Circulating MicroRNA Profiles for Detection of Peripheral Arterial Disease
Small New Biomarkers for Cardiovascular Disease

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Periphera Arterial Disease
Periphera arterial disease (PAD) is a clinical condition caused by an atherosclerotic process affecting the arteries of the limbs. PAD has many similarities with the atherosclerotic process in coronary artery disease and shares similar risk factors, including male sex, age, diabetes mellitus, smoking, hypertension, high cholesterol, and renal insufficiency. Furthermore, PAD is known to be associated with a reduction in functional capacity and quality of life, as well as an increased risk for myocardial infarction, stroke, death, and a major cause of limb amputation. However, because PAD is often observed with comorbid conditions, such as hypertension, dyslipidemia, diabetes mellitus, cigarette smoking, and physical inactivity, the pathophysiology of PAD is certainly complex and multifactorial. For this reason, a better physiological understanding of the pathogenesis and treatment options for patients with PAD is necessary. In general, the goal of medical therapy in patients with PAD is to reduce the risk of future cardiovascular morbidity and mortality in patients with high ischemic risk and to improve walking distance and functional status in patients with intermittent claudication. Despite major improvements in surgical endovascular techniques, PAD is still associated with high mortality and morbidity. Unfortunately, most patients are diagnosed late and are not treated optimally. A blood test for PAD, if sufficiently sensitive and specific, would be expected to improve recognition and treatment of PAD-affected patients, but biomarkers to predict the development of PAD are lacking although recently OxPL/apoB levels are demonstrated the extracellular presence of circulating miRNAs to be positively associated with risk of PAD.

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Circulating MicroRNAs
Traditionally, cellular processes and linked gene control were considered to occur via a relatively simple mechanism. Genomic DNA is transcribed into coding mRNA strands that are subsequently translated into proteins, finally carrying out cellular tasks. However, recent discoveries of a new class of noncoding RNAs revealed a change in this thinking, in which many small RNA molecules are not translated into protein but regulate cellular behavior via different mechanisms. Among these molecules, microRNAs (miRNAs) are the best understood and studied class of small RNAs. miRNAs are an abundant class of short, noncoding RNA molecules that are ≈22 nucleotides in length, which regulate gene expression through inhibition of the translation of target genes. It is generally accepted that miRNAs guide processes and cellular functions through precise titration of gene dosage not only for a single gene but also controlling the levels of a large cohort of gene products. Not surprisingly, miRNA expression is altered in cardiovascular disease and may thereby limit and impair cardiovascular repair responses.

The first evidence and suggestion for the essential general role of miRNAs in normal mammalian development and in the cardiovascular system came from analysis of mice lacking the miRNA-processing enzyme Dicer that resulted in embryonic lethality between E12.5 and E14. Dicer deficiency results in the lack of production of mature miRNAs and resulted in severe vascular developmental defects caused by impaired blood vessel formation, which was confirmed by conditional ablation of Dicer from endothelial or vascular smooth muscle cells that resulted in defective blood vessel development. Moreover, in mice experiments lacking miR-143 and miR-145, neointima and atherosclerotic lesion development were reduced, demonstrating that individual miRNAs can direct the smooth muscle cell fate.

In addition to their cellular presence, more and more reports demonstrate the extracellular presence of circulating miRNAs that can potentially be used as biomarkers for several cardiovascular diseases. One of the straightforward and best studied populations for the use of miRNAs is to diagnose myocardial infarction. Several miRNAs have been identified that increase myocardial damage in a population of acute coronary syndrome–suspected patients, thereby having even additional diagnostic power instead of traditional superior markers as high-sensitive troponin. For atherosclerotic disease, miRNA signatures were previously used to identify patients exhibiting atherosclerotic coronary artery disease in general and those at risk for acute coronary syndrome, thereby identifying miR-135a and miR-147 for coronary artery disease and miR-134, miR-198, and miR-370 for acute coronary syndrome.

In this issue of Circulation Cardiovascular Genetics, Stather et al aimed to determine whether circulating miRNAs are differentially expressed in patients having PAD. These first
attempts to identify a human miRNome (miRNA transcriptome-wide) signature for PAD, thereby identifying potential factors involved in the pathogenesis, or being functional active during PAD worsening, are crucial to permit new therapeutic approaches. Moreover, there is a need for minimally invasive biomarkers for the diagnosis of PAD, the early detection of patients at risk, and the management of disease progression. In the study of Stather et al., patients were selected on the basis of both symptomatic and radiological evidence of PAD in the legs, thereby including symptoms of intermittent claudication, and with TransAtlantic InterSociety Classification II type B or type C lesions, but excluding several confounding factors. An miRNA panel was obtained from peripheral blood cells and was based on abundant presence and significance scoring. They subsequently studied and validated 12 miRNAs, including let 7e, miR-15b, miR-16, miR-20b, miR-25, miR-26b, miR-27b, miR-28-5p, miR-126, miR-195, miR-335, and miR-363, of which miR-16, miR-363, and miR-15b had the best predictive values (area under the curve >0.92; P<0.001). In addition, by algorithm-searching and pathway-enrichment analysis, they identified inversely correlated candidate target genes.

**Potential Impact**

Because this is one of the first attempts to create an miRNAs signature for patients with PAD, several limitations should be considered for the final impact of the identified panel. The authors are well aware that their cohorts are relatively small and detect many different miRNAs to find potential candidates and can easily lead to false-positive findings because statistical correction for multiple testing of their miRNA panel is hard. Moreover, no extended imaging of the vasculature is performed to exclude generalized atherosclerosis in patients, exclude atherosclerosis in controls, or phenotype the limited number of patients, thereby maybe explaining the variations in expression levels and affecting potential clinical impact for specific PAD findings. Only biomarkers that have unique information with additional incremental value, relative to other prognostic indicators available to the clinician, will be useful. Although a biomarker may seem prognostically important in a statistical model, demonstrating that it can impact on and guide therapy is a long way to go. miRNAs that were identified by Stather et al are mainly downregulated, which might be a problem for clinical use as a biomarker, but interestingly they also suggest that the identified miRNAs are taken up by cells in the system. Also the association studies in which miRNAs are linked to predicted mRNA levels are a first step of identification and potential understanding of mechanisms playing a role. However, because they focused on pathways with a clear involvement in vascular physiology, this will probably not lead to new targets for drug development.

A more general remark for the use of circulating miRNAs as biomarkers is the lack of consensus on their isolation procedures and clear normalization factors needed. U6 and RNU48 were confirmed as stable reference genes, but whether these are stable enough in a clinical setting is not clear yet. Moreover, currently RNA isolations and polymerase chain reaction amplification steps are needed for their detection, which is laborious and time-consuming. Therefore, a quick detection technique needs to be developed.

These critical notes are needed to reflect current findings and could be made for all new biomarker discovery studies, which per definition need extensive follow-up studies to validate their usefulness. These observations, presented by Stather et al., to characterize and predict patients with PAD by comparing circulating miRNAs signatures is a nice and important study in this new and intriguing area of research. By creating better biomarkers for different cardiovascular diseases, the potential diagnosis and subsequent treatments can improve and thereby realize more personalized care.

**Conclusions**

Current findings reported by Stather et al are of importance because blood miRNAs can be monitored easily, and, therefore, the miRNAs identified potentially represent a convenient and minimally invasive tool for the diagnosis of PAD and patient stratification and may complement existing methods. Moreover, predicting the level and progression of atherosclerosis and the risk of secondary cardiovascular events, such as stroke, myocardial infarction, or claudication, is of major importance in clinical practice to stratify patients with generalized atherosclerosis and to improve disease management. Index scores or prediction models incorporating these miRNAs will now have to be tested in larger patient populations, but results are promising for the use of these novel types of biomarkers.

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**Disclosures**

None.

**References**


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