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Assessment of peripheral vascular endothelial function in the ambulatory setting

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Abstract: Until now, peripheral vascular endothelial function testing has been performed in research laboratories under highly controlled conditions, thus limiting its clinical applicability. In this study, we evaluated endothelial function in two peripheral vascular beds before and during reactive hyperemia in an outpatient clinic setting. The brachial artery was imaged with a portable ultrasound device and changes in vessel diameter were expressed as percent flow-mediated dilation (%FMD). Pulse wave amplitude of the finger was detected by peripheral arterial tonometry (PAT) and PAT hyperemia was defined as the maximal plethysmographic recording compared to baseline. Sixty individuals (43 men) were enrolled with an average age 53 $\pm$ 2 years (mean $\pm$ SE). The 31 individuals with more than two cardiac risk factors (CRF) had lower FMD (7.0 $\pm$ 1.1%) and PAT hyperemia (2.1 $\pm$ 0.9) compared to the 29 individuals with 0–2 CRF (FMD 11.3 $\pm$ 0.8%, PAT hyperemia 2.4 $\pm$ 0.1; $p < 0.05$ for both). The 32 individuals with coronary artery disease (CAD) had lower FMD (6.8 $\pm$ 1.1%) and PAT hyperemia (2.0 $\pm$ 0.1) compared to the 28 individuals without CAD (FMD 11.5 $\pm$ 0.8%, PAT hyperemia 2.4 $\pm$ 0.1; $p < 0.05$ for both). Thus, peripheral vascular endothelial function testing in the ambulatory setting correlates with the extent of CAD risk and the presence or absence of CAD. In conclusion, these data suggest that peripheral vascular endothelial function testing is feasible in ambulatory patients, and this is an important next step in bringing this technology to clinical applicability.

Key words: ambulatory; brachial artery ultrasound; endothelium; flow-mediated dilation; peripheral arterial tonometry

Introduction

Abnormal endothelial function is an early marker of vascular disease and increased cardiovascular risk.[1] Assessment of vasomotor tone provides physiologic information regarding the bioavailability of nitric oxide, an important endogenous anti-atherosclerotic agent.[2,3] Endothelial dysfunction is a diffuse disease process, and therefore, non-invasive assessment of the peripheral vasculature may be helpful for the evaluation of vascular relaxation in large patient populations. Brachial artery ultrasound testing and peripheral arterial tonometry (PAT) are methods for quantifying vascular function during reactive hyperemia.[4,5] Brachial artery vascular testing utilizes high-resolution ultrasound to measure changes in vascular dimensions, while PAT records finger arterial pulse wave amplitude. Our group and others have shown that peripheral vascular reactivity correlates with cardiovascular risk factors (CRF) and coronary artery disease (CAD).[6] Thus far, assessment of vascular endothelial function has been tested in research laboratories under highly controlled conditions, thus limiting its use in clinical practice. Devices are now available that are small and portable enough to be used in the ambulatory clinic setting. We have recently validated two of these approaches, a portable vascular ultrasound machine and PAT, and demonstrated that they provide similar assessment of endothelial function as standard approaches.[5,7] In this study, we sought to evaluate the feasibility and utility of non-invasive testing of peripheral vascular endothelial function in the ambulatory care setting.

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Methods

Individuals over 18 years of age with a prior evaluation for CAD (either by cardiac catheterization or nuclear imaging stress testing) presenting to the outpatient

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Preventive Cardiovascular Center at Tufts-New England Medical Center (Boston, MA, USA) were enrolled. Prior to their arrival at the clinic, these individuals had no knowledge of this study and were not instructed to withhold their medications, food or caffeine. Individuals were clinically stable without active chest discomfort, recent myocardial infarction, unstable angina or congestive heart failure, and were excluded if they had significant valvular heart disease (greater than mild stenosis or regurgitation) or left ventricular dysfunction (ejection fraction <40%). All individuals provided written informed consent and the protocol was approved by the institutional investigation review board.

The presence or absence of the following CRF were assessed in each individual: males >55 years or females >65 years, hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or being on an antihypertensive medication), hyperlipidemia (total serum cholesterol >220 mg/dl or taking lipid-lowering medication), diabetes mellitus (treated with an oral hypoglycemic agent and/or insulin or having fasting glucose levels >126 mg/dl), family history of CAD (having first or second degree relatives with premature cardiovascular disease), postmenopause, and smoking (more than five cigarettes per day within the last month). CAD was defined as >50% stenosis of a major coronary vessel by angiography or the presence of ischemia or infarction on nuclear myocardial perfusion imaging.

Endothelial function testing was performed in an outpatient examination room following the regularly scheduled outpatient appointment. We followed the brachial artery ultrasound imaging protocol similar to that originally described by Celermajer and according to present guidelines.[3,8] Longitudinal brachial artery images were obtained with a high-resolution (up to 11 MHz) linear-array vascular transducer using portable ultrasound equipment (SonoSite, Titan; Bothell, WA, USA). Following a 10-minute equilibrium period, baseline two-dimensional images of the right brachial artery were obtained. A blood pressure cuff (Hokanson; Bellevue, WA, USA) placed proximal to the imaging transducer on the upper arm was inflated to supra-systolic pressure for 5 minutes. Percent flow-mediated dilation (%FMD) of the brachial artery was defined as the maximal brachial artery diameter after 60 seconds of reactive hyperemia compared to the baseline vessel diameter.

PAT (Itamar-Medical; Caesarea, Israel) was performed simultaneously with brachial artery evaluation by placing thimble-shaped pneumatic probes on the index finger of the hand undergoing hyperemia testing and the contralateral index finger as previously described.[5,9] The probes were connected to isolated volume reservoirs, which buffer pressure changes and provide a uniform pressure field. PAT hyperemia was automatically calculated at the conclusion of the study as the ratio of the average baseline pulse wave amplitude during reactive hyperemia compared with the pre-occlusion baseline. This ratio was normalized to the concurrent signal from the contralateral, non-ischemic hand to account for any systemic changes during upper extremity testing. Evaluation of endothelial function with brachial and finger testing occurred once during a clinic visit; no follow-up evaluation was performed to assess variability of the techniques.

Continuous variables were compared using a Student's *t*-test and are presented as mean ± standard error of the mean (SEM). The Spearman rank test was used to assess for potential univariate correlation. Sensitivity and the predictive value of FMD and PAT were determined to evaluate CAD in this ambulatory setting. In all analyses, a *p*-value <0.05 was considered statistically significant.

Results

Sixty individuals (43 men) with an average age of 53 ± 2 years completed the peripheral endothelial function testing. Characteristics of the study population are listed in Table 1. Approximately one half of the population had CAD. Less than half of the individuals were on statin therapy and their last meal was, on average, 2 ± 0.5 hours prior to arrival to the outpatient clinic. The mean FMD and PAT were 9.1 ± 0.8% and 2.2 ± 0.6, respectively. The 31 individuals with more than two CRF had lower FMD (7.0 ± 1.1%) and PAT hyperemia (2.1 ± 0.9) compared with the 29 individuals with 0–2 CRF (FMD 11.3 ± 0.8%, PAT hyperemia 2.4 ± 0.1; $p < 0.05$ for both). There was a significant relationship between the number of CRF

Table 1 Study population characteristics.

Variable	$n = 60$
Age (years)	53 ± 2
Men	43 (72%)
Systemic hypertension	23 (38%)
Diabetes mellitus	10 (17%)
Hyperlipidemia	33 (55%)
Tobacco abuse	21 (35%)
Family history of coronary disease	8 (13%)
Coronary artery disease	32 (53%)
Left ventricular ejection fraction (%)	59 ± 2
Body mass index (kg/m^2)	29 ± 3
Total cholesterol (mg/dl)	158 ± 5
High-density lipoprotein cholesterol (mg/dl)	44 ± 3
Low-density lipoprotein cholesterol (mg/dl)	90 ± 4
Triglycerides (mg/dl)	125 ± 10
Angiotensin converting enzyme inhibitor	11 (18%)
Aspirin	31 (52%)
Beta-blocker	18 (30%)
Calcium channel blocker	4 (7%)
Nitroglycerin	6 (10%)
Statin	24 (40%)

Data expressed as mean ± SEM; number (%).

and FMD ($R = 0.4$; $p < 0.01$) and PAT hyperemia ($R = 0.3$; $p < 0.01$). The 32 individuals with CAD had lower FMD (6.8 ± 1.1%) and PAT hyperemia (2.0 ± 0.1%) compared with the 28 without CAD (FMD 11.5 ± 0.8%, PAT hyperemia 2.4 ± 0.1%; $p < 0.05$ for both; Figures 1 and 2). There was a significant relationship between FMD and PAT hyperemia in both the CAD and non-CAD populations ($R = 0.3$; $p < 0.05$ for both). Using FMD and PAT cut-points (11% and 2.7%, respectively) to maximize the predictive value for the evaluation of CAD, the sensitivity and negative predictive value for FMD were 83% and 74%, respectively, while for PAT hyperemia, they were 92% and 78%, respectively.

Discussion

For over a decade, researchers have explored a variety of methods to assess endothelium-dependent vascular reactivity in the coronary and peripheral circulations. While coronary artery endothelial function testing provides a direct assessment of the vascular bed of most clinical importance,[10,11] this methodology is limited due to cost and procedural risks and, therefore, is not available for everyday use in clinical practice. Since atherosclerosis is a diffuse disease process and there is significant correlation between abnormalities in the central and peripheral vascular beds, peripheral vascular testing for endothelial function has evolved as a more feasible testing modality for assessing larger patient populations.[12,13] Evaluation of endothelium-dependent vasomotion by high-resolution brachial artery ultrasound is a widely used method in research studies, and previous reports have noted a correlation between abnormalities in FMD of the brachial artery and the presence of CAD by coronary angiography as well as perfusion imaging.[6] In addition, peripheral vascular dysfunction is predictive of cardiovascular events,[14,15] and an augmented endothelial response following a medical intervention appears to improve cardiovascular risk.[16] Brachial artery imaging is typically performed with standard ultrasound equipment, although we have recently shown that FMD of the brachial artery may be assessed with portable technology.[7]

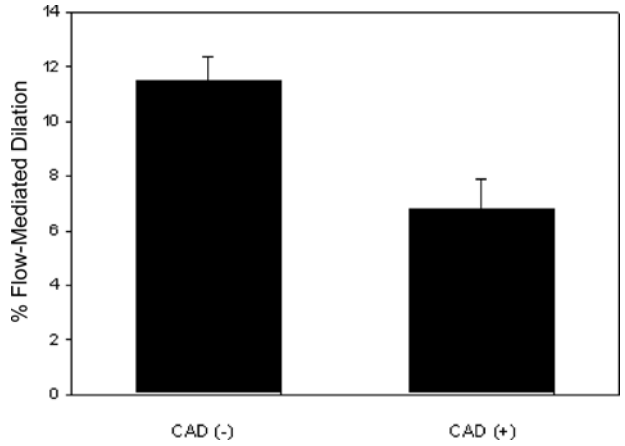

Figure 1 Differences between individuals without coronary artery disease (CAD–) and those with coronary artery disease (CAD+) for percent flow-mediated dilation (%FMD) of the brachial artery; $p < 0.05$.

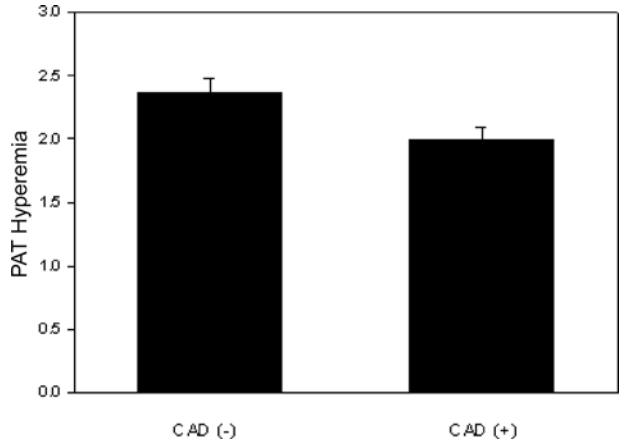

Figure 2 Differences between individuals without coronary artery disease (CAD−) and those with coronary artery disease (CAD+) for finger peripheral arterial tonometry (PAT); $p < 0.05$.

In addition to large vessel ultrasound evaluation, other non-invasive modalities to assess peripheral vascular endothelial function, such as PAT, are actively being investigated as markers of vascular health and may play a role in the evaluation of cardiovascular risk. Assessment of pulse wave amplitude of the finger by PAT correlates with brachial artery imaging and coronary artery endothelial function testing, and appears to capture a nitric oxide-mediated pathway related to flow-mediated vasodilation.[17] Thus far, PAT hyperemia has been shown to be an adequate surrogate marker to assess for changes in vascular function over time[18]; however, the prognostic importance of this test remains unclear.

Owing to labile changes in endothelial function related to diet, timing of the test and medications, as well as environmental influences such as temperature and noise, assessment of vascular endothelial function has been reserved for well-controlled research laboratories and has not been evaluated in the ambulatory setting. In this study, we performed a pilot study to evaluate the feasibility of performing non-invasive vascular studies to predict the presence or absence of CRF and CAD in stable outpatients presenting for routine cardiovascular evaluation. In 60 individuals, we found that testing vascular reactivity in the large and small vessels of the upper extremity is practical and correlates with the extent of CAD risk and the presence or absence of CAD. In addition, ambulatory FMD and PAT testing correlate only modestly with each other, possibly because the two modalities are testing different vascular beds. There is potential for

these tests to be helpful in tailoring medical therapy in at-risk individuals, or perhaps in following individuals longitudinally after specific interventions. Thus, the findings in this paper support the potential use of non-invasive vascular endothelial function testing in ambulatory populations.

Several limitations of this study deserve comment. First, assessment of CRF is inherently imperfect, in part because some individuals are undergoing aggressive treatment with medications or lifestyle changes, while others are not. Second, we enquired only about traditional CRF, and did not examine 'novel' risk factors, which may also play a role in endothelial function and atherosclerosis. Third, endothelial function testing typically takes place in tightly controlled environments while withholding medications and food, but these variables are difficult to control in the ambulatory setting. Our study sought to determine, in part, whether the effect of not controlling these factors was so large that the test would no longer be useful. The current findings suggest that these tests of endothelial function are indeed adequate in the ambulatory setting. Furthermore, it is in the clinic environment that physicians have the ability to address and potentially modify cardiovascular risk, which may have a significant impact on patient care. Fourth, we did not perform follow-up studies on this population to test for the variability of these techniques. Finally, the population studied was relatively small. Larger studies are warranted to test the predictive value of these tests in the ambulatory setting.

The assessment of ambulatory endothelial function is likely to be a clinically useful tool with many potential applications. For example, endothelial function testing may be helpful to identify patient subgroups at higher risk for cardiovascular events, many of whom do not qualify for medical therapy according to present guidelines. Ambulatory testing may have a role in defining the vascular response to various medical therapies or to aid preoperative risk assessment. Bringing endothelial function testing to the ambulatory arena may be helpful when screening low cardiovascular risk individuals, thereby obviating the need for more elaborate testing. Thus, physiologic vascular testing of the endothelium in the outpatient clinical setting is feasible and has the potential to evolve into an important adjunctive tool for evaluating cardiovascular risk and response to treatment.

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