherosclerosis in healthy adults. *Int J Clin Pract* 2005; 59:1276–1282.
9. Mercuro G, Vitale C, Cerquetani E, Zoncu S, Deidda M, Fini M, Rosano GM. Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk. *Am J Cardiol* 2004; 94:932–935.
10. Grundy SM. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. *J Am Coll Cardiol* 2006; 47:1093–1100.
11. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes* 2005; 54:3252–3257.
12. Hamburg NM, Larson MG, Vita JA, Vasan RS, Keyes MJ, Widlansky ME, Fox CS, Mitchell GF, Levy D, Meigs JB, Benjamin EJ. Metabolic syndrome, insulin resistance, and brachial artery vasodilator function in Framingham Offspring participants without clinical evidence of cardiovascular disease. *Am J Cardiol* 2008; 101:82–88.
13. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* 2008; 359:1811–1821.
14. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39:257–265.
15. The Committee of Establishing the Definition of the Diagnosis of Metabolic Syndrome in Japan. *J Jpn Soc Int Med* 2005; 94:188–203 (In Japanese).
16. Esen AM, Akcakoyun M, Esen O, Acar G, Emiroglu Y, Pala S, Kargin R, Karapinar H, Ozcan O, Barutcu I. Uric acid as a marker of oxidative stress in dilatation of the ascending aorta. *Am J Hypertens* 2011; 24:149–154.
17. Vlachopoulos C, Xaplanteris P, Vyssoulis G, Bratsas A, Baou K, Tzamou V, Aznaouridis K, Dima I, Lazaros G, Stefanadis C. Association of Serum Uric Acid Level With Aortic Stiffness and Arterial Wave Reflections in Newly Diagnosed, Never-Treated Hypertension. *Am J Hypertens* 2011; 24: 33–39.
18. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, Krotova K, Block ER, Prabhakar S, Johnson RJ. Hyperuricemia induces endothelial dysfunction. *Kidney Int* 2005; 67:1739–1742.
19. Butler R, Morris AD, Belch JJ, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 2000; 35:746–751.
20. Yiginer O, Ozcelik F, Inanc T, Aparci M, Ozmen N, Cingozbay BY, Kardesoglu E, Suleymanoglu S, Sener G, Cebeci BS. Allopurinol improves endothelial function and reduces oxidant-inflammatory enzyme of myeloperoxidase in metabolic syndrome. *Clin Res Cardiol* 2008; 97:334–340.
21. Tavil Y, Kaya MG, Oktar SO, Sen N, Okyay K, Yazici HU, Cengel A. Uric acid level and its association with carotid intima-media thickness in patients with hypertension. *Atherosclerosis* 2008; 197:159–163.
22. Ishizaka N, Ishizaka Y, Toda E, Nagai R, Yamakado M. Association between serum uric acid, metabolic syndrome, and carotid atherosclerosis in Japanese individuals. *Arterioscler Thromb Vasc Biol* 2005; 25:1038–1044.
23. Kawamoto R, Tomita H, Oka Y, Ohtsuka N. Relationship between serum uric acid concentration, metabolic syndrome and carotid atherosclerosis. *Intern Med* 2006; 45:605–614.
24. Libby P, Ridker PM, Hansson GK; Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 2009; 54:2129–2138.
25. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003; 42:1149–1160.
26. Ter Avest E, Stalenhoef AF, de Graaf J. What is the role of non-invasive measurements of atherosclerosis in individual cardiovascular risk prediction? *Clin Sci* 2007; 112:507–516.
27. Kasprzak JD, Klosinska M, Drozdz J. Clinical aspects of assessment of endothelial function. *Pharmacol Rep* 2006; 58 Suppl:33–40.
28. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002; 106:3143–3421.

JACC: CARDIOVASCULAR INTERVENTIONS
© 2011 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.

VOL. 4, NO. 10, 2011
ISSN 1936-8798/$36.00
DOI: 10.1016/j.jcin.2011.05.025

STATE-OF-THE-ART PAPER

Vascular Inflammation and Repair

CME

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

Teruo Inoue, MD, PhD,* Kevin Croce, MD, PhD,†‡ Toshifumi Morooka, MD,§ Masashi Sakuma, MD,‖ Koichi Node, MD,‖ Daniel I. Simon, MD§

Tochigi and Saga, Japan; Boston and West Roxbury, Massachusetts; and Cleveland, Ohio

JACC: CARDIOVASCULAR INTERVENTIONS CME

This article has been selected as this issue's CME activity, available online at http://interventions.onlinejacc.org/ by selecting the CME tab on the top navigation bar.

Accreditation and Designation Statement

The American College of Cardiology Foundation (ACCF) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

The ACCF designates this journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit(s)™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Method of Participation and Receipt of CME Certificate

To obtain credit for this CME activity, you must:

1. Be an ACC member or *JACC: Cardiovascular Interventions* subscriber.
2. Carefully read the CME-designated article available online and in this issue of the journal.
3. Answer the post-test questions. At least 2 out of the 3 questions provided must be answered correctly to obtain CME credit.
4. Complete a brief evaluation.
5. Claim your CME credit and receive your certificate electronically by following the instructions given at the conclusion of the activity.

CME Objective for This article: After reading this paper, the reader should be able to: recognize the various contributors to early and late DES thrombosis; assess the favorable and adverse effects of DES on vascular inflammation, neointimal proliferation, re-endothelialization, and endothelial function; discuss the role of bone marrow-derived stem cells in restenosis and vascular repair as well as the role of local vascular inflammation on stem cell recruitment; and describe novel strategies to reduce smooth muscle proliferation and enhance re-endothelialization in next-generation DES.

CME Editor Disclosure: *JACC: Cardiovascular Interventions* CME Editor Habib Samady, MB, ChB, FACC, has research grants from the Wallace H. Coulter Foundation, Volcano Corp., St. Jude Medical, Forrest Pharmaceuticals Inc., and Pfizer Inc.

Author Disclosure: This work was supported in part by grants from the National Heart, Lung, and Blood Institute to Dr. Simon (HL85816, HL57506 MERIT Award, HL73852) and to Dr. Croce (1K08HL086672); a Future Leaders in Cardiovascular Medicine Fellowship Grant to Dr. Croce; an award from the Michael Lerner Foundation to Dr. Croce; a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a grant from Kimura Foundation to Drs. Inoue and Node; and by a research grant from the Japan Foundation of Cardiovascular Research to Drs. Inoue and Node. Dr. Simon is on the advisory board and is a consultant for Cordis/Johnson & Johnson and Medtronic Vascular; and is a consultant for Daiichi-Sankyo. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Medium of Participation: Print (article only); online (article and quiz).

CME Term of Approval:

Issue Date: October 2011
Expiration Date: September 30, 2012

From the *Department of Cardiovascular Medicine, Dokkyo Medical University, Tochigi, Japan; †Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; ‡Veteran's Administration Healthcare System, West Roxbury, Massachusetts; §University Hospitals Harrington-McLaughlin Heart and Vascular Institute, Case Western Reserve University School of Medicine, Cleveland, Ohio; and the

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 10, 2011
OCTOBER 2011:1057–66

Vascular Inflammation and Repair

Implications for Re-Endothelialization, Restenosis, and Stent Thrombosis

The cellular and molecular processes that control vascular injury responses after percutaneous coronary intervention involve a complex interplay among vascular cells and progenitor cells that control arterial remodeling, neointimal proliferation, and re-endothelialization. Drug-eluting stents (DES) improve the efficacy of percutaneous coronary intervention by modulating vascular inflammation and preventing neointimal proliferation and restenosis. Although positive effects of DES reduce inflammation and restenosis, negative effects delay re-endothelialization and impair endothelial function. Delayed re-endothelialization and impaired endothelial function are linked to stent thrombosis and adverse clinical outcomes after DES use. Compared with bare-metal stents, DES also differentially modulate mobilization, homing, and differentiation of vascular progenitor cells involved in re-endothelialization and neointimal proliferation. The effects of DES on vascular inflammation and repair directly impact clinical outcomes with these devices and dictate requirements for extended-duration dual antiplatelet therapy. (J Am Coll Cardiol Intv 2011;4:1057–66) © 2011 by the American College of Cardiology Foundation

Drug-eluting stents (DES) substantially reduce angiographic and clinical restenosis by 70% across broad patient and lesion subsets and decrease repeat target lesion interventions. The prototypical antiproliferative DES agents sirolimus (CYPHER stent, Cordis, Miami Lakes, Florida), paclitaxel (Taxus stent, Boston Scientific, Natick, Massachusetts), zotarolimus (Endeavor stent, Medtronic, Minneapolis, Minnesota), and everolimus (Xience stent, Abbott and Boston Scientific) have potent antimitotic actions that strongly inhibit smooth muscle proliferation and matrix production (1–3) and thus reduce neointimal formation and restenosis. Despite efficacy in reducing neointimal proliferation and restenosis, DES failure and restenosis still occurs and is more frequent in the settings of diabetes mellitus and during treatment of restenotic lesions, bypass grafts, and bifurcations (4–6). In addition to restenosis, concern has arisen about the potential for late thromboses or very late thromboses after DES implantation, and this concern has led to extended-duration dual antiplatelet therapy (7–9). Mechanisms of stent thrombosis might vary, depending on the timing of the event (10). Acute stent thrombosis (within 24 h of implantation) and early stent thrombosis (within 30 days) are likely related to mechanical issues with the stent, inadequate platelet inhibition, or pro-thrombotic patient risk factors. In contrast, late stent thrombosis (up to 1 year) and very late stent thrombosis (after 1 year) have been attributed to delayed re-endothelialization and inhibition of vascular repair. The potential for delayed re-endothelialization and inhibition of vascular repair is particularly important after implantation of DES, because the antiproliferative agents used to prevent smooth muscle cell proliferation also delay re-endothelialization in the stented segment (11,12). Angioscopic (13) and pathological (11,12,14,15) evidence suggests that there is delayed arterial healing with DES, compared with bare-metal stents (BMS), because DES-treated arteries have more histological evidence of incomplete re-endothelialization, chronic inflammatory cell infiltration, fibrin deposition, and platelet activation. It is important to recognize that inflammatory and thrombotic pathways share common signaling pathways and that inflammatory responses promote activation of the clotting cascade and stimulate platelet activation (reviewed in Croce and Libby [16]). Experimental studies also suggest that delayed arterial healing and DES-associated inflammation is greatest at sites of overlapping DES with placement of multiple stents (17). The finding of increased inflammation in areas of stent overlap suggests a possible molecular mechanism to explain higher stent thrombosis rates that are associated with overlapping stents.

‖Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga, Japan. This work was supported in part by grants from the National Heart, Lung, and Blood Institute to Dr. Simon (HL85816, HL57506 MERIT Award, HL73852) and to Dr. Croce (1K08HL086672); a Future Leaders in Cardiovascular Medicine Fellowship Grant to Dr. Croce; an award from the Michael Lerner Foundation to Dr. Croce; a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a grant from Kimura Foundation to Drs. Inoue and Node; and by a research grant from the Japan Foundation of Cardiovascular Research to Drs. Inoue and Node. Dr. Simon is on the advisory board and is a consultant for Cordis/Johnson & Johnson and Medtronic Vascular; and is a consultant for Daiichi-Sankyo. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. The first two authors contributed equally to this work.

Manuscript received August 20, 2010; revised manuscript received February 22, 2011, accepted May 3, 2011.

In addition to antiproliferative drug-associated delayed healing with DES, stent-induced or polymer-induced inflammation has also been identified as a possible contributor to stent thrombosis, especially because late and very late stent thrombosis occurs long after antiproliferative dugs have been eluted from the polymer (18–20). Inflammatory responses to drug, stent, or polymer might result from nonspecific innate immune responses, which have a predominance of monocyte/macrophage infiltrates, or might be related to antigen-specific adaptive immune hypersensitivity responses typified by infiltration of eosinophils, B-cells, and T-cells (reviewed in Byrne et al. [21]). Several studies have also implicated DES-polymer-induced inflammation in the pathobiology of restenosis and stent thrombosis (18,19). Currently, the 4 stent platforms approved for use by the U.S. Food and Drug Administration use different nonerodible polymeric coatings for drug delivery, and experimental animal studies suggest that biological compatibility, immunogenicity, and thrombogenicity might vary among specific polymeric compounds (22). The next generations of DES represent an attempt to reduce the possibility of polymer-induced inflammation, delayed arterial healing, restenosis, and stent thrombosis through use of polymers that have better biocompatibility and/or are biodegradable.

Emerging evidence indicates that compared to BMS, DES impair endothelial function in arterial segments distal to the stented site (23,24). Even 6 months after implantation of DES, artery segments distal to the DES show abnormal vasoreactivity (25–27). DES-associated abnormalities in endothelial function could be related to delayed vascular repair and not the DES drug itself, because the kinetics of DES are such that the drugs are completely eluted within months after implantation (28–31). It is possible, however, that in certain circumstances drug accumulation in the arterial wall (32) and the lipophilic core of stented atheroma results in prolonged drug retention/release and ongoing vascular dysfunction. The mechanism of DES-associated endothelial dysfunction is not established, and recent studies have demonstrated that there is variability in the severity of DES-associated endothelial dysfunction among specific DES agents (33–35). It is unclear whether DES-associated vascular dysfunction influences clinical outcomes after DES implantation. One small study demonstrated impaired endothelial function in patients presenting with in-stent restenosis, compared with matched control subjects (36); however, this association will require validation in larger prospective investigations.

Because of the potential for delayed re-endothelialization and repair with DES, concern was raised about possible increased mortality and late stent thrombosis following DES implantation (reviewed in Garg and Mauri [7]). Because of the insufficient power of individual trials to assess the low-incidence events of late and very late stent thrombosis, multiple meta-analyses were performed to evaluate the risk of stent thrombosis in patients treated with DES versus BMS (37–41). These meta-analyses and subsequent analyses of stent registry data (42–45) demonstrated nearly equivalent risk of stent thrombosis (approximately 0.5%) in patients treated with DES or BMS. A small increase in the risk of late and very late stent thrombosis on the order of 1% to 2% cannot be excluded, however, because available data have insufficient power to evaluate this very rare event.

Analyses of stent thrombosis and outcomes with DES are further complicated by significant differences in stent structure, drug delivery polymers, and antiproliferative drugs among the rapidly expanding panel of DES. In addition, complex biology controls vascular repair after percutaneous coronary intervention (PCI). Understanding the common and differential molecular pathways that regulate re-endothelialization versus restenosis will provide a biological context for rational use of DES and will enable development of new DES technologies that can inhibit neointimal proliferation and preserve or even promote endothelial repair. In the following sections, we will highlight key cellular and molecular pathways that regulate vascular injury and repair in the setting of percutaneous coronary revascularization, and we will discuss the role of DES in modulating vascular repair processes.

Abbreviations and Acronyms

BMS = bare-metal stent(s)
DES = drug-eluting stent(s)
EPC = endothelial progenitor cell
G-CSF = granulocyte colony-stimulating factor
PCI = percutaneous coronary intervention
SDF = stromal cell-derived factor
SMPC = smooth muscle progenitor cell

Role of Inflammation in Restenosis and Vascular Repair

Stent placement leads to mechanical injury that induces substantial local inflammation, which stimulates vascular smooth muscle cell proliferation and extracellular matrix deposition, resulting in neointimal thickening and restenosis (46,47). Vascular inflammation after PCI involves complex interactions between multiple vascular cell types, and under normal circumstances, the cellular and molecular processes that control vascular injury responses direct repair and vascular healing. In pathological conditions, dysregulation of vascular repair results in persistent vascular inflammation, neointimal proliferation, and restenotic obstruction of the stent lumen.

Immediately after PCI, platelets, neutrophils, and monocytes play a central role in the initial inflammatory response (47,48). Platelets and fibrin deposit on the de-endothelialized vessel wall and recruit leukocytes to the injured vessel segment through a cascade of cell adhesion molecules that direct leukocyte attachment and transmigration across surface-adherent platelets (49). The initial tethering and

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 10, 2011
OCTOBER 2011:1057–66

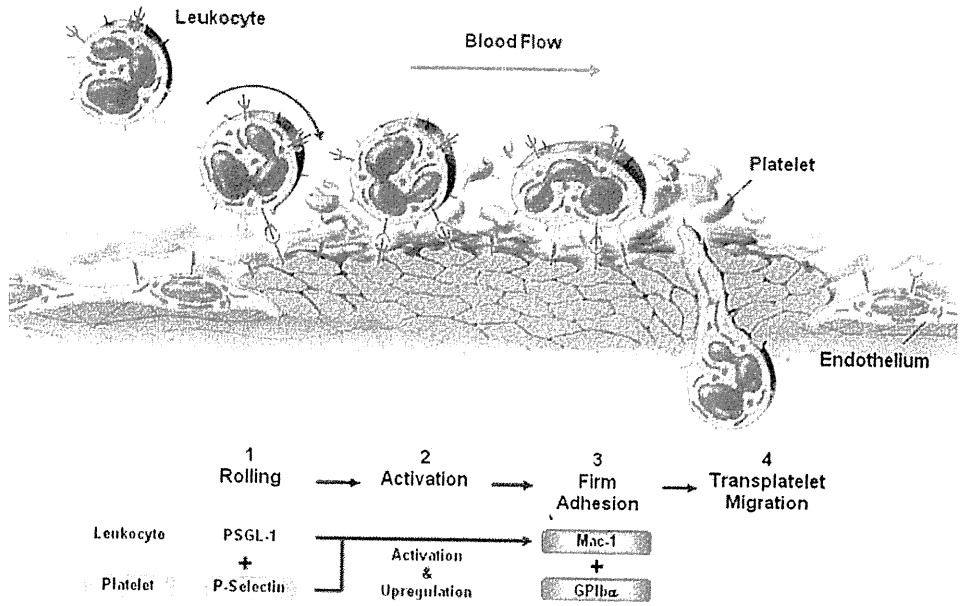

Figure 1. **Transplatelet Leukocyte Migration**

At the site of stent implantation after percutaneous coronary intervention, endothelial cells are denuded, and the subendothelial matrix is exposed to flowing blood. Platelets and fibrinogen immediately adhere to the surface of the injured vessel. A multistep cascade of platelet and leukocyte adhesion molecules direct leukocyte adhesion to the adherent platelets in a process termed "secondary capture." Leukocyte capture and rolling are mediated by interaction between platelet P-selectin and leukocyte P-selectin glycoprotein ligand (PSGL)-1. Arrest and firm adhesion are mediated by platelet glycoprotein (GP) Ib-alpha and leukocyte Mac-1. Chemokines stimulate transmigration into the extraluminal tissue.

rolling of leukocytes on platelets is mediated through binding of the leukocyte receptor P-selectin glycoprotein ligand-1 to platelet P-selectin (50–52). Rolling leukocytes stop and firmly attach to adherent platelets when the leukocyte integrin Mac-1 (CD11b/CD18) binds to platelet glycoprotein Ib-alpha (53) or to fibrinogen bound to the platelet glycoprotein IIb/IIIa (Fig. 1) (54). A direct role for Mac-1 in leukocyte adhesion after mechanical injury has been demonstrated in several experimental studies where Mac-1 targeting reduces neointimal thickening after experimental angioplasty (55,56). Clinical studies of patients undergoing PCI further support the premise that Mac-1 and platelet-mediated leukocyte adhesion (also termed "secondary capture") plays an important role in vascular inflammation and restenosis after coronary stenting. We have previously shown that, compared with circulating neutrophils, Mac-1 surface expression is significantly increased in the neutrophils obtained from the coronary sinus of patients who underwent PCI within the preceding 48 h and that high levels of Mac-1 expression are associated with angiographic late lumen loss and increased risk of restenosis (57–60). Increased Mac-1 expression also correlates with increased expression of P-selectin on the surface of platelets obtained from the coronary sinus after PCI (57–60).

Role of Bone Marrow-Derived Stem Cells in Restenosis and Vascular Repair

Emerging research is demonstrating that bone marrow-derived progenitor cells play an important role in vascular inflammation responses and in vascular repair. Endothelial progenitor cells (EPCs) mobilized from bone marrow into peripheral blood promote endothelial regeneration and postnatal neovascularization (61,62). In contrast to the potential protective effects of EPCs, it has been hypothesized that smooth muscle progenitor cells (SMPCs), which are also mobilized from bone marrow, migrate to the sites of vascular injury where they contribute to smooth muscle cell expansion and neointimal proliferation (63–65).

The precise function of EPCs and SMPCs once they home to sites of vascular inflammation is controversial. Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Transdifferentiation of CD34-positive cells into EPC or SMPC lineages depends on the local environment; ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization (61,66), and inflammatory

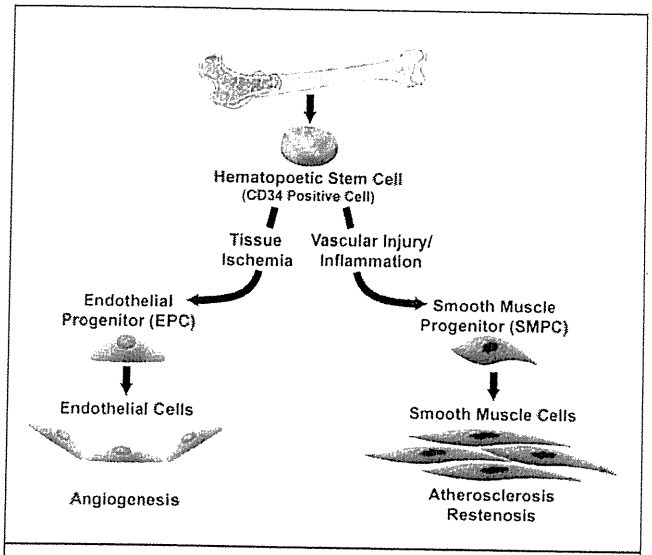

Figure 2. **Differentiation of Bone Marrow-Derived Stem Cells**

Previously, CD34-positive cells were believed to be committed to develop into EPCs; however, further study demonstrated that the CD34 surface antigen actually identifies undifferentiated bone marrow-derived stem cells that have the ability to differentiate into EPC and SMPCs. Ischemic conditions signal differentiation toward EPC phenotypes to promote re-endothelialization. Inflammatory conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation.

conditions signal differentiation toward SMPC phenotypes that promote neointimal proliferation (63) (Fig. 2).

Several studies have implicated CD34-positive progenitor cells in vascular injury responses after PCI. Circulating CD34-positive cells are increased in the days after acute myocardial infarction, and characterization of these circulating cells suggests that they have an EPC-like phenotype, raising the possibility that CD34-positive EPC-like cells are mobilized to promote angiogenesis in the ischemic myocardium. In contrast to ischemia-mediated mobilization, SMPC-like CD34-positive cells increase after PCI in patients with chronic coronary artery disease, presumably in response to inflammatory mediators produced at sites of stent implantation (67). In this setting, elevated levels of circulating CD34-positive cells are associated with increased rates of restenosis, suggesting possible involvement in neointimal formation (68).

We have also demonstrated that molecular signals generated at sites of local arterial inflammation promote the mobilization of CD34-positive stem cells (69). In our study, the number of CD34-positive cells in the peripheral blood increased Day 7 to 14 after PCI, and patients who received BMS had significantly more CD34-positive cells than those who received DES (Fig. 3A) (69,70). Granulocyte colony-stimulating factor (G-CSF) and Mac-1 levels were significantly reduced in patients who underwent implantation of DES, compared with those who received BMS, suggesting that the antiproliferative stent drug attenuated inflammatory cell activation (Fig. 3B) (69). This observation is consistent with our hypothesis that inflammatory signals generated at sites of coronary injury mobilize bone marrow-derived progenitor cells involved in vascular repair. To further elucidate the role of CD34-positive cells in vascular injury and repair after PCI, we isolated circulating CD34-positive progenitor cells from patients who received DES and BMS and performed in vitro differentiation assays (Fig. 4) (69). In most patients, a proportion of the cultured CD34-positive cells differentiated into both CD31-positive endothelial-like cells and into alpha-actin-positive cells with features suggestive of smooth muscle cell lineage. Several other observations were made. First, the number of differentiated colonies that formed from the CD34-positive cells correlated with the extent of restenosis during angiographic follow-up. Second, patients with more angiographic restenosis had more CD34-positive cells that differentiated into alpha-actin containing SMPC-like cells. Third, implantation of sirolimus-eluting stents resulted in reduced differentiation of CD34-positive cells into CD31-positive cells and reduced differentiation into alpha-actin-positive cells with smooth muscle cell features. This finding is consistent with in vitro data demonstrating that sirolimus inhibits differentiation of human bone marrow-derived stem cells into endothelial or smooth muscle cells (71,72).

Several lines of evidence support the premise that PCI induces local inflammatory signals that mobilize bone marrow-derived CD34-positive stem cells and that these cells have the ability to differentiate along endothelial or smooth muscle cell lines. In the setting of vascular injury, there seems to be a balance between endothelial-like stem cell responses that favor re-endothelialization and smooth muscle-like stem cell responses that promote restenosis (Fig. 2). Furthermore, it seems that, compared with BMS, sirolimus-eluting stent implantation attenuates production of local inflammatory signals that promote stem cell mobilization and differentiation into smooth muscle-like cells that contribute to neointimal proliferation. In the future, targeted pharmacological therapies might be able to promote reparative progenitor cell responses and/or inhibit responses that result in excess neointimal proliferation.

Local Vascular Inflammation Signals Stem Cell Recruitment

As described in the preceding text, inflammatory and hematopoietic cytokines produced locally at sites of vascular inflammation direct mobilization of stem cells from the bone marrow. Vascular-derived molecules involved in stem cell mobilization include G-CSF, matrix metalloproteinase-9, and stromal cell-derived factor (SDF)-1.

G-CSF, a potent hematopoietic cytokine produced by endothelium and immune cells, is expressed at sites of

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 10, 2011
OCTOBER 2011:1057–66

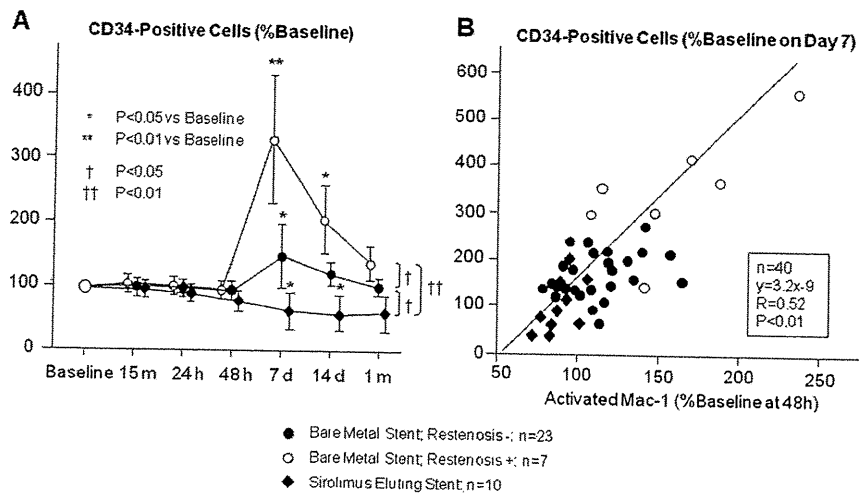

Figure 3. **CD34-Positive Cell Counts and CD34-Positive Cell Mac-1 Expression After PCI**

(**A**) Circulating CD34-positive cells increase after percutaneous coronary intervention (PCI). The highest levels of CD34-positive cells were seen in the peripheral blood of patients who received bare-metal stents that went on to have restenosis at 6-month (m) angiographic follow-up (Bare-Metal Stent Restenosis +). Implantation of drug-eluting stent was associated with a significant reduction in the number of circulating CD34-positive cells. (**B**) Neutrophil Mac-1 expression correlates with mobilization of CD34-positive cells. Forty-eight hours after PCI, neutrophils were harvested from the coronary sinus of patients who had coronary stents implanted. Neutrophil Mac-1 expression was quantified by flow cytometry. Neutrophil Mac-1 expression at 48 h correlated with circulating levels of CD34-positive cells 7 days (d) after PCI, demonstrating that higher levels of local vascular inflammation are associated with increased systemic CD34-positive progenitor cell mobilization. Data are expressed as percentage change of the baseline values. Adapted, with permission, from Inoue et al. (69).

vascular injury (73). G-CSF promotes stem cell proliferation and mobilization, and it has been hypothesized that, after PCI and/or myocardial infarction, G-CSF signals production and homing of reparative stem cells that promote angiogenesis and myocardial repair. Clinical evaluation of systemic G-CSF therapy after myocardial infarction failed to show benefit in limiting infarct size or in improving left ventricular function, despite its experimental effects on stem mobilization (74,75). It is possible that the nonselective mobilization of both EPCs and SMPCs by G-CSF might limit its therapeutic value for treating restenosis and promoting vascular repair.

Neutrophil-derived matrix metalloproteinase-9 is another inflammatory mediator that has a role in stem cell mobilization (76). Matrix metalloproteinase-9 is secreted locally in response to inflammatory inputs, including ligand binding to the leukocyte integrin Mac-1 (77). Matrix metalloproteinase-9 is required for G-CSF and chemokine-induced mobilization of hematopoietic stem cells from the bone marrow (78,79) and provides a mechanism through which inflamed vascular beds generate systemic signals that promote bone marrow-derived stem cell mobilization.

SDF-1 is a member of the CXC group of chemokines that plays a role in stem cell plasticity and engraftment (80). SDF-1 is expressed by smooth muscle cells at sites of atherosclerosis and vascular inflammation. SDF-1 signals the bone marrow to mobilize Sca-1^+ lineage progenitor cells that home to sites of vascular injury where the progenitor cells adopt smooth muscle cell phenotypes. In experimental models, SDF-1 directly regulates neointimal smooth muscle cell content, and inhibition of SDF-1 function decreases neointimal formation (80). Therapies targeting SDF-1 function could potentially inhibit restenosis after PCI.

Modulating Vascular Injury and Repair: New Frontiers in DES Technology

Current-generation DES agents prevent restenosis by inhibiting smooth muscle cell proliferation. In developing the next generation of DES agents it might be possible to harness differential drug effects on smooth muscle cell proliferation versus re-endothelialization in a manner that could accelerate repair. Vascular endothelial growth factor has attracted attention as a DES agent that could promote endothelial regeneration and angiogenesis (81). Proof-of-concept investigations have demonstrated that vascular endothelial growth factor gene-eluting stents accelerate re-endothelialization and reduce in-stent neointimal area in animal models (82). Another new strategy to promote vascular repair after PCI involves the use of antibodies (83) or peptides (84) that bind membrane receptors on circulating endothelial progenitor cells. This strategy promotes capture of these cells to accelerate healing (83). CD34 antibody-coated stents have been implanted in human

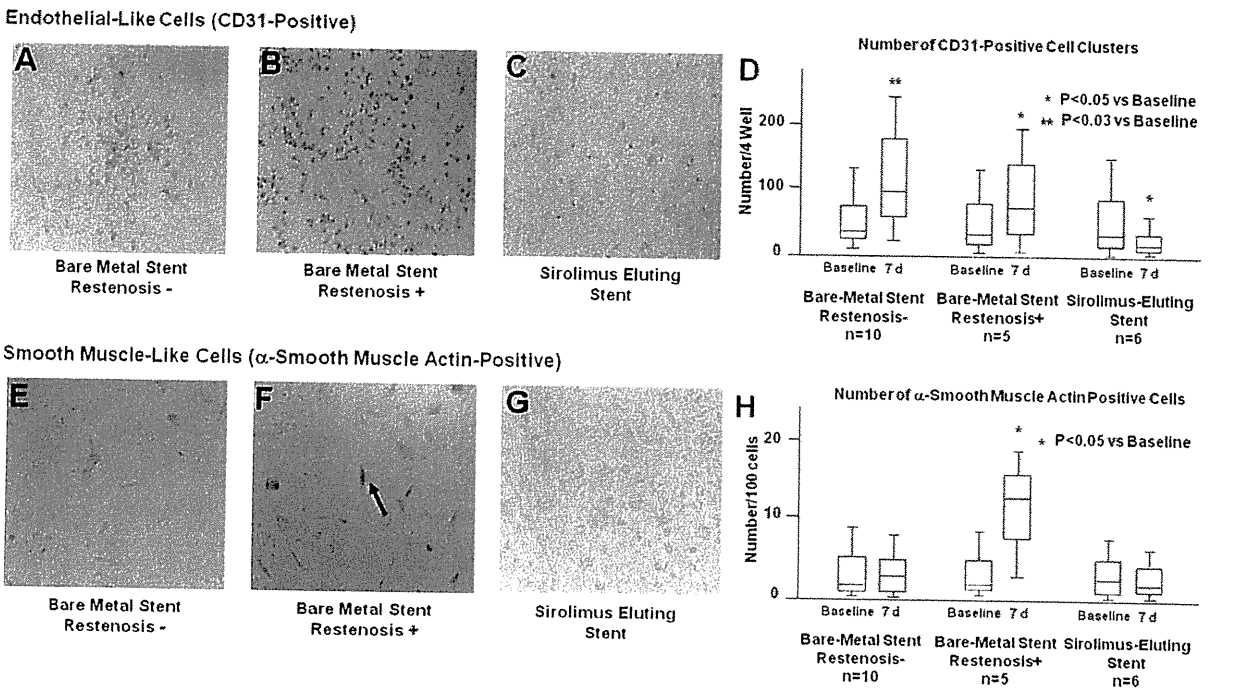

Figure 4. **Differentiation of Patient-Derived CD34-Positive Stem Cells Into Endothelial-Like and Smooth Muscle-Like Cells After PCI**

Circulating CD34-positive stem cells were isolated from peripheral blood of patients 7 days (d) after implantation of bare-metal stent (BMS) or sirolimus-eluting stent (SES). Immunohistochemical staining for CD31 (**A to D**). (**A**) BMS without restenosis, (**B**) BMS with restenosis, (**C**) SES, (**D**) quantification of CD31-positive cell clusters. Patients who received BMS had similar differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, regardless of whether they went on to have restenosis at 6-month angiographic follow-up. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into CD31-positive endothelial-like cells, compared with patients that received BMS. Actin staining (**E to H**). (**E**) BMS without restenosis, (**F**) BMS with restenosis, (**G**) SES, (**H**) quantification of actin positive cells. Patients who received BMS and went on to have restenosis at 6-month angiographic follow-up had increased numbers of CD34-positive stem cells that differentiated into actin-positive smooth muscle-like cells. Patients who received SES had a significant reduction in the differentiation of CD34-positive stem cells into actin-positive smooth muscle-like cells, compared with patients that received BMS. **Arrow** denotes representative actin-positive cell. Adapted, with permission, from Inoue et al. (69).

coronary arteries in the multicenter HEALING (Healthy Endothelial Accelerated Lining Inhibits neointimal Growth) II pilot trial and in later follow-up studies (85,86). The long-term safety and efficacy of this pro-healing stent technology awaits further evaluation in randomized trials.

In addition to DES technology itself, adjunctive systemic medications might also influence stem cell homing and the balance between re-endothelialization and neointimal proliferation. Interestingly, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) were recently shown to promote EPC proliferation in vitro (87) and increase the number of circulating EPCs in patients with coronary artery disease (88). Despite initial optimism that statins might favorably influence arterial healing after DES implantation, enthusiasm has tempered after release of data showing that high doses of statins started before PCI and continued thereafter increased EPC mobilization but did not increase circulating $CD34^+$ cells and did not improve the angiographic outcome after implantation of a bioengineered EPC-capture stent (89).

Thiazolidinediones, which are used to treat diabetes, function by activating peroxisome proliferator activating receptor transcription factors. Several thiazolidinedione agents increase the number of EPCs in both circulating blood and bone marrow and reduce EPC apoptosis in a phosphatidylinositol 3-kinase-dependent manner (90). Although there are several potential vasculoprotective actions of statins and thiazilidinediones, further clinical investigation will be required to determine whether these medications will positively influence vascular repair, resulting in reduced rates of restenosis and enhanced re-endothelialization after PCI.

Conclusions

Percutaneous coronary intervention results in mechanical injury that induces vascular inflammation. Vascular inflammation involves complex interactions between endothelial cells, smooth muscle cells, platelets, and inflammatory cells, including neutrophils, monocytes, and lymphocytes. Signaling molecules produced by cells at the site of vascular injury

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 10, 2011
OCTOBER 2011:1057–66

stimulate mobilization of bone marrow-derived EPCs and SMPCs, which are recruited to the sites of vascular inflammation. The cellular and molecular processes that control vascular injury responses direct repair and vascular healing; however, dysregulation of these responses can result in adverse arterial remodeling, neointimal proliferation, and restenosis. Drug-eluting stents effectively reduce neointimal proliferation but they slow re-endothelialization and healing. Drug-eluting stents also seem to influence the mobilization, homing, and differentiation of reparative stem cells. Despite the potential for DES-induced delayed vascular healing, clinical trial investigations have demonstrated similar safety of DES and BMS in the setting of extended dual antiplatelet therapy. In the future, improved DES technologies have the potential to abolish restenosis and further improve stent safety by inhibiting maladaptive neointimal proliferation while promoting re-endothelialization and repair.

Reprint requests and correspondence: Dr. Kevin J. Croce, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, NRB 740, Boston, Massachusetts 02132. E-mail: kcroce@partners.org.

REFERENCES

1. Gouëffic Y, Potter-Perigo S, Chan CK, et al. Sirolimus blocks the accumulation of hyaluronan (HA) by arterial smooth muscle cells and reduces monocyte adhesion to the ECM. Atherosclerosis 2007;195:23–30.
2. Hilker M, Buerke M, Guckenbiehl M, et al. Rapamycin reduces neointima formation during vascular injury. VASA 2003;32:10–3.
3. Park J, Ha H, Ahn HJ, et al. Sirolimus inhibits platelet-derived growth factor-induced collagen synthesis in rat vascular smooth muscle cells. Transplant Proc 2005;37:3459–62.
4. Bhatia V, Bhatia R, Dhindsa M. Drug-eluting stents: new era and new concerns. Postgrad Med J 2004;80:13–8.
5. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. Circulation 2005;111:2257–73.
6. Lemos PA, van Mieghem CA, Arampatzis CA, et al. Post-sirolimus-eluting stent restenosis treated with repeat percutaneous intervention: late angiographic and clinical outcomes. Circulation 2004;109:2500–2.
7. Garg P, Mauri L. The conundrum of late and very late stent thrombosis following drug-eluting stent implantation. Curr Opin Cardiol 2007;22:565–71.
8. McFadden EP, Stabile E, Regar E, et al. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. Lancet 2004;364:1519–21.
9. Webster MW, Ormiston JA. Drug-eluting stents and late stent thrombosis. Lancet 2007;370:914–5.
10. Jaffe R, Strauss BH. Late and very late thrombosis of drug-eluting stents: evolving concepts and perspectives. J Am Coll Cardiol 2007;50:119–27.
11. Joner M, Finn AV, Farb A, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 2006;48:193–202.
12. Nakazawa G, Finn AV, Joner M, et al. Delayed arterial healing and increased late stent thrombosis at culprit sites after drug-eluting stent placement for acute myocardial infarction patients: an autopsy study. Circulation 2008;118:1138–45.
13. Kotani J, Awata M, Nanto S, et al. Incomplete neointimal coverage of sirolimus-eluting stents: angioscopic findings. J Am Coll Cardiol 2006;47:2108–11.
14. Finn AV, Joner M, Nakazawa G, et al. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. Circulation 2007;115:2435–41.
15. Finn AV, Nakazawa G, Joner M, et al. Vascular responses to drug eluting stents: importance of delayed healing. Arterioscler Thromb Vasc Biol 2007;27:1500–10.
16. Croce K, Libby P. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol 2007;14:55–61.
17. Finn AV, Kolodgie FD, Harnek J, et al. Differential response of delayed healing and persistent inflammation at sites of overlapping sirolimus- or paclitaxel-eluting stents. Circulation 2005;112:270–8.
18. Nebeker JR, Virmani R, Bennett CL, et al. Hypersensitivity cases associated with drug-eluting coronary stents: a review of available cases from the Research on Adverse Drug events And Reports (RADAR) project. J Am Coll Cardiol 2006;47:175–81.
19. Virmani R, Guagliumi G, Farb A, et al. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious? Circulation 2004;109:701–5.
20. Pallero MA, Talbert Roden M, Chen YF, et al. Stainless steel ions stimulate increased thrombospondin-1-dependent TGF-beta activation by vascular smooth muscle cells: implications for in-stent restenosis. J Vasc Res 2010;47:309–22.
21. Byrne RA, Joner M, Kastrati A. Polymer coatings and delayed arterial healing following drug-eluting stent implantation. Minerva Cardioangiol 2009;57:567–84.
22. Wilson GJ, Nakazawa G, Schwartz RS, et al. Comparison of inflammatory response after implantation of sirolimus- and paclitaxel-eluting stents in porcine coronary arteries. Circulation 2009;120:141–9, 1–2.
23. Fuke S, Maekawa K, Kawamoto K, et al. Impaired endothelial vasomotor function after sirolimus-eluting stent implantation. Circ J 2007;71:220–5.
24. Shin DI, Kim PJ, Seung KB, et al. Drug-eluting stent implantation could be associated with long-term coronary endothelial dysfunction. Int Heart J 2007;48:553–67.
25. Maekawa K, Kawamoto K, Fuke S, et al. Images in cardiovascular medicine. Severe endothelial dysfunction after sirolimus-eluting stent implantation. Circulation 2006;113:e850–1.
26. Togni M, Windecker S, Cocchia R, et al. Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. J Am Coll Cardiol 2005;46:231–6.
27. Hofma SH, van der Giessen WJ, van Dalen BM, et al. Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. Eur Heart J 2006;27:166–70.
28. Tesfamariam B. Drug release kinetics from stent device-based delivery systems. J Cardiovasc Pharmacol 2008;51:118–25.
29. Kamath KR, Barry JJ, Miller KM. The Taxus drug-eluting stent: a new paradigm in controlled drug delivery. Adv Drug Deliv Rev 2006;58:412–36.
30. Waugh J, Wagstaff AJ. The paclitaxel (TAXUS)-eluting stent: a review of its use in the management of de novo coronary artery lesions. Am J Cardiovasc Drugs 2004;4:257–68.
31. McKeage K, Murdoch D, Goa KL. The sirolimus-eluting stent: a review of its use in the treatment of coronary artery disease. Am J Cardiovasc Drugs 2003;3:211–30.
32. Raman VK, Edelman ER. Coated stents: local pharmacology. Semin Interv Cardiol 1998;3:133–7.
33. Shin DI, Seung KB, Kim PJ, et al. Long-term coronary endothelial function after zotarolimus-eluting stent implantation. A 9 month comparison between zotarolimus-eluting and sirolimus-eluting stents. Int Heart J 2008;49:639–52.
34. Kim JW, Suh SY, Choi CU, et al. Six-month comparison of coronary endothelial dysfunction associated with sirolimus-eluting stent versus paclitaxel-eluting stent. J Am Coll Cardiol Intv 2008;1:65–71.
35. Hamilos MI, Ostojic M, Beleslin B, et al. Differential effects of drug-eluting stents on local endothelium-dependent coronary vasomotion. J Am Coll Cardiol 2008;51:2123–9.
36. Thanyasiri P, Kathir K, Celermajer DS, Adams MR. Endothelial dysfunction and restenosis following percutaneous coronary intervention. Int J Cardiol 2007;119:362–7.
37. Bavry AA, Kumbhani DJ, Helton TJ, Bhatt DL. Risk of thrombosis with the use of sirolimus-eluting stents for percutaneous coronary

intervention (from registry and clinical trial data). Am J Cardiol 2005;95:1469–72.
38. Bavry AA, Kumbhani DJ, Helton TJ, Bhatt DL. What is the risk of stent thrombosis associated with the use of paclitaxel-eluting stents for percutaneous coronary intervention?: A meta-analysis. J Am Coll Cardiol 2005;45:941–6.
39. Cutlip DE, Windecker S, Mehran R, et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation 2007;115:2344–51.
40. Moreno R, Fernández C, Hernández R, et al. Drug-eluting stent thrombosis: results from a pooled analysis including 10 randomized studies. J Am Coll Cardiol 2005;45:954–9.
41. Stettler C, Wandel S, Allemann S, et al. Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. Lancet 2007;370:937–48.
42. Ong AT, Hoye A, Aoki J, et al. Thirty-day incidence and six-month clinical outcome of thrombotic stent occlusion after bare-metal, sirolimus, or paclitaxel stent implantation. J Am Coll Cardiol 2005;45:947–53.
43. Ong AT, Serruys PW, Aoki J, et al. The unrestricted use of paclitaxel-versus sirolimus-eluting stents for coronary artery disease in an unselected population: one-year results of the Taxus-Stent Evaluated at Rotterdam Cardiology Hospital (T-SEARCH) registry. J Am Coll Cardiol 2005;45:1135–41.
44. Urban P, Gershlick AH, Guagliumi G, et al. Safety of coronary sirolimus-eluting stents in daily clinical practice: one-year follow-up of the e-Cypher registry. Circulation 2006;113:1434–41.
45. Williams DO, Abbott JD, Kip KE, DEScover Investigators. Outcomes of 6906 patients undergoing percutaneous coronary intervention in the era of drug-eluting stents: report of the DEScover Registry. Circulation 2006;114:2154–62.
46. Tanaka H, Sukhova GK, Swanson SJ, et al. Sustained activation of vascular cells and leukocytes in the rabbit aorta after balloon injury. Circulation 1993;88:1788–803.
47. Welt FG, Rogers C. Inflammation and restenosis in the stent era. Arterioscler Thromb Vasc Biol 2002;22:1769–76.
48. Welt FG, Edelman ER, Simon DI, Rogers C. Neutrophil, not macrophage, infiltration precedes neointimal thickening in balloon-injured arteries. Arterioscler Thromb Vasc Biol 2000;20:2553–8.
49. Evangelista V, Manarini S, Rontondo S, et al. Platelet/polymorphonuclear leukocyte interaction in dynamic conditions: evidence of adhesion cascade and cross talk between P-selectin and the b2 integrin cd11b/cd18. Blood 1996;88:4183–94.
50. Hamburger SA, McEver RP. Gmp-140 mediates adhesion of stimulated platelets to neutrophils. Blood 1990;75:550–4.
51. Larsen E, Celi A, Gilbert GE, et al. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. Cell 1989;59:305–12.
52. McEver RP, Cummings RD. Role of psgl-1 binding to selectins in leukocyte recruitment. J Clin Invest 1997;100:S97–103.
53. Simon DI, Chen Z, Xu H, et al. Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med 2000;192:193–204.
54. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. Blood 1996;88:146–57.
55. Rogers C, Edelman ER, Simon DI. A mAb to the beta2-leukocyte integrin Mac-1 (CD11b/CD18) reduces intimal thickening after angioplasty or stent implantation in rabbits. Proc Natl Acad Sci U S A 1998;95:10134–9.
56. Simon DI, Dhen Z, Seifert P, Edelman ER, Ballantyne CM, Rogers C. Decreased neointimal formation in Mac-1(−/−) mice reveals a role for inflammation in vascular repair after angioplasty. J Clin Invest 2000;105:293–300.
57. Inoue T, Sakai Y, Hoshi K, Yaguchi I, Fujito T, Morooka S. Lower expression of neutrophil adhesion molecule indicates less vessel wall injury and might explain lower restenosis rate after cutting balloon angioplasty. Circulation 1998;97:2511–8.
58. Inoue T, Sakai Y, Morooka S, Hayashi T, Takayanagi K, Takabatake Y. Expression of polymorphonuclear leukocyte adhesion molecules and its clinical significance in patients treated with percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 1996;28:1127–33.
59. Inoue T, Sohma R, Miyazaki T, Iwasaki Y, Yaguchi I, Morooka S. Comparison of activation process of platelets and neutrophils after coronary stent implantation versus balloon angioplasty for stable angina pectoris. Am J Cardiol 2000;86:1057–62.
60. Inoue T, Uchida T, Yaguchi I, Sakai Y, Takayanagi K, Morooka S. Stent-induced expression and activation of the leukocyte integrin Mac-1 is associated with neointimal thickening and restenosis. Circulation 2003;107:1757–63.
61. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–6.
62. Murohara T, Ikeda H, Duan J, et al. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 2000;105:1527–36.
63. Sata M, Saiura A, Kunisato A, et al. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med 2002;8:403–9.
64. Caplice NM, Bunch TJ, Stalboerger PG, et al. Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. Proc Natl Acad Sci U S A 2003;100:4754–9.
65. Strauss BH, MacLeod DC, de Feyter PJ, et al. Analysis of VNTR loci amplified by the polymerase chain reaction for investigating the origin of intimal smooth muscle cells in a coronary artery lesion developing after heart transplantation in man. Am Heart J 1993;125:1176–80.
66. Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 2001;103:634–7.
67. Shintani S, Murohara T, Ikeda H, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001;103:2776–9.
68. Schober A, Hoffmann R, Oprée N, et al. Peripheral cd34+ cells and the risk of in-stent restenosis in patients with coronary heart disease. Am J Cardiol 2005;96:1116–22.
69. Inoue T, Sata M, Hikichi Y, et al. Mobilization of cd34-positive bone marrow-derived cells after coronary stent implantation: impact on restenosis. Circulation 2007;115:553–61.
70. Elemer GS, Edgington TS. Two independent sets of monoclonal antibodies define neoepitopes linked to soluble ligand binding and leukocyte adhesion functions of activated alpha M beta 2. Circ Res 1994;75:165–71.
71. Fukuda D, Sata M, Tanaka K, Nagai R. Potent inhibitory effect of sirolimus on circulating vascular progenitor cells. Circulation 2005;111:926–31.
72. Imanishi T, Kobayashi K, Kuki S, Takahashi C, Akasaka T. Sirolimus accelerates senescence of endothelial progenitor cells through telomerase inactivation. Atherosclerosis 2006;189:288–96.
73. Chen X, Kelemen SE, Autieri MV. Expression of granulocyte colony-stimulating factor is induced in injured rat carotid arteries and mediates vascular smooth muscle cell migration. Am J Physiol Cell Physiol 2005;288:C81–8.
74. Zohlnhöfer D, Ott I, Mehilli J, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. JAMA 2006;295:1003–10.
75. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the magic cell randomised clinical trial. Lancet 2004;363:751–6.
76. Starckx S, Van den Steen PE, Wuyts A, Van Damme J, Opdenakker G. Neutrophil gelatinase B and chemokines in leukocytosis and stem cell mobilization. Leuk Lymphoma 2002;43:233–41.
77. Wize J, Sopata I, Smerdel A, Maśliński S. Ligation of selectin L and integrin CD11b/CD18 (Mac-1) induces release of gelatinase B (MMP-9) from human neutrophils. Inflamm Res 1998;47:325–7.
78. Heissig B, Hattori K, Dias S, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002;109:625–37.
79. Pelus LM, Bian H, King AG, Fukuda S. Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. Blood 2004;103:110–9.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 4, NO. 10, 2011
OCTOBER 2011:1057–66

80. Schober A, Knarren S, Lietz M, Lin EA, Weber C. Crucial role of stromal cell-derived factor-1alpha in neointima formation after vascular injury in apolipoprotein e-deficient mice. Circulation 2003;108:2491–7.
81. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995;1:27–31.
82. Walter DH, Cejna M, Diaz-Sandoval L, et al. Local gene transfer of phvegf-2 plasmid by gene-eluting stents: an alternative strategy for inhibition of restenosis. Circulation 2004;110:36–45.
83. Kutryk MJ, Kuliszewski MA. In vivo endothelial progenitor cell seeding for the accelerated endothelialization of endovascular devices. Am J Cardiol 2003;92:94–8.
84. Blindt R, Vogt F, Astafieva I, et al. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. J Am Coll Cardiol 2006;47:1786–95.
85. Aoki J, Serruys PW, van Beusekom H, et al. Endothelial progenitor cell capture by stents coated with antibody against CD34: the Healing-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First in Man) Registry. J Am Coll Cardiol 2005;45:1574–9.
86. Miglionico M, Patti G, D'Ambrosio A, Di Sciascio G. Percutaneous coronary intervention utilizing a new endothelial progenitor cells antibody-coated stent: a prospective single-center registry in high-risk patients. Catheter Cardiovasc Interv 2008;71:600–4.
87. Assmus B, Urbich C, Aicher A, et al. HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. Circ Res 2003;92:1049–55.
88. Vasa M, Fichtlscherer S, Adler K, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation 2001;103:2885–90.
89. den Dekker WK, Houtgraaf JH, Onuma Y, et al. Final results of the HEALING IIB trial to evaluate a bio-engineered CD34 antibody coated stent (Genous™Stent) designed to promote vascular healing by capture of circulating endothelial progenitor cells in CAD patients. Atherosclerosis 2011 Jun 25 [E-pub ahead of print]; doi:10.1016/j.atherosclerosis.2011.06.032.
90. Gensch C, Clever YP, Werner C, Hanhoun M, Böhm M, Laufs U. The PPAR-gamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. Atherosclerosis 2007;192:67–74.

Key Words: inflammation ■ re-endothelialization ■ restenosis ■ stent thrombosis.

To participate in this CME activity by taking the quiz and claiming your CME credit certificate, please go to **http://interventions.onlinejacc.org/** and select the CME tab on the top navigation bar.

International Journal of Cardiology 150 (2011) 338–374

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letters to the Editor

Sex differences with respect to clinical characteristics, treatment, and long-term outcomes in patients with heart failure

Miyuki Tsuchihashi-Makaya [a,*,1], Sanae Hamaguchi [a,1], Shintaro Kinugawa [a,1], Kazutomo Goto [a,1], Daisuke Goto [a,1], Tomoo Furumoto [a,1], Satoshi Yamada [a,1], Hisashi Yokoshiki [a,1], Akira Takeshita [b,1,2], Hiroyuki Tsutsui [a,1]

[a] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Futsukaichi Saiseikai Hospital, Futsukaichi, Japan

ARTICLE INFO

Article history:
Received 17 March 2011
Accepted 19 March 2011
Available online 16 April 2011

Keywords:
Heart failure
Sex
Prognosis

The effect of sex on the etiology, risk factors, comorbidities, treatment and prognosis in patients with heart failure (HF) encountered in routine clinical practice in Asian populations has not been well described. The objective of the present study was to elucidate sex differences with respect to the clinical characteristics, treatment, and prognosis of HF patients treated in routine clinical practice settings using the Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) database, which is a nationwide registry for hospitalized patients with HF in Japan.

JCARE-CARD enrolled 2675 patients hospitalized for HF at 164 participating hospitals from January 2005 to June 2006. HF was diagnosed by the simultaneous presence of at least two major criteria or one major criterion in conjunction with two minor criteria by use of the criteria from the Framingham Heart Study [1]. For each patient, baseline data recorded on the form were: demography; HF causes; comorbidities; complications; clinical status; echocardiographic findings; plasma B-type natriuretic peptide (BNP); and treatments. Long-term follow-up data could be obtained from 2305 patients after hospital discharged. Mean post-discharge follow-up was 2.3 ± 0.7 years.

To evaluate the effects of sex on the outcomes, the propensity score which is one of the most widely employed covariate adjustment methods was used to adjust the confounding factors between sex and the outcomes. Multiple covariate Cox regression analyses were used to assess the association of sex with long-term outcomes using all variables and individual propensity scores.

JCARE-CARD collected data from 2675 patients hospitalized with HF, of which 1598 (60%) were male and 1077 (40%) were female. Table 1 provides a comparison of demographic and clinical characteristics for the entire cohort according to sex. Female patients were a mean of 5.7 years older than male patients. Ischemic etiology was more common in males than in females (36% vs 26%). Hypertensive etiology was more common in females than in males (27% vs 23%). Females were more likely to have hypertension, hyperlipidemia, and anemia than males. However, renal failure, hyperuricemia, COPD, and smoking were more frequent in males. These findings of the different clinical characteristics between male and female are similar to the results of other registries in US [2,3]. Mean LVEF was significantly higher in females. Females had a significantly higher level of BNP in serum upon hospital admission, although there was no significant difference in New York Heart Association (NYHA) class. A lower proportion of female patients received ACE inhibitors (32.1% vs 40.9%), β-blockers (44.5% vs 51.6%), antiarrhythmics (13.6% vs 18.6%), aspirin (44.5% vs 49.0%), and warfarin (36.0% vs 43.9%), but a higher proportion received a calcium channel blocker (28.3% vs 23.1%).

There were 282 deaths from any cause (20.5%) in males and 192 (20.7%) in females ($P = 0.836$) The prevalence of cardiac mortality (12.5% vs 12.9%; $P = 0.729$), sudden cardiac deaths (3.3% vs 3.3%; $P = 0.729$), and hospitalization due to the worsening of HF (36.2% vs 36.4%; $P = 0.985$) were also comparable between males and females (12.5% vs 12.9%; $P = 0.729$). After adjustment for multiple variables predictive of mortality after hospital discharge, there was no significant difference in all-cause mortality between males and females (adjusted hazard ratio [HR] 0.97, 95% confidence interval (CI) 0.80–1.19, $P = 0.774$). Risk of cardiac mortality (adjusted HR 1.06; 95% CI 0.82–1.36; $P = 0.665$), sudden cardiac mortality (adjusted HR 1.04; 95% CI 0.64–1.69; $P = 0.870$), and hospitalization due to the worsening of HF (adjusted HR 1.05; 95% CI 0.90–1.22; $P = 0.529$) were also similar between males and females.

Despite the low rate of prescription of drugs to females, their prognosis was equal to that of males. Possible explanations of this finding might be the sex-related differences of the pathophysiology of HF [4], psychosocial factors [5], and life circumstances (e.g., support from the family and partner as well as social services). Consequently, to explain sex differences in the prognoses for HF, a multidimensional

* Corresponding author at: Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan. Tel.: +81 11 706 6970; fax: +81 11 706 7874.
E-mail address: miyuki_t@cardiol.med.kyushu-u.ac.jp (M. Tsuchihashi-Makaya).
[1] For the JCARE-CARD Investigators.
[2] Dr. Akira Takeshita died on 15 March 2009.

Table 1
Baseline demographic and clinical characteristics.

Characteristics	All ($n=2675$)	Male ($n=1598$)	Female ($n=1077$)	P
Age, years	71.0 ± 13.4	68.7 ± 13.3	74.4 ± 12.7	<0.001
Older than 65 years, %	72.5	66.0	82.2	<0.001
Body mass index, kg/m^2	22.3 ± 4.1	22.7 ± 4.0	21.7 ± 4.3	<0.001
Causes of HF, %				
Ischemic	32.0	36.3	25.6	<0.001
Hypertensive	24.6	22.8	27.3	0.008
Cardiomyopathic	21.9	26.2	15.5	<0.001
Valvular	27.7	21.8	36.6	<0.001
Undetermined	15.7	14.2	17.9	0.010
History, %				
Previous myocardial infarction	26.9	31.7	19.9	<0.001
Hypertension	52.9	51.2	55.4	0.036
Diabetes mellitus	29.9	31.1	28.2	0.116
Hyperlipidemia	24.8	22.9	27.6	0.006
Renal failure	11.7	13.0	9.9	0.014
Serum creatinine, mg/dL	1.4 ± 1.2	1.5 ± 1.3	1.2 ± 1.0	<0.001
Hyperuricemia	46.8	51.9	39.3	<0.001
Stroke	15.0	15.3	14.5	0.594
Anemia	20.8	17.5	25.6	<0.001
Hemoglobin, g/dL	12.0 ± 3.2	12.6 ± 3.7	11.2 ± 2.3	<0.001
COPD	6.7	8.5	4.1	<0.001
Smoking	37.7	57.4	9.2	<0.001
Atrial fibrillation	35.2	36.5	33.4	0.103
Sustained VT/Vf	6.2	7.2	4.8	0.013
Prior hospitalization due to HF	48.3	50.0	45.9	0.044
PCI	17.7	21.0	12.8	<0.001
CABG	9.2	11.7	5.6	<0.001
Valve surgery	6.7	5.9	7.9	0.053
NYHA class on hospital admission				
I	1.2	1.4	0.8	
II	11.4	12.5	9.8	0.523
III	44.6	43.7	45.8	0.173
IV	42.9	42.4	43.5	0.189
Echocardiographic data on hospital admission				
LV EDD, mm	56.1 ± 10.5	58.7 ± 10.0	52.2 ± 10.1	<0.001
LV ESD, mm	44.1 ± 12.5	46.9 ± 12.1	39.8 ± 11.7	<0.001
LVEF, %	42.2 ± 17.6	39.4 ± 17.1	46.5 ± 17.6	<0.001
<40%	49.7	56.5	39.2	
40–50%	16.0	15.8	16.4	0.001
>50%	34.2	27.6	44.4	<0.001
Plasma BNP on hospital admission, pg/ml	871.3 ± 970.2	851.4 ± 843.7	900.8 ± 1132.5	0.003

HF, heart failure; COPD, chronic obstructive pulmonary disease; VT, ventricular tachycardia; VF, ventricular fibrillation; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; NYHA, New York Heart Association; LV, left ventricular; EDD, end-diastolic dimension; ESD, end-systolic dimension; EF, ejection fraction; BNP, B-type natriuretic peptide.

study that includes physiological and psychosocial aspects must be conducted.

In conclusions, there were several differences with respect to clinical characteristics and treatment between males and females with HF. However, the effect of sex on outcomes was not found during long-term follow-up. Further investigation is needed to reveal novel mechanisms, therapeutic strategies, and effective management in males and females.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [6].

References

[1] Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. Circulation 1993;88:107–15.

[2] Fonarow GC, Abraham WT, Albert NM, et al. Age- and gender-related differences in quality of care and outcomes of patients hospitalized with heart failure (from OPTIMIZE-HF). Am J Cardiol 2009;104:107–15.

[3] Galvao M, Kalman J, DeMarco T, et al. Gender differences in in-hospital management and outcomes in patients with decompensated heart failure: analysis from the Acute Decompensated Heart Failure National Registry (ADHERE). J Card Fail 2006;12:100–7.

[4] Konhilas JP, Leinwand LA. The effects of biological sex and diet on the development of heart failure. Circulation 2007;116:2747–59.

[5] Faller H, Stork S, Schowalter M, et al. Depression and survival in chronic heart failure: does gender play a role? Eur J Heart Fail 2007;9:1018–23.

[6] Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2010;144:1–2.

0167-5273/$ – see front matter
doi:10.1016/j.ijcard.2011.03.042

Journal of Cardiology (2011) 58, 165–172

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jjcc

Original article

Rationale and design of the Japanese Heart Failure Outpatients Disease Management and Cardiac Evaluation (J-HOMECARE)

Miyuki Tsuchihashi-Makaya (PhD)[a,*], Hisashi Matsuo (MD, PhD)[b], Shigeo Kakinoki (MD, PhD)[c], Shigeru Takechi (MD, PhD)[d], Hiroyuki Tsutsui (MD, PhD, FJCC)[a], for the J-HOMECARE Investigators[1]

[a] *Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[b] *Department of Cardiology, Keiwakai Ebetsu Hospital, Ebetsu, Japan*
[c] *Department of Cardiology, Otaru Kyokai Hospital, Otaru, Japan*
[d] *Department of Cardiology, Date Red Cross Hospital, Date, Japan*

Received 12 April 2011; received in revised form 18 April 2011; accepted 21 April 2011

KEYWORDS
Heart failure;
Disease management;
Psychological status;
Prognosis;
Quality of life

Summary
Background: Although many studies have demonstrated the efficacy of disease management programs on mortality, morbidity, quality of life (QOL), and medical cost in patients with heart failure (HF), no study has focused on psychological status as an outcome of disease management. In addition, very little information is available on the effectiveness of disease management programs in other areas than the USA and Europe.
Methods: The Japanese Heart Failure Outpatients Disease Management and Cardiac Evaluation (J-HOMECARE) is a randomized controlled trial in which 156 patients hospitalized with HF will be randomized into usual care or a home-based disease management arm receiving comprehensive advice and counseling by visiting nurses during the initial 2 months and telephone follow-up for the following 4 months after discharge. This study evaluates depression and anxiety (Hospital Anxiety and Depression Scale), mortality, readmission due to HF, and QOL (Short Form-8). Data are collected during index hospitalization and then 2, 6, and 12 months after discharge. This study started in December 2007, and the final results are expected in 2011.
Conclusion: The J-HOMECARE will provide important information on the efficacy of disease management for psychological status as well as the effective components of disease management for patients with HF. (ClinicalTrials.gov number, NCT01284400).

* Corresponding author at: Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan. Tel.: +81 11 706 6970; fax: +81 11 706 7874.
E-mail address: miyuki_t@cardiol.med.kyushu-u.ac.jp (M. Tsuchihashi-Makaya).
[1] See Appendix A.

0914-5087/$ – see front matter © 2011 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.jjcc.2011.04.004

Introduction

Heart failure (HF) is one of the leading causes of death and hospitalization in developed countries. It is often associated with multiple co-morbidities and complications, as well as impaired quality of life (QOL). Although many therapeutic options have reduced mortality and morbidity in patients with HF [1–4], frequent re-hospitalization due to worsening HF, low QOL [5], and psychological problems remain a critical issue [6]. Our previous studies demonstrated that poor follow-up as well as psychosocial distress such as anxiety was an independent predictor associated with hospitalization due to worsening HF [7,8].

To improve outcomes of HF patients, a variety of disease management programs have been developed and tested over the past 25 years [9–11]. These programs include HF clinics, home-based intervention, and tele-monitoring. The key components of all of these interventions were education and counseling, symptom monitoring by a nurse, accessibility of healthcare provider in case of problems, optimization of medication, and social support service after discharge. They have been reported to decrease rehospitalization due to worsening HF, increase time to first major event, decrease medical costs, and improve QOL [12]. However, some studies have failed to support these positive findings, by reporting negative or inconclusive results [13,14]. In addition, the differences in national healthcare systems raise questions about the suitability and comparability of these programs in different countries. To the best of our knowledge, no trials have been conducted to evaluate the effect of disease management programs in other countries other than the USA, Europe, and Australia. Moreover, almost all previous studies have evaluated the effects of disease management on mortality, readmission due to HF, QOL, and medical costs. Even though psychosocial distress, including depression and anxiety, is common among patients with HF and is a high risk for mortality and morbidity in HF [8,15], there is no trial to assess the efficacy of disease management programs for the psychosocial status of HF patients.

The Japanese Heart Failure Outpatients Disease Management and Cardiac Evaluation (J-HOMECARE) is a randomized controlled trial to evaluate the efficacy of home-based disease management programs compared with usual care in improving psychosocial status, mortality, HF hospitalization, and QOL in Japanese HF patients.

Study design

Overview

J-HOMECARE is a multicenter, randomized, efficacy trial designed to evaluate the efficacy of home-based disease management programs on psychosocial status and QOL as well as mortality and morbidity as compared to usual care in Japanese HF patients. This study has been approved by the Ethics Committee of Hokkaido University Graduate School of Medicine. Recruited patients with HF were randomized into usual care and home-based disease management groups between December 2007 and March 2010. Patients undergo their respective J-HOMECARE treatment for 6 months and are then followed up for an additional 6 months. All data collection was scheduled to end in March 2011.

Study objectives

The primary objective of J-HOMECARE is to determine the effectiveness of interventions, as compared to that of usual care, on psychological status, including depression and anxiety, in HF patients. The secondary objective is to determine the effectiveness of interventions, compared to that of usual care, on all-cause death, cardiac death, sudden cardiac death, readmission due to decompensated HF, and QOL.

Study patients and baseline assessment

The process of the trial is shown in Fig. 1. All study candidates are required to have had a hospital admission for HF with symptoms and signs of HF and a pre-existing history of chronic HF [New York Heart Association (NYHA) II–IV]. Eligible patients must be at least 18 years of age. Reasons for exclusion from the study are as follows: end-stage HF defined as requiring mechanical support or continuous intravenous inotropic support; a serious life-threatening illness with a life-expectancy of <6 months; stroke within the last 3 months; cognitive dysfunction; substance abuse or psychotic disorder; patients whose physician or nurses refused access.

After informed consent has been obtained from eligible patients, they are randomized on a 1:1 basis, to either usual care or a home-based disease management program.

Baseline and all annual examinations consist of: (1) clinical characteristics including height, body weight, pulse, and blood pressure; (2) etiology of HF; (3) risk factors such as hypertension, diabetes mellitus, dyslipidemia, smoking habits, and/or alcohol drinking habits; (4) comorbidities such as prior myocardial infarction (MI), atrial fibrillation, ventricular arrhythmias, hyperuricemia, chronic kidney disease, anemia, stroke, chronic obstructive pulmonary disease, locomotor disability, prior percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG); (5) severity of HF [NYHA functional class, brain natriuretic peptide (BNP)], and echocardiography; (6) treatment at hospital discharge; and (7) a questionnaire assessing depression, anxiety, QOL, and physical activity (Table 1).

Intervention protocol

Enrolled patients receive comprehensive discharge education using a booklet provided by a cardiologist, nurse, dietitian, or pharmacist. This booklet provides knowledge and information on pathophysiology, medical treatment, diet, physical activity, lifestyle modification, self-measurement of body weight, self-monitoring of worsening HF, and emergency contact methods (Fig. 2). Follow-up assessments were performed 1, 2, 6, and 12 months after discharge.

A home-based disease management program consists of home visit by nurse to provide symptom monitoring, education, and counseling and telephone follow-up by nurse in addition to routine follow-up by cardiologist (Table 2). A home visit is made within 14 days after discharge from