Circulating micro-RNAs as biomarkers of coronary artery disease: is it ready for primetime or still a work in progress?

Surovi Hazarika, Brian H. Annex

Division of Cardiovascular Medicine, Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, USA

Correspondence to: Brian H. Annex, MD. Division of Cardiovascular Medicine, Robert M. Berne Cardiovascular Research Center, University of Virginia, PO Box 800158, Charlottesville, VA 22908, USA. Email: Bha4n@virginia.edu.

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MicroRNAs (miRs) are small non-coding RNAs that play a significant role in epigenetic regulation of gene expression (1,2). Several miRs have been established to play crucial roles in a spectrum of different cardiovascular diseases (3,4). Since the initial identification of detectable levels of circulating miRs in plasma (5-7), circulating miRs have become the spotlight of investigation as potential biomarkers for several diseases. Micro-RNAs in circulation have two important features of ideal biomarkers: they are remarkably stable and safe from degradation by RNases, and they are easily measured in the circulation using sensitive and specific quantitative PCR techniques. Thus, circulating miRs can be linked to specific disease processes or to specific tissue and/or cell types.

In a recent study in *International Journal of Cardiology*, O Sullivan *et al*. did microRNA profiling from plasma samples from a cohort of control subjects, patients with stable coronary artery disease (CAD) and patients presenting with ST-elevation myocardial infarction (STEMI). The authors used a set of selected 22 miRs based on miRs with known role in cardiovascular biology, and identified four miRs (miR15a-5p, miR16-5p, miR93-5p and miR146a-5p) that were differentially expressed between controls subjects versus patients with stable CAD. Out of these miRs, miR 146a-5p was significantly decreased in stable CAD *vs*. control, while the rest of the miRs were found to be elevated in stable CAD compared to controls. The authors also found one miR (miR499a-5p) that was significantly elevated in patients with STEMI compared to control subjects. To adjust for traditional risk factors, the authors used a stepwise logistic regression model using all Framingham Heart Study (FHS) risk factors, and miR-93-5p remained significantly different between controls *vs*. stable CAD groups. Similarly, using a stepwise logistic regression incorporation using all FHS risk factors, the authors found miR-499a-5p was significantly elevated in patients with STEMI compared to controls. Further analysis using ROC curves showed that all four miRs that differed between control *vs*. stable CAD groups were significant predictors of stable CAD (AUCs of 0.67, 0.65, 0.68), while the miR-93-5p was found to be a better predictor based on the AUC curve of 0.75. In addition, addition of miR-93-5p to the FHS risk factors enhanced the discriminatory ability of FHS risk factors model to detect stable CAD. Similarly, the discriminatory ability of the FHS risk factors to detect STEMI was significantly enhanced with the addition of miR-499a -5p to the model.

This study by O Sullivan *et al*. provides an exciting step towards identification of potential circulating biomarkers for CAD. However, several notes of caution need to be considered. In the comparisons made by O Sullivan *et al*., the miRs were quantitated using "normalized expression" levels, adjusted to the average Cp of all expressed miRs, and therefore do not reflect absolute copy numbers. Given the prediction models were done using relative levels of miRs in plasma, it is possible that the prediction models may differ

based on the normalization method used.

An interesting finding was the value of miR93-5p expression to the traditional FHS risk factors to improve ability to detect stable CAD. This can be clinically useful and warrants further evaluation. It is interesting that the finding did not hold in control *vs*. STEMI subjects, and the significance of this remains unknown. STEMI is a diagnosis made based on clinical presentation and EKG-based criteria, and given the time constraints of definitive treatment for STEMI (8,9); the utility of a blood test is very limited, except for unusual cases where diagnosis can be confounded. Even in these situations, given the very high sensitivity and specificity of the currently available biomarkers for myocardial injury such as cardiac troponins (10), the potential clinical utility of miR-499a-5p is questionable. An ideal biomarker should provide diagnostic and prognostic information that is specific and incremental to existing clinical and demographic data, and in the context of STEMI, the findings from miR-499a-5p falls short of existing cardiac biomarkers. miR-499 is encoded by an intronic region of the myosin heavy chain gene (11). Therefore, circulating levels of miR-499 likely reflect the pathogenic process of myocardial damage. Given that the comparisons in this study were specifically made between controls and patients with STEMI, it is unclear if miR-499 is specific to STEMI, or a reflection of myocyte injury in response to any form of acute myocardial infarctions. Given similar findings of miR-499 from other studies in patients with acute myocardial infarction (12-15), miR-499 may have some clinical utility in detecting any form of myocardial infarctions at a time frame before cardiac troponins are detectable as shown by Wang *et al*. (15), or if a troponin negative, but miR-499 positive group is established to have true myocardial damage.

In selecting a panel of limited miRs with known role in cardiovascular disease, O Sullivan *et al*. used a candidate biomarker approach in this study. This approach allows for stronger statistical analysis of the selected miRs, but this limits the identification of potential unknown or novel biomarkers. In addition, the possibility that combined changes in a panel of miRs may have a better predictive ability for diagnosis of CAD over a single miR was not explored in the current study.

Finally, miRs in circulation can exist as free micro-RNAs, in exosomes or micro-particles, in protein-bound complexes, or in lipid complexes (16). In this study by O Sullivan *et al*., the authors examined the total circulating miRs, but it is prudent to consider that micro-RNAs from each of these circulating fractions may give different information compared to miRs from total circulating fraction. miRs from these fractions may provide alternate approaches to identify circulating miRs as biomarkers, and give crucial information regarding pathophysiology of a disease process.

A biomarker is a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions (17). Circulating miRs hold promise as potential biomarkers that can be indicative of these processes, but as knowledge of different circulating forms of miRs are still evolving, the identification of an ideal miR as a biomarker of CAD remains a work in progress.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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