Endothelial Dysfunction and Cardiovascular Risk Prediction in Peripheral Arterial Disease
Additive Value of Flow-Mediated Dilation to Ankle-Brachial Pressure Index

Gregorio Brevetti, MD; Antonio Silvestro, MD; Vittorio Schiano, MD; Massimo Chiariello, MD

Background—Endothelial dysfunction plays a key role in atherogenesis. We prospectively investigated the impact of noninvasive measurement of endothelial function on cardiovascular risk in peripheral arterial disease (PAD). The study was specially aimed at assessing whether brachial artery flow-mediated dilation (FMD) added to the predictive value of ankle-brachial pressure index (ABPI).

Methods and Results—Of 131 patients monitored for a mean of 23±10 months, 18 had a coronary event, 12 a cerebrovascular event, and 9 a peripheral event. The median FMD was lower in patients with an event than in those without (5.8% versus 7.6%, P<0.05), whereas vasodilation to nitroglycerin was similar in the two groups. The cardiovascular event rate was higher in patients with FMD below the median versus those with FMD above the median (P<0.001 by log-rank test). In a Cox proportion hazard model, independent predictors of events were FMD below the median (P<0.01), ABPI below the median (P<0.01), and previous stroke (P<0.02). Similar results were obtained when peripheral events were excluded from the analysis. Below-median ABPI and FMD combined was more accurate in predicting risk (relative risk [RR] 13.0; 95% CI, 3.0 to 56.2; P<0.01) than ABPI (RR, 6.4; 95% CI, 1.4 to 29.1; P<0.02) and FMD (RR, 4.8; 95% CI, 1.1 to 23.3; P<0.05) alone.

Conclusions—A low brachial artery FMD is an independent predictor of cardiovascular risk in patients with PAD and adds to the prognostic value of ABPI, which is currently the most powerful prognostic indicator in PAD. (Circulation. 2003;108:2093-2098.)

Key Words: peripheral vascular disease • endothelium • cardiovascular diseases • prognosis

Endothelial dysfunction, by predisposing to thrombosis, leukocyte adhesion, and smooth muscle cell proliferation,1 plays a pivotal role in the development, progression, and clinical manifestations of atherosclerosis. It is associated with risk factors also in the absence of overt atherosclerosis,2 promotes progression of the atherosclerotic lesion,3,4 and is more pronounced in arteries with lesions responsible for acute coronary syndromes.5,6 Consequently, endothelial function has been defined an “excellent barometer” of vascular health7 and can be used to gauge cardiovascular risk. In fact, endothelial function, measured as the vasodilating response of resistance or conduit vessels to stimuli that induce release of nitric oxide,8,9 is related to atherosclerosis-induced clinical events in a variety of cardiovascular conditions.10–13 In particular, ultrasound measurement of brachial artery flow-mediated dilation (FMD) during reactive hyperemia provides prognostic information about cardiovascular events in patients with chest pain11 and in those undergoing surgery for vascular disease.13,14

Patients with peripheral arterial disease (PAD) and intermittent claudication have a reduced FMD,14,16 which is exacerbated by acute ischemic exercise.16 This impairment in endothelial function is related to the severity of the circulatory failure in the affected limb and with increased plasma markers of inflammation,17,18 both of which are independent predictors of cardiovascular risk.19–22 Despite the promise of FMD, only one study has assessed the predictive value of FMD in patients with vascular disease.14 However, that study included subjects with a broad range of vascular alterations and did not provide information about the subgroup of patients with PAD. In the present study, we prospectively investigated the relation between brachial artery FMD and cardiovascular risk in patients with symptomatic PAD of the lower limbs, a condition associated with high rates of cardiovascular morbidity and mortality.19,23,24 We specially aimed at investigating whether brachial artery FMD adds to the predictive value of the ankle-brachial pressure index (ABPI), which is the most consistent and powerful prognostic indicator in PAD.19–21
Methods

Patients
We studied 131 consecutive patients with evidence of PAD at Doppler examination. All of them were referred to our laboratory for leg pain while walking and had ABPI <0.90, which decreased by at least 15% after treadmill exercise. Exclusion criteria were recent interventions for coronary or peripheral artery disease (<6 months), recent (<3 months) unstable angina, myocardial infarction or stroke, decompensated heart failure, malignant neoplasia and significant hepatic, and renal or inflammatory disease. All women were postmenopausal, and none was receiving hormone replacement therapy. None of the patients had rest pain, ulceration, or gangrene in the affected limb(s). In the first 65 patients of our series, FMD was originally measured to assess the relation between endothelial function and both adhesion molecules and exercise. All the 65 patients included in those two studies regularly attended our vascular laboratory for routine follow-up visits and thus were included in the present study. All participants gave written informed consent to the study, which was approved by our institutional ethics committee.

Study Protocol
Clinical history and risk factors were assessed at the first evaluation. Smokers included current or former smokers. Hyper-tension was diagnosed if systolic arterial pressure exceeded 140 mm Hg and/or diastolic arterial pressure exceeded 90 mm Hg or if the patient used antihypertensive drugs. Hyperlipidemia was diagnosed if plasma total cholesterol exceeded 240 mg/dL, plasma LDL cholesterol exceeded 130 mg/dL, plasma triglycerides exceeded 200 mg/dL, or if the patient used lipid-lowering drugs. Diabetes mellitus was diagnosed if plasma fasting glucose exceeded 120 mg/dL, or if the patient used hypoglycemic agents. For each subject, the Framingham risk score (FRS) and the relative 10-year estimated coronary risk was calculated by the most recent FRS equation. Endothelial function in the form of FMD was measured according to recent guidelines by ultrasound (Image Point Hx, Hewlett Packard), using a 7.5-MHz linear-array transducer. Briefly, FMD was assessed by measuring, by ultrasound unit electronic calipers, the change in brachial artery diameter after 60 seconds of reactive hyperemia compared with baseline measurements after deflation of a cuff placed around the forearm that had been inflated to 50 mm Hg above systolic blood pressure for 5 minutes. The diameter increase after sublingual nitroglycerin spray (0.4 mg) was used as a measure of endothelium-independent vasodilation. The response of the vessel diameter to reactive hyperemia and nitroglycerin was expressed as a percent change relative to the diameter immediately before cuff inflation and to the diameter immediately before drug administration, respectively. In our laboratory, the intraobserver variability for repeated measurements of resting arterial diameter is 0.01±0.02 mm. When reactive hyperemia studies are performed on two different days, the between-occasion, within-subject variability for repeated measurements of resting arterial diameter is 1.5±0.7%. We measured ABPI with the use of a Doppler ultrasound probe. The systolic pressure in the right and left posterior tibial artery and in the right brachial artery was measured twice. The average of the two measurements was used to calculate ABPI. The lower ABPI of the two legs was used as predictor for future cardiovascular events. All drugs were discontinued for ≥18 hours before the study, which was carried out in the morning, after an overnight fast, in a quiet room at a constant temperature of 21±1°C. All subjects abstained from smoking and intake of caffeine-containing food or beverages for at least 12 hours before the study.

Assessment of Cardiovascular Events
Patients were contacted for follow-up examination at intervals of 3 months. Nine patients did not regularly attend our outpatient clinic and, thus, follow-up data were obtained by periodic telephone interviews. Medical records and death certificates of all patients who had an event were obtained and validated by a cardiologist unaware of the FMD and ABPI results. The minimum follow-up period was 6 months. The occurrences of cardiac (fatal and nonfatal acute myocardial infarction, unstable angina, and coronary revascularization procedures), cerebrovascular (fatal and nonfatal stroke, transient ischemic attack, and carotid surgery), and peripheral (critical limb ischemia and revascularization procedures of the lower limbs) events were prospectively assessed. Myocardial infarction was defined by an increase of at least 2-fold in creatine kinase-MB with typical ECG changes; unstable angina by hospitalization due to angina pectoris of Brawnnwald classification IIB or IIIB; ischemic stroke by clinical evidence of stroke without intracranial hemorrhage; transient ischemic attack by a diagnosis of any sudden focal neurological deficit that cleared completely within 24 hours; critical limb ischemia by the occurrence of rest pain or trophic lesions in the leg. Percutaneous transluminal peripheral angioplasty counted only when performed in a de novo leg stenosis during follow-up. Similarly, peripheral bypass revascularization was defined as the need for surgical intervention for symptomatic deterioration occurring at least 1 year after FMD measurement. Carotid surgery counted only when performed because of de novo critical stenosis during follow-up. For patients who had more than 1 event, only the first was considered in the analysis.

Statistical Analysis
Cumulative event rates were estimated with Kaplan-Meier survival curves, and probability values were calculated with the log-rank test. For Kaplan-Meier analysis, brachial artery FMD and non–endothelium-mediated dilation were divided into values below and above the median. Cox proportional hazard regression models were then used to control for confounders identified by univariate analysis (inclusion criteria $P<0.1$). To evaluate whether FMD measurement added to the predictive value of ABPI, we divided the study population into 4 groups: patients with both ABPI and FMD higher than their respective median value, those with ABPI higher than the median and FMD lower than the median, those with ABPI lower than the median and FMD higher than the median, and those with both ABPI and FMD below their respective median value. FMD is expressed as median and 25th, 75th percentiles because of its skewed distribution. Other variables are expressed as mean±SD or n (%). Comparisons of baseline characteristics between patients with and those without cardiovascular events at follow-up were made by $t$ test for unpaired samples or $\chi^2$ test, as appropriate.

Results

Patients
In the 131 patients studied, the mean duration of follow-up was 22.7±10 months (median, 21 months). During this time, 39 (29.7%) had a cardiovascular event: 7 (5%) had an acute myocardial infarction (3 were fatal), 9 (7%) unstable angina, 2 (1%) coronary revascularization procedures, 7 (5%) ischemic stroke, 3 (2%) transitory ischemic attack, 2 (1%) carotid surgery, 4 (3%) peripheral revascularization procedures, and 5 (4%) critical limb ischemia. On univariate analysis, FMD, ABPI, hypertension, and previous stroke were significantly associated with cardiovascular events (Table 1). In particular, FMD was lower in patients with events during follow-up than in those without events (5.8% [4.7; 7.2] versus 7.6% [5.3; 9.9], $P<0.05$). The use of antiplatelet and lipid-lowering agents, ACE inhibitors, and other cardiovascular drugs was comparable in patients with and those without cardiovascular events (Table 1).
TABLE 1. Baseline Characteristics of Patients With PAD, With and Without a Cardiovascular Event at Follow-Up

<table>
<thead>
<tr>
<th>Cardiovascular Event</th>
<th>With (n=39)</th>
<th>Without (n=92)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.3±9</td>
<td>63.2±10</td>
<td>0.553</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>34 (87%)</td>
<td>84 (91%)</td>
<td>0.470</td>
</tr>
<tr>
<td>BMI</td>
<td>26.1±1.7</td>
<td>25.8±1.2</td>
<td>0.378</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>35 (90%)</td>
<td>89 (97%)</td>
<td>0.104</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>27 (68%)</td>
<td>46 (50%)</td>
<td>0.043</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>25 (64%)</td>
<td>44 (48%)</td>
<td>0.088</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>14 (36%)</td>
<td>25 (27%)</td>
<td>0.318</td>
</tr>
<tr>
<td>FRS (10-y predicted risk &gt;20%)</td>
<td>16 (41%)</td>
<td>43 (47%)</td>
<td>0.548</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>12 (31%)</td>
<td>27 (29%)</td>
<td>0.871</td>
</tr>
<tr>
<td>Previous stroke, n (%)</td>
<td>3 (8%)</td>
<td>1 (1%)</td>
<td>0.045</td>
</tr>
<tr>
<td>BMI</td>
<td>26.1±1.7</td>
<td>25.8±1.2</td>
<td></td>
</tr>
<tr>
<td>NTG-mediated dilation %</td>
<td>10.2 [8.7; 11.6]</td>
<td>10.8 [9.1; 12.2]</td>
<td>0.549</td>
</tr>
<tr>
<td>ACE inhibitor therapy, n (%)</td>
<td>17 (43%)</td>
<td>28 (30%)</td>
<td>0.122</td>
</tr>
<tr>
<td>Lipid-lowering therapy, n (%)</td>
<td>22 (56%)</td>
<td>40 (43%)</td>
<td>0.175</td>
</tr>
<tr>
<td>Calcium-antagonist therapy, n (%)</td>
<td>20 (51%)</td>
<td>37 (40%)</td>
<td>0.200</td>
</tr>
<tr>
<td>Nitrate therapy, n (%)</td>
<td>17 (43%)</td>
<td>35 (38%)</td>
<td>0.492</td>
</tr>
<tr>
<td>Antiplatelet therapy, n (%)</td>
<td>29 (74%)</td>
<td>79 (86%)</td>
<td>0.177</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; MI, myocardial infarction; and NTG, nitroglycerin.

Brachial Artery Vascular Reactivity and Prognosis

As shown in Figure 1, the incidence of cardiovascular events during follow-up was significantly higher in patients with FMD below the median (6.7%) than in those with FMD above the median (P=0.004 by log-rank test). A similar result was obtained when peripheral events were excluded from the analysis (Figure 1). Conversely, when the population was divided according to the median value of nitroglycerin-mediated dilation, event-free survival was not associated with endothelium-independent vasodilation (P=0.29).

The variables that met the entry criterion (P<0.1 at univariate analysis) and were included in the Cox proportional hazard model were hyperlipidemia, hypertension, previous stroke, ABPI, and FMD. In this analysis, FMD below the median, ABPI below the median (0.65), and previous stroke were significantly associated with cardiovascular events (Table 2). When entered in the equation as continuous variables, both FMD (relative risk [RR], 0.870; 95% CI, 0.782 to 0.967; P=0.01) and ABPI (RR, 0.965; 95% CI, 0.943 to 0.987; P=0.002) remained independent predictors, thus showing that the higher the values of each variable, the lower the cardiovascular risk. Additionally, when peripheral events were excluded from the analysis, FMD maintained its

**TABLE 2. Independent Predictors of Cardiovascular Events**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite end point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD &lt; median</td>
<td>2.66</td>
<td>1.29–5.49</td>
<td>0.008</td>
</tr>
<tr>
<td>ABPI &lt; median</td>
<td>3.44</td>
<td>1.63–7.28</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>4.75</td>
<td>1.35–16.10</td>
<td>0.015</td>
</tr>
<tr>
<td>All events except peripheral events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD &lt; median</td>
<td>3.62</td>
<td>1.56–8.40</td>
<td>0.003</td>
</tr>
<tr>
<td>ABPI &lt; median</td>
<td>2.85</td>
<td>1.26–6.43</td>
<td>0.012</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>5.58</td>
<td>1.53–20.28</td>
<td>0.009</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>2.46</td>
<td>1.10–5.53</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Figure 1. Kaplan-Meier analysis of event-free survival curves refer to patients with FMD above and below the median. Left, Analysis of any cardiac, cerebrovascular, and peripheral event. Right, All except peripheral events.
prognostic impact on cardiovascular events (Table 2). To verify whether the predictive value of FMD was independent of a composite risk index, we performed a Cox analysis including FMD, ABPI, and FRS. In this analysis, only FMD and ABPI showed an independent association with future cardiovascular events.

ABPI, FMD, and Clinical Outcome
Kaplan-Meier curves for the four subgroups of patients delineated according to ABPI and FMD median values are shown in Figure 2. The analysis showed that FMD improved the prognostic value of ABPI, and this was confirmed by Cox analysis (Figure 3). Compared with patients with both ABPI and FMD above the median, those with ABPI above the median but FMD below the median had an independent association with future cardiovascular events.

The accuracy of FMD and ABPI were similar, being 61% and 66%, respectively. For FMD below the median, the positive predictive value was 41% whereas the negative predictive value of FMD above the median was 81%. The corresponding values for ABPI were 45% and 86%. Noteworthy, when both ABPI and FMD were higher than their respective median, the negative predictive value increased to 95%.

Discussion
This study demonstrates that brachial artery FMD has a prognostic impact on the cardiovascular risk in patients with PAD of the lower limb. The long-term predictive value of brachial artery FMD was recently demonstrated in a setting of vascular patients different from that of our study. Actually, that study investigated only patients undergoing vascular surgery for a broad range of conditions. The series included patients with PAD who underwent revascularization of the lower limbs, but a subanalysis of this group was not provided. Our study included a less severe population of patients with PAD, not requiring vascular surgery, and the results indicate that the predictive value of FMD was independent of the classic risk factors and of previous cardiovascular events. Furthermore, a low FMD remained an independent predictor of worse clinical outcome, even after adjustment for ABPI, which is the most powerful prognostic indicator in PAD. Importantly, our data demonstrate that the addition of FMD to ABPI measurement improves the global risk prediction in such patients. These results have several pathophysiological and clinical implications.

First, the observation that clinical outcome was associated with FMD, which depends on nitric oxide synthesis, and not with nitroglycerin-mediated vasodilation, indicates a specific relation between endothelial dysfunction and cardiovascular risk in PAD. In patients with PAD, impaired endothelial function has been associated with established risk factors, increased inflammation, and with the oxidative stress induced by acute ischemic exercise. Therefore, endothelial cells appear to integrate the injury imposed by exposure to classic and novel risk factors. This process probably leads to a reduced bioavailability of nitric oxide, a key mediator of many antiatherothrombotic effects. According to this scenario, endothelial dysfunction could represent the link between several atherogenic factors and the risk of cardiovascular events.

Second, the finding that the association between reduced FMD and poor prognosis was independent of classic risk factors appears to be further confirmation that in PAD, risk factors are poorly associated with future cardiovascular events when adjusted for measures of atherosclerotic disease severity. Furthermore, it suggests that improvement of endothelial function could be a specific target for intervention aimed at improving the prognosis of these patients.

However, the most important finding of this study is that FMD measurement adds to the predictive value of ABPI in...
determining risk for future cardiovascular events. Our data suggest that FMD may serve to stratify the cardiovascular risk both in patients with ABPI >0.65 (median value in our PAD population) and in those with ABPI <0.65. Among the latter, FMD contributed to the identification of a subgroup of patients at very high risk. Indeed, in patients with both FMD and ABPI below the median, the relative risk for future cardiovascular events was double that in patients in whom only ABPI was below the median. These findings have important diagnostic and prognostic implications because, to date, ABPI is acknowledged to be the most consistent and powerful predictor of the clinical outcome in PAD. In our study, the positive and negative predictive values of FMD were similar to those found for ABPI; however, the addition of a FMD above the median to an ABPI above the median increased the negative predictive value to 95%. Taken together, data of the present study indicate that FMD is a promising technique that may reflect an independent measure of the cardiovascular risk; however, standardization of the method and additional prospective studies are needed before this modality can become a part of a routine clinical assessment of cardiovascular risk.

Study Limitations
The results of this study cannot be extended to all subjects with PAD because we enrolled only patients with intermittent claudication. Thus, investigations are required to assess the prognostic impact of FMD in patients with critical limb ischemia and in those with asymptomatic PAD. In particular, FMD measurement could be very important in the latter, who represent the majority of the PAD population and who have a cardiovascular risk not much lower than symptomatic patients. With respect to our assessment of whether FMD added to the predictive value of ABPI, the number of subjects in each of the four subgroups delineated by FMD and ABPI median value is small. Therefore, although the combination of low ABPI and low FMD emerged as a better predictor of cardiovascular risk than ABPI and FMD alone, our findings should be verified in larger studies. Moreover, although all consecutive patients undergoing brachial artery FMD measurement were included in the follow-up analysis, for 65 patients originally studied for other goals, long-term evaluation was not a prespecified end point at the time vascular reactivity was assessed. Thus, the results of this hypothesis-generating study need to be confirmed by a larger, multicenter trial, using broader inclusion criteria.

Conclusions
This study demonstrates that reduced FMD is an independent predictor for increased cardiovascular risk in patients with intermittent claudication. More importantly, we provide the first demonstration that measurement of brachial artery vasoreactivity can add to the standard cardiovascular risk prediction. Indeed, in our population, FMD improved the prognostic value of ABPI, the most powerful marker of cardiovascular risk in PAD. Therefore, FMD measurement may be particularly useful to identify a subgroup of claudics at increased risk. We hope that our data will prompt studies aimed at assessing whether patients with PAD with severe endothelial dysfunction could represent a target population for ACE inhibitors and statins, which have been shown to improve endothelial function, and reduce the cardiovascular risk.

References
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