NOVEL IMMUNOASSAY APPROACH TO INVESTIGATE microRNA BIOMARKERS IN ACUTE MYOCARDIAL INFARCTION

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Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target mRNAs. They are involved in a wide range of biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation, and also in pathophysiology of many diseases. Numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of CVD. Monitoring the level of specific miRNA together with protein-based biomarkers may represent an efficient tool for diagnosis of CVD and prognosis estimation.

miR-1 is highly expressed in the cardiovascular system. Recent studies have revealed that its expression is dysregulated in heart under cardiovascular disease conditions such as proliferative vascular disease, cardiac hypertrophy and heart failure or ischemic heart disease. miR-126 and miR-223 are involved in endovascular inflammation and platelet activation and have been described as biomarkers in the diagnosis of coronary artery disease.

The aim of our study was to investigate potential predictive value of selected miRNAs. A cohort of 140 samples of patients with acute myocardial infarction (AMI) and 100 samples of healthy persons were obtained from the PRAGUE-18 study.

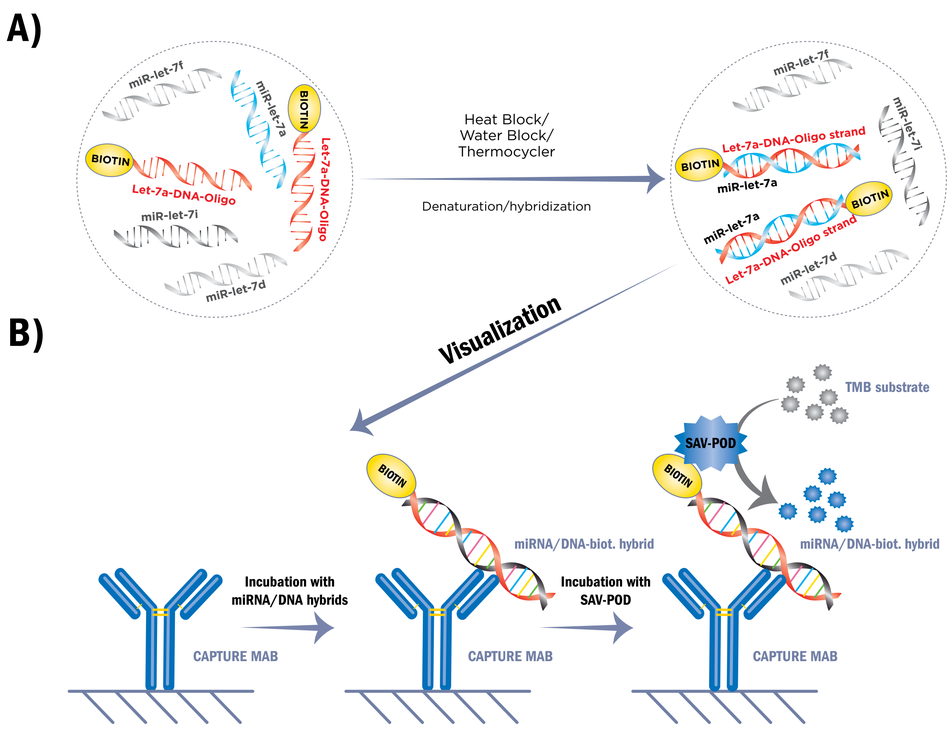
Method

Recently, we have introduced miREIA, a novel method for quantification of miRNA based on enzyme immunoassay format. The novel approach involves hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/RNA hybrids are then transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. After washing, the solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized (after another washing step) by a chromogenic substrate.
Four miREIAs were developed to quantify miRNAs (miR-1-3p,miR-126-3p,miR-223-3p and cel-miR-39-3p) isolated from the whole blood. Typical analytical characteristics (miR-223-3p assay) were: LOD = 0.13 amol/µl, intraassay CV 8 %, dilution recovery 102 %, spiking recovery 98 %.

Conclusion

- We are introducing a novel method for absolute quantification of microRNA in clinical samples without reverse transcription and amplification steps.
- miREIA method exhibits excellent analytical characteristics and strong correlation with qRT-PCR method
- This method is bringing an opportunity to analyze miRNA using conventional available immunoassay equipment and thus speed-up utilization of miRNA biomarkers in clinical and laboratory practice.

miRNA quantification

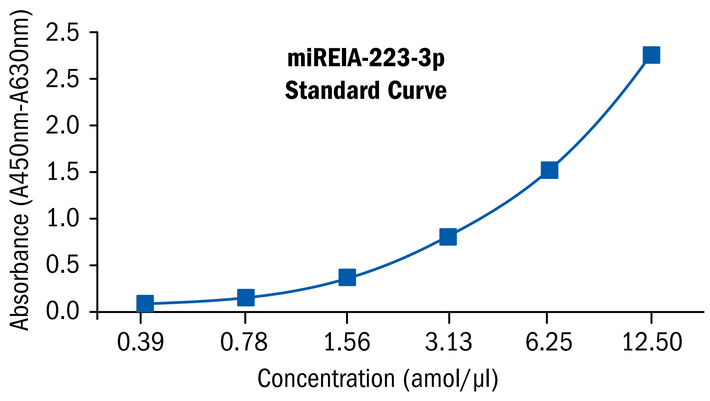


Hybridization of miRNA to complementary biotinylated DNA(A).
Defined amount of specific biotin-labeled DNA oligonucleotide is hybridized to miRNA isolated from blood sample.

miREIA – miRNA enzyme immunoassay (B)
The hybridization mixture is transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched RNA/DNA-biotin hybrids. In the next step, the solid phase is washed and subsequently incubated with streptavidin-HRP conjugate. Finally, the resulting complexes are visualized by chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB).

miREIA-223-3p

C) Calibration range



The calibration range of the miREIA-223-3p assay is 0.39-12.5 amol/ul.

D) Dilution linearity

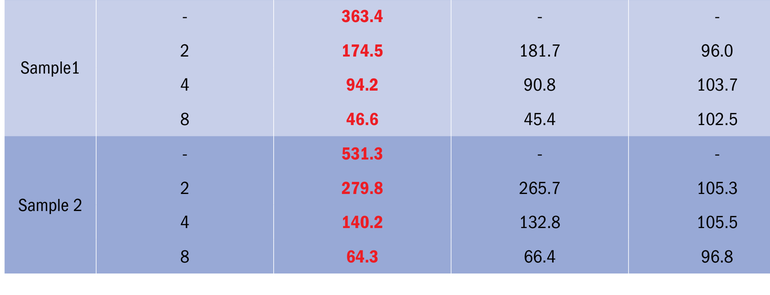
Two microRNA isolates from whole blood samples were serially diluted.

Sample	Sample dilution	Observed concentration amol/µl	Expected concentration amol/µl	Recovery O/E (%)
Sample1	-	363.4	-	-
	2	174.5	181.7	96.0
	4	94.2	90.8	103.7
	8	46.6	45.4	102.5
Sample 2	-	531.3	-	-
	2	279.8	265.7	105.3
	4	140.2	132.8	105.5
	8	64.3	66.4	96.8

Recovery was determined to be 96– 106%

E) Comparison of methods – miREIA versus qRT-PCR (E)

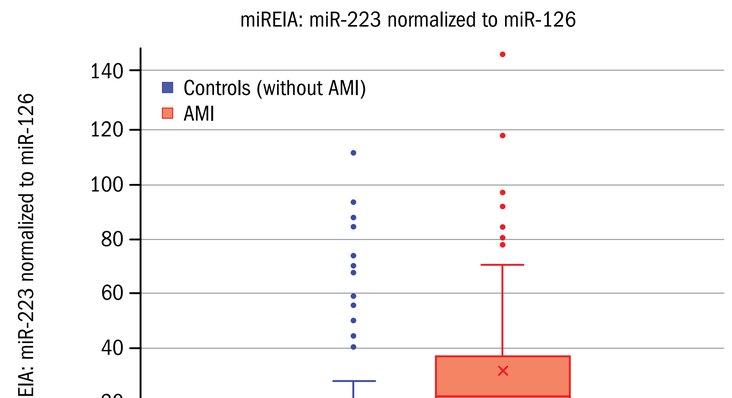
Synthetic non-human cel-miR-39-3p was added to 139 whole blood samples during the process of RNA isolation. After RNA isolation, concentration of exogenous cel-miR-39-3p was measured using immunoassay cel-miR-39-3p miREIA (BioVendor) and qRT-PCR (TaqMan Advanced miRNA Assay, ThermoFisher).

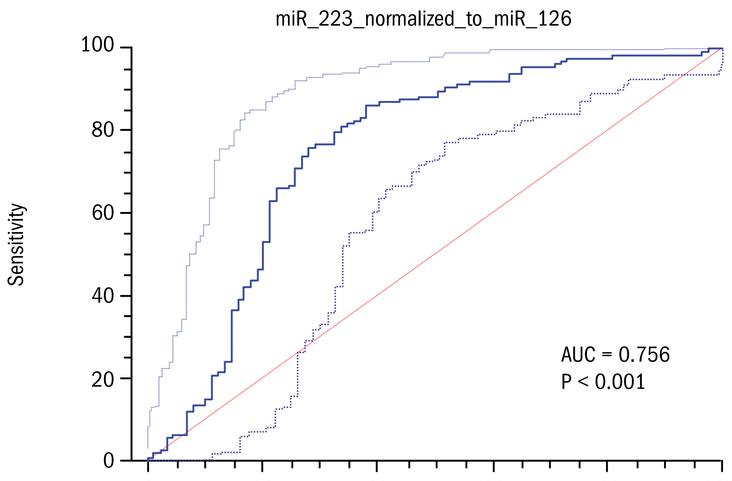


We found a strong correlation between concentration of cel-miR-39-3p detected by miREIA and qRT-PCR, the Pearson correlation coefficient being >0.68.

F) Measurement of miR-223 in patient with AMI and Control group (F)

We isolated RNA from 89 whole blood samples of subjects without acute myocardial infarction and 139 whole blood samples of patients with acute myocardial infarction. We measured concentration of miR-223-3p using miREIA kit. Observed concentrations of miR-223-3p were normalized by miR-126-3p. Normalized concentration of miR-223-3p in subjects with AMI was significantly elevated when compared with Controls (p<0,000001).





For evaluation of diagnostic potential of normalized miR-223-3p, we constructed the receiver operating characteristic (ROC) curves. The ROC curves reflected strong separation between groups with and without acute myocardial infarction, with an AUC 0.76, a sensitivity of 76.3 and specificity of 71.9.

References:

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