Platelets, endothelium, and circulating microRNA-126 as a prognostic biomarker in cardiovascular diseases: per aspirin ad astra

Elena Cavarretta¹, Giovanni Alfonso Chiariello², and Gianluigi Condorelli³*  

¹Department of Medical-Surgical Sciences and Biotechnology, Sapienza University of Rome, Italy; ²Institute of Genetics and Biomedical Research (IRGB), National Research Council of Italy, Rozzano (MI), Italy; and ³University of Milan, Humanitas Clinical and Research Center, Via Manzoni 56, 20089, Rozzano (MI), Italy

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This editorial refers to ‘Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease’¹, by H.C. de Boer et al., on page 3451

MicroRNAs (miRNAs) have been proven to be key regulators of the cardiovascular system, orchestrating physiological development, inflammation, hypertrophy, ischaemia/reperfusion injury, angiogenesis, atherosclerosis, apoptosis, and fibrosis.¹ In addition to their presence within cells, miRNAs can be found in the bloodstream because, in contrast to naked RNA, circulating miRNAs are highly resistant to RNase degradation on account of (i) their transport in exosomes, apoptotic bodies, or microparticles; (ii) their association with lipoprotein complexes, such as HDL; or (iii) their binding to protein complexes, such as the Argonaute family or nucleophosmin.² Different tissues contribute to the circulating pool of miRNAs, with a significant percentage probably originating from blood and inflammatory cells. Interestingly, the levels of circulating miRNAs have been found to be altered in pathologies such as acute myocardial infarction (AMI),³ heart failure,⁴ coronary artery disease (CAD),⁵ and type 2 diabetes mellitus (T2DM).⁶ This occurs, for example, because cell damage, such as the necrosis of cardiomyocytes in AMI, causes the release of cellular miRNAs into the bloodstream: in fact, cardiac-specific miRNAs (such as miR-1, miR-133a, and miR-208b) are found to be elevated in blood cardiomyocytes in AMI, causes the release of cellular miRNAs into the bloodstream: in fact, cardiac-specific miRNAs (such as miR-1, miR-133a, and miR-208b) are found to be elevated in blood after AMI, correlating well with troponin T serum levels.⁷ Extracellular miRNAs, therefore, are gaining interest because of their potential as biomarkers. Currently, however, their use as diagnostic biomarkers is still limited,⁸ with other laboratory analyses being more easily conducted than the quantitative PCR-based assays needed to identify circulating miRNAs. The use of miRNAs as prognostic biomarkers, predicting cardiovascular disease risk before clinical onset, would seem to be a more appealing approach.

On this point, miR-126 (also called angiomiR-126 because of its importance for the vasculature) holds promise as a prognostic biomarker of vascular damage and endothelial dysfunction. This miRNA—which is highly expressed in endothelial cells (ECs) and platelets—signals for proangiogenic effects on ECs and, thus, acts as a pivotal regulator for the maintenance of endothelial homeostasis and vascular integrity.² The level of circulating miR-126 was found to be decreased in a glucose-dependent fashion in T2DM patients, resulting in impaired vascular endothelial growth factor (VEGF) facilitation.⁸ A down-regulated blood level of miR-126 was also demonstrated in CAD patients.⁴ In contrast, however, a positive association between circulating miR-126 and fatal myocardial infarction has been reported more recently,³ raising the questions of whether abnormal platelet functioning might be the major contributor to the blood miR-126 level in T2DM patients and whether antiplatelet therapy—or medication in general—might have been a confounding factor in the assessment of circulating miRNA profiles in the previous studies. de Boer et al.¹⁰ now try to answer these questions by highlighting the role of platelets as a source of circulating miR-126 and the effect of aspirin treatment on the plasma level of this and other platelet-derived miRNAs.

Aspirin determines irreversible inhibition of cyclo-oxygenase 1, which normally acts upon arachidonic acid to produce prostaglandin G₂, prostaglandin H₂, and subsequently thromboxane A₂ (Figure 1); deficient thromboxane A₂ synthesis then results in a decreased ability of platelets to aggregate. Patients with T2DM have an abnormally high platelet reactivity that can be inhibited by the administration of aspirin. First, de Boer et al.¹⁰ show that activation of healthy ex vivo platelets promotes the release of miR-126 from intracellular stores and that this is event is inhibited by aspirin. The authors then demonstrate in T2DM patients that the plasma level of miR-126 is significantly correlated with that
of soluble P-selectin (sP-sel), a measure of platelet activation. Moreover, to assess the contribution of EC-derived miR-126, the authors measure plasma levels of von Willebrand factor (vWF). vWF is produced exclusively by ECs and megakaryocytes, and has important roles in mediating platelet adhesion and aggregation, facilitating blood clotting factor VIII, and in regulating angiogenesis. The high shear stress on the endothelial wall allows conformational changes within vWF that expose platelet binding sites. vWF is essential in initiating platelet adhesion in the extracellular matrix, either by a direct mechanism, in which fibrin binds to the glycoprotein (GP)IIb–IIIa complex, or by an indirect mechanism, in which vWF serves as a bridging agent between fibrin and platelet GPIb. The authors do not find a correlation between the levels of vWF and miR-126 or sP-sel, indicating that their results were not altered by EC activation or damage. In pathologic states of platelet aggregation, therefore, a high circulating miR-126 level probably reflects platelet activation more than endothelial dysfunction.

The authors also take into account how pharmacodynamics impact the level of circulating miR-126, demonstrating that in aspirin responders (i.e. patients in which sP-sel decreases upon treatment with aspirin), the ratio of miR-126 levels measured after aspirin and after placebo is significantly lower than that in non-responders (i.e. patients that do not have a reduction in sP-sel after aspirin treatment). The confounding effects of antiplatelet drugs and any other medication that might affect aggregation, such as statins, should therefore be taken into account to obtain robust data on circulating miRNAs.

Another very recent paper has described the substantial contribution of platelets to the pool of circulating miRNAs and how antiplatelet drugs affect levels. Considering the different antiplatelet drugs commercially available, there is a need for patient care optimization, appropriate dose individualization, and monitoring to guide drug therapy. The assessment of platelet-derived circulating miRNAs could potentially be used to these ends.

Circulating miRNAs are appealing biomarkers, but we are still far from having a definite picture of their presence and regulation. Moreover, because circulating miRNAs can be found in various blood fractions—miRNAs can be associated with microvesicles/apoptotic bodies or with blood proteins—analytical challenges hamper their accurate detection and quantification. Before miRNAs can enter clinical practice as a diagnostic/prognosis aid.

**Figure 1** Molecular mechanism of aspirin action on platelets and the presence of circulating microRNAs in the bloodstream contained in vesicles (exosomes, microparticles, microvesicles, apoptotic bodies) or associated with proteins or lipoproteins. AA, arachidonic acid; COX-1, cyclo-oxygenase 1; GP, glycoprotein; IP3, inositol-1,4,5-trisphosphate; PG, prostaglandin; TxA2, thromboxane A2; TxaS1, thromboxane A synthase 1; vWF, von Willebrand factor.
for cardiovascular disease, it will be imperative to obtain standardized and detailed expression profiles of healthy and pathological tissues while also considering the influence of drugs and their metabolism (which cell types produce the miRNAs and in what percentages, how they are released, how are they transported, which cells pick them up from the bloodstream, through which pathways are they cleared, what is their activity on target cells, etc.). Importantly, a recent Request for Application of the National Institutes of Health has stimulated the submission of grants trying to cover these unanswered questions. In addition, better understanding of miRNAs might lead to their use as therapeutic targets or as gene expression modulators for therapeutic intervention, aspects which have not been extensively tested yet in vivo. It still remains to be proven whether miR-126 is the ideal vascular injury biomarker candidate, but it indeed offers interesting insight into and novel points of view on cardiovascular disease.

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**References**