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Predictive Value of Noninvasively Determined Endothelial Dysfunction for Long-Term Cardiovascular Events in Patients With Peripheral Vascular Disease

Noyan Gokce, MD, FACC,* John F. Keaney, Jr, MD, FACC,* Liza M. Hunter, ANP,* Michael T. Watkins, MD,† Zoran S. Nedeljkovic, MD,* James O. Menzoian, MD,‡ Joseph A. Vita, MD, FACC*

Boston, Massachusetts

OBJECTIVES The goal of this study was to prospectively examine the long-term predictive value of brachial-artery endothelial dysfunction for future cardiovascular events.

BACKGROUND Brachial-artery endothelial function is impaired in individuals with atherosclerosis and coronary risk factors. The prospective relation between endothelial function determined by brachial-artery ultrasound and long-term cardiovascular risk is unknown.

METHODS We examined brachial-artery endothelial function using ultrasound in 199 patients with peripheral arterial disease before elective vascular surgery. Patients were prospectively followed with an average follow-up of 1.2 years after surgery.

RESULTS Thirty-five patients had an event during follow-up, including cardiac death (5 patients), myocardial infarction (17 patients), unstable angina (10 patients), or stroke (3 patients). Preoperative endothelium-dependent flow-mediated dilation (FMD) was significantly lower in patients with an event (4.4 ± 2.8%) compared with those without an event (7.0 ± 4.9%, $p < 0.001$), whereas endothelium-independent vasodilation to nitroglycerin was similar in both groups. In a Cox proportional-hazards model, independent predictors of events included age ($p = 0.003$), more invasive surgery (surgery other than carotid endarterectomy, $p = 0.02$), and impaired brachial-artery endothelial function ($p = 0.002$). Risk was approximately nine-fold higher in patients with FMD <8.1% (lower two tertiles) compared with those in the upper tertile (odds ratio 9.5; 95% confidence interval 2.3 to 40).

CONCLUSIONS Impaired brachial-artery endothelial function independently predicts long-term cardiovascular events in patients with peripheral arterial disease. The findings suggest that noninvasive assessment of endothelial function using brachial-artery FMD may serve as a surrogate end point for cardiovascular risk. (J Am Coll Cardiol 2003;41:1769–75) © 2003 by the American College of Cardiology Foundation

The vascular endothelium plays a central role in the regulation of vascular tone, thrombosis, and inflammation through elaboration of a number of paracrine factors that act locally in the arterial wall (1). Endothelial dysfunction is associated with coronary risk factors, angiographically evident coronary artery disease (CAD), and is pathophysiologically linked to acute cardiovascular syndromes (2). Recent studies have shown that impaired vasodilation assessed via intra-arterial infusion of the endothelium-dependent vasodilator acetylcholine in a coronary or peripheral artery identifies individuals at increased risk for future cardiovascular disease events (3–7). However, the invasive nature of such studies has limited the widespread clinical application of those techniques.

Noninvasive ultrasound assessment of brachial-artery flow-mediated dilation (FMD) has emerged as a broadly applicable method for the study of nitric oxide-dependent endothelial function (8,9). Brachial endothelial function is impaired in patients with coronary disease and its risk factors and improves in response to treatments known to reduce cardiovascular disease risk (2). On the basis of these findings, investigators have proposed that brachial-artery FMD might prove useful as a surrogate marker for cardiovascular risk (8,10).

In support of this proposal, we previously demonstrated that impaired brachial-artery FMD independently predicts short-term (30-day) perioperative cardiovascular events in patients undergoing vascular surgery (11). However, it remains unclear whether this short-term predictive value for perioperative events will extend to long-term spontaneous events in these individuals after they have recovered from the immediate stress of vascular surgery. The present study sought to address this question by completing long-term follow-up of vascular surgery patients who have undergone noninvasive testing of endothelial function.

From the *Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine; †Surgical Service, Massachusetts General Hospital; and the ‡Department of Surgery, Boston University School of Medicine, Boston, Massachusetts. Supported by a Specialized Center of Research in Ischemic Heart Disease grant from the NIH (HL55993) and NIH grants HL60886 and HL52936. Dr. Gokce is the recipient of a Mentored Patient-Oriented Research Career Transition Award from the National Institutes of Health (K23 HL04425). Dr. Watkins is supported by NIH grant HL48152 and a grant from the Research Administration of the Department of Veterans Affairs. Dr. Keaney is an Established Investigator of the American Heart Association.

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Abbreviations and Acronyms
CAD = coronary artery disease
FMD = flow-mediated dilation
MI = myocardial infarction
NTG = nitroglycerin

METHODS

Patient population. Consecutive patients with peripheral vascular disease undergoing nonemergent vascular surgery at Boston Medical Center and the Veterans Administration Boston Healthcare System were eligible for participation in the study. Patients with unstable angina, recent (within one month) myocardial infarction (MI) or stroke, decompensated heart failure, or clinically significant ventricular arrhythmias were excluded. All patients provided written informed consent as approved by the institutional review boards. We previously reported the results of 30-day follow-up on 187 of these patients (11).

Study protocol. Up to one month before surgery, brachial-artery reactivity was assessed as previously described (11,12). Endothelium-dependent FMD of the brachial artery was examined noninvasively using an established method of high-resolution vascular ultrasound with an upper arm cuff position. Briefly, brachial-artery two-dimensional and pulsed Doppler flow velocity signals were obtained above the antecubital crease with a 7.5-MHz linear array transducer using a vascular ultrasound system (140A, Toshiba American Medical Systems, Tustin, California). Hyperemia was induced by inflating a blood pressure cuff on the proximal portion of the arm to occlude arterial flow (>200 mm Hg) for 5 min and then rapidly deflating the cuff. After pulse-Doppler recordings, hyperemic two-dimensional images were obtained 60 s after cuff deflation. After a 10-min rest period to allow restoration of baseline conditions, nonendothelium-dependent brachial-artery dilation was assessed by obtaining two-dimensional images before and 3 min after administration of sublingual nitroglycerin (NTG) (0.4 mg). Nitroglycerin was omitted if the patient refused, had a history of migraine headaches, systolic blood pressure <100 mm Hg, previous adverse reaction to nitrates, or critical carotid artery stenosis. An investigator blinded to image sequence and clinical information performed off-line analysis of digitized end-diastolic images (13).

Assessment of cardiovascular events. Patients were followed prospectively after surgery. Cardiovascular events were defined to include cardiac death, MI, unstable angina, or nonhemorrhagic stroke as previously described (11,14). In our prior study (11), serum troponin level was measured within three days of surgery in all subjects, and we counted troponin elevation in the absence of other clinical criteria for MI as an event. However, routine measurement of troponin during this long-term follow-up study was not feasible or clinically relevant. To maintain consistency between definitions of short- and long-term events in this present study, an isolated perioperative troponin elevation in the absence of other clinical criteria for MI was not considered an event. Medical records were reviewed, and telephone calls were placed to the patient or family to identify additional events that occurred after discharge. Three cardiologists blinded to ultrasound results adjudicated all events by review of medical records. One event per patient was included in the analysis. For patients with more than one event, the coded event was selected using the hierarchical order listed above.

Statistical analysis. The prospectively determined primary end point was any cardiovascular event, as defined in the previous text. We compared FMD, NTG-mediated dilation, and clinical characteristics in the groups of patients with and without an event using the Student *t* test (for continuous variables), the chi-square test (for categorical variables), and the Fisher exact test (for categorical variables when the expected cell count was <5). Kaplan-Meier analysis with the log-rank test and Breslow test (weighing the results for number of "at-risk" patients) was used to plot and compare event-free survival distributions according to tertile of brachial-artery FMD. A Cox proportional-hazards model was used to control for the potentially confounding variables that differed between patients with and without an event using an inclusion criterion of $p < 0.10$ (15). Potential confounding variables were age, gender, race, diabetes mellitus, hypertension, chronic renal insufficiency (creatinine >1.2 mg/dl), known coronary disease, known congestive heart failure, history of smoking, history of hypercholesterolemia, white blood count, hematocrit, angiotensin-converting enzyme inhibitor therapy, beta-blocker therapy, NTG-mediated dilation, baseline brachial-artery diameter, extent of reactive hyperemia, and type of operation. The noncollinear variables that met the inclusion criterion were age, carotid surgery, FMD, diabetes mellitus, and smoking. Because the Kaplan-Meier survival curves for the lowest and middle tertiles of FMD overlapped and differed from the highest tertile, patients were dichotomized in this multivariate analysis as having high (upper tertile) or low (lower two tertiles) FMD. The analyses were completed using SPSS for Windows version 10.1 (SPSS Inc., Chicago, Illinois). Data are presented as mean ± SD.

RESULTS

Study patients. A total of 199 patients were enrolled in the study and underwent a vascular operation, including carotid endarterectomy (48 patients), femoral-popliteal or other peripheral bypass (108 patients), aortic aneurysm repair (25 patients), or limb amputation (18 patients). Patients were prospectively followed for an average of 1.2 ± 0.8 years (median, 390 days; range, 1 to 1,020 days). During this follow-up period, 35 patients had a cardiovascular event including cardiac death (5 patients), MI (17 patients), unstable angina (10 patients), and stroke (3 patients). Fifteen of these events occurred 30 or more days after surgery.

Table 1. Clinical Characteristics

	No Event (n = 164)	Event (n = 35)	p Value
Age (yrs)	65 ± 11	70 ± 10	0.005
Gender (% female)	21	31	0.17
Race (% African American)	21	20	0.25
Diabetes mellitus (%)	42	60	0.05
Hypertension (%)	68	77	0.27
Chronic renal insufficiency (%)	25	30	0.49
Known coronary disease (%)	34	40	0.47
History of heart failure (%)	6	11	0.99*
Current smoking (%)	42	26	0.07
Known hypercholesterolemia (%)	53	67	0.15
Total/HDL cholesterol	4.6 ± 1.4	4.8 ± 2.2	0.59
Triglycerides (mg/dl)	165 ± 97	203 ± 263	0.54
Creatinine (mg/dl)	1.2 ± 1.6	1.5 ± 1.8	0.38
White blood cell count (1,000/μl)	8.5 ± 2.9	9.0 ± 3.3	0.37
Carotid surgery (%)	27	11	0.05
ACE inhibitor therapy (%)	37	51	0.12
Statin therapy (%)	40	49	0.33
Beta-blocker therapy (%)	45	60	0.11

Data are mean ± SD or percent as indicated. *By Fisher exact test.
ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein.

The clinical characteristics of patients with and without an event are displayed in Table 1. Patients with a cardiovascular event were older, and there were strong trends for diabetes mellitus and more invasive surgical procedures in patients with events.

Brachial-artery ultrasound results. For the entire group of patients, average FMD was 6.6 ± 4.7% (n = 199), and average NTG-mediated dilation was 10.5 ± 6.3% (n = 65). Brachial-artery parameters for the patients with and without a cardiovascular disease event are displayed in Table 2. As shown, patients with an event had significantly lower FMD. The extent of reactive hyperemia and responses to NTG were similar in the two groups. The proportion of individuals who received NTG was similar in the event (n = 11, 31%) and nonevent groups (n = 54, 33%). Univariate negative correlates of FMD were larger baseline vessel diameter ($p < 0.001$), renal insufficiency ($p = 0.03$), white blood cell count ($p = 0.01$), and diabetes mellitus ($p = 0.001$).

Predictive value of endothelial function. As shown in the upper panel of Figure 1, event-free survival differed according to tertile of FMD. Notably, the survival curves overlapped for the patients in the lower two tertiles. Table 3 displays the number of cardiovascular disease events when patients are dichotomously categorized as having low FMD (lower two tertiles, ≤8.1%) or high FMD (upper tertile, >8.1%), and the event-free survival curves are shown in the middle panel in Figure 1. As shown, patients with low FMD had significantly more events. In the Cox proportional-hazards model, the independent predictors of cardiovascular disease events were older age, noncarotid surgery, and lower brachial-artery FMD (Table 4).

As shown in Figure 1, there is a large downward step in survival resulting from a single event at 960 days, which reflects the relatively few "at-risk" patients with this duration of follow-up. However, poor FMD remained a predictor of events when the analysis was weighed by number of "at-risk" patients ($p = 0.0008$). Furthermore, if the analysis is truncated to <2 years (n = 163, 33 events), poor FMD remained a predictor of events in the Kaplan-Meier analysis ($p = 0.0005$) and, by Cox proportional-hazards analysis, poor FMD ($p = 0.005$) and age ($p = 0.004$) are independent predictors of events.

If FMD is expressed as absolute change in diameter (a variable that is independent of baseline diameter), rather than as percent change in diameter, the results are similar with impaired FMD ($p = 0.007$), noncarotid surgery ($p = 0.03$), and older age ($p = 0.004$) as the independent predictors of events. When the subset of patients who received NTG was analyzed separately (n = 65), all 11 patients with an event had low FMD, and FMD ($p = 0.02$) and age ($p = 0.01$) were independent predictors of events.

Table 2. Brachial Artery Parameters

	No Event	Event	p Value
Baseline brachial diameter (mm)	3.8 ± 0.7	4.0 ± 0.6	0.19
Reactive hyperemia (% increase)	480 ± 320	550 ± 430	0.31
Flow-mediated increase in diameter (mm)	0.26 ± 0.17	0.17 ± 0.11	<0.001
Flow-mediated dilation (%)	7.0 ± 4.9	4.4 ± 2.8	<0.001
Nitroglycerin-mediated dilation (%)	10.9 ± 6.5	8.8 ± 5.3	0.32

Data are mean ± SD.

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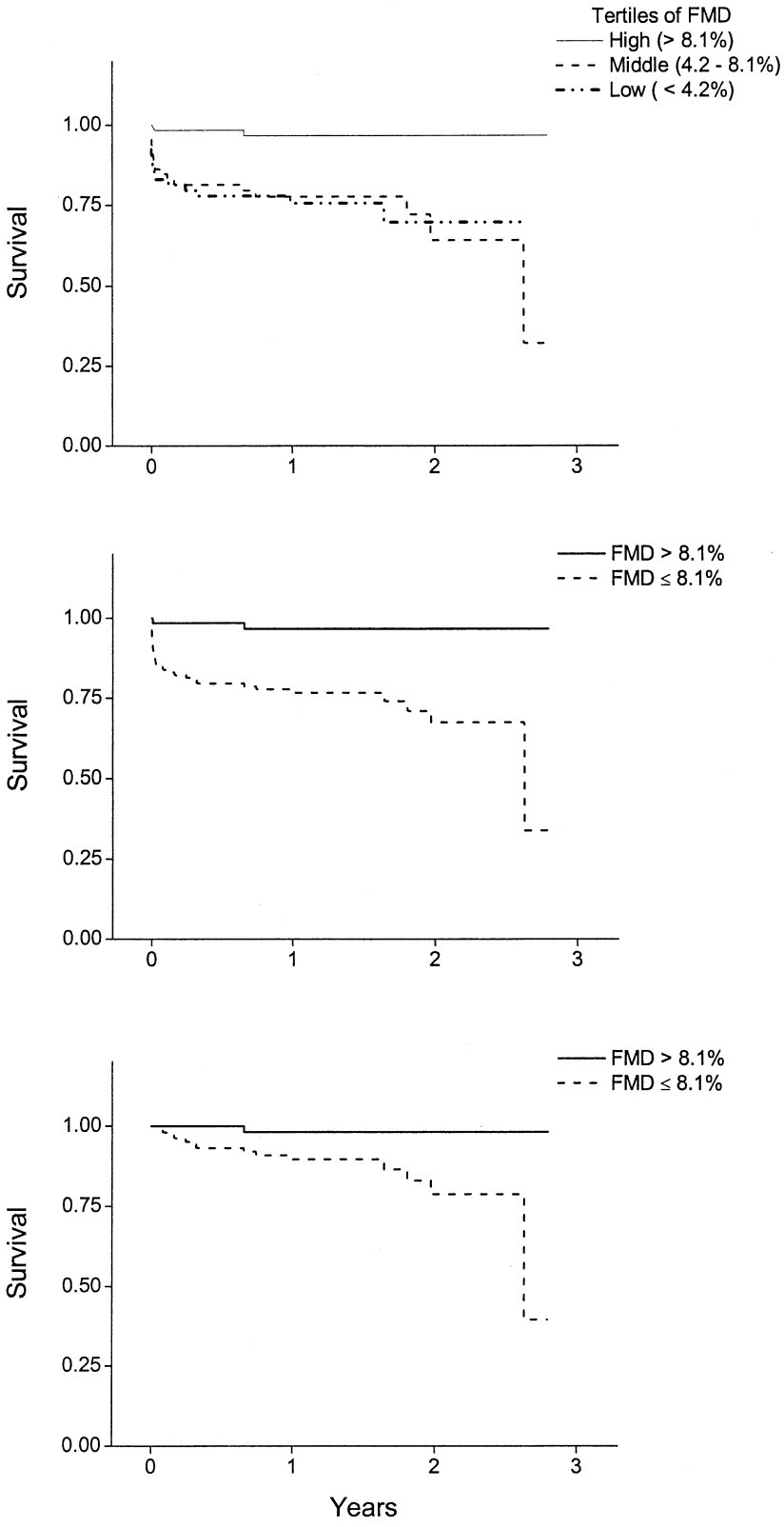

Figure 1. Kaplan-Meier plots showing survival according to brachial-artery flow-mediated dilation (FMD). The **upper panel** displays survival according to tertile of FMD. Overall, survival differed according to tertile of FMD ($p = 0.0005$). By multiple pairwise comparison, the survival curves for the lowest and middle tertiles did not differ ($p = 0.87$), but both differed from the highest tertile by log-rank test ($p = 0.0003$ and $p = 0.0001$, respectively). The **middle panel** displays survival for patients in the highest tertile and for the combination of the middle and lowest tertiles. The survival curves differed by log-rank test ($p = 0.0001$). In the **lower panel**, the analysis included only those events that occurred more than 30 days after vascular surgery and displays survival curves for patients in the highest tertile and for the combination of the middle and lowest tertiles. The survival curves differed by log-rank test ($p = 0.006$).

Table 3. Numbers of Events According to FMD

	FMD		
	Low and Middle Tertiles (≤8.1%)	High Tertile (>8.1%)	p Value
Number of subjects	133	66	
Cardiac death	5	0	0.11
Myocardial infarction	16	1	0.01
Unstable angina	9	1	0.17
Stroke	3	0	0.55
Any event	33	2	< 0.001

FMD = flow-mediated dilation.

Because it could be argued that events that occur during and immediately after vascular surgery may have a different pathogenesis than spontaneously occurring events, we also examined the relation between endothelial dysfunction and cardiovascular events that developed more than 30 days after surgery. When considering only the 15 events that took place 30 or more days after surgery, 14 (93%) occurred in patients with FMD ≤8.1%. As shown in the lower panel of Figure 1, event-free survival differed in the highest tertile compared with the combined middle and lowest tertiles. After controlling for age and noncarotid surgery, endothelial dysfunction remained an independent predictor of events with an odds ratio of 9.6 (95% confidence interval, 1.2 to 74, p = 0.03).

DISCUSSION

This prospective study demonstrated that impaired FMD of the brachial artery is a strong independent predictor of cardiovascular events in patients with peripheral arterial disease. The predictive value of FMD was independent of the extent of reactive hyperemia and the response to an exogenous source of nitric oxide (NTG), suggesting that the findings are not due to variation in the stimulus for vasodilation or the function of vascular smooth muscle. Thus, the study supports a pathophysiologic link between endothelial dysfunction and cardiovascular events.

Previous invasive coronary studies examined the relation between endothelial dysfunction and cardiovascular risk. During a 28-month follow-up of 157 patients, Suwaidi et al. (3) demonstrated that coronary endothelial dysfunction in the absence of obstructive lesions was associated with increased cardiovascular events. Schachinger et al. (4) reported that impaired vasodilator responses to both endothelium-dependent and -independent agonists predicted events in 147 patients undergoing cardiac catheterization over a 7.7-year follow-up period. In a study involving 308 patients with a 46-month follow-up period, Halcox et al. (5) reported more events in patients with blunted coronary vascular responses.

Invasive studies in the brachial artery also provide prognostic information about cardiac risk. Perticone et al. (7) reported that hypertensive patients with blunted forearm microvascular responses to intra-arterial acetylcholine infusion are more likely to experience adverse outcomes. Similarly, Heitzer et al. (6) demonstrated that CAD patients with reduced forearm dilator responses to intra-arterial acetylcholine developed more cardiovascular events over a 4.5-year follow-up period.

Table 4. Independent Predictors of a Postoperative Event

	Odds Ratio	95% CI	p Value
Low flow-mediated dilation	9.5	2.3–40	0.002
Surgery other than carotid endarterectomy	3.4	1.2–9.7	0.02
Age (per decade)	1.7	1.2–2.4	0.003

CI = confidence interval.

Two prior studies have investigated the predictive value of endothelial function using ultrasound-based methodology. Neunteufl et al. (16) completed a retrospective study in 73 patients undergoing cardiac catheterization and demonstrated that a brachial-artery FMD of <10% is associated with increased likelihood of MI, coronary angioplasty, or coronary bypass surgery during five-year follow-up. However, the majority of events in that study were revascularization procedures, and the predictive value of endothelial dysfunction for this outcome was lost after controlling for extent of CAD. Finally, we recently reported that FMD <8.1% predicts short-term (within 30 days) cardiovascular events in patients undergoing vascular surgery (11).

The present study prospectively examined the important question of whether brachial-artery endothelial dysfunction identifies patients at risk for spontaneously occurring events over the long term. We found that nearly all of the events occurred in the patients with FMD below the cut point of 8.1%. Consistent with this observation was the finding that endothelial dysfunction had independent predictive value even when events occurring within 30 days of surgery were excluded from analysis.

It might be reasonable to question whether perioperative and spontaneous events during chronic follow-up have different pathogenic mechanisms. For example, the perioperative state is associated with fluid shifts, pain, and other stresses that lead to changes in heart rate, blood pressure, and activation of the sympathetic nervous system. Such changes could provoke rupture of a vulnerable plaque or increase myocardial oxygen demand to the point that it exceeds supply in the setting of fixed coronary stenoses. Although such changes would likely be less frequent or less severe during chronic follow-up compared with the immediate postoperative period, they still can occur during the physical and emotional stress of normal life. Under such circumstances, changes in blood pressure, heart rate, and sympathetic tone would be more likely to provoke an acute coronary syndrome or stroke if endothelial dysfunction and/or other factors have impaired normal regulation of vascular homeostasis. This scenario is supported by the current findings that endothelial dysfunction predicts cardiovascular events with both short- and long-term follow-up.

An important issue to consider is why endothelial dysfunction in the arm predicts ischemic events in remote locations such as the coronary or carotid circulations. Endothelial dysfunction has been proposed to be a "barometer" of vascular health that integrates the overall effects of risk factors and intrinsic defense mechanisms (10). Most traditional coronary risk factors (e.g., hypercholesterolemia, diabetes mellitus, hypertension) and novel risk factors (e.g., hyperhomocysteinemia, systemic infection, chronic inflammation) are systemic in nature and might be expected to affect vascular beds throughout the body. This concept is supported by several studies showing that impaired FMD in the brachial artery predicts vascular dysfunction in the coronary circulation (17,18). The systemic nature of endothelial dysfunction is also supported by studies demonstrating the predictive value of invasively determined endothelial function in forearm resistance vessels (6,7). Thus, it is not entirely unexpected that endothelial dysfunction detected noninvasively in the arm has utility as a tool to assess the generalized functional integrity of blood vessels throughout the body (10,19,20).

One implication of the present study is the potential role of noninvasive examination of endothelial function in the clinical assessment of individual patients. The present finding that impaired brachial-artery FMD has long-term prognostic value suggests that the technique might be used to frame therapeutic decisions about a particular patient's care, although more studies are needed before the technique can be introduced into clinical practice. For example, it will be important to examine larger and more diverse populations and to evaluate the relative negative and positive predictive values of this methodology compared with other surrogate markers for cardiovascular risk, including preoperative stress testing. There is currently considerable center-to-center variation in how FMD is measured, and there is need for a standardized approach (8,21). Although the applicability of this methodology for individual patients remains undefined, this present study provides strong evidence for the clinical relevance of brachial-artery FMD. Thus, the findings support the use of the methodology as a surrogate cardiovascular end point for investigation of potential new interventions in studies involving groups of subjects.

Regarding limitations, the present study examined subjects with established atherosclerosis. Thus, the results should not be generalized to lower risk populations. In addition, only a subset of patients received NTG, and there was a numerical, but nonsignificant, difference in NTG-mediated dilation between the event and no-event groups. We cannot exclude the possibility that this difference would have been significant with a larger sample size. However, our finding that FMD remained an independent predictor of events after controlling for the NTG response in this subset of patients is consistent with a specific impairment of endothelial function. Finally, low FMD remained an independent predictor of events when the analysis was truncated at two years, suggesting that the relatively modest number of subjects with longer follow-up does not skew the results.

In summary, this prospective study of patients with peripheral arterial disease demonstrates that brachial-artery FMD is an independent predictor of long-term cardiovascular events. The results suggest that patients with endothelial dysfunction continue to have increased risk for adverse cardiac events subsequent to the short-term stress of vascular surgery. These findings add to the growing body of evidence that endothelial dysfunction may contribute to the pathogenesis of cardiovascular disease. In addition, this study provides evidence that noninvasive assessment of endothelial function in the brachial artery has utility as a surrogate marker for cardiovascular disease risk.

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Reprint requests and correspondence: Dr. Joseph A. Vita, Section of Cardiology, Boston Medical Center, 88 East Newton Street, Boston, Massachusetts 02118. E-mail: jvita@bu.edu.

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