Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

miR-142 is a Sensitive Biomarker for the Diagnosis and Prognosis of Acute Myocardial Infarction

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SUMMARY

Background: **Acute myocardial infarction (AMI) is myocardial necrosis caused by acute and persistent ischemia and hypoxia of coronary arteries. AMI is one of the most common diseases in European countries and over 1.5 million AMI patients die of it in the United States annually. A collection of studies proposed that certain microRNAs play crucial roles in the onset and development of AMI.**
Methods: **Ninety-four AMI patients and 83 non-AMI healthy controls were recruited from Zhongda Hospital, Southeast University between July 2015 and September 2017. Serum samples were collected at admission and the expression of miR-142 was detected using real-time quantitative polymerase chain reaction (RT-qPCR) assays.**
Results: **miR-142 expression was markedly elevated in serum samples of AMI patients compared with the 83 non-AMI healthy controls. miR-142 expression was positively correlated with creatine kinase-KB (CK-MB; r = 0.6731, p = 0.0021) and troponin (r = 0.7138, p = 0.0013). The area under the curve (AUC) of miR-142, CK-MB, and troponin for the diagnosis of AMI were 0.9185, 0.8172, and 0.8717, respectively. Overall survival analysis implied that high miR-142 expression may predict poor survival (log-rank test, p = 0.0146).**
Conclusions: **miR-142 may be a diagnostic and prognostic indicator for AMI, and therefore, it may contribute to AMI clinicopathologic prediction.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.191019)

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Manuscript accepted November 3, 2019

KEY WORDS

acute myocardial infarction, miR-142, diagnosis, prognosis, biomarker

INTRODUCTION

Acute myocardial infarction (AMI) is one of the most common and lethal cardiovascular diseases worldwide [1]. In China, AMI-related deaths increased 5.6-fold in 2014 compared to that in 1987 [2]. Accurate and early diagnosis and intervention are important for clinical outcome and prognosis, which will be improved by identifying high-sensitive indicators. To date, conventional and reliable indicators such as creatine kinase-MB (CK-MB) and troponins have been widely used for early diagnosis [3]. However, the accuracy and sensitivity of them remain to be improved. Hence, it is urgent to seek novel and multiple biomarkers in order to improve sensitivity, specificity, and accuracy in the diagnosis

and prognosis.

MicroRNAs (miRNAs) are a group of regulatory small and non-coding RNAs which may contribute to various cellular processes, including cell proliferation, apoptosis, invasion, migration, differentiation, and stress response [4-6]. In the cardiovascular system, a number of miRNAs play crucial roles in cardiovascular pathogenesis and development, such as hypertension, coronary heart disease, and acute myocardial infarction [7-9]. Recent studies have indicated miR-142 may function as a potential biomarker in cardiovascular diseases [10,11]. However, the diagnosis and prognosis values of miR-142 in acute myocardial infarction have not been elucidated yet.

The aim of the present study was to investigate the role of miR-142 in the diagnosis and prognosis of AMI.

MATERIALS AND METHODS

Patients

Ninety-four AMI patients and 83 non-AMI healthy volunteers were recruited between July 2015 and September 2017 at Zhongda Hospital, Southeast University. The clinicopathological features were shown in Table 1. Fasting venous blood samples were collected into tubes from AMI and healthy volunteers at admission. After being centrifuged at 3,000 rpm at 4°C for 10 minutes, the isolated supernatant was then centrifuged at 12,000 rpm at 4°C for another 10 minutes. The processed serum samples were stored at -80°C before analysis.

The present study was approved by the Ethics Committee of Zhongda Hospital, Southeast University and written consent was obtained from each participant before the experiments.

Real-time quantitative polymerase chain reaction (RT-qPCR) assay.

Total RNA was extracted from serum samples of AMI and non-AMI patients using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., USA) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using miScript reverse transcription kit (Applied Biosystem; Thermo Fisher Scientific, Inc., USA) and the expression of miR-142 was determined by SYBR Premis Ex Taq (Takara, Japan) following the instructions using a 7300 Real-Time PCR System (Applied Biosystem; Thermo Fisher Scientific, Inc., USA). The thermal cycling conditions were described as follows: initial denaturation at 94°C for 30 seconds, 40 cycles of 94°C for 5 seconds, and 62°C for 10 seconds. U6 functioned as internal control. The expression levels of miR-142 were normalized to the level of U6 and the fold change of miR-142 level was calculated using the $2^{-\Delta\Delta Ct}$ method. All the procedures above were conducted in triplicate. The primer sequences were as follows: miR-142-5p forward 5'-TGCGGTGTAGTGTTTCCTACTT-3' and reverse, 5'-CCAGTGCAGGGTCCGAGGT-3'; U6 forward 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (IBM, USA). All data were presented as mean ± standard deviation (SD). Student's *t*-test was used to distinguish miR-142 expressions in AMI and non-AMI groups. Spearman's correlation analysis was used to analyze the correlations between miR-142 and CK-MB and troponin. Receiver operating characteristic (ROC) curve assays were used to determine the diagnostic value of miR-142, CK-MB, and troponin in AMI. Survival curve analysis was carried out using Kaplan-Meier and analyzed with long-rank test in order to elucidate the prognostic value of miR-142 in AMI. A level of $p < 0.05$ was considered to be statistically significant.

RESULTS

miR-142 was markedly elevated in AMI patients relative to healthy controls

miR-142 expressions in 94 AMI patients and 83 non-AMI healthy controls were measured using RT-qPCR assay. Compared with non-AMI healthy controls, miR-142 levels were significantly higher in serum samples of AMI patients with a fold change at about 3.94 (Figure 1, $p < 0.01$).

Correlations with miR-142 with CK-MB and troponin

In order to further evaluate the diagnostic value of miR-142 in AMI, we analyzed whether the expressions of miR-142 were correlated with the known diagnostic parameters CK-MB and troponin. As demonstrated in Figure 2A and 2B, the level of miR-142 was positively related to CK-MB ($r = 0.6731$, $p = 0.0021$) and troponin ($r = 0.7138$, $p = 0.0013$).

The diagnostic accuracy of miR-142 in AMI

To determine the diagnostic accuracy of miR-142, receiver operating characteristic curve (ROC) analysis was performed on data from 94 AMI patients and 83 non-AMI healthy controls. The ROC curve of miR-142 had an area under curve (AUC) of 0.9185 compared with CK-MB with an AUC of 0.8172, and troponin with an AUC of 0.8717 (Figure 3). These results suggested that miR-142 may function as a diagnostic biomarker for AMI, and its accuracy was more sensitive than CK-MB and troponin.

miR-142 was a crucial biomarker for AMI prognosis

As demonstrated in Figure 4, AMI patients who had high miR-142 showed shorter survival times than those with low expression ($p = 0.0146$).

Table 1. Clinicopathological features of all the participants.

Features	AMI (n = 94)	Non-AMI (n = 83)	p
Age (years)	66.5 ± 11.2	67.5 ± 9.6	0.53
Male/Female (%)	51.61	50.91	0.92
Diabetes (%)	41.49	43.37	0.77
Hypertension (%)	52.13	49.40	0.68
Hyperlipemia (%)	26.60	25.30	0.82
Smoking (%)	28.72	9.64	0.00
TC (mg/dL)	4.12 ± 0.10	3.67 ± 0.08	0.00
TG (mg/dL)	1.61 ± 0.08	1.65 ± 0.11	0.00
HDL (mmol/L)	40.81 ± 11.47	36.83 ± 12.31	0.03
LDL (mmol/L)	99.50 ± 34.22	103.62 ± 29.15	0.41
CK-MB (ng/mL)	16.97 ± 4.01	12.53 ± 3.18	0.00
Troponin (ng/mL)	17.24 ± 3.25	11.13 ± 3.15	0.00
miR-142 (fold)	3.94 ± 1.48	1.36 ± 0.52	0.00

CK-MB - creatine kinase MB.

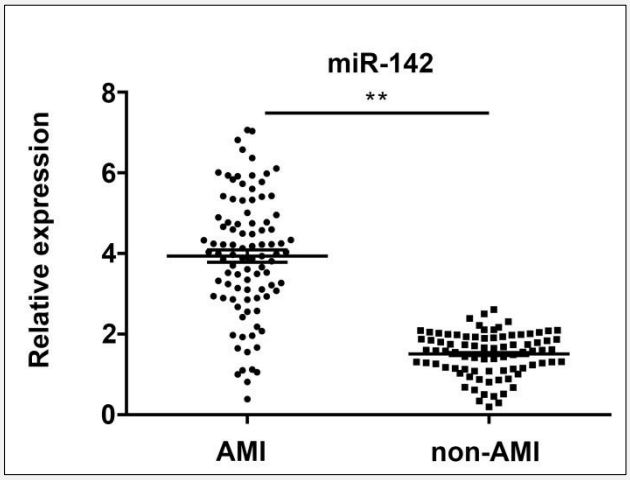

Figure 1. The expressions of miR-142 in serum samples of AMI and non-AMI healthy controls.

**** - p < 0.01, AMI vs. non-AMI. miR-142, microRNA-142. AMI - acute myocardial infarction, non-AMI - non-AMI healthy controls.**

DISCUSSION

To date, biomarkers have crucial functions in early diagnosis and prognosis in cardiac diseases including AMI [12]. As known, CK-MB and troponin are established sensitive diagnostic indicators for AMI [13]. However, these conventional biomarkers may either lack sensitivity or may not have sufficient specificity which reduces the accuracy of diagnosis. Hence, a multiple biomarker strategy should be established and new biomarkers should be identified in order to improve shortcomings and add accuracy concerning early diagnosis.

Recently, it was reported that miRNAs are sensitive and

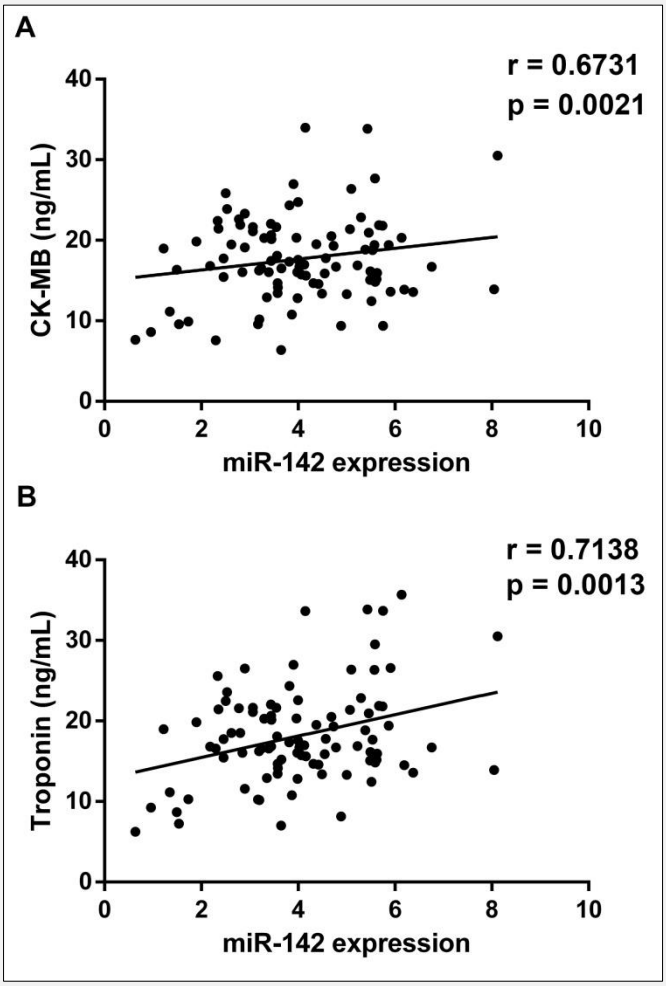

Figure 2. Correlation analysis of miR-142 with CK-MB (A) and troponin (B).

miR-142, microRNA-142; CK-MB, creatine kinase-MB; AMI, acute myocardial infarction.

potential biomarkers for early diagnosis or prognosis of cardiovascular diseases, including AMI [14,15]. Meanwhile, numerous studies pointed out that dysregulation of certain miRNAs in tissues were related to the progression and development cardiovascular diseases [16, 17]. In the AS realm, some miRNAs such as miR-208, miR-499, and miR-133 were found up-regulated in AMI using microarray or qRT-PCR analysis; whereas, miR-150 and miR-126 were found down-regulated [18]. Furthermore, certain dysregulated miRNAs were elucidated to be involved in the diagnosis or prognosis of AMI as well [19,20]. In the present study, the results demonstrated that miR-142 expression was markedly increased in serum samples from AMI patients as compared with healthy volunteers.

Conventional biomarkers for AMI diagnosis include CK-MB and troponin. Hence, the sensitivity and specificity of miR-142 was compared with these routine biomarkers. To determine the correlation between miR-142 and CK-MB and troponin, we measured the expressions of miR-142, CK-MB, and troponin at admission. Notably, the expression of miR-142 was positively correlated with CK-MB and troponin. Afterward, the ROC analysis revealed the AUC of 0.9185, 0.8172, and 0.8717, respectively, for miR-142, CK-MB, and troponin. The ROC results demonstrated that miR-142 may have high-sensitivity and accuracy concerning AMI diagnosis compared with traditional indicators. Finally, the Kaplan-Meier survival analysis revealed that AMI patients with high miR-142 expression had poorer prognosis compared with those who had lower miR-142 expression.

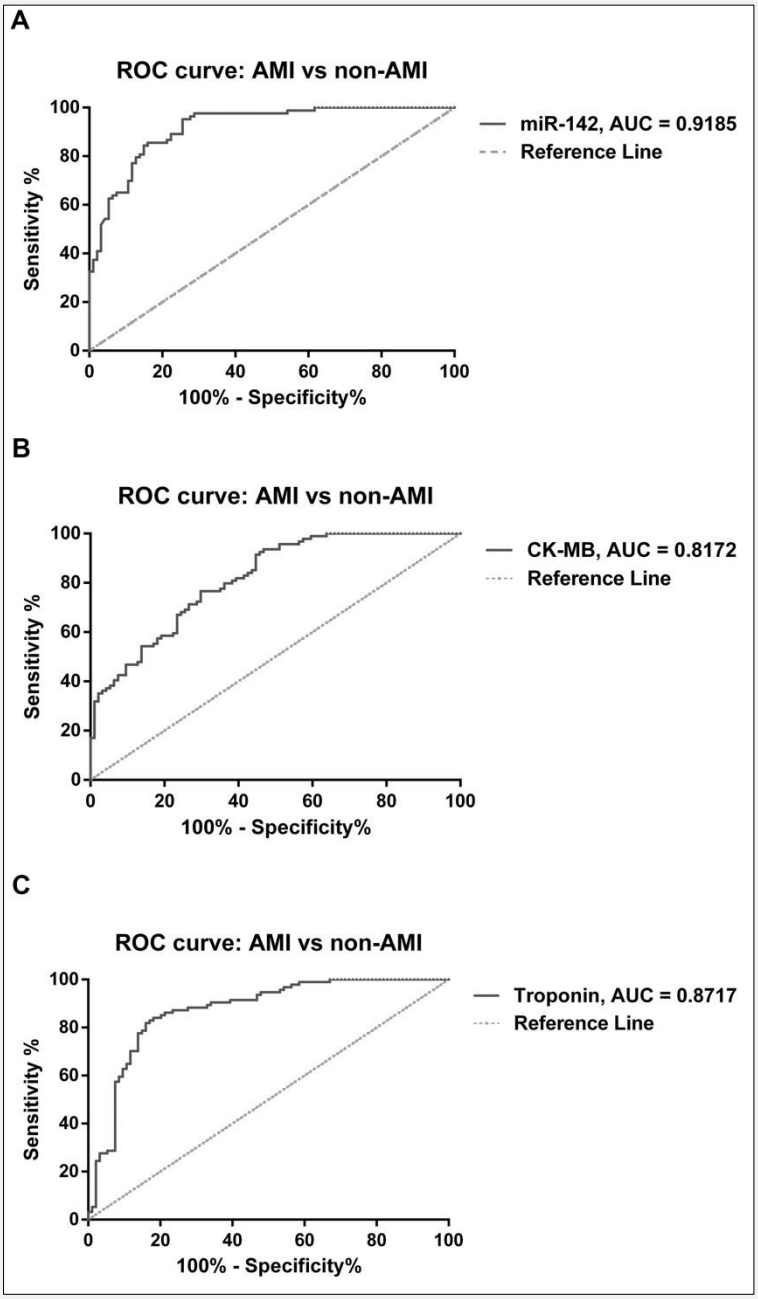

Figure 3. ROC curve analysis for miR-142, CK-MB and troponin discriminate AMI from non-AMI.

ROC, receiver operating characteristic; miR-142, microRNA-142; CK-MB, creatine kinase-MB; AMI, acute myocardial infarction; non-AMI, non-AMI healthy controls.

CONCLUSION

Our study confirms that miR-142 may function as a candidate biomarker for early diagnosis and prognosis for AMI.

Source of Funding:
The National Nature Science Foundation of the People's Republic of China (Grant No. 81600227 for GY).

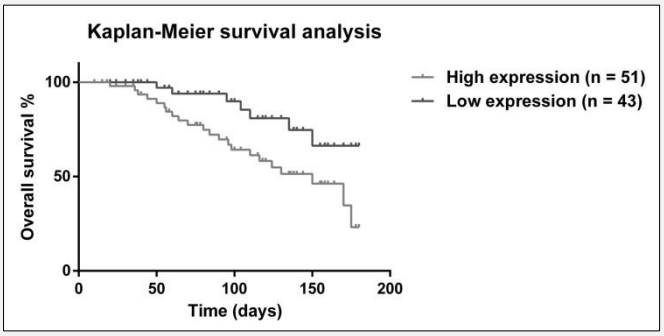

Figure 4. Kaplan-Meier curve of 94 patients with AMI, based on miR-142 level at admission (long-rank tests).

miR-142, microRNA-142; AMI, acute myocardial infarction.

Declaration of Interest:

The authors report no competing financial interests.

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