Table 1—*Baseline demographics by treatment*

	eGFR ≥90 mL/min/1.73 m^2			eGFR 60–89 mL/min/1.73 m^2			eGFR <60 mL/min/1.73 m^2		
	Aspirin	Nonaspirin	*P*	Aspirin	Nonaspirin	*P*	Aspirin	Nonaspirin	*P*
n	248	270		661	712		342	290	
Age (years), mean (SD)	61 (9)	58 (10)	0.0001	65 (10)	65 (10)	0.6	68 (9)	69 (8)	0.7
Male, *n* (%)	127 (51)	130 (48)	0.5	386 (58)	398 (56)	0.4	184 (54)	150 (52)	0.6
Hypertension, *n* (%)	120 (48)	131 (49)	0.97	376 (57)	399 (56)	0.8	242 (71)	198 (68)	0.5
Dyslipidemia, *n* (%)	140 (56)	132 (49)	0.09	339 (51)	362 (51)	0.9	195 (57)	168 (58)	0.8
Laboratory measurements									
Glycated hemoglobin (%), mean (SD)	7.5 (1.7)	7.4 (1.4)	0.3	7.0 (1.3)	6.9 (1.2)	0.2	7.0 (1.3)	6.9 (1.1)	0.1
Serum creatinine level (mg/dL), mean (SD)	0.5 (0.09)	0.5 (0.1)	0.2	0.7 (0.1)	0.7 (0.1)	0.8	1.1 (0.5)	1.0 (0.2)	0.2
Dipstick-positive proteinuria, *n* (%)	32 (13)	35 (13)	0.96	68 (10)	72 (10)	0.9	76 (23)	63 (22)	0.9
Blood pressure (mmHg), mean (SD)									
Systolic	134 (16)	134 (16)	0.6	135 (14)	134 (14)	0.07	138 (15)	136 (15)	0.1
Diastolic	77 (10)	77 (10)	0.9	77 (9)	76 (9)	0.01	77 (9)	76 (10)	0.1
Medications for diabetes, *n* (%)									
Sulfonylurea	145 (58)	146 (54)	0.3	384 (58)	382 (54)	0.1	205 (60)	177 (61)	0.8
α-Glucosidase inhibitor	90 (36)	80 (30)	0.1	222 (34)	238 (33)	0.95	105 (31)	94 (32)	0.6
Biguanides	38 (15)	56 (21)	0.1	82 (12)	96 (13)	0.6	47 (14)	34 (12)	0.4
Insulin	42 (17)	46 (17)	0.97	87 (13)	80 (11)	0.3	36 (11)	34 (12)	0.6
Thiazolidinediones	10 (4)	23 (9)	0.04	31 (5)	31 (4)	0.8	21 (6)	11 (4)	0.2
Medication for hypertension and dyslipidemia, *n* (%)									
Calcium channel blocker	73 (29)	75 (28)	0.7	214 (32)	239 (34)	0.6	149 (44)	123 (42)	0.8
Angiotensin receptor blocker	36 (15)	41 (15)	0.8	135 (20)	137 (19)	0.6	94 (27)	88 (30)	0.4
Angiotensin-converting enzyme inhibitor	30 (12)	39 (14)	0.4	96 (15)	104 (15)	0.96	52 (15)	52 (18)	0.4
β-Blocker	10 (4)	14 (5)	0.5	40 (6)	49 (7)	0.5	25 (7)	24 (8)	0.7
α-Blocker	4 (1.6)	5 (1.9)	1	25 (4)	22 (3)	0.5	24 (7)	11 (4)	0.08
Statins	69 (28)	61 (23)	0.2	159 (24)	179 (25)	0.6	91 (27)	87 (30)	0.3
History of smoking, *n* (%)	113 (46)	109 (40)	0.2	311 (47)	282 (40)	0.005	140 (41)	100 (34)	0.1

(P = 0.03), as shown in Supplementary Fig. 2. The group of patients with eGFR ≥90 mL/min/1.73 m^2 was used as the reference group in the analysis of the association between the level of eGFR and primary end points. The incidence of primary end points was significantly higher in patients with mildly reduced eGFR 60–89 mL/min/1.73 m^2 (HR 1.6 [95% CI 1.0–2.7]; P = 0.048) and in patients with eGFR <60 mL/min/1.73 m^2 (2.0 [1.2–3.5]; P = 0.0066).

Efficacy of low-dose aspirin therapy on primary and secondary end points in diabetic patients with reduced GFR

In 1,373 patients with eGFR 60–89 mL/min/1.73 m^2 (661 patients in the aspirin group and 712 patients in the nonaspirin group), a total of 85 primary end points (any atherosclerotic event) occurred: 30 in the aspirin group and 55 in the nonaspirin group (HR 0.57 [95% CI 0.36–0.88]; P = 0.011) (Fig. 1*B*). The Cox proportional hazard model demonstrated significant interaction between mild renal dysfunction (eGFR 60–89 mL/min/1.73 m^2) and aspirin use (P = 0.02). However, there was no significant difference in the incidence of primary end points in patients with eGFR ≥90 mL/min/1.73 m^2 (nine events in the aspirin group and 11 events in the nonaspirin group; 0.94 [0.38–2.3]) (Fig. 1*A*), or those with eGFR <60 mL/min/1.73 m^2 (29 events in the aspirin group and 19 in the nonaspirin group; 1.3 [0.76–2.4]) (Fig. 1*C*). Adjusting for age, hypertension, dyslipidemia, and history of smoking, low-dose aspirin significantly reduced primary end points in patients with eGFR 60–89 mL/min/1.73 m^2 (0.53 [0.34–0.83]; P = 0.0052), and not in patients with eGFR ≥90 or <60 mL/min/1.73 m^2 (eGFR ≥90 mL/min/1.73 m^2: 0.87 [0.36–2.14]; eGFR <60 mL/min/1.73 m^2: 1.24 [0.69–2.23]).

The secondary end point of atherosclerotic/ischemic events occurred in 26 patients in the aspirin group and in 50 patients in the nonaspirin group, among the patients with eGFR 60–89 mL/min/1.73 m^2 (HR 0.54 [0.33–0.86]; P = 0.010) (Supplementary Table 2). The incidence of atherosclerotic/ischemic events was similar between the aspirin and nonaspirin groups in both categories of patients with eGFR of at least 90 mL/min/1.73 m^2 (1.15 [0.45–2.95]; P = 0.76) and those with eGFR <60 mL/min/1.73 m^2 (1.29 [0.70–2.43]; P = 0.42) (Supplementary Table 2). In the structural and hemorrhagic events, the benefit of aspirin was not observed in any category of patients stratified by eGFR.

Efficacy of low-dose aspirin therapy on primary end points in subgroups

As reported previously, the incidence of primary end points was significantly lower in the aspirin group than in the nonaspirin group in the subgroup of patients aged 65 years or older (Fig. 2) (10). In the subgroups of patients aged 65 years or older whose eGFR was 60–89 mL/min/1.73 m^2, low-dose aspirin

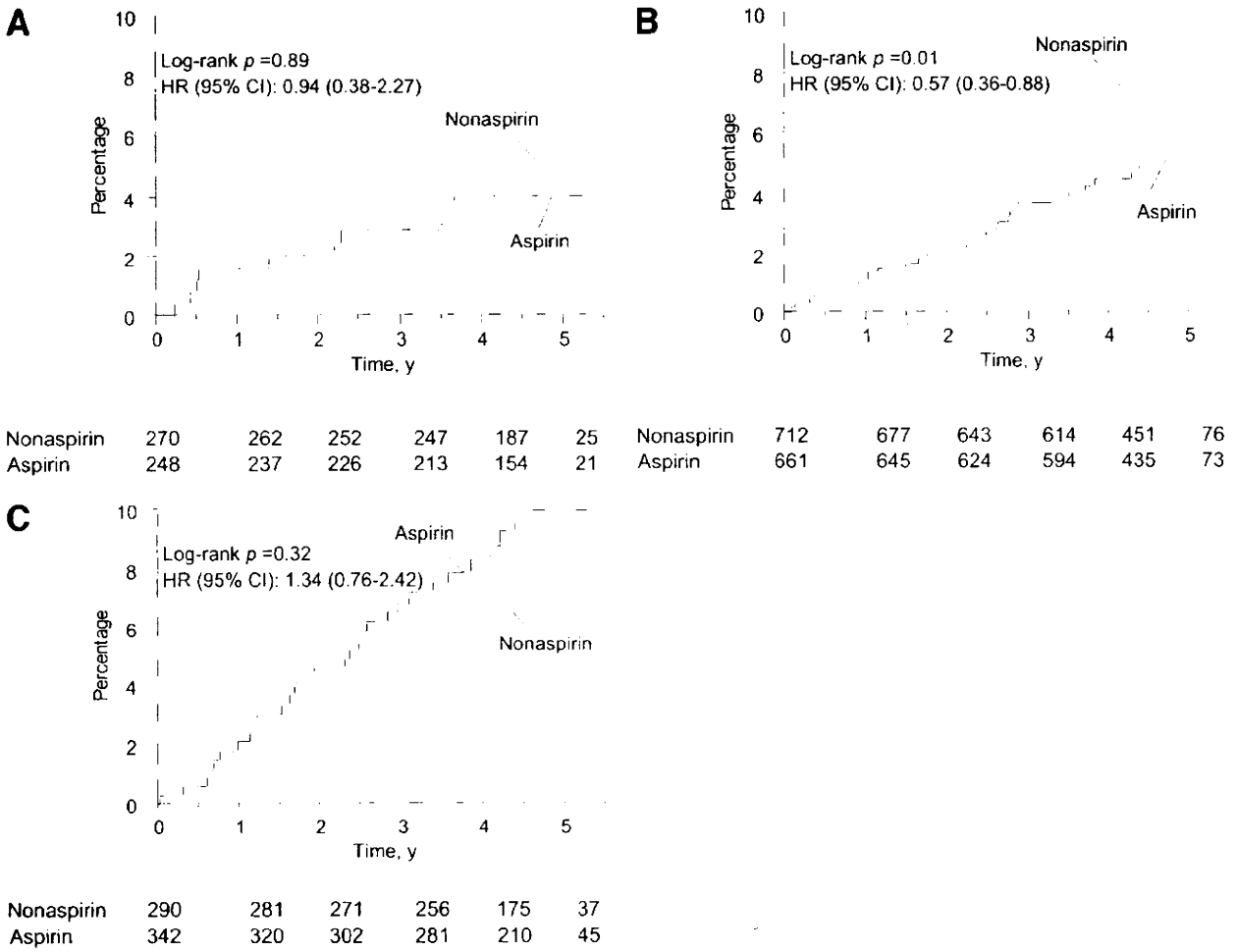

Figure 1—*Percentage of primary end points by category of patients with eGFR of at least 90 mL/min/1.73 m^2 (A), 60–89 mL/min/1.73 m^2 (B), or <60 mL/min/1.73 m^2 (C).*

therapy reduced primary end points by 52% (HR 0.48 [95% CI 0.27–0.82]; P = 0.007) (Fig. 2). In the subgroups of male or female, there was no significant difference in the primary end point between the aspirin and nonaspirin group (data not shown).

Safety

Incidence of the composite of gastrointestinal bleeding and cerebral bleeding was very low and was similar between the aspirin (three gastrointestinal bleeding events and four cerebral bleeding events) and nonaspirin (two gastrointestinal bleeding events and four cerebral bleeding events) groups in the group of patients with eGFR 60–89 mL/min/1.73 m^2.

CONCLUSIONS—In the present subgroup analysis of JPAD, a prospective, randomized, clinical trial of low-dose aspirin versus nonaspirin groups for primary prevention in Japanese type 2 patients with diabetes, low-dose aspirin therapy reduced the incidence of atherosclerotic events in diabetic patients with eGFR 60–89 mL/min/1.73 m^2, but not in those with either eGFR <60 mL/min/1.73 m^2 or at least 90 mL/min/1.73 m^2. In the subgroup of patients with eGFR 60–89 mL/min/1.73 m^2, there was no increase in serious gastrointestinal and cerebral bleeding in the aspirin group compared with the nonaspirin group.

There has been rapidly growing interest in the relation between renal dysfunction and atherosclerotic events in general populations as well as patients at risk for CV events. This is the first subanalysis to clarify the efficacy of aspirin in reducing atherosclerotic risk in patients stratified according to eGFR in patients with diabetes. The current study provides new information that eGFR may be useful to identify candidates for aspirin therapy among Japanese patients with diabetes. We used the new three-variable Japanese equation for GFR, which is more closely correlated with the inulin clearance than the Modification of Diet in Renal Disease equation in the Japanese population (14). It is not clear, however, that eGFR-based identification can be applied to the Caucasian population, because GFR in the Japanese population is lower than that in Caucasians (15). Furthermore, some previous studies in Western populations had reported that Modification of Diet in Renal Disease equation or Cockcroft-Gault formula underestimated GFR in patients with diabetes, and that eGFR was not a predictor of mortality (16,17). Further studies are therefore necessary to confirm the usefulness of eGFR in this strategy.

The progressive increase in CV risk with worsening eGFR found in Japanese patients with diabetes in this study is consistent with previous data (8,18). It has been hypothesized to be related to factors associated with renal damage,

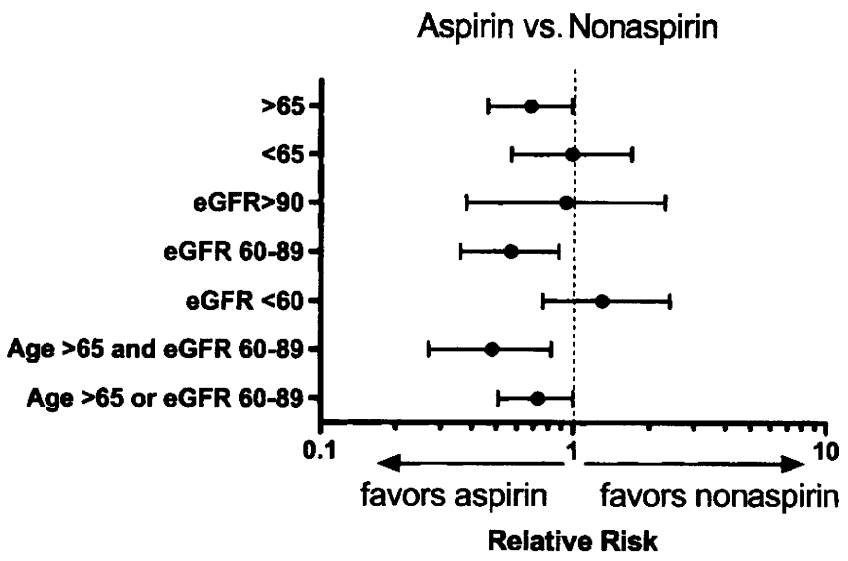

Figure 2—*Subgroup analysis of incidence of primary end points.*

including anemia, oxidative stress, derangements in calcium-phosphate homeostasis, inflammation and conditions promoting coagulation (18). The possible mechanism for the beneficial effect of low-dose aspirin therapy on the prevention of atherosclerotic events in the subgroup of patients with eGFR 60–89 mL/min/1.73 m^2, is inhibition of thrombus formation via blocking thromboxane-dependent platelet activation. Recently, aspirin also has been found to have protective effects on oxidative stress-induced endothelial dysfunction in vivo, which may be involved in the prevention of atherosclerotic events (19,20). However, it is not clear why low-dose aspirin therapy could not prevent atherosclerotic events in the subgroup of patients with eGFR <60 mL/min/1.73 m^2. Given that aspirin in a daily dose of 100 mg or less is associated with a higher incidence of aspirin resistance in patients with diabetes or renal dysfunction (21,22), one possible explanation is that the dose of aspirin is too low to inhibit platelet activation in these patients. Another possible explanation is that in patients with advanced kidney disease, atherosclerotic events are predominantly caused by factors such as renal anemia, derangement of calcium-phosphate homeostasis, and other unknown renal-related factors that are not influenced by aspirin.

During preparation of this manuscript, the subanalysis of the Hypertension Optimal Treatment (HOT) study was published about the efficacy of the low-dose aspirin for primary prevention in patients with chronic kidney disease (23). This study showed that low-dose aspirin is beneficial for preventing major CV disease in patients with eGFR <45 mL/min/1.73m^2 (aspirin, 11/264; placebo 32/272; HR 0.34 [95% CI 0.17–0.67]), and not in those with eGFR ≥60 mL/min/1.73m^2 (aspirin, 233/7517; placebo, 252/7461; 0.91 [0.76–1.09]) (23). The result seems inconsistent with our result; however, the HOT study enrolled patients with diastolic hypertension, and the rate of diabetic patients was only 8%. The underlying mechanisms were unclear, but the difference in patients' characteristics, especially coexisting with diabetes, might affect the aspirin effect.

Along with progression of renal damage in diabetes, GFR is normal or increases to above the normal level in the early period and then gradually decreases. The proportion of patients with eGFR of at least 90 mL/min/1.73 m^2 in the JPAD trial was 21% of the total patients enrolled, which was higher than the 13% prevalence for this eGFR category observed in the general Japanese population (15). The proportion with eGFR <60 mL/min/1.73 m^2 in the JPAD trial was 25%, which was also higher than the 16% prevalence in the general population (15). This distributional difference in eGFR is probably explained by the characteristic progression pattern of diabetic renal damage. In the current study, eGFR <60 mL/min/1.73 m^2—a usual cutoff value—was associated with increased atherosclerotic risk, but in addition, eGFR 60–89 mL/min/1.73 m^2 was also associated with increased risk for any atherosclerotic events.

This study has a few limitations. With the nonblinded design, differential ascertainment is possible; however, end point classification was conducted by a blinded, independent committee that was unaware of the group assignments. Second, we used the eGFR instead of direct measurement of GFR using an exogenous marker, such as inulin clearance. Equations for estimating GFR have limited precision compared with measured GFR. However, for practical reasons, many large trials have used eGFR calculated by the Modification of Diet in Renal Disease equation or Cockcroft-Gault formula. Third, our population enrolled only 20 patients with eGFR <30 mL/min/1.73 m^2 and no patients receiving hemodialysis, so we could not analyze the effect of aspirin in these categories of patients. Finally, we did not measure the rate of urinary albumin excretion, a factor that may drive the documented independent effect of the baseline eGFR on CV outcomes.

In conclusion, the current study demonstrated that low-dose aspirin therapy reduced the risk of atherosclerotic events in type 2 diabetic patients with eGFR 60–89 mL/min/1.73 m^2. The results suggest that eGFR may be useful for risk stratification in the primary prevention strategy with aspirin. However, as these results are from a post hoc subgroup analysis, they should be viewed as hypothesis generating and should be investigated further in additional studies.

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Y.S. conducted the trial, interpreted and analyzed data, and wrote the manuscript. T.M. performed all statistical analyses. H.O. conducted the trial, contributed to discussion, and reviewed and edited the manuscript. M.N., S.U., N.D., H.J., M.W., H.S., and S.S. researched data. S.O. contributed to discussion and reviewed and edited the manuscript. Y.A. reviewed and edited the manuscript.

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ORIGINAL ARTICLE

Safety and benefits of a tablet combining losartan and hydrochlorothiazide in Japanese diabetic patients with hypertension

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This study was conducted to determine the effects of a tablet combining losartan/hydrochlorothiazide (L/HCTZ) in comparison with losartan alone in Japanese diabetic patients with hypertension. Thirty consecutive Japanese diabetic patients with hypertension were randomly assigned to group A, receiving losartan alone for the first 3 months, then L/HCTZ for the next 3 months, or group B, receiving L/HCTZ for the first 3 months, then losartan alone for the next 3 months. Clinical and biological parameters were obtained before, and 3 and 6 months after the start of this study. The decreases in systolic and diastolic blood pressure (BP) during treatment with L/HCTZ were significantly greater than in treatment with losartan alone. Both treatments significantly and similarly decreased urinary albumin excretion, the cardio-ankle vascular index (CAVI) and augmentation index (AI). There was no significant difference in metabolic change during both the mono- and combination pharmacotherapies. The tablet combining L/HCTZ significantly reduced systolic and diastolic BP compared with the losartan monotherapy, and offered benefits similar to losartan monotherapy for albuminuria, arterial stiffness assessed by the CAVI and AI, and metabolic effects. Thus, the L/HCTZ tablet could be a useful drug for Japanese diabetic patients with hypertension.
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Keywords: albuminuria; angiotensin; arterial stiffness; blood pressure; diuretics

INTRODUCTION

Achievement of the target blood pressure (BP) is the most crucial objective of antihypertensive treatment. More than two-thirds of hypertensive patients will require two or more antihypertensive agents from different classes to control their BP.[1,2] Patients with diabetes or renal disease will need a greater intensity of antihypertensive treatment, on an average of 2.6 to 4.3 different classes of antihypertensive agents to attain a BP goal of lower than 130/80 mm Hg.[3] The angiotensin II type 1 receptor blocker (ARB) is currently one of the most widely used first-line antihypertensive drugs, especially for diabetic patients with hypertension based on the evidence that it slows the progressive deterioration of kidney function in patients with diabetic nephropathy.[4] However, it is often difficult to achieve the target BP with dose titration of ARB alone, and other antihypertensive medications are often required to provide sufficient BP control in addition to an ARB.

Hydrochlorothiazide (HCTZ) is a diuretic that has been a standard antihypertensive drug prescribed worldwide because of its cost and efficacy in lowering BP based on the available evidence including the ALLHAT trial[5] and NICS-EH trial.[6] The guidelines of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure,[7] the European Society of Hypertension[8] and Japanese Society of Hypertension[9] also recommend combination therapy comprising ARB and thiazide-type diuretics for hypertensive patients. However, add-on HCTZ may have some adverse effects on electrolyte, glucose, lipid and uric acid metabolism, especially in Japanese diabetic patients with hypertension.

The aim of this crossover study was to examine the effects of a tablet combining losartan and HCTZ (L/HCTZ) on urinary albumin excretion (UAE), arterial stiffness, and BP and its adverse effects on the metabolic changes in comparison with losartan alone in Japanese diabetic patients with hypertension. UAE was evaluated as a surrogate marker for cardiovascular morbidity and mortality,[10] and arterial stiffness was assessed by the cardio-ankle vascular index (CAVI) and augmentation index (AI).

METHODS

Study population and design

The subjects of this study comprised 30 consecutive Japanese diabetic patients who had untreated hypertension or uncontrollable hypertension treated with medications except for renin–angiotensin system (RAS) inhibitors. Hypertension was defined as a clinic systolic BP of > 140 mm Hg at any time and/or a

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clinic diastolic BP of >90 mm Hg at any time. Patients with serious refractory hypertension defined as more than 120 mm Hg in diastolic BP, history of acute myocardial infarction, stroke, or any other cardiovascular events within 6 months, heart failure with NYHA grade III, or grade IV, history of gout or hyperuricemia at the beginning of this study, kidney dysfunction defined as a serum creatinine (Cr) level of more than 2 mg per 100 ml, liver dysfunction defined as a serum transaminase level more than 3 times higher than normal, bilateral renal artery stenosis, secondary, or malignant hypertension, polycystic kidney disease, congenital kidney deformities, solitary kidney, pregnancy or probable pregnancy, history of allergy to the medication in this study, or those considered inappropriate were excluded from the study. The glomerular filtration rate was estimated by the MDRD equation modified by a Japanese coefficient, as follows:

$$\text{eGFR} = 0.741 \text{ if male gender (or } 0.742 \text{ if female gender)} \times 175 \times \text{Age}^{-0.203} \times \text{Cr}^{-1.154}$$

(where eGFR=estimated glomerular filtration rate, Age=age (years old); and

$$\text{Cr} = \text{serum Cr level (mg per 100 ml)}$$

This study was designed as a crossover study. All patients were randomly assigned either to group A (receiving losartan for the first 3 months, then L/HCTZ for the next 3 months) or group B (receiving L/HCTZ for the first 3 months, then losartan alone for the next 3 months). Five patients (three patients in group A and two patients in group B) were dropped out of the study mainly due to the concern about adverse effects of the medication such as diabetes and hyperuricemia. The doses of losartan and L/HCTZ were fixed throughout the study at 50 mg day^{-1} and 50 mg per 12.5 mg day^{-1}, respectively, which are typical doses administered in Japanese patients. Clinical and biological parameters were obtained before the start of the study, as well as 3 and 6 months after. During the study period, previous medications and therapies except RAS blockers and diuretics were continued. To achieve the target BP of <130/80 mm Hg, amlodipine was added at a dose of 2.5 mg day^{-1}, and the dose was subsequently increased by 2.5 mg increments at intervals of 4 weeks to a maximum dose of 10 mg day^{-1}. The study was approved by the review board of Keio University Medical School Hospital, and written informed consent was obtained from every subject.

Serum levels of Cr, cystatin C, potassium, uric acid, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, and glycoalbumin, and plasma levels of active renin concentrations (ARC), and aldosterone were measured in venous blood samples drawn in the morning after an overnight fast on the same days as those in which CAVI, AI and BP measurements were taken.

Cardio-ankle vascular index

The CAVI was measured using a VaSera VS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) as described previously.[11] Cuffs are applied to the bilateral upper arms and ankles, with the subject lying supine and the head held in the midline position. ECG electrodes are placed on both wrists, and a microphone for detecting heart sounds is placed on the sternum. Patients rested in the supine position for at least 10 min before the start of monitoring. The CAVI was calculated with the following formula:

$$\text{CAVI} = a\{(2\rho/\Delta P) \times \ln(\text{Ps}/\text{Pd})\,\text{PWV}^2\} + b$$

where Ps is systolic BP, Pd is diastolic BP, ΔP is Ps−Pd, ρ is blood density and a and b are constants. The number of patients in this study was assumed to be statistically sufficient because the estimated required sample size for pulse wave velocity (PWV) is 15.6 in each group with an α-error of 0.05 and a power of 0.8.

Augmentation index

The AI was measured using an automated tonometric device (HEM-9000AI; Omron Healthcare, Kyoto, Japan) as described previously.[12] Peripheral pressure waveforms were recorded over 30 s from the radial artery at the wrist with the subjects in a sitting position after at least 5 min rest. The AI was calculated with the following formula:

$$\text{AI} = (\text{late systolic BP} - \text{diastolic BP (DBP)})/(\text{systolic BP} - \text{DBP}) \times 100\,(\%)$$

Urinary albumin excretion

Urinary albumin excretion was evaluated on the basis of the mean albumin-to-creatinine ratio in three nonconsecutive overnight urine samples. Urinary concentrations of albumin and Cr were determined using a turbidimetric immunoassay with a Superior-Microalbumin kit (DPC, Tokyo, Japan) and with the Jaffé reaction using an autoanalyzer.

Statistical analyses

Analyses were performed with StatView 5.0. software (SAS Institute, Cary, NC, USA). The χ^2-test and Fisher's exact test were used to analyze the proportion of patients who achieved target BP. The changes in biological parameters were analyzed with a one-way analysis of variance for repeated measures combined with the Dunnett and Tukey–Kramer *post hoc* tests. A *P*-value <0.05 was considered significant. Data are reported as means ± s.d.

RESULTS

All patients in this study had untreated hypertension or hypertension, which had been inadequately treated for more than a month with medications other than RAS inhibitors. Patient characteristics in baseline values were as follows: age, 53 ± 11 years; the number of male gender, 20; body mass index, 25.2 ± 4.5 kg m^{-2}; waist circumference, 88 ± 13 cm; serum Cr, 0.82 ± 0.16 mg per 100 ml; eGFR, 75.2 ± 14.1 ml min^{-1} per 1.73 m^2; cystatin C, 0.74 ± 0.09 mg l^{-1}; serum potassium, 4.31 ± 0.24 mEq l^{-1}; serum uric acid, 5.9 ± 1.4 mg per 100 ml; serum total cholesterol, 211 ± 34 mg per 100 ml; serum triglyceride, 161 ± 153 mg per 100 ml; serum high-density lipoprotein cholesterol, 54 ± 13 mg per 100 ml; serum low-density lipoprotein cholesterol, 125 ± 34 mg per 100 ml; blood sugar, 109 ± 24 mg per 100 ml; glycoalbumin, 15 ± 2.9%; plasma ARC, 9.4 ± 8.5 pg ml^{-1}; plasma aldosterone concentration, 147 ± 59 pg ml^{-1}; UAE, 25.7 ± 42.4 mg gCr^{-1}; CAVI, 8.7 ± 1.1; AI, 87.2 ± 11.6%; systolic BP, 156 ± 16 mm Hg; diastolic BP, 98 ± 16 mm Hg.

To achieve the target BP of <130/80 mm Hg, amlodipine was added during the losartan treatment in two patients at a dose of 5 and 10 mg day^{-1}, respectively. Figure 1 illustrated the changes in the metabolic effects with both drugs. There was no significant change in metabolic parameters with either treatment.

Figure 2 illustrated the changes in clinical parameters with both drugs. The plasma ARC significantly increased from 9.4 ± 8.5 to 70.5 ± 72.2 pg ml^{-1} during the L/HCTZ treatment. The plasma ARC after the treatment with L/HCTZ was significantly greater than that after losartan alone. UAE significantly decreased from 25.7 ± 42.4 to 11.5 ± 17.0 mg gCr^{-1} during the losartan treatment, whereas UAE significantly decreased from 25.7 ± 42.4 to 6.1 ± 13.6 mg gCr^{-1} during the L/HCTZ treatment, although there was no significant difference in UAE values between both treatment periods. The CAVI significantly decreased from 8.7 ± 1.1 to 8.0 ± 1.3 during the losartan treatment, and significantly decreased from 8.7 ± 1.1 to 7.7 ± 1.3 during the L/HCTZ treatment. There was no significant difference in the CAVI between both treatments. The AI significantly decreased from 87.2 ± 11.6 to 77.2 ± 13.9 during the losartan treatment, and significantly decreased from 87.2 ± 11.6 to 77.5 ± 9.6 during the L/HCTZ treatment. There was no significant difference in the AI values between both treatment periods. The systolic BP significantly decreased from 156 ± 16 to 137 ± 14 mm Hg during the losartan treatment, whereas the systolic BP significantly decreased from 156 ± 16 to 130 ± 10 mm Hg during the L/HCTZ treatment. The systolic BP

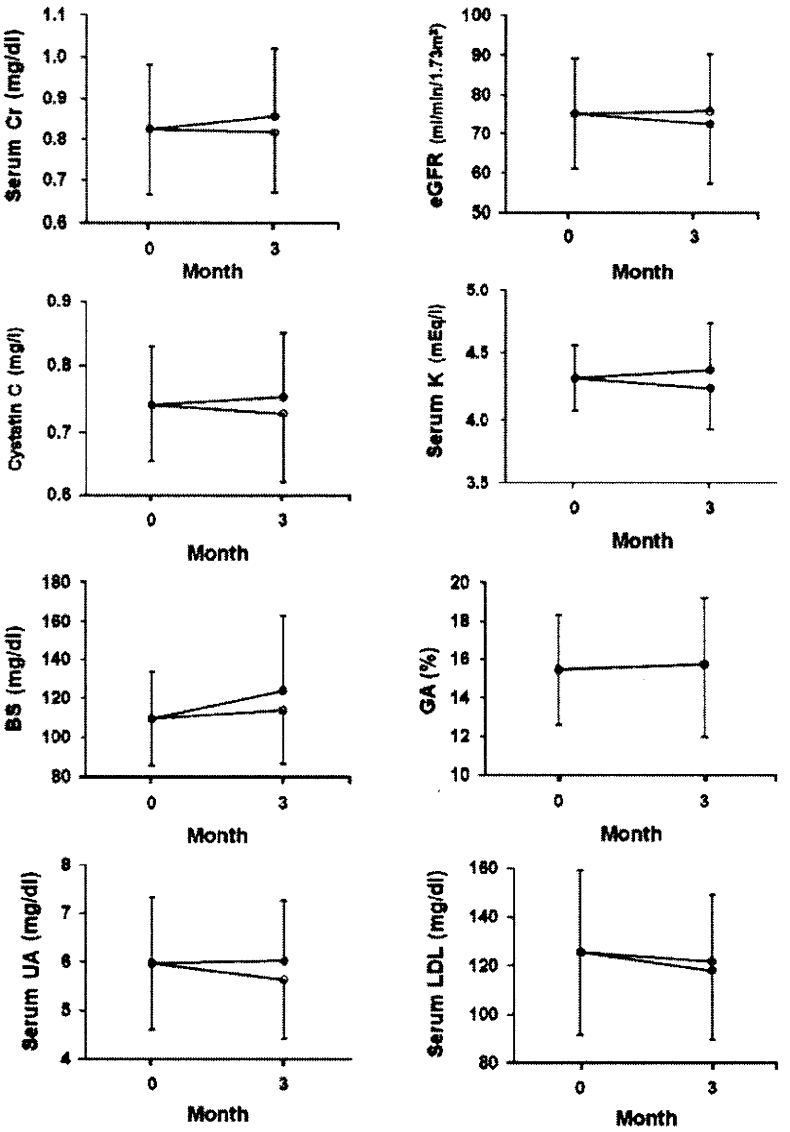

Figure 1 Serum creatinine (Cr), estimated glomerular filtration rate (eGFR), serum cystatin C, serum potassium, blood sugar (BS), glycoalbumin (GA), serum uric acid (UA), serum low-density lipoprotein cholesterol (LDL-C), at baseline, after 3 months of treatment with losartan alone (open circles, n=25) and with L/HCTZ (closed circles, n=25).

after the treatment with L/HCTZ was significantly lower than that after the treatment with losartan alone. The diastolic BP significantly decreased from 98 ± 16 to 87 ± 6 mm Hg during the losartan treatment, whereas the diastolic BP decreased from 98 ± 16 to 81 ± 8 mm Hg during the L/HCTZ treatment. The diastolic BP during the treatment with L/HCTZ was significantly lower than that during the treatment with losartan alone. The percentage of patients having achieved target BP of < 130/80 mm Hg during the treatment with losartan alone and L/HCTZ was 12 and 32%, respectively, and were not statistically different between both treatment periods. There were no significant changes in other parameters during either treatment period.

DISCUSSION

This study demonstrated that the treatment with the L/HCTZ tablet provided a significantly great reduction in BP compared with losartan alone, consistent with the previous studies in which ARB+HCTZ combination therapy produced more significant BP reduction than monotherapy with ARB or diuretics.[13] This might have derived from the enhanced suppression of RAS, as we observed the significantly greater increase of ARC with L/HCTZ treatment than losartan alone. On the other hand, diuretics have been reported to have some adverse effects on the metabolism, such as insulin resistance, hyperuricemia and electrolyte disturbances.[14] In this study, however, the elevation in serum levels of glucose and uric acid and the reduction in serum potassium level during the treatment with L/HCTZ were similar to those during the treatment with losartan alone. As it was expected that the use of losartan and HCTZ in combination would counteract each other's potential adverse effects, which occur when they are given as a monotherapy, most of the undesirable metabolic side effects of thiazide were minimized by the combination with losartan.

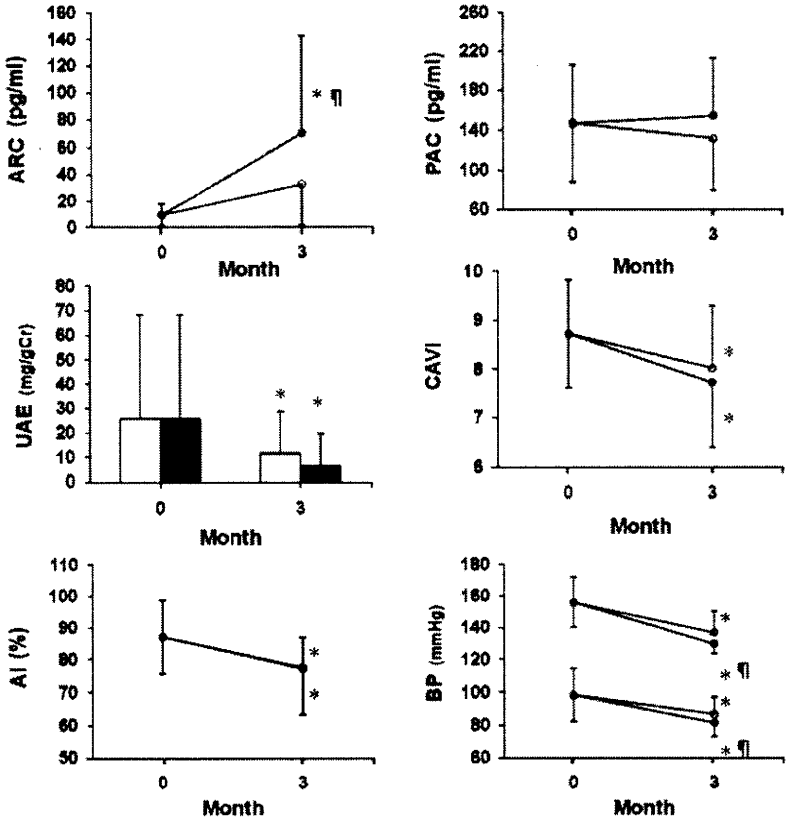

Figure 2 Plasma active renin concentration (ARC) and plasma aldosterone concentration (PAC) serum brain natriuric peptide (BNP), urinary albumin excretion (UAE), the cardio-ankle vascular index (CAVI), augmentation index (AI) and blood pressure (BP) at baseline, after 3 months of treatment with losartan alone (open circles, n=25) and with L/HCTZ (closed circles, n=25). *$P<0.05$ *vs.* the baseline. ¶$P<0.05$ *vs.* the losartan alone.

Albuminuria is an important predictor of cardiovascular events and of progression to end-stage renal disease in diabetic patients with hypertension.[15,16] In this study, the reduction in UAE with the 3-month combination therapy with 50 mg of losartan and 12.5 mg of HCTZ was similar to that with the losartan alone. This was inconsistent with a recent study showing that add-on 6-month treatment with a low sodium diet or 25 mg of HCTZ further decreased proteinuria in patients treated with 100 mg of losartan.[17] As our study demonstrated that the BP reduction during the L/HCTZ tablet was significantly greater than the losartan alone, more extended observation period might have provided the significant difference in proteinuria between both treatments.

Both treatments with losartan and L/HCTZ significantly and similarly improved the CAVI, which reflects arterial stiffness with less dependency on BP compared with PWV,[11] and AI, which is a marker for the magnitude of arterial wave reflections. Previous studies have demonstrated that ARB improves arterial stiffness assessed by PWV independently of lowering BP in diabetic patients[18] and in hypertensive patients,[19] and that thiazide diuretics have a limited effect on arterial stiffness.[20,21] In addition, angiotensin-converting enzyme inhibitors and ARBs have been reported to reduce AI in hypertensive patients,[22] and monotherapy with HCTZ has been reported not to decrease AI even if it reduced BP to an extent similar to ARB.[23] On the basis of these evidences, ARB has a BP-independent benefit on vascular wall properties. Thus, the improvement of the CAVI and AI might result from the RAS blockade but not from the reduction in BP or HCTZ treatment. As arterial stiffness is a powerful and independent risk factor for mortality in cardiovascular events,[24] L/HCTZ could be one of the useful antihypertensive drugs with cardiovascular protective properties. Although previous studies have demonstrated the benefits of a fixed-dose angiotensin-converting enzyme inhibitor–diuretic combination for AI compared with diuretic monotherapy,[25] there have not been any studies which examined the effects of a fixed-dose ARB–diuretic combination on CAVI or AI in comparison with diuretic alone. Further studies will be needed to elucidate the difference between the two treatments.

There were some other limitations in interpreting the results of this study. First, the trial population was comparatively small in number. Second, we did not compare the L/HCTZ tablet with dose-titrated ARBs. Thus, this study did not provide a definitive conclusion regarding the superiority of the L/HCTZ tablet *vs.* losartan alone in reducing BP. However, as no difference in adverse effects was observed during the treatment periods, the L/HCTZ tablet is a safe and useful antihypertensive drug in diabetic patients with hypertension. Finally, prognostic events were not examined. Further studies will be needed to confirm the benefits of the L/HCTZ therapy.

In conclusion, the treatment with the tablet combining L/HCTZ exerted a greater reduction in BP than losartan monotherapy, and decreased albuminuria and arterial stiffness assessed by CAVI and AI to the levels similar to losartan monotherapy. As metabolic adverse effects were similar in the L/HCTZ treatment and losartan treatment, the L/HCTZ tablet could be a safe and potent antihypertensive drug in Japanese diabetic patients with hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Digital Assessment of Endothelial Function and Ischemic Heart Disease in Women

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Objectives We investigated the utility of digital reactive hyperemia peripheral arterial tonometry (RH-PAT) in predicting ischemic heart disease (IHD), including obstructive coronary artery disease (CAD) and nonobstructive coronary artery disease (NOCAD), in women.

Background IHD is the leading cause of mortality, and its pathogenesis is diverse in women. Fingertip RH-PAT is a new device that provides noninvasive, automatic, and quantitative evaluation of endothelial dysfunction.

Methods RH-PAT was measured using Endo-PAT2000 (Itamar Medical, Caesarea, Israel) before cardiac catheterization in 140 stable women scheduled for hospitalization to examine chest pain. NOCAD was diagnosed by angiography with measurement of coronary blood flow and cardiac lactate production during intracoronary acetylcholine provocation test and cardiac scintigraphy with stress tests.

Results Sixty-eight women (49%) had obstructive CAD and 42 women (30%) had NOCAD. RH-PAT indexes were significantly attenuated in both obstructive CAD and NOCAD as compared with non-IHD (n = 30) (obstructive CAD: median 1.57, interquartile range [IQR] 1.42 to 1.76; NOCAD: median 1.58, IQR 1.41 to 1.78; non-IHD: median 2.15, IQR 1.85 to 2.48, $p < 0.001$). By multivariate logistic regression analysis, only RH-PAT index was significantly associated with IHD, including obstructive CAD and NOCAD (odds ratio 0.51; 95% confidence interval: 0.38 to 0.68; $p < 0.001$). In receiver-operating characteristic analysis, RH-PAT index was a significant predictor of IHD (area under the curve 0.86; $p < 0.001$). Furthermore, only RH-PAT was useful for the prediction of NOCAD after excluding obstructive CAD (area under the curve 0.85; $p < 0.001$; RH-PAT index of <1.82 had 81% sensitivity and 80% specificity).

Conclusions RH-PAT indexes were significantly attenuated in women with IHD. Digital RH-PAT can predict patients with IHD, especially NOCAD before angiography. RH-PAT is potentially useful for identifying high-risk women for IHD. (Endothelial Dysfunction and Coronary Artery Spasm; NCT00619294) (J Am Coll Cardiol 2010;55:1688–96)

Coronary artery disease (CAD) is the leading cause of mortality in post-menopausal women (1). Women with clinical features of myocardial ischemia remain a clinical challenge due to the diverse pathogenic mechanisms of ischemic heart diseases (IHD) in women. The WISE (Women's Ischemia Syndrome Evaluation) study found no significant coronary artery stenoses (i.e., <50% stenosis) in any major coronary artery in 69% of women suspected of having IHD (2). Nonobstructive coronary artery disease (NOCAD) also presents a high risk for women with myocardial ischemia (3–5).

See page 1697

Thus, cardiovascular prognosis of women with NOCAD is not benign, and there is a need for effective strategies for the identification and treatment of these patients.

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The pathogenesis of NOCAD is unclear at present, but it could be due to physiological abnormality in coronary circulation, coronary spastic angina, coronary microvascular spasm, microcirculatory insufficiency, or diffuse arteriosclerosis. The assessment of NOCAD is not yet established enough. Coronary spastic angina can be diagnosed only by the acetylcholine-provocation test during coronary angiography (CAG) (6), and microvascular coronary spasm can be diagnosed by measurements of coronary blood flow and myocardial lactate production during acetylcholine-provocation test (7). Abnormal residual function of the oxygen supply due to microcirculatory insufficiency and diffuse arteriosclerosis can be evaluated by the measurement of coronary blood flow during adenosine-provocation and stress myocardial scintigraphy (8,9). Because plural complicated vascular function tests are necessary, NOCAD is not fully diagnosed definitely in the routine clinical practice.

Vascular endothelial dysfunction is found from the early phase of arteriosclerosis to the advanced atheroma resulting in obstructive CAD (10). Furthermore, regarding NOCAD, not only coronary spastic angina, but also microvascular spasm and microcirculatory insufficiency have been shown to be associated with coronary endothelial dysfunction (7,11,12), thus it could be possible to identify all these patients with IHD by physiological examination and evaluating endothelial dysfunction.

Endothelial dysfunction in peripheral arteries is assessed by forearm flow-mediated vasodilation (13). However, the results of forearm flow-mediated vasodilation can vary due to technical problems encountered during measurement, and thus forearm flow-mediated vasodilation is not standardized among institutions (14). Kuvin et al. (15) described a new method to evaluate endothelial dysfunction called reactive hyperemia peripheral arterial tonometry (RH-PAT). It is a noninvasive, automatic, and quantitative clinical test for digital measurement of hyperemic response. Using this test, the Framingham Heart Study reported that RH-PAT indexes correlated inversely with various cardiovascular risk factors (16), indicating the practical usefulness of RH-PAT test.

We hypothesized that endothelial function measured by fingertip RH-PAT is impaired in patients with IHD, including NOCAD, and that the RH-PAT indexes can predict the presence of IHD, especially NOCAD, in women complaining of chest pain.

Methods

Study population and protocol. One hundred fifty-eight consecutive stable post-menopausal women with angina-like chest pain who were referred and scheduled for hospitalization at Kumamoto University Hospital between August 2006 and April 2009 for CAG were registered. We excluded 17 patients for the following reasons: severe aortic valve regurgitation or stenosis (n = 2), hypertrophic cardiomyopathy (n = 6), uncontrolled hypertension (n = 1), severe collagen disease (n = 6), and neuromuscular disease (n = 2).

Abbreviations and Acronyms

ACh-CBF = acetylcholine-induced increase in coronary blood flow ratio
Ad-CFR = adenosine-induced coronary flow reserve
AUC = area under the curve
CAD = coronary artery disease
CAG = coronary angiography
IHD = ischemic heart disease
NOCAD = nonobstructive coronary artery disease
RH-PAT = reactive hyperemia peripheral arterial tonometry
ROC = receiver-operating characteristic
SPECT = single-photon emission computed tomography

RH-PAT was monitored using Endo-PAT2000 (Itamar Medical, Caesarea, Israel) on the day before CAG. CAG and RH-PAT studies were performed in the fasting state in the early morning after >3-day discontinuation of vasodilators. Cardiologists blinded to the results of the RH-PAT performed cardiac catheterization. On the basis of the results of CAG and after excluding 1 patient for incomplete cardiac catheterization data, patients with atherosclerotic organic coronary artery stenosis (≥50%) were diagnosed as having obstructive CAD, whereas those with no significant epicardial coronary artery stenosis (<50%) on CAG (NOCAD suspected) underwent acetylcholine-provocation test. Patients who showed myocardial ischemia during acetylcholine-provocation test were divided into 2 groups: patients with epicardial coronary spasm and patients with microvascular spasm, those with coronary blood flow decrease by acetylcholine provocation without epicardial coronary spasm. Patients with negative results in the acetylcholine-provocation test were further examined by adenosine-induced coronary flow reserve and stress thallium-201 single-photon emission computed tomography (SPECT). Patients with abnormal results of these tests were diagnosed as having microcirculatory insufficiency, and patients who had no abnormal results were defined as the nonischemic heart disease group (non-IHD group) (Fig. 1). The Reynolds Risk Score was calculated for each patient as described previously (17).

Risk factors for cardiovascular disease were defined as current smoking (smoking within 1 year), hypertension (>140/90 mm Hg or taking antihypertensive medication), dyslipidemia (high-density lipoprotein cholesterol <40 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, or triglycerides ≥150 mg/dl or taking medication for dyslipidemia), and diabetes mellitus (symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl, fasting plasma glucose concentration ≥126 mg/dl, 2-h plasma glucose concentration ≥200 mg/dl during 75-g oral glucose tolerance test, or taking medication for diabetes mellitus).

Written informed consent was obtained from each patient before participation. The study was conducted in accordance with the guidelines approved by the ethics committee of our institution.

RH-PAT. The principle of RH-PAT has been described previously (18). Briefly, a blood pressure cuff was placed on 1 upper arm, while the contralateral arm served as a control.

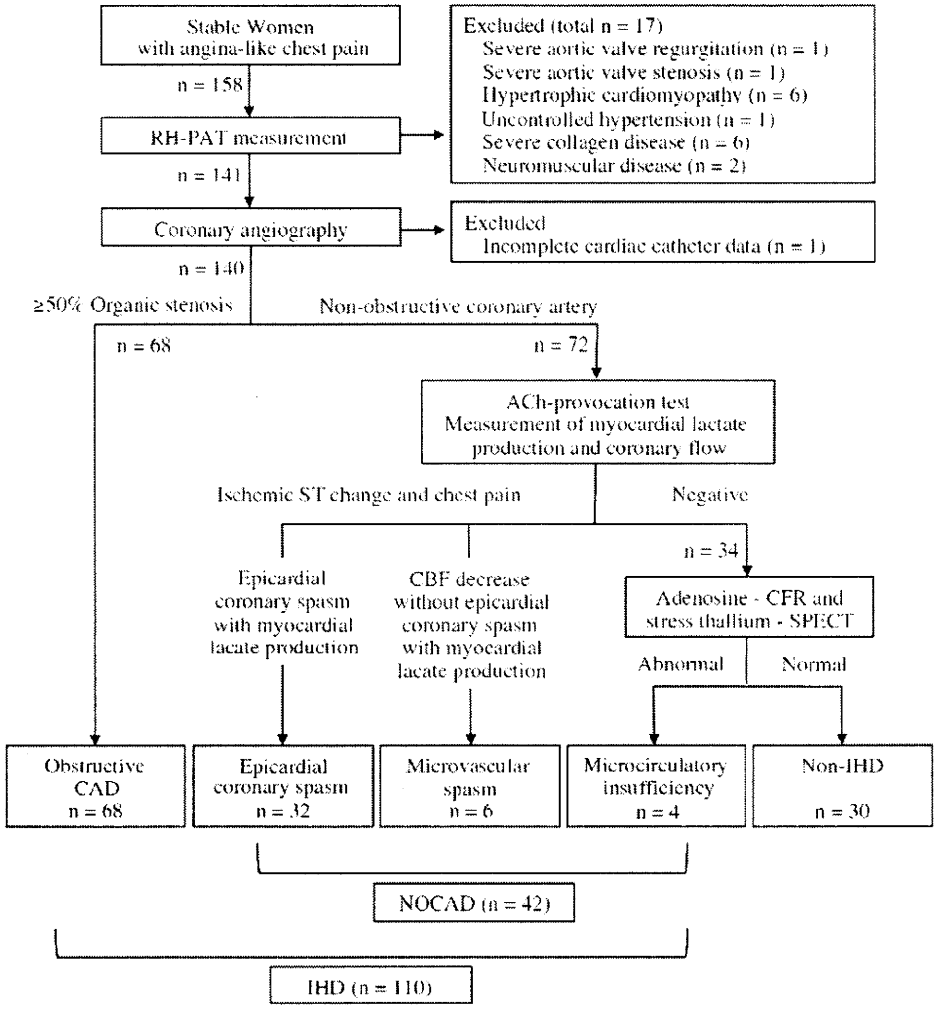

Figure 1 **Flow Chart of Patient Enrollment Used in the Present Study**

ACh = acetylcholine; CAD = coronary artery disease; CBF = coronary blood flow; CFR = coronary flow reserve; IHD = ischemic heart disease; NOCAD = nonobstructive coronary artery disease; RH-PAT = reactive hyperemia peripheral arterial tonometry; SPECT = single-photon emission computed tomography.

PAT probes were placed on 1 finger of each hand. After a 5-min equilibration period, the cuff was inflated to 60 mm Hg above the systolic pressure or 200 mm Hg for 5 min and then deflated to induce reactive hyperemia.

The RH-PAT data were digitally analyzed online (Endo-PAT2000 software version 3.0.4). The RH-PAT index reflects the extent of reactive hyperemia and was calculated as the ratio of the average amplitude of PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the average amplitude of PAT signal of a 2.5-min time period before cuff inflation (baseline) (control arm, B; occluded arm, D). Thus RH-PAT index = (C/D)/(A/B) × baseline correction.

Cardiac catheterization. After baseline CAG, patients without obstructive CAD underwent the acetylcholine-provocation test. A 6-F catheter was placed in the coronary sinus to sample blood for measurement of lactate concentrations. A 0.014-inch Flow-Wire Doppler flow probe (REF 1400J-FloWire, Volcano, San Diego, California) was inserted into the proximal side of the left anterior descending coronary artery, and acetylcholine was injected into the left coronary artery as described previously (6,19). Briefly, incremental doses (20, 50, and 100 μg) of acetylcholine were injected into the left coronary artery, and angiography was performed 1 min after each injection. Then, 50 μg of acetylcholine was injected into the right coronary artery, followed by angiography. At baseline and acetylcholine-induced coronary spasm or 1 min after the maximum dose of acetylcholine was injected into the left coronary artery, paired samples of 2 ml of blood were collected simultaneously from the main trunk of the left coronary artery and coronary sinus to measure the myocardial lactate extraction rate, as reported previously (20).

Using Flow-Wire, we measured changes in coronary blood flow in response to low-dose acetylcholine (20 μg

injected into the left coronary artery) to determine coronary endothelial function (21):

Acetylcholine-induced increase in coronary blood flow ratio (ACh-CBF) = acetylcholine-induced hyperemia coronary blood flow/baseline coronary blood flow, where coronary flow = π(average peak velocity/2)(vessel diameter/2)^2

At the end of the acetylcholine test, nitroglycerin was injected into each coronary artery when acetylcholine-induced coronary spasm did not resolve spontaneously within 5 min, angina chest pain persisted for more than 2 min, or upon the development of ischemia-related hemodynamic instability. After intracoronary nitroglycerin, adenosine (150 μg/kg/min) was injected intravenously until maximal hyperemia was achieved. Then coronary flow reserve was calculated using the following equation:

Adenosine-induced coronary flow reserve (Ad-CFR) = adenosine-induced hypermia coronary blood flow/baseline coronary blood flow

Coronary spasm was defined as >90% lumen narrowing of the epicardial coronary artery associated with chest pain, transient ST-segment depression (>0.1 mV) or elevation (>0.1 mV) from baseline levels occurring at 60 to 80 ms after the J point, and lactate production in the coronary circulation. Microvascular spasm was defined as decreased coronary blood flow during acetylcholine provocation associated with chest pain, transient ST-segment depression or elevation, and myocardial lactate production without epicardial coronary spasm. In a subject without positive results in acetylcholine-provocation test, we defined the patients who showed Ad-CFR <3.0 and myocardial perfusion abnormality in adenosine-provocation thallium SPECT as having microcirculatory insufficiency.

Statistical analysis. The results of normally distributed continuous variables were expressed as mean (SD), whereas those with skewed distribution were expressed as the median value (interquartile range [IQR]). Continuous variables were analyzed by the unpaired *t* test and Mann-Whitney *U* test, as appropriate. Categorical variables were presented by percent, and intergroup comparisons were analyzed by chi-square test (and Fisher exact test). Pearson's correlation coefficient was used for evaluation of possible association between ln[RH-PAT index] and ln[ACh-CBF]; ln[RH-PAT index] and ln[Ad-CFR]. Associations between the presence of IHD or NOCAD and other significant parameters in simple logistic analysis were analyzed by multiple logistic regression analysis with the forced entry method, and the Hosmer-Lemeshow

Table 1 Baseline Clinical Characteristics of 140 Women With Chest Pain

	Non-IHD	Obstructive CAD	NOCAD
n	30	68	42
Age (yrs)	63 (10)	73 (9)*	64 (10)†
Body mass index (kg/m^2)	23 (3)	24 (5)	24 (4)
Hypertension (%)	53	84*	62†
Diabetes (%)	20	50*	29†
Dyslipidemia (%)	67	91*	64†
Current smoking (%)	3	7	14
Family history of CAD (%)	3	19	21*
Systolic blood pressure (mm Hg)	124 (17)	131 (20)	130 (17)
Diastolic blood pressure (mm Hg)	77 (13)	72 (12)	78 (12)†
Fasting blood glucose (mg/dl)	89 [85–94]	97 [88–115]	92 [86–105]
Hemoglobin A1c (%)	5.5 (0.6)	6.1 (1.2)*	5.6 (1.0)†
HOMA-IR	1.0 [0.8–1.7]	1.4 [1.0–2.5]*	1.4 [0.8–1.8]
Total/high-density lipoprotein cholesterol ratio	3.5 (1.2)	3.5 (1.1)	3.3 (0.9)
Triglycerides (mg/dl)	92 [65–123]	101 [76–136]	99 [80–139]
Left ventricular ejection fraction (%)	66 (7)	65 (7)	66 (5)
B-type natriuretic peptide (pg/ml)	18 [10–26]	48 [21–120]*	20 [12–30]†
High-sensitivity C-reactive protein (mg/l)	0.5 [0.3–1.0]	1.2 [0.5–2.8]*	0.5 [0.3–0.9]†
Reynolds Risk Score (%)	1.3 [0.5–3.5]	5.6 [2.6–10.2]*	1.8 [1.0–4.0]†
Mild-to-moderate coronary atherosclerosis (%)	33	—	43
Aspirin (%)	27	90*	46†
HMG-CoA reductase inhibitors	33	79*	38†
Calcium-channel blockers (%)	46	71*	49†
ACE-I or ARB (%)	27	62*	29†
Beta blockers (%)	3	46*	0†

Data are mean (SD), median [25th to 75th percentile range], or %. Mild-to-moderate coronary atherosclerosis: >25% but <50% coronary stenosis. *Significantly different from non-IHD. †Significantly different from obstructive CAD.

ACE-I = angiotensin-converting enzyme inhibitors; ARB = angiotensin II receptor blockers; CAD = coronary artery disease; HMG-CoA = 3-hydroxy-3-methylglutanyl coenzyme A; HOMA-IR = homeostasis model assessment insulin resistance; IHD = ischemic heart disease; NOCAD = nonobstructive coronary artery disease.

goodness-of-fit statistic was calculated. Receiver-operating characteristics (ROC) curves were constructed for Reynolds Risk Score and RH-PAT index. The area under the curve (AUC), sensitivity, and specificity were calculated to predict the ability to detect subjects with obstructive CAD, NOCAD, and IHD, with an AUC value of 0.50 indicating no accuracy and a value of 1.00 indicating maximal accuracy. AUC values are compared using an algorithm suggested by DeLong et al. (22,23). We defined optimal thresholds of RH-PAT index by maximizing the sum of sensitivity and specificity (24). A p value of <0.05 denoted statistical significance; all tests were 2-tailed. Statistical analyses were performed using SPSS version 17.0J (SPSS Inc., Tokyo, Japan) and STATA version 10 (Stata Corp., College Station, Texas).

Results

Clinical characteristics of stable women with chest pain. CAG indicated that 68 patients (49%) had obstructive CAD (30 patients had single-vessel disease, 38 patients had

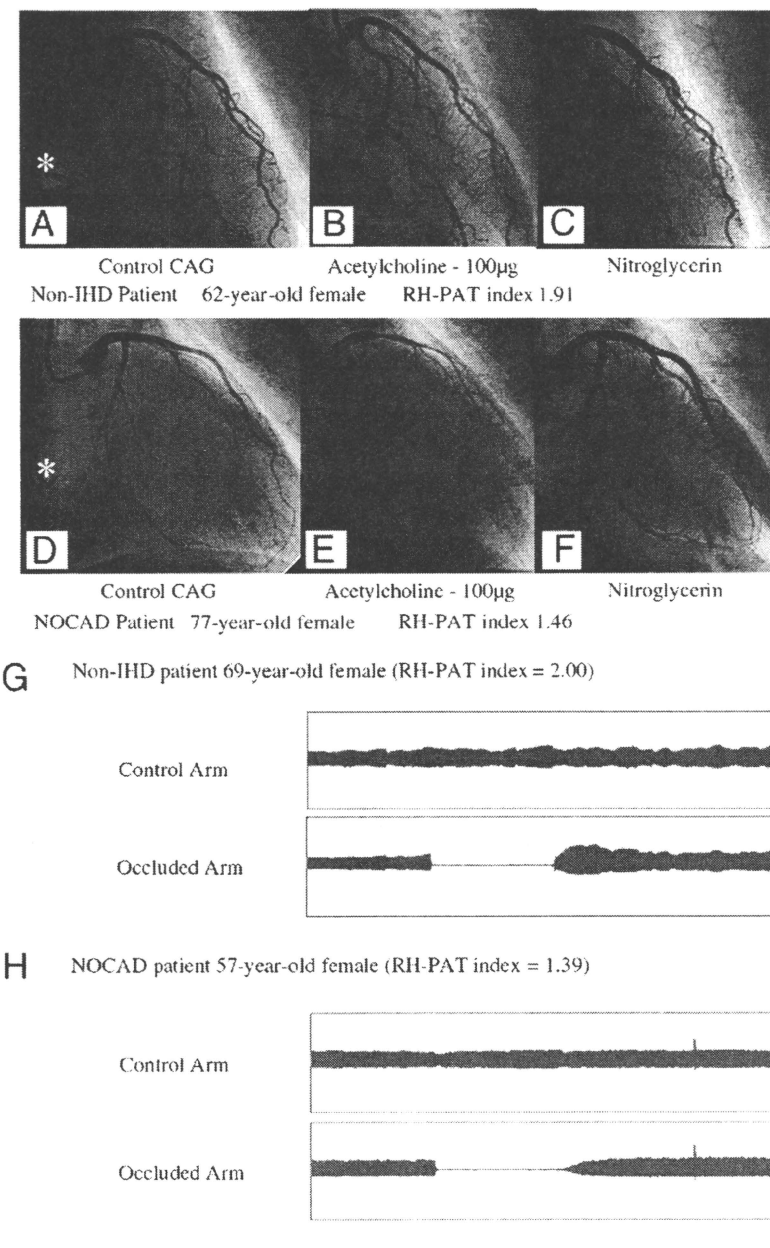

Figure 2 **Acetylcholine-Provocation Test**

(A to C) Non-IHD group. **(D to F)** Epicardial coronary spasm group. **(E)** Coronary artery spasm after intracoronary injection of acetylcholine. *Sampling catheter in the coronary sinus. RH-PAT results of representative **(G)** non-IHD and **(H)** NOCAD cases. Abbreviations as in Figure 1.

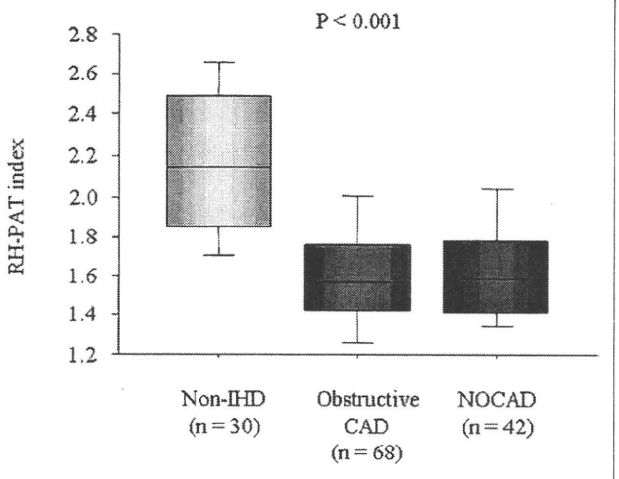

Figure 3 **RH-PAT Index and IHD**

Box-and-whisker plots of RH-PAT indexes. In these plots, **lines within the boxes** represent median values; the **upper and lower lines of the boxes** represent 25th and 75th percentiles, respectively; and the **upper and lower bars outside the boxes** represent the 90th and 10th percentiles, respectively. Abbreviations as in Figure 1.

multivessel disease), and 72 patients (51%) were suspected to have NOCAD (44 patients had no stenosis, 28 patients had mild-to-moderate coronary atherosclerosis [25% to 50% coronary artery stenosis]) (Table 1). Among the latter group (n = 72; NOCAD suspected), coronary spasm was induced in 32 patients (15 with ST-segment elevation, 17 with ST-segment depression) and microvascular spasm was induced in 6 patients (1 with ST-segment elevation, 5 with ST-segment depression). Among another 34 patients without positive results in acetylcholine-provocation test, 4 patients were diagnosed as having microcirculatory insufficiency, and 30 patients had no evidence of myocardial ischemia (non-IHD group) (Fig. 1).

Patients in the obstructive CAD group were significantly older, had a higher prevalence of conventional cardiovascular risk factors, higher homeostasis model assessment insulin resistance, B-type natriuretic peptide, high-sensitivity C-reactive protein, and Reynolds Risk Score. In contrast, patients in the NOCAD group were not significantly different from those in the non-IHD group, except for family history of CAD (Table 1).

RH-PAT indexes and IHD including obstructive CAD and NOCAD. Figure 2 shows representative records of RH-PAT signals and CAG in patients with non-IHD and NOCAD. RH-PAT indexes were lower in patients with obstructive CAD and NOCAD than in non-IHD patients (non-IHD: median 2.15 [IQR 1.85 to 2.48]; obstructive-CAD: median 1.57 [IQR 1.42 to 1.76]; NOCAD: median 1.58 [IQR 1.41 to 1.78]; $p < 0.001$) (Fig. 3). There was no significant difference in RH-PAT indexes between NOCAD and obstructive CAD groups.

RH-PAT indexes and ACh-CBF among patients with NOCAD. We were successful in obtaining complete coronary flow data from 51 patients without obstructive CAD. ACh-CBF was attenuated in patients with NOCAD relative to the non-IHD group (NOCAD: median 1.52 [IQR 1.23 to 1.90]; non-IHD: median 2.12 [IQR 1.82 to 2.42]; $p < 0.001$). However, Ad-CFR was comparable in the 2 groups (NOCAD: median 3.25 [IQR 3.01 to 3.58]; non-IHD: median 3.09 [IQR 2.78 to 3.60]; $p = 0.36$). There was a significant correlation between ln[RH-PAT index] and ln[ACh-CBF] ($r = 0.52$; $p < 0.001$), but not Ad-CFR ($r = 0.21$; $p = 0.12$).

RH-PAT indexes and presence of IHD. Simple logistic regression analysis demonstrated that age, B-type natriuretic peptide, Reynolds Risk Score, and RH-PAT index significantly predicted the presence of IHD in women complaining of chest pain (Table 2). Multiple logistic regression analysis identified only RH-PAT index as the

Table 2 **Logistic Regression Analysis for the Presence of Ischemic Heart Disease in Female Patients Complaining of Chest Pain**

	Simple Regression			Multiple Regression		
Variable	OR	95% CI	p Value	OR	95% CI	p Value
Age (per yr)	1.06	1.02–1.10	0.004	0.99	0.92–1.07	0.81
Body mass index (per kg/m^2)	1.06	0.95–1.18	0.28			
Current smoking (yes)	3.22	0.40–26.0	0.27			
Systolic blood pressure (per mm Hg)	1.02	1.00–1.05	0.06			
Diastolic blood pressure (per mm Hg)	0.98	0.95–1.01	0.24			
Ln[Fasting blood glucose] (per 0.1)	1.23	0.96–1.57	0.10			
Total/High-density lipoprotein cholesterol (per 1)	0.95	0.65–1.42	0.81			
Ln[Triglycerides] (per 0.1)	1.03	0.94–1.12	0.54			
Ln[B-type natriuretic peptide] (per 0.1)	1.05	1.01–1.09	0.01	1.03	0.98–1.09	0.21
Ln[High-sensitivity C-reactive protein] (per 0.1)	1.01	0.99–1.03	0.31			
Left ventricular ejection fraction (per %)	0.98	0.92–1.05	0.60			
Ln[Reynolds Risk Score] (per 0.1)	1.07	1.04–1.11	<0.001	1.06	1.00–1.13	0.05
Ln[RH-PAT index] (per 0.1)	0.50	0.38–0.65	<0.001	0.51	0.38–0.68	<0.001

CI = confidence interval; OR = odds ratio; RH-PAT = reactive hyperemia peripheral arterial tonometry; other abbreviations as in Table 1.

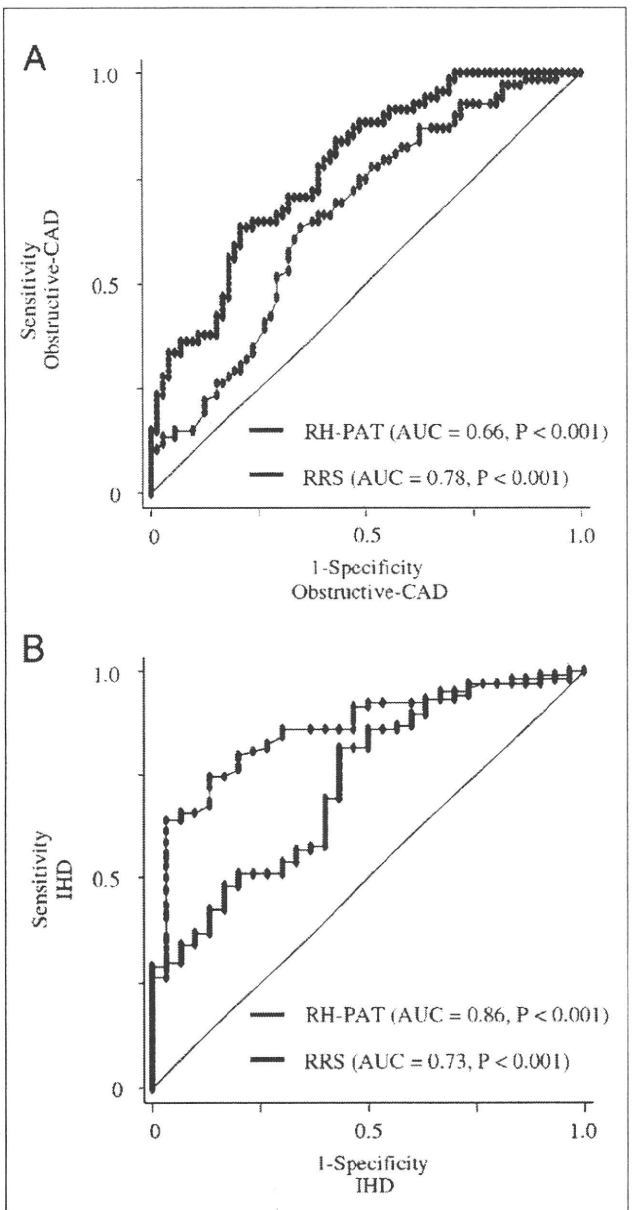

Figure 4 **ROC Curves to Identify Obstructive CAD and IHD**

Receiver-operating characteristic (ROC) curves for the Reynolds Risk Score (RRS) and RH-PAT index to identify patients with obstructive CAD and patients with IHD in stable women complaining of chest pain. AUC = area under the curve; other abbreviations as in Figure 1.

significant predictor of IHD, including obstructive CAD and NOCAD (odds ratio: 0.51; 95% confidence interval [CI]: 0.38 to 0.68; p < 0.001) (Table 2). Hosmer-Lemeshow goodness of fit chi-square and p are 12.1 and 0.15, respectively. Furthermore, we found that only RH-PAT index was also a significant factor for predicting NOCAD in women complaining of chest pain by multiple logistic regression analysis (odds ratio: 0.78; 95% CI: 0.63 to 0.96; p = 0.02). Hosmer-Lemeshow goodness of fit chi-square is 11.4 and the p value is 0.18.

ROC analysis for Reynolds Risk Score and RH-PAT index to predict patients with obstructive CAD and IHD in women complaining of chest pain. ROC curves were constructed to assess the ability of Reynolds Risk Score and RH-PAT index to predict obstructive CAD and IHD. The AUC for detection of obstructive CAD was 0.78 (95% CI: 0.70 to 0.85; p < 0.001) of Reynolds Risk Score and 0.66 (95% CI: 0.57 to 0.75; p < 0.001) of RH-PAT index (Fig. 4A). The AUC for detection of IHD, including obstructive CAD and NOCAD, was 0.73 (95% CI: 0.63 to 0.83; p < 0.001) of Reynolds Risk Score and 0.86 (95% CI: 0.79 to 0.93; p < 0.001) of RH-PAT index (Fig. 4B). There was no significant (borderline) difference between AUCs of RH-PAT index and Reynolds Risk Score for prediction of obstructive CAD (p = 0.06) or IHD (p = 0.05). Reynolds Risk Score was comprehensively useful for prediction of obstructive CAD, and RH-PAT index was particularly useful for prediction of overall IHD. Using an RH-PAT index cutoff value of <1.82, the sensitivity and specificity for the detection of IHD were 80% and 80%, respectively.

RH-PAT index has high ability to predict NOCAD, particularly in patients without obstructive CAD. We can diagnose obstructive CAD simply on anatomical examination such as CAG or multidetector computed tomographic CAG. Therefore, we examined whether RH-PAT was useful for a prediction of NOCAD after excluding the presence of obstructive CAD. We found that only the RH-PAT index was a significantly associated factor of NOCAD in women without obstructive CAD by simple logistic regression analysis (odds ratio 0.49; 95% CI: 0.35 to

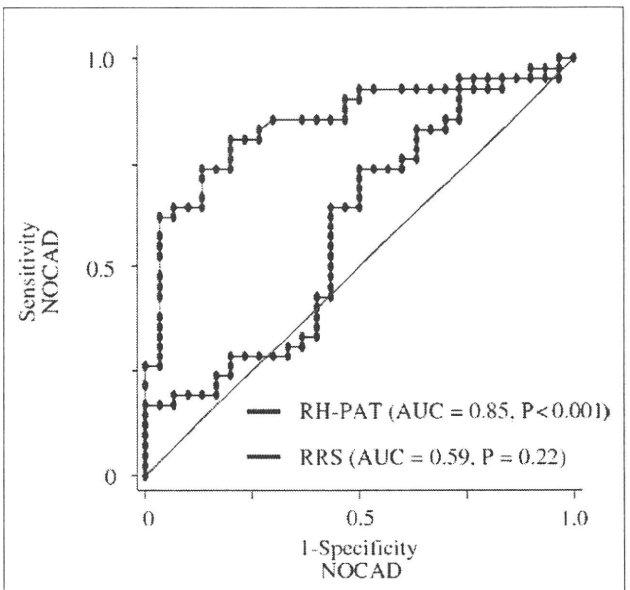

Figure 5 **ROC Curves to Identify NOCAD**

Receiver-operating characteristic (ROC) curves for Reynolds Risk Score (RRS) and RH-PAT index to identify patients with NOCAD among women without obstructive CAD. Abbreviations as in Figures 1 and 4.

0.69; $p < 0.001$). Among women without obstructive CAD, Reynolds Risk Score could not predict NOCAD (AUC 0.59; 95% CI: 0.45 to 0.73; $p = 0.22$), but RH-PAT index could significantly predict NOCAD (AUC 0.85; 95% CI: 0.76 to 0.94; $p < 0.001$) (Fig. 5). The AUC of RH-PAT index for prediction of NOCAD was significantly higher compared with Reynolds Risk Score ($p = 0.003$). The cutoff value of RH-PAT index of <1.82 had sensitivity of 81% and specificity 80% for prediction of NOCAD in patients without obstructive CAD.

Discussion

The present study of stable women complaining of angina-like symptoms showed significant impairment of digitally recorded endothelial function in patients with IHD, especially NOCAD, and such impairment was equivalent to that seen in patients with organic obstructive CAD. Reynolds Risk Score and RH-PAT index significantly predicted IHD. RH-PAT index was particularly superior in predicting NOCAD. In other words, noninvasive measurement of RH-PAT can predict patients with IHD, including NOCAD, before angiography. Thus, RH-PAT is potentially useful for identification of women at high risk for IHD.

Over the years, cardiovascular mortality has declined substantially, but this improvement in prognosis had been restricted to men (25,26). IHD is sometimes not diagnosed correctly in women; therefore, the prognosis of such women is worse than that of men (2). It is important that more women are diagnosed correctly with IHD and receive appropriate treatment. NOCAD is more common in women, and women have been recognized recently as a high-risk population (3–5). The underlying mechanisms of NOCAD are not fully known, though it is likely caused by a multitude of pathogenic mechanisms (3). Women with myocardial ischemia are still a clinical challenge. Obstructive CAD could be diagnosed by CAG (anatomical examination), but NOCAD cannot be simply diagnosed with anatomical tests. Although the Reynolds Risk Score predicts obstructive CAD well, it may not be useful for prediction of NOCAD in women. The design of a new and noninvasive test for the assessment of NOCAD is strongly desirable. Coronary endothelial dysfunction plays an important pathogenic role in NOCAD (7,11,12,27). The present study demonstrated that noninvasive digital assessment of peripheral endothelial function by RH-PAT significantly predicted IHD, particularly NOCAD. Thus, RH-PAT can potentially provide useful and noninvasive clinical assessment of IHD, including NOCAD, in women. We could noninvasively provide superior identification of high-risk patients for IHD with a combination of the physiological examination with RH-PAT and the anatomical examination with computed tomography.

The vascular endothelium plays a crucial role in regulation of vasomotor tone, thrombosis, and platelet adhesion (10,13), and endothelial dysfunction is a significant risk factor of future cardiovascular events (28,29). Invasive measurement of coronary vasodilator response by acetylcholine infusion is an established method for assessment of coronary endothelial function (21). On the other hand, digital RH-PAT can evaluate peripheral endothelial function, and the results are correlated with traditional and metabolic cardiovascular risk factors (15,16,18). Bonetti et al. (30) demonstrated that RH-PAT index significantly predicted coronary endothelial dysfunction. Similar to their study, we also found that RH-PAT index was correlated significantly with ACh-CBF. In addition, we also found that RH-PAT index was significantly attenuated in patients with NOCAD who were diagnosed by simultaneous measurement of coronary flow and cardiac lactate production. Measurement of RH-PAT index, which can reflect coronary endothelial function, may also have a useful predictive value for future cardiovascular events. Recently, Rubinshtein et al. (31) reported that assessment of peripheral vascular function by RH-PAT in addition to the Framingham Risk Score may be useful for identification of risk for cardiac events.

Our results showed significant impairment of RH-PAT index in women with IHD. Endothelial dysfunction could be a modifiable risk factor of future cardiovascular events and a potential clinical therapeutic target. Because RH-PAT is a noninvasive, quantitative, and repeatable test, the values of RH-PAT index could be used for evaluation of vascular condition and treatment efficacy.

Study limitation. The predictive value of RH-PAT index in NOCAD is limited to a modest number of patients and needs confirmation in larger patient populations.

Conclusions

Digital fingertip endothelial function was significantly impaired in post-menopausal women with NOCAD equivalent to those with obstructive CAD. RH-PAT noninvasively predicted the presence of IHD, especially NOCAD, before CAG. RH-PAT is a potentially useful clinical test and can effectively help to identify high-risk women with chest pain.

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