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FOCUS ISSUE: BIOMARKERS IN CARDIOVASCULAR DISEASE

# Biomarkers of Peripheral Arterial Disease

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**Atherosclerotic arterial occlusive disease affecting the lower extremities is also known as peripheral artery disease (PAD). This disorder affects 8 to 12 million individuals in the U.S. and is increasingly prevalent in Europe and Asia. Unfortunately, most patients are not diagnosed and are not optimally treated. A blood test for PAD, if sufficiently sensitive and specific, would be expected to improve recognition and treatment of these individuals. Even a biomarker panel of moderate sensitivity and specificity for PAD could refine risk stratification to select individuals for diagnostic vascular examination. Alternatively, biomarkers for PAD may be useful in determining prognosis, the risk for progression, or the response to therapy. Finally, the discovery of biomarkers associated with PAD may provide novel insights into the pathophysiology of PAD and new therapeutic avenues to pursue. Biomarkers may be derived from studies of the genome, transcriptome, proteome, or metabolome. The focus of this review is on proteomic biomarkers associated with PAD. (J Am Coll Cardiol 2010;55:2017–23) © 2010 by the American College of Cardiology Foundation**

The prevalence of lower-extremity peripheral artery disease (PAD), assessed using the ankle-brachial blood pressure index (ABI), has been estimated to be 10% to 20% in individuals older than 65 years of age in community-based studies (1–4). Even greater prevalence is observed in individuals attending general medicine practices, in which 20% to 30% of patients age 50 years and older have the disease (5,6). Peripheral arterial disease causes limb pain with exertion, reduces functional capacity and quality of life (7), and is frequently associated with coronary, cerebral, and renal artery disease (8). Individuals with PAD are at increased risk for acute cardiovascular events such as myocardial infarction, cerebrovascular attack, aortic aneurysm rupture, and vascular death, as well as ischemic ulceration and amputation (9,10). This increased risk for cardiovascular morbidity and mortality is seen even in patients without symptoms (11).

Aggressive medical treatment of risk factors can substantially reduce the mortality and morbidity of PAD (12). Unfortunately, PAD is underdiagnosed and undertreated, with most patients not receiving optimal management, including therapies proven to reduce mortality such as antiplatelet agents, statins, and converting enzyme inhibitors (13). Suboptimal physician recognition and management of the condition is in part because of poor public awareness of PAD (14), inadequate training and tools for primary physicians, a lack of remuneration for screening (15), and the absence of the classic symptom complex in a majority of the patients (16). Classical intermittent claudication (i.e., exertional leg discomfort relieved by rest) is only noted by 10% to 30% of patients with PAD (7,13). Musculoskeletal disease or neuropathy commonly coexist with PAD and confound the clinical picture (7). Accordingly, clinical assessment for PAD has a relatively poor predictive value (<10%) (17). Structured questionnaires such as the Edinburgh Claudication Questionnaire have improved sensitivity and specificity compared with clinician assessment (18), but these questionnaires only identify patients with classical symptomatology. Because the current recognition of PAD is suboptimal, and because effective therapy that improves mortality is available for these individuals, an efficacious strategy to screen the population for PAD is highly appealing.

## PAD: The Case for Screening

Compared with angiography, the ABI can detect hemodynamically significant lesions with a sensitivity of 80% to 95% and a specificity of 95% to 100% (19,20). Furthermore, the ABI has independent prognostic value beyond the Framingham risk factors (21). The ABI is calculated from Doppler-derived measurements of the systolic pressure at the brachial and ankle arteries. By convention, for each lower extremity, the higher of the 2 ankle artery pressures is used for the ABI calculation. The ABI for that extremity is the higher ankle pressure divided by the higher of the 2 brachial artery pressures.

Targeted screening with ABI is recommended by all professional vascular societies, including the American College of Cardiology (22). The American College of Cardi-

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**Abbreviations and Acronyms**

**ABI** = ankle-brachial blood pressure index

**$\beta_2$M** = $\beta_2$ microglobulin

**CAD** = coronary artery disease

**CRP** = C-reactive protein

**MD** = mass spectroscopy

**PAD** = peripheral artery disease

ology/American Heart Association guidelines support ABI screening in high-risk patients (defined as individuals age <50 years with diabetes and 1 other atherosclerosis risk factor, those age 50 to 69 years with a history of smoking or diabetes, individuals age ≥70 years, those with leg symptoms with exertion or ischemic rest pain, and those with an abnormal lower-extremity pulse examination) (22). Also, the American Diabetes Association recommends annual screening for PAD in diabetics (23).

Despite the abundant evidence supporting the value of the ABI, and despite careful studies that have revealed suboptimal recognition of individuals with PAD and inadequate utilization of therapies that reduce mortality, there is resistance to adopting the ABI as a screening tool. The U.S. Preventive Services Task Force has given the practice a "D" level recommendation (i.e., in their opinion, routinely providing the service to asymptomatic patients is ineffective or harm from the test may outweigh benefits). Also, the American Academy of Family Physicians recommends against the use of the test in asymptomatic persons (24).

These opinions are contrary to the recommendations of vascular specialty societies and have been convincingly rebutted (15). In brief, these unfortunate recommendations are driven by the concern that screening may lead to unnecessary tests and increased risk from subsequent invasive studies or procedures. However, of much greater concern is the very real cost to the health care system and to the patient of not identifying individuals with PAD. The primary purpose in screening for PAD is to identify individuals at high risk of vascular events (8,9,25–27) to target them for aggressive risk reduction interventions (28–33). Unfortunately, most patients with PAD are currently not diagnosed and are not receiving therapies that can improve their prognosis (13,34).

## Beyond the ABI

Among vascular specialists, there is widespread recognition of the value of the ABI and evidence-based documentation of its sensitivity and specificity. However, a practical concern is that most primary practitioners lack the specialized equipment and trained personnel to perform ABI measurements in the office setting. In the absence of an effective screening strategy in the primary practitioner's office, all individuals at risk could be referred for a formal vascular laboratory evaluation. This would be a costly screening strategy.

The number of individuals that should be screened for PAD (i.e., all smokers who are >50 years of age, all patients with diabetes who are >50 years of age, and all individuals who are >70 years of age) represents approximately 60 million individuals in the U.S. An alternative screening approach would be to develop a blood biomarker, or panel of biomarkers, that could stratify the risk for individuals in the primary practitioner's office. Such a panel could be assessed by a blood draw in the office and would optimally identify a smaller subset of patients for vascular evaluation. Such an approach could reduce the overall cost of screening while improving recognition and proper management. This alternative diagnostic paradigm requires progress toward developing novel biomarkers of PAD.

## Challenges in Discovering New Biomarkers

There are hurdles to the discovery of any new blood protein biomarkers. The most daunting problem is the great diversity of the proteome (i.e., plasma contains approximately 10,000 plasma proteins and even more protein fragments) and its dynamic range (approximately 10 orders of magnitude difference between the least and most abundant proteins) (35). The discovery process is complicated by the fact that the 22 most abundant proteins, such as albumin and the immunoglobulins, constitute approximately 99% of the total proteome mass (36). However, it is the low-abundance proteins that are often of the greatest interest as novel disease markers. Any technology to profile the plasma proteome in an informative manner must be able to delve deeply into the proteome and to discriminate differences in the levels of low-abundance proteins. For example, cardiac markers such as troponin are found in the nanomolar range, whereas cytokines are in the femtomolar range.

Another important issue is confounding by medications or associated diseases. Careful phenotyping of the subjects is critical for proteomic discovery, and the control group should be matched for variables already known to influence disease risk and outcome. Renal or hepatic disease may influence the excretion or metabolism of a biomarker. Other disorders may influence the level of a biomarker by pathophysiologic processes unrelated to the disease of interest (e.g., infection increases the plasma level of the cardiovascular biomarker C-reactive protein [CRP]). Technical details such as how the blood is drawn, processed, and stored can substantially affect the findings and lead to spurious results if the samples from different patient groups are not treated similarly. For example, multiple freeze-thaws while samples are studied cause protein degradation, introducing artifactual peaks in mass spectroscopic analyses.

Despite these challenges, the field of cardiovascular proteomics continues to develop rapidly, and a range of collaborative initiatives have been undertaken. The National Institutes of Health/National Heart, Lung and Blood Institute has funded several centers for cardiovascular proteomics (37). The Human Proteome Organization has recently initiated a plasma proteome project (38). The early phase of the project has reported the identification of approximately 345 cardiovascular disease-related proteins in human