Serum Circulating miR-150 is a Predictor of Post-Acute Myocardial Infarction Heart Failure

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Summary
Patients with ischemic heart disease are associated with poor prognosis, and their number has increased globally. Therefore, biomarkers that could predict post-acute myocardial infarction (AMI) heart failure (HF) would be helpful to guide appropriate treatment. Based on the diagnosis on admission and results of echocardiogram performed on admission and 1 year after discharge, the current study recruited 54 patients with post-AMI HF, 59 patients with post-AMI non-HF, and 59 healthy controls. Eight candidate microRNAs (miRs) were screened through real-time quantitative PCR. Serum circulating miR-150 level in the post-AMI HF group was significantly lower than the post-AMI non-HF group (0.4 ± 0.3 versus 0.7 ± 0.3, P < 0.001). Further analysis showed that serum circulating miR-150 level was associated with ejection fraction (EF) 1 year after discharge (P < 0.001). Receiver operating characteristic curve (ROC) analysis found that area under the ROC (AUC) was 0.616 (95%CI = 0.511-0.721, P = 0.034) when BNP was used to predict post-AMI HF, whereas AUC improved to 0.764 (95%CI = 0.674-0.855, P < 0.001) when miR-150 was used. The combination of BNP and miR-150 significantly improved the AUC to 0.807 (95%CI = 0.727-0.886, P < 0.001). Finally, multivariate logistic regression analysis revealed that either LVEF on admission or serum circulating miR-150 level was independently associated with post-AMI HF. Serum circulating miR-150 is a novel biomarker to predict post-AMI HF. Further large sample prospective clinical research is needed to validate its role in the future.

Key words: Myocardial biomarker, Ischemic heart disease, Prognosis

Coronary artery disease (CAD) is the primary cause of morbidity and mortality in cardiovascular disease, and acute myocardial infarction (AMI) is the most critical condition observed in patients with CAD. The treatment of myocardial infarction has recently demonstrated rapid progress; for example, the use of percutaneous coronary intervention (PCI) and evidence-based medications have greatly reduced the AMI morbidity rate. On the other side, although patients with AMI successfully put through the acute phrase, several of these cases develop into chronic ischemic heart disease. Currently, the number of ischemic heart disease patients has surged globally, leading to a huge social and economic burden. Hence, it is urgent to look for biomarkers that are associated with the development of post-AMI heart failure (HF), facilitating the on-time treatment decision making.

Recent studies have found that various types of cells can release microRNAs (miRs) that are stable in the serum, namely circulating miRs. Serum circulating miRs were shown to be potential biomarkers in cardiovascular diseases. Serum circulating miR-133a and miR-208a levels altered significantly during myocardial infarction (MI) acute phase, and thus, became potential biomarkers of AMI diagnosis and post-AMI prognosis prediction. Serum p53 responsive miRs levels, including miR-194, and miR-34a, elevated more dramatically in the early convalescent stage post-AMI that later led to HF, suggesting these miRNAs were reliable predictors of post-AMI HF. However, they were not validated in other AMI cohorts with different ethnic and genetic background. In our current study, MI-related serum circulating miRs (miR-29a, miR-133a, miR-208b, miR-499, miR-150, miR-194, miR-192, and miR-34a) were compared between patients with post-AMI HF and those with post-AMI non-HF through real-time quantitative PCR to investigate their predictive role of post-AMI HF.

Methods

Study subjects: The study protocol was approved by Institutional Review Board at the Second Affiliated Hospital,

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Zhejiang University School of Medicine. Written informed consent was obtained from all participants. Patients with AMI were recruited according to the third universal definition of MI\(^1\) and ejection fraction (EF) by echocardiography. The detailed inclusion criteria included the following: 1) Symptoms of ischemia; 2) New or presumed new significant ST-segment/T wave (ST-T) changes or new left bundle branch block (LBBB); 3) Development of pathological Q waves in the ECG; 4) Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, and EF > 50% on admission. The exclusion criteria were as follows: 1) History of previous MI; 2) Revascularization in the past 24 months; 3) Active malignant tumor; 4) Active rheumatic disease. Because only echocardiography data were available in 1 year follow-up, the clinical status of EF preserved HF (EFpHF) could not be accurately defined in patients with EF > 50%. Hence, in the present study, HF specifically refers to EF reduced HF (EFrHF). All participating patients with AMI presented with normal EF (EF > 50%) before discharge, and were divided into 2 groups according to EF 1 year after discharge, namely post-AMI HF group (LVEF ≤ 35%) and post-AMI non-HF group (LVEF > 50%). Age and sex matched healthy population were selected as controls. The clinical data including a complete medical history, physical exam, lab tests, and image studies were systematically reviewed.

**Candidate serum circulating miRs screening:** Through literature mining, 8 candidate miRs (miR-29a, miR-133a, miR-208b, miR-499, miR-150, miR-194, miR-192, and miR-34a) were selected as potential predictors of post-AMI HF. Small sample clinical studies have shown that alteration of these circulating miRs were related to the poor prognosis in patients with AMI,\(^9\)-\(^13\)) such as short-term major adverse cardiovascular events (MACE), left ventricular remodeling, LVEF among others (Figure 1).

**Serum circulating miRs detection:** Peripheral blood of patients with AMI were collected using PAXgene\(^TM\) blood RNA tubes (BD Bioscience, San Jose, US) on admission (post-AMI day 1). Total RNA were extracted from blood serum via standard procedures using miRNeasy kit (Qiagen, Germany). RNA quality and concentration were examined using NANOdrop2000 (Thermo Scientific, Waltham, US). cDNA were obtained using miRCURY LNA Universal cDNA synthesis kit (Qiagen) according to the manufacturer’s instruction. Real-time quantitative PCR was performed with Step One Plus (Applied Biosystems, Waltham, US), using SYBR Green master mix (Applied Biosystems). Small nuclear RNA U6 served as the internal control. U6 variation was insignificant between groups based on mean CT values using one-way ANOVA (\(P = 0.892\)), suggesting it was an acceptable internal control in our study. Healthy controls served as reference controls. Primers for U6 and 8 candidate miRs were synthesized by Takara, Japan. According to the delta CT method, the relative expression of individual miR was normalized to U6 and was calculated as \(\Delta Ct = \text{mean CtmiRNA} - \text{mean CtU6}\). Changes in miRs delta CT of patients with AMI were presented as a fold change from the delta CT of the reference controls and calculated as \(FC = \)
Echocardiography: All patients with AMI underwent echocardiography before discharge (post-AMI day 5-6) and 1 year after discharge. Echocardiographic examinations were performed with patients in the left lateral decubitus position using a Vivid 7 cardiac ultrasound machine (General Electric, United States) equipped with a 3.5-MHz transducer. Echocardiographic analysis was performed by an experienced clinical echocardiographer, who was blinded to the clinical information of the patients. Conventional measurements included left ventricular internal diameters at end diastole (LVIdD), interventricular septum thickness (IVSd), EF by biplane Simpson’s method and left atrial diameters.

Statistics: Continuous variables are expressed as mean ± standard deviation; categorical variables are presented as absolute and percentage numbers. The Student’s t-test or Mann-Whitney U test was used to test significance between groups depending on their distributions. Chi-square test or Fisher’s exact test was used for categorical variables. Pearson Correlation Coefficient was used to analyze correlations between serum circulating miRs levels and post-AMI LVIdD and EF. Receiver operating characteristic (ROC) curve was used to analyze the accuracy of BNP and miR-150 of predicting post-AMI HF. The cutoff values of BNP, miR-150, and BNP + miR-150 were determined by calculating Yonden’s Index from ROC curve. Multivariate logistic regression analysis were performed for evaluating the relationship between the presence of 1 year post-AMI HF and risk factors or biomarkers, including age, BNP, miR-150, and LVEF on admission. A P-value of < 0.05 was considered significant. All statistical analyses were performed using the SPSS statistical package (v.17.0).

Results

Demographic and clinical features of study subjects: In-hospital patients with AMI during 2015-2016 were retrospectively reviewed. Overall, 113 patients with AMI, including 54 patients with post-AMI HF patients and 59 with post-AMI non-HF (Table I), and 59 age- and sex-matched healthy controls were recruited. All participants were Chinese Han ethnicity. No significant differences were found between the post-AMI HF and post-AMI non-HF groups on baseline demographic information and medical history. In total, 70.4% and 60.0% of patients were diagnosed with ST-segment elevated MI (STEMI) in the post-AMI HF and post-AMI non-HF groups respectively; nevertheless, the differences were insignificant. TnT (1.6 ± 1.1 versus 1.2 ± 0.9 ng/mL, \( P = 0.061 \)) and BNP (109.1 ± 55.8 versus 87.0 ± 48.1 ng/μL, \( P = 0.027 \)) levels were higher in the post-AMI HF group than that in the post-AMI non-HF group, suggesting that myocardial injury was likely to be more severe in the post-AMI HF group. All patients with AMI received dual anti-platelet and statin therapy; 77.8% and 69.5% patients received β-blocker \( (P = 0.319) \), and 81.5% and 89.8% patients re-
received ACEI/ARB ($P = 0.204$) in post-AMI HF and post-AMI non-HF group, respectively. 92.6% and 88.1% patients received revascularization in post-AMI HF and post-AMI non-HF group, respectively ($P = 0.425$).

Comparison of echocardiographic results: No significant differences were found in LVEF between the post-AMI HF and post-AMI non-HF groups before discharge (59.4 ± 4.8 versus 61.6 ± 4.7%, $P = 0.059$), suggesting that no LV dysfunction was initially present in either group. However, follow-up echocardiogram performed 1 year after discharge revealed that LVEF in the post-AMI HF group was significantly lower than post-AMI non-HF group (31.4 ± 3.3 versus 60.5 ± 4.0%, $P < 0.001$), whereas LVIDd was higher in the former (5.8 ± 0.5 versus 4.7 ± 0.3 cm, $P < 0.001$), indicating left ventricular (LV) remodeling paralleled with contraction impairment in the post-AMI HF group (Table II).

Comparison of serum circulating miRs levels: Real-time quantitative PCR found that serum levels of 7 (miR-29a, miR-133a, miR-208b, miR-499, miR-194, and miR-34a) out of 8 candidate miRs were elevated in all patients with AMI, when compared with the control group, whereas no differences were detected between the post-AMI HF and post-AMI non-HF groups (Table III, Figure 2). The serum level of miR-150, which was the only miR declined in patients with AMI, was significantly lower in the post-AMI HF group than the post-AMI non-HF group (0.4 ± 0.3 versus 0.7 ± 0.3, $P < 0.001$), suggesting miR-150 was potentially associated with the development of post-AMI HF.

Correlation between serum circulating miR-150 level and clinical parameters: Pearson Correlation Coefficient test found that only follow-up LVEF estimated 1 year after discharge correlated with serum circulating miR-150 level ($P < 0.001$) (Table IV). Further analysis with other parameters revealed that LVEF at 1 year after discharge was correlated with BNP ($P < 0.001$), LVEF on admission ($P < 0.001$), LVIDd on admission ($P < 0.001$), and LVIDd at 1 year discharge ($P < 0.001$), suggesting worse LV remodeling at admission was strongly associated with post-

AMI indicates acute myocardial infarction; HF, heart failure; LVIDd, left ventricular internal diameter at end diastole; and LVEF, left ventricular ejection fraction. *$P < 0.05$. **$P < 0.001$.
AMI HF.

Role of serum circulating miR-150 as a predictor of post-AMI HF: ROC curve was introduced to compare the accuracy of traditional biomarker BNP and novel biomarker miR-150 on predicting post-AMI HF (Table V, Figure 3). It was found that area under the ROC curve (AUC) for BNP alone was 0.616 (95%CI = 0.511-0.721, \( P = 0.034 \)) and that for miR-150 alone improved to 0.764 (95%CI = 0.674-0.855, \( P < 0.001 \)); nevertheless, this improvement was not significant (\( P = 0.296 \)). However, combined use of BNP and miR-150 significantly improved AUC to 0.807 (95%CI = 0.727-0.886, \( P < 0.001 \)) when compared with BNP (\( P = 0.004 \)) or miR-150 (\( P = 0.006 \)) alone, which suggested that this combination was most effective in predicting post-AMI HF.

Serum circulating miR-150 was independently associated with post-AMI HF: Multivariate logistic regression analysis including age, BNP, miR-150, and LVEF on admission revealed that either LVEF on admission or serum circulating miR-150 level was independently associated with the presence of post-AMI HF, suggesting a powerful role of serum circulating miR-150 on predicting post-AMI HF (Table VI).

Discussion

In our current study, through real-time quantitative PCR, serum circulating miR-150 was found to be differentially expressed between the post-AMI HF group and post-AMI non-HF groups. Further, the correlation test revealed that miR-150 was closely associated with LVEF 1 year after discharge. Finally, ROC curve analysis unveiled

Table IV. Correlation Analysis between Serum Circulating miR-150 Levels and Various Clinical Parameters of Patients with AMI

| Index                          | \( r \) value | \( P \) value |
|--------------------------------|---------------|---------------|
| TnT                            | 0.182         | 0.053         |
| BNP                            | 0.088         | 0.354         |
| LVEF (on admission)            | 0.093         | 0.307         |
| LVIDd (on admission)           | 0.024         | 0.552         |
| LVEF (at 1 year follow-up)     | 0.538         | < 0.001*      |
| LVIDd (at 1 year follow-up)    | 0.179         | 0.056         |

TnT indicates troponin T; BNP, brain natriuretic peptide; LVIDd, left ventricular end-diastolic internal diameter; and LVEF, left ventricular ejection fraction. *\( P < 0.05 \).

Table V. AUC of BNP, miR-150, and BNP Combined with miR-150 on Predicting Post-AMI HF

| Variable              | AUC      | 95% CI     | \( P \) value | Cut off value |
|-----------------------|----------|------------|---------------|---------------|
| BNP                   | 0.616    | 0.511-0.721| 0.034*        | 110           |
| miR-150               | 0.764    | 0.674-0.855| < 0.001*      | 0.3           |
| BNP ± miR-150         | 0.807    | 0.727-0.886| < 0.001*      | 0.553         |

AUC indicates area under the ROC curve; BNP, brain natriuretic peptide; and CI, confident interval.
that miR-150 alone improved the predicting accuracy of post-AMI HF when compared with traditional biomarker BNP. Furthermore, a combination of miR-150 and BNP were shown to be the most powerful predictor of developing post-AMI HF.

miR-150, located in human Chromosome 19q13, was highly expressed in lymph node and spleen, playing an important role in the immunogenesis, hematopoiesis, and embryogenesis. Therefore, the deregulated expression of miR-150 might result in autoimmune diseases and hematopoietic malignancies. However, the role of miR-150 in cardiovascular diseases was less investigated. A clinical study has found reduced serum circulating miR-150 level was associated with LV remodeling following first STEMI event. Another recent study has found serum circulating miR-150 level was significantly dysregulated in patients with acute HF when compared with those with stable HF, suggesting that it was a potential biomarker for the severity of HF. In our study, miR-150 expression was found to be downregulated following MI and tightly associated with impaired LV contractility; this was consistent with previous studies. In future, a large sample prospective cohort study is warranted to validate the predictive power of post-AMI HF for this novel biomarker as well as long-term prognosis, such as MACE and survival rate.

It was consistently reported that miR-150 was a protective factor against myocardial injury, cardiac hypertrophy, or myocardial fibrosis through various mechanisms. In an MI mouse model, miR-150 acted as a key modulator in the migration of monocytes and production of proinflammatory cytokines by targeting CXCR4 and thereby exerted a protective effect against an MI-induced myocardial injury. Another study has identified miR-150 as a potential regulator of cell death in the process of an MI-induced myocardial injury. A study on rat MI model has further revealed that miR-150 interacted with long non-coding RNAZFAS1, exerting an anti-apoptotic effect against myocardial injury by targeting C reactive protein (CRP). Hearts that overexpressed miR-150 were resistant to cardiac hypertrophy and fibrosis through downregulation of serum response factor. Transverse aortic constriction mouse model suggested that miR-150 was a key regulator of pressure overload-induced cardiac fibrosis by targeting c-Myb. We found downregulation of miR-150 was significantly associated with post-AMI LV dysfunction; however, the precise mechanism remains unclear, possibly through multiple pathways as previously reported. Functional studies using cellular and animal models are needed to further elucidate the role of miR-150 in the development of MI and HF.

**Table VI. Multivariate Logistic Regression Analysis on Post-AMI HF**

|     | β    | SE   | Wald | P    | OR   | 95% CI for OR |
|-----|------|------|------|------|------|--------------|
| Age | −0.020 | 0.022 | 0.830 | 0.362 | 0.980 | 0.938-1.024  |
| BNP | 0.005  | 0.005 | 0.960 | 0.327 | 1.005 | 0.995-1.015  |
| LVEF (on admission) | −0.287 | 0.069 | 17.215 | < 0.001 | 0.751 | 0.656-0.860  |
| miR-150 | 0.210  | 0.047 | 19.984 | < 0.001 | 1.233 | 1.125-1.352  |

BNP indicates brain natriuretic peptide; LVEF, left ventricular ejection fraction; SE, standard error; OR, odds ratio; and CI, confident interval.

**Conclusion**

Due to funding and time limitation, our current retrospective study involved a small sample, in which serum circulating miR-150 was screened out as potential predictor of post-AMI HF. Future studies with larger sample cohorts are necessary to further validate the usefulness of miR-150 as a novel biomarker of post-AMI prognosis.

**Disclosures**

**Conflicts of interest:** The authors report no relationships that could be construed as a conflict of interest.

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