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Prognostic Role of Flow-Mediated Dilation and Cardiac Risk Factors in Post-Menopausal Women

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Objectives The aim of this study was to examine the association between brachial artery flow-mediated dilation (FMD) and cardiovascular events in a cohort of initially asymptomatic post-menopausal women, with adjustment for the presence of the major cardiovascular risk factors.

Background Conventional major cardiovascular risk factors (cigarette smoking, hypercholesterolemia, hypertension, and diabetes) fail to explain nearly 50% of cardiovascular events. Defining the magnitude of future risk for the development of clinical events is a major focus of effective primary prevention. Evaluation of endothelial function, utilizing the noninvasive measurement of the brachial artery FMD, may serve as a screening tool to individualize high-risk patients.

Methods We conducted a prospective study on 2,264 post-menopausal women, age 54 ± 6 years. The length of the follow-up was 45 ± 13 months (range 6 to 65 months).

Results During observation, 90 major events were recorded. Risk-adjusted relative risk values resulted 1.0, 1.33 (95% confidence interval [CI] 1.09 to 4.09), and 4.42 (95% CI 2.97 to 8.01) for women in the higher, intermediate, and lower tertile of FMD, respectively ($p < 0.0001$ for trend). The event rate for women in the lower tertile (FMD ≤4.5%) was greater than the combined event rate noted in the other 2 tertiles (women in the lower tertile accounted for 51 events [56.6% of total events]). When added to age and other conventional cardiovascular risk factors (smoking habits, presence of hypercholesterolemia, history of diabetes, hypertension), FMD contributed significantly to the model predicting cardiovascular events (likelihood ratio chi-square change: 10.22; $p < 0.0001$).

Conclusions In post-menopausal women, the knowledge of FMD provided incremental prognostic information regarding the risk of developing cardiovascular events. (J Am Coll Cardiol 2008;51:997–1002) © 2008 by the American College of Cardiology Foundation

Cardiovascular diseases (CVDs) are the primary cause of death in all industrialized countries, and will most likely remain the leading cause of death well into the 21st century (1–3). Prospective epidemiologic studies have established the association between major risk factors (age, cigarette smoking, hypercholesterolemia, diabetes, and hypertension) and the development of cardiovascular (CV) events (4–7). However, it has been estimated that these risk factors fail to explain up to 50% of the CVD morbidity and mortality (2,4,8). According to Framingham data, nearly 50% of all CV events occur in individuals with no previous history of symptomatic heart diseases (9–11). Thus, defining the magnitude of future risk for the development of clinical CVD is a major focus of effective primary prevention.

One proposed screening strategy for searching the patient susceptible to an acute CV event (defined as "vulnerable patient" by Naghavi et al. [12]) uses the noninvasive measurement of the flow-mediated dilation (FMD) in the brachial artery, utilizing a high-resolution ultrasound method ("endothelial dysfunction, as measured by impaired FMD in the brachial artery, can aid in the detection of pan-arterial vulnerability and may serve as a screening tool" [12]).

The impairment of endothelial function is the primary etiology implicated in the origin and development of atherosclerotic CVD (13). Since 1992, the noninvasive measurement of brachial artery FMD has been used to evaluate endothelial function (14), which is considered a marker of CVD risk (15–18).

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Abbreviations and Acronyms

CV = cardiovascular
CVD = cardiovascular disease
FMD = flow-mediated dilation
NO = nitric oxide
TIA = transient ischemic attack
t-PA = tissue-type plasminogen activator
u-PA = urokinase-type plasminogen activator

The aim of this study was to examine the association between FMD and CV events in a cohort of initially asymptomatic post-menopausal women, with adjustment for the presence of the major CV risk factors.

Methods

Patient selection. We conducted a prospective study on 2,264 post-menopausal women, age 55 ± 6 years (range 47 to 60 years), all self-referred to the "Bene Essere Donna" center, an institution dedicated to the study, prevention, and treatment of menopause-related disorders. This service, located in a third-level university hospital, is open to all women providing they are in their post-menopausal period (post-menopausal status was defined as the absence of menstruation for at least 6 months and/or by a follicle-stimulating hormone blood level >40 IU/l and 17 beta-estradiol levels <120 pmol/l) and are ≤60 years of age. These women, who are initially drawn to the center through local media advertising, have free access and can make queries or obtain advice about particular symptoms they are having by scheduling an appointment beforehand. Physical examination variables measured at baseline included body weight, height, waist circumference, and systolic and diastolic blood pressure. Patient history, 12-lead electrocardiogram, and echocardiogram were used to exclude past or present heart disease. At baseline each participant had fasting blood tests for levels of glucose and total cholesterol. Participants provided questionnaire data concerning life-style potential risk factors for CVD (cigarette smoking, hypercholesterolemia, diabetes, and hypertension). Hypercholesterolemia was defined as a total cholesterol level of >200 mg/dl or the use of cholesterol-lowering medications. Individuals were considered to be diabetic patients in the presence of a fasting blood glucose ≥126 mg/dl or if they reported using oral hypoglycemic agents and/or insulin; and hypertensive in presence of high blood pressure values (>140/90 mm Hg) or if they reported a history of high blood pressure and use of antihypertensive medications.

Patients with a past medical history of chest pain, diagnostic coronary angiography, coronary revascularization, myocardial infarction, stroke or transient ischemic attack (TIA), and previous or current use of hormone replacement therapy were excluded from the study.

The length of the follow-up was 45 ± 13 months (range 6 to 65 months). All participants gave their written informed consent to participate in this study, which had been approved by the science and ethics committee of our institution.

Determination of CV events. Events in this study were defined as the occurrence of 1 of the following: 1) cardiac-related death; 2) myocardial infarction; and 3) revascularization procedure (catheter-based or surgically), TIA, and stroke. Only confirmed events were included in this analysis, and only 1 event per person was counted in the order of its occurrence. Efforts were made to determine the cause of death: review of hospital records and death certificates as well as conversations with family members. Deaths unrelated to heart disease were excluded from this analysis. Reported events were verified in all cases with medical records and/or death certificates.

Evaluation of FMD. Endothelial function was evaluated through the measure of the FMD performed on the brachial artery. Flow-mediated dilation was measured unaware of the clinical data. The technique for assessing brachial artery FMD has been described in detail elsewhere (14,19–22). Briefly, FMD was assessed in the subject's right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22°C to 25°C) by a Acuson 128 XP/10 mainframe (Acuson, Mountain View, California) with a 7.5-MHz linear array transducer. The brachial artery was longitudinally imaged ~5 cm proximal to the antecubital crease, where the clearest image was obtained, and the diameter at end diastole was measured (the mean of 3 measurements was used in the analysis). A pressure cuff, placed on the forearm (distal to the target artery), was inflated until no blood flow was detected through the brachial artery with the Doppler probe. After 5 min, the cuff was released, and this was followed by an increase in blood flow. This phenomenon increased shear stress, which served as the stimulus to induce dilation. After cuff release, the diameter of the brachial artery was measured at 45, 60, 90, 180, and 300 s. The maximum diameter in any of these measurements was used in the calculation of FMD according to the following formula: (maximum diameter during reactive hyperemia − diameter at baseline)/diameter at baseline × 100.

In our laboratory, the methodology has an interobserver variability in diameter measurements of 0.45 ± 0.25%, yielding a coefficient of variation of 1.34% and a coefficient of repeatability of 0.8%. The day-to-day variability was calculated on 50 consecutive women of our center, and resulted in 0.64 ± 0.35% in brachial artery diameter measurements.

Statistical analysis. Descriptive statistics were used to summarize the baseline characteristics, CV risk factors information, and FMD. Women were categorized for the primary analysis on the basis of the severity of endothelial dysfunction, according to the tertile cut points of FMD. Baseline values of all studied characteristics were compared across tertiles using a nonparametric test for trend across the ordered groups (Jonckheere-Terpstra test).

Student *t* test or chi-square test, when appropriate, was used in the comparison of women who sustained any CV events versus women without events.

The associations between risk factors, FMD, and CV events were examined by use of the Pearson chi-square test, multivariate logistic regression, and Cox proportional hazards regression. Hierarchical Cox proportional hazards regression was used to compare 2 nested models, including: 1) age and other major risk factors (smoking habits, the presence of diabetes, hypertension, and hypercholesterolemia), and 2) age, major risk factors, and FMD. The likelihood ratio chi-square statistics were obtained to determine whether the addition of FMD contributed significantly to the models predicting CV events. The initial model included only age and other conventional risk factors. Flow-mediated dilation was subsequently added to the model. We computed crude and multiple-adjusted hazard ratios (and 95% confidence intervals) as a measure of the relative risks of CV events for decreasing percent of FMD, with the highest tertile as the referent. Differences between the different strata of women were evaluated with the chi-square test (linear-by-linear association). The significance level was set at a value of $p < 0.05$.

Results

The baseline characteristics of our population are illustrated in Table 1; the 2,264 women were divided in 3 groups according to the tertiles of FMD.

During the follow-up period, 91 events were reported. After 1 death unrelated to heart disease had been excluded, 90 confirmed CV events (47 TIAs, 21 ischemic strokes, 9 acute myocardial infarctions, 10 hospitalizations for coronary revascularization, 3 cardiac-related deaths) were used in the analysis.

Compared with those without events, women who sustained any event were older (mean age 57 ± 6 years vs. 52 ± 7 years, $p < 0.001$) and had a higher prevalence of smoking (31.1% [28 of 90] vs. 17.6% [384 of 2,174], $p < 0.001$), diabetes (13.3% [12 of 90] vs. 3.49% [76 of 2,174], $p < 0.0001$), hypertension (45.5% [41 of 90] vs. 20.1% [437 of 2,174], $p < 0.0001$), and hypercholesterolemia (26.6% [24 of 90] vs. 18.3% [398 of 2,174], $p < 0.01$).

The univariate and multivariate association between the risk factors, FMD, and CV events is shown in Table 2. Controlling for age and other risk factors (smoking habits, presence of hypercholesterolemia, history of diabetes, hypertension), FMD was significantly associated with the incidence of CV events.

In the hierarchical Cox proportional hazards regression analysis, FMD resulted as a significant contributor to the model predicting CV events (likelihood ratio chi-square change = 10.22, which represents a strong significant change in goodness-of-fit measure; $p < 0.0001$).

The event rate and the relative risk for CV events at increased tertile of FMD were graded as shown in Table 3. The event rate among patients in the lowest tertile of FMD was greater than the combined event rate observed in the other 2 tertiles (women in the lower tertile accounted, in fact, for 51 events [56.6% of total events]).

Discussion

Atherosclerosis, the leading cause of morbidity and mortality in Western countries, is a systemic disease that infiltrates the arterial wall before it cause significant obstruction of blood flow and ischemic symptoms. In the last decade, the screening of asymptomatic individuals to detect latent disease has become a topic of great interest. The rationale for investigating the presence of pre-clinical atherosclerosis resides in the fact that more than one-half of all CV events take place in previously asymptomatic patients (23,24). Thus, in prevention, the emphasis has recently been focused on the identification of high-risk individuals with a high-risk of developing CV events (25,26). In our opinion, 2 critical issues of this topic deserved an answer. First of all, does the knowledge of the FMD supply an added value to our ability to predict the risk of developing CV events, and, if yes, to what degree can FMD predict events in our specific population? To attempt to answer these questions, we evaluated a large population of initially asymptomatic postmenopausal women with risk factors for atherosclerosis. Our results show that FMD adds prognostic information

Table 1 Baseline Characteristics of the Study Participants Divided in Groups According to the Tertiles of FMD

	3° (Higher) Tertile	2° (Intermediate) Tertile	1° (Lower) Tertile	p Value*
n	754	755	755	—
Levels of FMD, %	≥8.1	4.6–8.0	≤4.5	—
BAD, cm	3.9 ± 0.6	4.0 ± 0.5	3.8 ± 0.7	NS
Age, yrs	53 ± 7	55 ± 7	56 ± 6	<0.001
Caucasian, %	100 (n = 754)	100 (n = 755)	100 (n = 755)	NS
Time from menopause, months	33 ± 13	52 ± 15	62 ± 15	<0.001
Cigarette use, %	12.4 (94 of 754)	16.0 (121 of 755)	26.0 (197 of 755)	<0.0001
Hypercholesterolemia, %	11.6 (88 of 754)	19.3 (146 of 755)	24.9 (188 of 755)	<0.0001
Diabetes, %	1.8 (14 of 754)	3.0 (23 of 755)	6.7 (51 of 755)	<0.0001
Hypertension, %	14.9 (113 of 754)	17.7 (134 of 755)	30.6 (231 of 755)	<0.0001

Data are expressed as mean ± 1 standard deviation or as a percentages. *Probability values for ordered differences across tertiles of flow-mediated dilation (FMD).

BAD = brachial artery diameter.

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Table 2 Univariate and Multivariate Association Between Risk Factors, FMD, and Cardiovascular Events in Our Population of 2,264 Women

	Univariate		Multivariate (6-Predictor Model)*	
	RR	95% CI	RR	95% CI
Age (yrs)	1.42†	1.16–2.44	1.23†	1.04–1.97
Smoking habits (yes/no)	1.77‡	1.20–2.66	1.22§	1.03–2.19
Hypercholesterolemia (yes/no)	1.44§	1.11–1.95	1.07‖	1.02–1.77
Diabetes (yes/no)	3.80†	2.06–6.00	2.96†	1.68–4.09
Hypertension (yes/no)	2.25‡	1.40–3.10	1.76‡	1.14–2.34
FMD (%)	1.31†	1.12–2.55	1.12†	1.04–2.00

*The 6-predictor Cox proportional hazards regression model included simultaneously all variables listed in the table (age, smoking habits, hypercholesterolemia, diabetes, hypertension); †$p < 0.0001$; ‡$p < 0.001$; §$p < 0.01$; ‖$p < 0.05$.
CI = confidence interval; FMD = flow-mediated dilation; RR = relative risk.

above and beyond knowledge of traditional risk factors. In fact, the ability to classify and predict an event improved once FMD was added to risk factors.

It is well known that FMD induced by reactive hyperemia is endothelium dependent (27,28); in other words, it depends on the ability of the endothelium to produce dilator-endowed substances, mainly nitric oxide (NO). It is well demonstrated that post-menopausal status is associated with a significantly reduced arterial NO activity (29). It is reasonable to conclude that our patients with a lower level of FMD had worse endothelial function, and some important pathological effects are influenced by endothelial dysfunction. Disruption of atherosclerotic plaques is the most frequent cause of acute thrombo-embolic events, and these events include death, acute coronary syndromes, and stroke (30). Endothelial dysfunction often is found in ruptured plaques, as well as in plaques that show superficial erosion (31–34). Further, the endothelium has an important antithrombotic role. Nitric oxide and prostacyclin act synergistically to prevent platelet adhesion and aggregation (35,36). The regulation of fibrinolysis is another important function of the normal endothelium. The 2 principal determinants of fibrinolysis are: 1) tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), which promote fibrinolysis; and 2) plasminogen activator inhibitor type 1, which inhibits t-PA and u-PA and enhances formation of thrombi. In normal blood vessels, a basal level of t-PA is secreted, which prevents thrombus formation in the absence of vascular injury (37). In the presence of endothelial dysfunction, enhanced platelet deposition, thrombus formation, and inhibition of dilation may also explain the high incidence of transient ischemic episodes in the cerebral vascular territory (21).

Risk assessment strategies. Despite extensive studies and development of several risk prediction models, traditional risk factors fail to predict development of CV events in a large group of cases (3,5,6,38). The extensive use of the most famous risk prediction model was proposed by the National Cholesterol Education Program III guidelines, in which the approach offered by the Framingham risk score to formulate a 10-year risk of CV events was embraced (39). Today it is clear that the Framingham risk score as well as several other risk factor assessment models (40,41) have been shown to predict long-term outcome in a large population, but may not be able to predict short-term risk for individual persons, and cannot provide clear indications for cardiologists to identify and treat, to prevent near future victims of acute CV events (42). Hence, there is a need to improve risk prediction. In our study, all of the established risk factors were predictive of CV events in the univariate and multivariate analysis, but when FMD was added to the model, it was a significant predictor of outcome and added independently to the risk prediction.

Atherosclerosis is a diffuse and systemic disease involving vascular, metabolic, and immune systems, with several local manifestations. Therefore, it is essential to precisely assess the total vulnerability burden of the vascular system, rather

Table 3 Cardiovascular Event Rates and Association Between FMD Tertiles and Cardiovascular Events in Our Population

	3° (Higher) Tertile (FMD ≥8.1%)	2° (Intermediate) Tertile (FMD From 4.6% to 8.0%)	1° (Lower) Tertile (FMD ≤4.5%)	p Value*
TIA, %	0.26 (2 of 754)	1.72 (13 of 755)	4.23 (32 of 755)	<0.0001
Ischemic stroke, %	0.39 (3 of 754)	1.05 (8 of 755)	1.32 (10 of 755)	<0.01
Nonfatal myocardial infarction, %	0.26 (2 of 754)	3.97 (3 of 755)	5.29 (4 of 755)	<0.05
Hospitalization for coronary revascularization, %	0.13 (1 of 754)	0.92 (7 of 755)	0.26 (2 of 755)	NS
Cardiac-related death, %	0	0	0.39 (3 of 755)	NS
Cardiovascular events (total), %	1.06 (8 of 754)	4.10 (31 of 755)	6.75 (51 of 755)	<0.0001
Relative risk (crude)	1.0	3.87	6.36	<0.0001
95% CI	Referent	2.05–8.55	4.26–11.50	—
Relative risk (adjusted for age and other conventional risk factors)	1.0	1.33	4.42	<0.0001
95% CI	Referent	1.09–4.09	2.97–8.01	—

*p is the overall significance between groups, calculated by chi-square test, linear-by-linear association.
TIA = transient ischemic attack; other abbreviations as in Table 2.

than search for a single, unstable plaque (42). According to this approach, the study of the endothelial function may be important, because an impaired endothelial dilator function was demonstrated during acute and chronic CVD (43–45), endothelial vasomotor function was defined as "the risk factor of the risk factors" because patients with CV risk factor have endothelial dysfunction (43), and, finally, vulnerable plaques in several arterial districts have multiple sites of active inflammation and oxidative stress, and this is associated with an impaired systemic endothelial dysfunction (46,47).

Study limitations. Our study has some limitations, in that it is an observational study, which shows an interesting association between FMD and risk of developing CV events.

An important limit of our study is that the admittance to our center is completely free for all women age <60 years. For this reason we constructed a relatively young age group. We built our activity to make every woman (with the only limitation of age) free to come to our center when she needs to have any problem solved, to ask questions, to receive suggestions, and so on. As a consequence, the incidence of CV risk factors and CV events noted in our sample of patients could be different as regards what would be registered in the general population of post-menopausal women.

Another limit is that in the present study we could not analyze the influence of some major risk factors, such as the presence of paroxysmal atrial fibrillation, the levels of high-density lipoprotein cholesterol, and the concentration of C-reactive protein. In addition, the predictive value of FMD still needs to be assessed in a model including these important markers of CVD risk.

Although the present data is encouraging, it is opportune to specify that the routine clinical use of the noninvasive evaluation of endothelial function on an individual basis may be premature, because the test is not still completely standardized and is costly and rather labor intensive. Moreover, the value of the present study is to provide biological insight rather than practical implications.

However, the study presents several strengths including the prospective design, the relatively large and homogeneous sample, and the strict follow-up of the participants. All of these factors encourage us to hypothesize a causal link between FMD and incident CV events in post-menopausal women.

Conclusions

In our study on the incidence of CV events, FMD was found to be an independent risk determinant and adds prognostic information above and beyond traditional risk factors.

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