Clinical Research

Circulating miRNAs Related to Long-term Adverse Cardiovascular Events in STEMI Patients: A Nested Case-Control Study

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ABSTRACT

Background: Long-term morbidity and mortality of patients with ST-segment-elevation myocardial infarction (STEMI) after primary percutaneous coronary intervention (PCI) remain substantial. Circulating microRNAs (miRNAs) play an important role in cardiovascular disease development. We aimed to identify circulating miRNAs associated with adverse cardiovascular events after acute myocardial infarction (AMI).

Methods: We performed a prospective, nested, case-control study of 932 patients with STEMI who underwent primary PCI. A 3-phase approach was conducted to screen candidate circulating miRNAs in 70 patients who subsequently experienced cardiac death, hospitalization for heart failure, or recurrent AMI (major adverse cardiovascular events [MACE] group) and in 140 patients matched for age, sex, time from symptom onset to blood collection and dual-antiplatelet therapy to the development of novel pharmaceuticals and interventional therapies, such as the use of reperfusion therapy, primary percutaneous coronary intervention (PCI), antithrombotic therapy, and secondary prevention.

Acute myocardial infarction (AMI) is a common cardiac emergency with a substantial risk for morbidity and mortality.1 Significant advances have been made in understanding the underlying pathophysiology of AMI, leading to the development of novel pharmaceuticals and interventional therapies, such as the use of reperfusion therapy, primary percutaneous coronary intervention (PCI), antithrombotic therapy, and secondary prevention.2

RÉSUMÉ

Introduction : La morbidité et la mortalité à long terme des patients ayant subi un infarctus du myocarde avec élévation du segment ST (STEMI) après l’intervention coronarienne percutanée (ICP) demeurent considérables. Les microARN (miARN) circulants jouent un rôle important dans le développement de la maladie cardiovasculaire. Nous avions pour objectif de déterminer les miARN associés aux événements cardiovasculaires indésirables après l’infarctus aigu du myocarde (IAM).

Méthodes : Nous avons réalisé une étude cas-témoins nichée dans une cohorte prospective de 932 patients qui ont eu un STEMI et subi une ICP primaire. Nous avons mené une approche en 3 phases pour dépister les miARN circulants candidats auprès de 70 patients qui avaient ultérieurement expérimenté la mort d’origine cardiaque, l’hospitalisation...
who did not report adverse cardiovascular events during 2-year follow-up (non-MACE group).

Results: We found that miR-26a-5p, miR-21-5p, and miR-191-5p levels were lower in the MACE group than in the non-MACE group (all $P < 0.001$). Multivariate conditional logistic regression analysis revealed that miR-26a-5p, miR-21-5p, and miR-191-5p levels were significantly inversely associated with incident primary composite outcomes (all adjusted $P < 0.01$). Importantly, the combination of these 3 miRNAs plus B-type natriuretic peptide clearly improved the risk scores recommended in the current guidelines, as determined with the use of C-statistics, net reclassification, and integrated discrimination.

Conclusions: Our study provides proof-of-concept in humans that circulating miRNAs are associated with increased rates of distinct cardiovascular events, suggesting that they can serve as effective prognostic biomarkers and therapeutic targets for patients with AMI.

Nevertheless, long-term morbidity and mortality for patients with AMI remain substantial. Previous studies report that 1-year mortality is as high as 7% and that ~ 22% of patients experience heart failure (HF) and recurrent ischemic events. Therefore, the identification of new pathways to further improve outcomes after infarction is crucial.

MicroRNAs (miRNAs) are small noncoding RNAs (~ 22 nucleotides in length) that recognize and bind to mRNA and inhibit protein translation and degrade mRNA. Accumulating evidence suggests that miRNAs act as major regulators in AMI development in that they exhibit distinct tissue expression profiles, orchestrate coronary atherosclerotic plaque phenotypes, and respond to myocardial ischemia-reperfusion (MI/R) injury, angiogenesis, and tissue repair. MiRNAs are also released into circulation, where they are stable owing to encapsulation in extracellular vesicles or by binding to transport proteins. Therefore, miRNAs are protected from degradation and can be reliably measured in blood samples.

Recently, experimental studies have shown that circulating miRNAs can be invoked as cell-cell communication regulators and paracrine signalling mediators in AMI. The characteristic change in circulating miRNA levels observed in individuals with AMI indicates that they might serve as biomarkers for prognosis.

Initially identified as potential diagnostic biomarkers of AMI, cardiac-enriched miRNAs were first investigated for prognostic purposes. Zile et al. found that circulating miR-1, miR-133a, and miR-208a levels were associated with left ventricular (LV) remodelling in a small cohort of patients with AMI. Eitel et al. reported that univariate analysis showed that STEMI patients with high miR-133a levels are at a significantly higher risk of a cardiovascular event; however, by adjusting for high-sensitivity troponin T and B-type natriuretic peptide (BNP), the association became insignificant. Goretti et al. reported that measuring miR-208 and miR-499 concentrations alone was insufficient to predict early and late mortality in AMI patients. Therefore, the use of cardiac-enriched miRNAs as a prognostic marker for AMI remains controversial.

However, non–cardiac-enriched miRNAs show potential predictive ability in patients with AMI. For example, levels of p53-responsive miRNAs (miR-192, miR-194, and miR-34a) were lower in patients with AMI who developed HF. In addition, Jakob et al. found that miR-26b-5p, miR-320a, and miR-660-5p were associated with cardiac death or recurrent AMI. These findings suggest that non–cardiac-enriched miRNAs might be useful for AMI prognosis; however, additional studies are warranted to confirm this possibility.

Therefore, the present study aimed to identify the association between data collected for circulating miRNAs from screening and validation processes and 2-year outcomes in patients with ST-segment-elevation myocardial infarction (STEMI) who received primary PCI.

Methods

Study design and participants

We performed a nested case-control study on patients from the ARSGB-ACS (A Registry Study on Genetics and Biomarkers of Acute Coronary Syndrome) trial (ClinicalTrials.gov registration no. NCT03752515). This trial included a prospective cohort of 932 patients with STEMI who were 18 years of age or older. The patients presented within 12 hours of chest pain onset and were treated with primary PCI at Beijing Anzhen Hospital, Capital Medical University, from June 2, 2015, to December 30, 2016. Detailed information for STEMI diagnosis is provided in the Supplemental Methods. We subsequently excluded patients with
comorbidities that significantly affected miRNA expression levels (Fig. 1; Supplemental Methods).

Participants were identified as having major adverse cardiovascular events (MACE) during the 2-year follow-up if they experienced cardiac death, hospitalization for HF, or recurrent AMI. Detailed information for study end points is provided in the Supplemental Methods.

We identified 70 patients with STEMI who later experienced cardiovascular events during the 2-year follow-up (MACE group). For every such case, two control subjects were randomly selected from patients with STEMI who remained free of reported cardiovascular events during follow-up. These control participants were matched to case participants by age (within 3 years), sex, time from symptom onset to blood collection (within 3 hours), and administration of dual-antiplatelet therapy (DAPT). We ultimately selected 140 control participants (non-MACE group) for whom information was available for all cardiovascular risk factors and a serum sample was available for measurement of circulating miRNAs.

The study protocol was approved by the Ethics Committee of Beijing Anzhen Hospital and carried out according to the principles of the Declaration of Helsinki. All patients provided written informed consent. The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) Equator checklist was used for this study (Supplemental Table S1).

Study phases

We divided 210 STEMI patients and 30 healthy control subjects (HCs) into the 3 phases of the study (Fig. 2), including the profiling, replication, and validation phases. Detailed information for these study phases is provided in the Supplemental Methods.

Statistical analysis

Data are presented as n (%) for categoric variables and as mean ± SD for continuous variables. For comparisons among groups, the chi-square test (or Fisher exact test when any expected count was < 5 for a 2 × 2 table) was used for categoric variables and the Student t test or Mann-Whitney U test was used for continuous variables. Conditional logistic regression and Cox proportional hazards regression were used to evaluate possible associations between circulating miRNAs and adverse clinical outcomes. Kaplan-Meier failure rates at 2 years are presented. The added predictive ability of miRNAs over and above reference models were assessed by means of C-statistics, category net reclassification improvement (NRI), and integrated discrimination improvement (IDI). Spearman rank correlation was used to compare miRNA levels with clinical variables. Detailed information for the statistical analysis is provided in the Supplemental Methods. Statistical tests were performed with the use of SPSS version 24 (IBM Corp, Armonk, NY) and R programming version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). A 2-sided P value of < 0.05 or a false discovery rate of < 0.1 was considered to be statistically significant. All authors had full access to and take full responsibility for the integrity of the data.

Results

Characteristics of the study participants

The characteristics of STEMI patients and HCs in the profiling and replication phase are presented in Supplemental Table S4. There were significant differences in triglyceride, high-density-lipoprotein cholesterol, and glucose levels between the groups (P < 0.05).

Patients in the MACE group who developed incident adverse cardiovascular events had slightly worse heart function (P < 0.001) and a lower LV ejection fraction (P < 0.001),...
were more likely to suffer from anterior myocardial infarction ($P < 0.001$), had a higher heart rate ($P = 0.003$), and had higher BNP ($P < 0.001$), creatinine ($P = 0.037$), and high-sensitivity cardiac troponin I ($P = 0.001$) levels than matched patients in the non-MACE group (Table 1).

**Proiling of circulating miRNAs in STEMI patients and healthy control subjects**

The results of the proiling phase showed that according to the screening criteria (Fig. 3A) of circulating miRNAs, 15 were down-regulated and only 1 was up-regulated in STEMI patients compared with HCs (Fig. 3B). Seven miRNAs eventually complied with the screening criteria (Fig. 3A), and all of them showed lower expression levels in the MACE group than in the non-MACE group (Fig. 3C). On the basis of the results of the 2 comparisons, 3 miRNAs (miR-26a-5p, miR-21-5p, and miR-191-5p) were chosen for the next stage of quantitative reverse-transcription polymerase chain reaction (qRT-PCR) detection.

**Replication and validation of circulating miRNAs**

In the replication phase, we determined that the expression levels of the 3 miRNAs were consistently lower in patients in the MACE group compared with those in the non-MACE group ($2.28e$; expression levels: 0.82 ± 0.39 vs. 1.12 ± 0.56 [$P < 0.001$] for miR-26a-5p; 1.17 ± 0.57 vs. 2.06 ± 1.43 [$P < 0.001$] for miR-21-5p; and 0.57 ± 0.38 vs. 0.92 ± 0.67 [$P < 0.001$] for miR-191-5p; Fig. 4B). Cox regression analysis results are provided in the Supplementary Results and Supplemental Table S6.

For all STEMI patients, correlation analysis results of the 3 miRNAs with clinical variables are provided in the Supplementary Results and Supplemental Table S7.

**Association of circulating miRNAs levels with adverse cardiovascular events**

To examine the relationship between circulating miRNAs and adverse cardiovascular events, samples of the proiling, replication, and validation phases (70 MACE and 140 non-MACE) were combined for analysis. Multivariate conditional logistic regression analysis using models that separately included the Thrombolysis in Myocardial Infarction (TIMI) risk score plus BNP, the Global Registry of Acute Coronary Events (GRACE) score plus BNP, and low miR-26a-5p, miR-21-5p, and miR-191-5p levels were associated with an increased risk of experiencing composite outcomes during the 2-year follow-up (all $P < 0.01$; Fig. 5A; Supplemental Table S8). Furthermore, Cox regression analysis achieved consistent results with conditional logistic regression analysis (Supplemental Table S9).

In addition, we evaluated the ability of circulating miRNAs to predict accurate cardiovascular events, because this might reect different pathophysiological features (Fig. 5, B-D; Supplemental Table S8). For cardiac death, which occurred in 27 patients (3.84%), miR-21-5p levels had the strongest association as determined by means of univariate and separated TIMI risk score plus BNP— and GRACE score plus BNP—adjusted analyses. As expected, miR-26a-5p and miR-21-5p levels were signicantly inversely associated with hospitalization for HF events (n = 45, 6.39%) independently from risk scores and BNP. For recurrent AMI, which occurred in 19 patients (2.70%), there was a possible association between miR-191-5p levels and outcome.

Kaplan-Meier analysis showed that patients below the median for the 3 miRNAs had a signicantly higher MACE rate than those above the median (all log-rank $P < 0.001$; Supplemental Fig. S2).

**Incremental prognostic value of circulating miRNAs**

Furthermore, adding the 3 miRNAs to the TIMI risk score plus BNP, GRACE score plus BNP, and clinical model plus BNP yielded the largest improvement in C-statistics, from 0.769 (95% conidence interval [CI] 0.695-0.842) to 0.857 (95% CI 0.806-0.907; $P = 0.002$), from 0.765 (95% CI 0.690-0.839) to 0.863 (95% CI 0.812-0.914; $P < 0.001$), and from 0.690 (95% CI 0.610-0.771) to 0.827 (95% CI 0.769-0.885; $P < 0.001$), respectively (Supplemental Table S10). Moreover, NRI and IDI analyses showed that the addition of the 3 miRNAs to the known predictors improved the classiication of events (Supplemental Table S10).
Circulating miRNAs in Acute Myocardial Infarction

**Table 1.** Characteristics of participants with or without major adverse cardiovascular events (MACE)

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 210)</th>
<th>Yes (n = 70)</th>
<th>No (n = 140)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.8 ± 7.5</td>
<td>61.8 ± 7.5</td>
<td>61.8 ± 7.5</td>
<td>Matched</td>
</tr>
<tr>
<td>Male sex</td>
<td>165 (78.6)</td>
<td>55 (78.6)</td>
<td>110 (78.6)</td>
<td>Matched</td>
</tr>
<tr>
<td>Current smoking</td>
<td>136 (64.8)</td>
<td>44 (62.9)</td>
<td>92 (65.7)</td>
<td>0.683</td>
</tr>
<tr>
<td><strong>History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>122 (58.1)</td>
<td>41 (58.6)</td>
<td>81 (57.9)</td>
<td>0.921</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>132 (62.9)</td>
<td>44 (62.9)</td>
<td>88 (62.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>70 (35.3)</td>
<td>25 (35.7)</td>
<td>45 (32.1)</td>
<td>0.605</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>22 (10.5)</td>
<td>10 (14.3)</td>
<td>12 (8.6)</td>
<td>0.202</td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from symptom onset to blood</td>
<td>8.0 ± 2.8</td>
<td>8.0 ± 3.1</td>
<td>8.0 ± 2.6</td>
<td>Matched</td>
</tr>
<tr>
<td>collection, h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior MI</td>
<td>89 (42.4)</td>
<td>44 (62.9)</td>
<td>45 (32.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>119.5 ± 18.3</td>
<td>118.0 ± 21.0</td>
<td>120.0 ± 16.9</td>
<td>0.433</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>77.3 ± 13.5</td>
<td>81.3 ± 14.0</td>
<td>75.3 ± 12.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Killip class &gt; 1</td>
<td>62 (29.5)</td>
<td>41 (58.6)</td>
<td>21 (15.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>51.7 ± 8.6</td>
<td>47.8 ± 9.9</td>
<td>53.6 ± 7.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Laboratory results (at admission)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.8 ± 1.4</td>
<td>1.7 ± 0.9</td>
<td>1.9 ± 1.5</td>
<td>0.564</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.5 ± 1.1</td>
<td>4.5 ± 0.9</td>
<td>4.5 ± 1.1</td>
<td>0.646</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.593</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.8 ± 0.9</td>
<td>2.9 ± 0.8</td>
<td>2.8 ± 1.0</td>
<td>0.576</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>8.2 ± 3.7</td>
<td>9.0 ± 4.5</td>
<td>7.8 ± 3.1</td>
<td>0.098</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>289.0 ± 577.1</td>
<td>558.7 ± 923.7</td>
<td>154.1 ± 148.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>14.6 ± 12.2</td>
<td>16.9 ± 12.9</td>
<td>13.5 ± 11.8</td>
<td>0.068</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>84.9 ± 35.9</td>
<td>97.9 ± 54.6</td>
<td>78.3 ± 18.3</td>
<td>0.037</td>
</tr>
<tr>
<td>hs-cTnI, ng/mL</td>
<td>10.3 ± 22.5</td>
<td>15.3 ± 26.2</td>
<td>7.8 ± 19.9</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>210 (100.0)</td>
<td>70 (100)</td>
<td>140 (100)</td>
<td>matched</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>184 (87.0)</td>
<td>62 (88.6)</td>
<td>122 (87.1)</td>
<td>0.767</td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>41 (19.5)</td>
<td>14 (20.0)</td>
<td>27 (19.3)</td>
<td>0.902</td>
</tr>
<tr>
<td>Any DAPT</td>
<td>210 (100)</td>
<td>70 (100.0)</td>
<td>140 (100)</td>
<td>matched</td>
</tr>
<tr>
<td>Statin</td>
<td>199 (94.8)</td>
<td>66 (94.3)</td>
<td>133 (95.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>128 (61.0)</td>
<td>42 (60.0)</td>
<td>86 (61.4)</td>
<td>0.841</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>160 (79.0)</td>
<td>56 (80.0)</td>
<td>110 (78.6)</td>
<td>0.810</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD or n (%).

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; DAPT, dual-antiplatelet therapy; HDL, high-density-lipoprotein; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; LDL, low-density-lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; SBP, systolic blood pressure.

**Discussion**

This was the first study to perform profiling, replication, and validation of miRNAs associated with long-term adverse cardiovascular outcomes with the use of a prospective cohort of patients with STEMI undergoing primary PCI. We identified 3 miRNAs (miR-26a-5p, miR-21-5p, and miR-191-5p) that predicted patients who experienced adverse cardiovascular events vs those without such events within 2 years after STEMI. The relationship between these 3 miRNAs and various poor outcomes suggests a distinct pathophysiologic mechanism of miRNA involvement in adverse LV remodelling and platelet activation after AMI (Supplemental Fig. S3).

Adverse LV remodelling after myocardial infarction constitutes the structural basis for ischemic HF and comprises complex short- and long-term changes in LV size, shape, function, and cellular and molecular composition. LV remodelling in the early phase is mainly due to apoptosis and necrosis of cardiomyocytes and MI/R injury caused by a large area of infarction. This results in thinning and dilation of the infarcted myocardial wall. In the late phase, LV remodelling is secondary to architectural rearrangement of the surviving myocardium that involves hypertrophy of myocytes, interstitial fibrosis, neovascularization, and LV dilation. MiRNA-targeted therapy may prove to be useful for preventing postinfarction remodelling. However, to date, there are no clinical therapies targeting these miRNAs in the field of cardiovascular disease.

The present study showed that miR-21-5p expression levels within 12 hours after symptom onset in STEMI patients were lower than those in HCs. This result is consistent with the observation by d’Alessandra et al. of circulating miRNAs in the early phase of AMI. Interestingly, experimental studies showed that miR-21 expression was significantly decreased in infarcted areas and increased in border areas in rat hearts during the early phase of AMI. Furthermore, miR-21 overexpression via adenovirus has a protective effect on models of myocardial infarction and MI/R injury caused by a large area of infarction. This results in thinning and dilation of the infarcted myocardial wall. In the late phase, LV remodelling is secondary to architectural...
These observations suggest that miR-21-5p plays an active role in the regulation of cardiac healing in the early stage. This is consistent with our finding that low miR-21-5p levels were associated with HF after AMI. A possible explanation for these results might be a reduction in circulating miR-21-5p levels due to the large amount of myocardial cell death in the early stage of myocardial infarction. At the same time, myocardial tissue might also lose the protective effect of miR-21-5p, resulting in an increased infarction area and MI/R injury.

Notably, although miR-21 has a protective effect in the early stage of myocardial infarction, miR-21 plays an

![Diagram](image)

**Figure 3.** Small RNA sequencing results. (A) Circulating small RNA sequencing results and screening process. (B) Volcano plot showing small RNA sequencing results for the comparison of patients with ST-segment-elevation myocardial infarction (STEMI) vs healthy control subjects (HCs). The dashed lines indicate (horizontal) false discovery rate (FDR) of 0.1 and (vertical) fold changes (FCs) of 2 and 0.5. (C) Volcano plot showing small RNA sequencing results for the comparison of STEMI patients with major adverse cardiovascular events (MACE) versus non-MACE STEMI patients. The dashed lines indicate (horizontal) a P value of 0.05 and (vertical) FCs of 2 and 0.5. Individual miRNAs are displayed by their FDR or P value and the corresponding FC with the intensity of blue indicating their mean expression (routine algorithms). PPCI, primary percutaneous coronary intervention.

![Diagram](image)

**Figure 4.** Serum miRNAs differentially expressed in replication and validation phases. The scatter/dot plots show the expression levels of miR-26a-5p, miR-21-5p, and miR-191-5p in (A) the replication-phase population and (B) the validation-phase population; the relative miRNA expression levels were normalized to U6, calculated by $2^{-\Delta\Delta Ct}$, then log10 transformed. HCs, healthy control subjects; MACE, major adverse cardiovascular events.
important role in fibrosis during LV remodelling in the late stage. Roy et al. showed that miR-21 levels were elevated in myofibroblast-infiltrated areas 7 days after MI/R and that they promoted metalloproteinase-2 via targeting of the phosphatase and tensin homolog. This process has also been validated in clinical trials, as shown by Liu et al., who observed that circulating miR-21 levels at 5 days after PCI were elevated in patients with AMI and LV remodelling after 1 year. Recent clinical trials, such as those by Hsu et al. and Li et al., showed that miR-26a-5p levels were lower in patients with AMI at the early stage than in healthy control subjects. Unfortunately, those authors did not explore the prognostic value of miR-26a-5p. In our study, we found that low miR-26a-5p levels were associated with HF. Importantly, miR-26a showed cell specificity during its involvement in LV remodelling. Icli et al. showed that miR-26a targeted the bone morphogenic protein—SMAD1 signalling axis and inhibited angiogenesis in a mouse model of AMI; furthermore, inhibition of miR-26a rapidly induced angiogenesis and reduced the size of AMI with improved heart function. Zhang et al. demonstrated that miR-26a/b repressed the 3' untranslated region of glycogen synthase kinase-3β and reduced the expression of hypertrophy genes, A-type natriuretic factor, and beta-major histocompatibility complex in cardiomyocytes in vitro. Wei et al. found that miR-26a inhibited nuclear factor κB activity in fibroblasts and

**Figure 5.** Odds ratios for adverse cardiac events, according to continuous variables of circulating miRNAs. Univariate (unadjusted) and multivariate (adjusted) odds ratios (ORs) obtained by conditional logistic regression analysis for (A) major adverse cardiac events (MACE), (B) cardiac death, (C) hospitalization for heart failure (HF), and (D) recurrent acute myocardial infarction (AMI) at 2-year follow-up according to the continuous variables (10-log transformation, expressed per 1 SD increase) of circulating miRNA levels. Model 1 was adjusted for Thrombolysis in Myocardial Infarction (TIMI) risk score and B-type natriuretic peptide (BNP), and model 2 was adjusted for Global Registry of Acute Coronary Events (GRACE) score and BNP. CI, confidence interval.
attenuated connective tissue growth factor collagen I gene expression, thereby improving myocardial fibrosis. Notably, these known pathologic processes occur at the late stage of LV remodelling and fail to reflect the role of miR-26a in the early stages. Our study suggests that miR-26a-5p is a protective factor in the early stage of AMI, but further research is necessary.

The present study supports previous studies\(^1\) reporting that miR-21 and miR-26a play important roles in LV remodelling after AMI and could become potential therapeutic targets. Notably, expression and the role of miR-21 and miR-26a are time dependent and tissue/cell specific, requiring a staged therapy strategy for different time points and different cells.\(^2\,^3,^9,^19,^29\)

In our study, miR-191-5p expression in AMI was decreased, which is consistent with previous reports.\(^25,\,^26\) Although no previous study had identified a relationship between miR-191-5p expression and cardiovascular function, we found that low miR-191-5p levels were associated with recurrent myocardial infarction events. Willeit et al.\(^1\) found that miR-191 expression was affected by the dose of anti-platelet drugs. This finding suggests that miR-191 reflects the activation of platelets, indicating its potential role in thrombotic events. However, further detailed investigations are necessary to identify the mechanism involved.

The experimental studies mentioned above suggest that differential expression of miR-26a-5p, miR-21-5p, and miR-191-5p is related to pathophysiologic mechanisms that trigger HF, recurrent myocardial infarction, and cardiac death. We also used network bioinformatics to show that these 3 miRNAs might be related to diseases such as AMI and HF (Supplemental Results; Supplemental Fig. S1). Consistently, miR-26a-5p, miR-21-5p, and miR-191-5p are good discriminators of MACE. The combination of these 3 miRNAs improved the discriminatory power in our study. Furthermore, our results suggest that miRNAs can be used as circulating biomarkers to provide prognostic information for patients with AMI. These risk scores do not include biomarkers related to the pathologic mechanisms that reflect the occurrence of adverse cardiovascular events after AMI.

Different circulating miRNAs have recently been described in clinical studies in relation to their association with adverse cardiovascular events after AMI (Supplemental Table S11). The present study includes several differences and certain advantages over previous research. First, we observed the occurrence of adverse cardiovascular events (cardiac death, hospitalization for HF, or recurrent AMI) over a relatively long period of follow-up care (within 2 years), whereas previous studies mostly examined surrogate outcomes over a short time period. Second, all blood samples were collected within 12 hours of symptom onset, conferring a greater chance of recording pathophysiologic changes in the early stages of AMI. In contrast, previous studies used samples collected days after AMI onset. Third, increasing evidence indicates that medication might alter circulating miRNA levels. Antiplatelet therapy was one of the important matching principles in our study. In addition, blood samples were collected immediately after diagnosis, at which time the patient had not yet been administered heparin. Therefore, the influence of heparin on miRNAs was avoided as much as possible.

Our study has certain limitations. First, it was a single-centre, nested, case-control study that manifested a potential selection bias in that circulating miRNA levels were detected in only 140 non-MACE patients. We assume that this limitation did not greatly affect our results because there were no significant differences in baseline characteristics between the 140 non-MACE patients and 494 non-MACE patients (Supplemental Table S5). At the same time, to compensate for the limitations of a single centre, we are establishing an independent external validation cohort, although not enough patients have been followed for 2 years until now. In the future, we will continue this study to verify the prognostic value of these 3 miRNAs in patients with AMI in an external validation cohort. Second, this study did not include patients with non–ST-segment-elevation myocardial infarction (NSTEMI). Our study aimed to examine circulating miRNA changes in the early stage of AMI. However, in this registered study, the average period of time from symptom onset to blood sampling was 48 hours in patients with NSTEMI. In addition, the pathophysiologic mechanisms of adverse cardiovascular events in patients with NSTEMI are mainly LV remodelling and platelet activation. Therefore, we speculate that circulating miRNAs have the same prognostic value in NSTEMI, but this possibility must be confirmed. Third, the qRT-PCR methodology used in this study does not allow for easy and rapid application in a clinical setting. However, further development of microfluidic technology might overcome this limitation in the future. Moreover, the normalization strategies for miRNA expression data should involve a combination of endogenous and exogenous normalization methods, an approach that should be considered in future studies.

**Conclusion**

Using a profiling, replication, and validation model, this study shows that decreased levels of miR-26a-5p, miR-21-5p, and miR-191-5p are associated with adverse cardiovascular events. These miRNAs might aid in prediction of long-term cardiovascular events after AMI.

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**Disclosures**

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Supplementary Material

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