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EDITORIAL

MicroRNA biomarkers for failing hearts?

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This editorial refers to 'Multivariate miRNA signatures as biomarkers for non-ischaemic systolic heart failure'†, by B. Vogel *et al.*, on page 2812

Vogel *et al.*[1] report findings from a case–control study looking at microRNA (miRNA) signatures of heart failure (HF). Initial screening was performed on a microarray platform: 883 miRNAs and miRNA* sequences were measured in 53 patients with diagnosis of non-ischaemic systolic HF with reduced left ventricular ejection fraction (LVEF <50%) and 39 controls. Importantly, cases and controls were free of significant coronary artery disease. The best performance was observed for miR-558, miR-122*, and miR-520d-5p. A combination of eight miRNAs further improved test sensitivity and specificity, achieving an area under the curve (AUC) of 0.81. MiRNA measurements were compared with N-terminal pro brain natriuretic peptide (NT-proBNP), an established biomarker for HF. Validation was performed in an independent but even smaller cohort of 14 non-ischaemic HF patients and 8 controls by quantitative real-time polymerase chain reaction (qPCR). Two out of nine biomarker candidates identified in the screening cohort achieved statistical significance in the validation cohort. Consistent with a previous report by Tijsen *et al.*,[2] miR-622 was elevated in HF. MiR-1228 has not been implicated in HF so far. Several small-scale studies have looked at association of miRNAs and HF in serum or plasma.[2–5] The present study stands out for providing miRNA data in full blood and for providing correlations of miRNAs with white blood cells.

Micro RNAs in full blood

The cellular origin of circulating miRNAs is still unknown.[6] Most of the miRNAs in full blood will undoubtedly originate from circulating cells.[7] Thus, the authors assessed the expression profiles of selected miRNAs in different white blood cells. For example, one of the lead candidate biomarkers, miR-519e*, was not detected in serum, and higher expression was observed in granulocytes, providing a possible link to inflammatory mechanisms in circulating leucocytes rather than the biology of the failing myocardium *per se*. Leucocytes, however, are not the only contributor of miRNAs in full blood. Anucleated cells, such as erythrocytes and platelets, harbour a broad repertoire of miRNAs.[6,7] While serum and platelet-poor plasma show good overlap in their miRNA content, platelet-rich plasma has ~50% more miRNAs than serum and platelet-poor plasma combined.[7] Thus, cell counts, including erythrocytes and platelets, should be taken into consideration when interpreting miRNA changes in full blood (*Figure 1*). Future studies will need to address to what extent different cells contribute to circulating miRNAs.

Cases and controls

There was no formal matching of cases and controls, resulting in unequal distributions of some relevant clinical variables. The HF patients were predominantly male, whereas genders were more equally distributed in the control group. Moreover, there was a significant difference in LDL levels in the validation cohort. A trend towards lower LDL ($P = 0.14$) was also observed in the screening cohort, most probably due to statin medication. The effect of statins on miRNA levels in full blood is currently unknown. Recent interventional studies demonstrated that medication can have profound effects, i.e. antiplatelet therapy reduces plasma miRNA levels due to an inhibition of platelet microparticle shedding.[8,9] If this were the case, then the effect of antiplatelet therapy on miRNAs in full blood may be less pronounced. On the other hand, all blood samples were obtained during coronary angiography. Heparin is commonly administered during coronary angiography and interferes with qPCRs, including measurements of miRNAs.[10,11] miRNA biomarkers are a rapidly emerging field, and many confounding factors are just about to be recognized. The design of future case–control studies has to address effects of medication in HF patients.

Study size

The relatively small size of the screening cohort is explained by the high costs of measuring 883 miRNAs and miRNA*sequences. Microarray-based high-throughput platforms have been used extensively for miRNA profiling. A variety of technologies have been developed,[12] and the microfluidic primer elongation arrays

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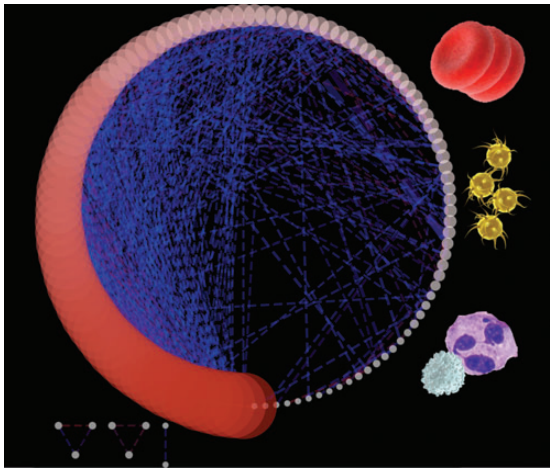

Figure 1 Cellular origin of circulating microRNAs (miRNAs). Circulating miRNAs are represented as nodes, with the size and redness of the circle corresponding to the centrality of each miRNA, i.e. its number of connections to other miRNAs. Relationships between miRNAs are shown as blue and red lines (low and high correlation, respectively). Although the miRNA content of anucleate cells such as erythrocytes and platelets is low compared with other cells, erythrocytes and platelets represent the two most abundant cell types in blood and are a major contributor to the miRNA pool.

used in the present study are particularly sensitive in assessing miRNA expression in low concentration RNA samples without pre-amplification. Nevertheless, as in all microarray data sets, besides quality control of the microarray experiment, same sample validation and confirmation of the array data is required[13] as discrepancies are known to occur.[1] Measurements by qPCR, the gold standard for miRNA measurements, were only performed in the validation cohort consisting of 14 HF patients and 8 controls. In the screening cohort, significant separation for event-free survival was obtained for miR-591e*, but this finding is preliminary given the low number of events (10 events in 43 patients).

Conclusions

Circulating miRNAs have emerged as novel biomarkers and potential signalling mediators of cardiovascular disease.[14,15] The study by Vogel *et al.*[1] should not be viewed as definitive but rather exploratory. It raises the bar for the number of miRNAs and miRNA* sequences that were assessed in patients with HF. The miRNA* sequences are attracting increasing interest as they are not just degraded but may also have functional activity.

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Conflict of interest: The authors filed patent applications on miRNAs as cardiovascular biomarkers.

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