# Micro-RNAs as promising biomarkers in cardiac diseases

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## miRNA, emerging and promising biomarkers in cardiology

miRNAs are endogenous, small (21-22 nucleotides), single-stranded, non-coding RNAs recently discovered (1). miRNAs complexed with Ago proteins (RISC complex) regulate gene expression by binding to reverse complementary sequences in their target mRNAs leading to mRNA degradation and/or repression of protein translation (2). Circulating miRNAs can be detected in serum or plasma and have been proposed as potential biomarkers for cardiovascular diseases, with high sensitivity (3).

Indeed, they are involved in multiple cellular functions (proliferation, migration, differentiation…) (4) and are therefore involved in cardiac and vascular development. A dysregulation in their expression has been suggested to be responsible for cardiovascular disease. Major implications were demonstrated in several pathologies as congenital cardiac disease (5), hypertrophy, fibrosis, arrhythmias and atherosclerosis (6).

Expression of miR-378 dramatically decreased as well as expression of miR-133 and miR-1 in mouse models of heart failure and in human tissues (7,8). Concerning cardiac fibrosis, several miRNAs were incriminated as miR-133, miR-21 and miR-29, involved in fibroblasts proliferation, collagen synthesis and Connective tissue growth factor (CTGF) signaling (9). In a rat model, miR-433 was consistently elevated in three various models of heart disease with prominent cardiac fibrosis (10).

The implication of miRNAs in heart failure was also demonstrated, particularly miR-25 whose increased expression can depress cardiac function (11). Interestingly, miRNAs secreted by cardiac fibroblasts act as paracrine mediators of cardiomyocyte hypertrophy (12).

Thereby, miRNAs appear as attractive biomarkers in cardiovascular field, easily assessed, with a robust stability in the plasma and an excellent sensitivity (13). A potential combination of several miRNAs can be considered to improve diagnostic performance.

However, few miRNA are tissue-specific and their plasma level can change depending on the physiological or the pathological situation, independently from the initial disease. Above all, there is a very large number of detectable miRNAs and only a few is likely to provide additional information compared to current validated biomarkers. Their specific interest in clinical settings both for diagnostic, prognostic and even therapeutic approaches remain largely under investigation.

## miRNA, promising in myocardial infarction as prognostic, diagnostic and therapeutic tools

In acute myocardial infarction, a rapid diagnostic is necessary to allow an immediate management of patients

**Table 1** Opened studies ongoing regarding diagnostic, prognostic or therapeutic effects of miRNA in myocardial infarction

| Country | Objectives | Primary endpoint | Number of patient | Estimated primary completion date | NCT |
|---|---|---|---|---|---|
| France | (I) To study how the macrophage functions after MI are changed by diabetes<br>(II) To determine the potential role of miRNAs contained in secreted MVs in the transition M1/M2 after MI | Level of expression markers | 20 | 2018 | NCT02768935 |
| China | To test the expression of microRNAs related to the syndromes after the intervention of Tongguan capsule | miRNAs spectrum | 100 | 2017 | NCT02850627 |
| Spain | To evaluate the prognostic value of circulating miRNAs in patients admitted for STEMI complicated with cardiogenic shock | Mortality | 142 | 2017 | NCT02691286 |
| Germany | To develop a biomarker protocol that combines the high sensitivity of cardiac Troponin T and the high specificity of mi-RNA profiles for early and safe identification of non-STEMI in ED patients | Mortality | Unknown | Unknown | NCT02116153 |
| China | To determine whether the expression level of miR-320a are effective as biomarker in evaluating the diagnosis, prognosis and treatment effects of coronary heart disease | Plasma expression of miR-320a in coronary heart disease patients | 400 | 2018 | NCT02751060 |

and ensure a better prognosis, limiting long-term consequences as remodelling and fibrosis leading to chronic heart failure.

Several miRNAs were identified as promising candidates to early detect patients with MI. Among them, miR-1, miR-133a, miR-133b, miR-208a, miR-499, miR-499-5p were advocated but further validation is required (14). A large study evaluated the level of six miRNAs in 1155 patients admitted for acute chest pain. In the 224 patients diagnosed with MI, the levels of miR-208b, miR-499 and miR-320a were significantly higher (15). Thereby, miRNAs as early diagnostic biomarkers in MI seems promising but have to be identified more precisely.

In the context of MI, a potential role of miRNAs as prognostic biomarkers was also raised.

Devaux *et al*. (15) reported that miR-208b predicted survival at 30 days but none of the miRNAs could predict long-term mortality. In a study including 407 patients with a suspected MI, miR-208b and miR-499-5p were identified as potential diagnostic biomarkers of MI with an area under the curve (AUC) around 0.8 and were also predictive factors for outcomes with a prognostic value comparable to cTnT (16).

Taken all these considerations, miRNAs provide promising therapeutic targets. In a pig model of reperfused MI, an intracoronary injection of an anti-miR-92a stimulates angiogenesis and could prevent cardiac remodelling (17). Recently, Gupta *et al*. demonstrated that miR-22, a key regulator of cardiac autophagy, could be an interesting target after myocardial infarction (18). Indeed, they show that miR-22 inhibition post-infarction improved cardiac function and inhibited cardiac remodelling in older mice but not young mice. Thereby, pharmacological inhibition of miR-22 could be promising, especially in older myocardium.

Opened ongoing studies regarding diagnostic, prognostic or therapeutic effects of miRNA in myocardial infarction are presented *Table 1*.

In this paper entitled "*Circulating miR-221-3p as a novel marker for early prediction of acute myocardial infarction*", Coskunpinar *et al*. aimed to identify potential miRNAs to predict early myocardial infarction (MI).

They set 3 objectives to answer:

(I) To compare the serum expression levels of miRNAs 1/in patients with AMI and control subjects with an acute atypical chest pain/dyspnoea and 2/in patients with STEMI and non- STEMI;

(II) To evaluate the potential of these miRNAs to be used as novel diagnostic biomarkers for AMI in patients admitted to emergency department for acute chest pain and/or dyspnoea;

 

(III) To investigate the relations between the serum levels of miRNAs with the serum levels of previously validated biomarkers, namely troponin I, cardiac risk scores and post-MI left ventricular functions.

In this study, 43 consecutive patients were included, all of them presented acute chest pain and/or dyspnoea, 27 were diagnosed acute myocardial infarction (AMI) and 16 were control subjects. The control subjects presented the same symptoms but without any diagnostic criteria for AMI, particularly no ECG modification and no rise of cardiac enzymes. The blood assessment was performed in all subjects within 4 h of onset of clinical symptoms and miRNAs expression levels were evaluated. Patients with AMI were compared to control patients and within the AMI group, STEMI patients were compared to NSTEMI patients.

The AMI group and control were comparable for baseline characteristics except for left ventricular function. Body mass index (BMI) was the only different baseline characteristics between the STEMI group and NSTEMI group, higher in the NSTEMI group (P=0.026).

The study highlighted three major points:

(I) Nine miRNAs were expressed differently between the control group and the AMI group, without any difference between STEMI and NSTEMI subgroups. Six of these miRNAs were upregulated while the other three were downregulated in patients with AMI. Moreover, the authors highlighted 2 miRNAs which were the most upregulated in the AMI group: miR-4290 and miR-221-3p with a fold regulation of 7.39 and 3.89 respectively. The most downregulated miRNA in this group was the miR-19b-1-5p with a fold regulation of –3.15;

(II) miR221 was significantly positively correlated with Troponin, GRACE and SYNTAX Score while significantly inversely correlated with left ventricular ejection fraction;

(III) miR-221-3p had the better discriminative value for the diagnosis of AMI with a ROC area under curve (AUC) of the level of 0.881 (95% CI: 0.774–0.987; P=0.002), close to AUC for Troponin (AUC: 0.954; 95% CI: 0.892–1.000; P=0.001).

These results are consistent with the upregulation of miR-221-3p in patients with atherosclerosis.

Here, the authors add a practical prognostic information of miRNAs demonstrating an association between the expression levels of mi-RNAs (miR-648, miR-4290, miR-3914, miR-221-3p, miR-127-5p) and cardiac scores as well as cardiac function assessed by echocardiography, in patients with AMI. Among these miRNAs, miR-221-3p had a high discriminative value and significant relations with Troponin, GRACE and SYNTAX score and left ventricular systolic function. Likewise, this biomarker may be useful in daily practice for early prediction of AMI and could provide a prognosis value in this context.

From a pathophysiological point-of-view, miR-221-3p is suggested to facilitate the development of vulnerable coronary plaques, coronary artery atherosclerosis and severe endothelial dysfunction by using molecular pathway such as Netrin/DCC induced pathway.

This study presents three main limitations: first, a very small number of patients were included. Secondarily, miRNAs expression levels were measured only once, no time-course or assessment after treatment to evaluate its effect are available. Importantly, the quantification of miRNA expression in different independent study cohorts was not performed and this will be a crucial step for further clinical development. Finally, it is striking that altogether the studies performed worldwide on plasma miRNA identification post AMI present a relatively poor overlap in the subset of differentially regulated miRNAs. This suggests potential methodological biases in the timing and handling of samples and quantification methods that will require to be solved in the future for efficient and reliable use of miRNAs detection in clinics. Alternatively, but not exclusively to the potential methodological biases, one can hypothesize that these differences in candidate miRNAs for AMI arise from different genetic backgrounds and environmental causes, which will generate a very interesting line of research for personalized medicine, a much awaited promise for the future in clinics.

Nevertheless, results presented in this study are innovative. The authors highlighted the miR-221-3p as a potential biomarker not only for early diagnostic of AMI but also for prognostic evaluation after AMI (because of its association with post-MI left ventricular systolic function). Further investigations are necessary to make clear the links between miRNAs products and pathophysiological mechanisms. Furthermore, larger clinical studies have to confirm the early predictive and the prognostic values of miR-221-3p in the context of MI in the aim to develop adapted therapeutic strategies. Finally, a key point for clinical development in this field of research will be to determine whether any of the identified miRNA can present at least an as high discriminating ROC value than

 

the validated Troponin I biomarker for AMI diagnostic.

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## Footnote

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

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