Original Paper

Circulating miR-181a as a Potential Novel Biomarker for Diagnosis of Acute Myocardial Infarction

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Key Words

miR-181a • Biomarker • AMI

Abstract

Background: In this study, we tested the hypothesis that miR-181a levels increase during acute myocardial infarction. We investigated circulating miR-181a as a potential novel biomarker for early diagnosis of acute myocardial infarction (AMI). **Methods:** From June 2014 to June 2016, 120 consecutive eligible patients with AMI ($n = 60$) or unstable angina (UA; $n = 60$) and 60 control subjects were enrolled. Plasma miR-181a levels were determined by quantitative reverse transcriptase-polymerase chain reaction. **Results:** Circulating miR-181a expression levels detected immediately after admission were higher in the AMI group than in the UA and control groups. Relative miR-181a levels in AMI patients were positively correlated with the concentrations of the creatine kinase-MB fraction and cardiac troponin I. Correlation analysis showed that plasma miR-181a was positively correlated with coronary Gensini score ($r = 0.573$, $P < 0.05$) and negatively correlated with left ventricular ejection fraction ($r = -0.489$, $P < 0.05$). Receiver operating characteristic curve analyses showed that plasma miR-181a was of significant diagnostic value for AMI (AUC, 0.834; 95% CI, 0.756–0.912, $P < 0.05$). **Conclusion:** Circulating miR-181a levels in patients with AMI were significantly changed in a time-dependent manner, indicating the value of plasma miR-181a as a novel biomarker for diagnosing AMI.

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Introduction

Acute myocardial infarction (AMI), one of the major causes of morbidity and mortality worldwide, is mainly caused by atherosclerosis, a chronic inflammatory response to

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cholesterol accumulation in the arterial wall [1]. Early diagnosis of AMI is crucial for successful treatment to protect against myocardial injury and preserve heart function [2]. AMI is a highly dynamic event associated with increased production of reactive oxygen species (ROS) [3]. The imbalance between ROS production and antioxidant defenses leads to a condition known as oxidative stress [4]. The most commonly recognized effect of increased oxidative stress is the oxidation and damage of macromolecules such as proteins, lipids, DNA, and enzymes involved in energy production, thereby contributing to cellular damage, energetic deficit, and accelerated cell death through apoptosis and necrosis [4]. Oxidative stress is a pivotal mechanism underlying myocardial ischemia injury, and inhibition targeting oxidative stress can decrease myocardial ischemia injury and preserve cardiac function [5]. Studying the possible association of oxidative stress-related biomarkers becomes relevant due to the involvement of these biomarkers in the atherosclerotic process, which may lead to the development of AMI. Early diagnosis of AMI is currently based on ischemia symptoms, physical examination, electrocardiographic examination (ECG), and circulating levels of cardiac troponins [6]. However, the measurement of circulating levels of cardiac troponins is not sufficiently sensitive and specific for use as a biochemical marker to discriminate AMI from other diseases, as slight increases may be associated with non-acute coronary syndrome and are limited to modest increases within the first few hours after AMI onset [7]. Therefore, more sensitive and specific novel biomarkers are needed to detect early AMI.

microRNAs (miRNAs) are small, endogenous, non-coding RNA molecules 21–23 nucleotides in length, which function as sequence-specific regulators of gene expression through translational repression and transcript cleavage by pairing with the 3'-untranslated regions (3'-UTRs) of target mRNAs [8]. Accumulating evidence has shown that miRNAs play crucial roles in numerous biological processes, including proliferation, differentiation, and apoptosis. In addition, they have been implicated in various cardiovascular diseases. Thus, circulating miRNAs may be useful as novel biomarkers for the diagnosis of AMI [9]. Furthermore, many miRNAs are remarkably stable and readily detectable in the plasma and other body fluids, and the levels of circulating miRNAs vary under specific physiological and pathological conditions, indicating that circulating miRNAs are ideal biomarkers of AMI [10].

Previous studies have shown that the levels of miR-1, miR-208a, miR-133a, and miR-499 are decreased in infarcted myocardium and increased in the plasma, indicating that the elevated circulating miRNAs are released from the infarcted heart [10]. The increased levels of plasma miRNAs after AMI reflect myocardial injury and parallel the extent of myocardial damage, as measured by cardiac troponin [10]. The area under the receiver operating characteristic (ROC) curve of miR-1 was 0.774 for AMI compared to the controls and there was a positive relationship between increased circulating miR-1 and serum creatine kinase-MB (CK-MB) [11]. A positive correlation was also reported between elevated miR-133 and cardiac troponin I (cTnI), indicating that miR-133 is superior to cTnI because confounding factors affect troponin I levels [12]. miR-208 was also shown to be involved in AMI, cardiomyocyte hypertrophy, fibrosis, and other cardiovascular diseases [13]. The increased levels of circulating miR-499 in patients with AMI were corroborated in rat and mouse models of AMI [14].

Previous studies showed that miR-181a plays important roles in various biological events, including development, differentiation, hematopoiesis, immune modulation, and muscle adaptation to exercise [15]. miR-181a is widely expressed in several tissues, including the brain, muscle, and bone marrow, and its levels were shown to change in response to stress [16, 17]. miR-181a is upregulated in osteosarcoma, and its overexpression promotes the proliferation and inhibits the apoptosis of osteosarcoma cells [18, 19]. Recently, miR-181a was suggested to be a novel and important regulator of autophagy, which is a cellular catabolic mechanism activated in response to stress conditions including starvation and hypoxia during AMI [20]. Moreover, we confirmed that miR-181a inhibited the oxidized low-density lipoprotein (ox-LDL)-induced immune inflammatory response in cultured bone marrow-derived dendritic cells (DCs) in our previous study [21]. However, the specific expression patterns of circulating miR-181a in patients with AMI and their clinical significance remain unknown. Hence, determining the roles of miR-181a in AMI

would not only assist in further understanding of the pathogenesis and progression of coronary artery disease (CAD), but would also reveal novel targets for the development of effective therapies. In this study, we evaluated whether miR-181a levels increase during AMI. Our aims were to investigate the expression of circulating miR-181a in the early phase of AMI and investigate the association between plasma miR-181a levels and the severity of AMI. In addition, we also assessed the ability of circulating miR-181a to identify and evaluate AMI.

Materials and Methods

Study Patients

From June 2014 to June 2016, 120 consecutive eligible patients with AMI ($n = 60$) or UA ($n = 60$) and 60 control subjects were enrolled. The AMI inclusion criteria were ischemic chest pain lasting >30 min, positive biochemical markers (cardiac troponin I [cTnI] > 0.1 ng/mL), presentation within 12 h after the onset of symptoms, electrocardiogram showing a new ST-segment elevation of 0.1 mV in at least two contiguous leads [22]. All AMI patients were diagnosed for the first time and underwent primary percutaneous coronary intervention (PCI). The diagnosis of UA was based on the new onset, worsening (in frequency, intensity, or duration), or resting angina associated with evidence of ST-segment elevation or depression without evidence of myocardial necrosis based on the rise in cardiac serum markers such as CK-MB and cardiac troponin T (cTnT) or cTnI [23]. Control volunteers with normal electrocardiogram findings matched for sex, age, smoking habit, hypertension, dyslipidemia, and diabetes and without a history of AMI or UA during the same period were enrolled as control subjects. Exclusion criteria included previous myocardial infarction (MI), previous use of thrombolytic agents for index MI, cardiomyopathy, cardiogenic shock, previous stroke within the past 6 months, and known bleeding diathesis. Clinical data, including baseline clinical characteristics, angiographic, and laboratory features, and clinical outcomes, were recorded for all patients. The procedures were performed in accordance with the Declaration of Helsinki of 1975, as revised in 1983. Informed consent was obtained from all patients, and the study protocol was approved by the ethics committee of Zhongshan Hospital, Fudan University, Shanghai, China.

Blood Sampling

Whole blood samples (3–5 mL) were collected from patients with AMI at the indicated time points (0 h, 6 h, 12 h, 24 h, 3 days, and 7 days) after the onset of symptoms. The initial blood sample collection time (0 h) was 120 min (IQR: 80–520 min) after the onset of chest pain symptoms (symptom-onset-to-door time). Additionally, after 0 h, other blood samples were obtained at $6 \text{ h} \pm 30 \text{ min}$ (6 h), $12 \text{ h} \pm 30 \text{ min}$ (12 h), $24 \text{ h} \pm 30 \text{ min}$ (24 h), $72 \text{ h} \pm 30 \text{ min}$ (3 d), and $168 \text{ h} \pm 30 \text{ min}$ (7 days). The “before PCI” blood sample collection time (“before PCI”) was 80 min (IQR: 62–112 min) after the patient’s admission to the emergency department (door-to-balloon time). The “after PCI” blood sample collection time (“after PCI”) was $48 \text{ h} \pm 30 \text{ min}$ after PCI. Whole blood samples were collected from UA patients immediately after hospitalization. Samples were obtained via venipuncture of an antecubital vein and were centrifuged at $10,000 \times g$ for 15 min at 4°C . After separation, plasma was frozen at -80°C until analysis, and 0.5 mL of plasma was used for RNA isolation. A full 12-h fasted lipid profile comprising total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and HDL-C was determined for each patient. cTnI, cTnT, creatine kinase (CK), and CK-MB were measured using the Access Immunoassay System (Beckman Coulter). Lipid hydroperoxide levels were evaluated based on the oxidation of Fe^{2+} to Fe^{3+} in the presence of xylenol orange and measured as the change in the absorbance at 560 nm [24]. Samples were calibrated using hydrogen peroxide (0.2–20 mmol/L). The limit of detection for this assay is 0.25 nmol/L [24].

Percutaneous coronary intervention (PCI) procedures

All coronary angiograms and PCI procedures were performed by experienced investigators who were blinded to the study, and transradial coronary intervention was the preferred operating approach. Coronary angiography and intervention were started within 30 min after admission using a radial artery approach. The following data and angiograms were estimated and collected by two operators: infarct-related artery, thrombolysis in myocardial infarction (TIMI) coronary flow before and after coronary intervention, extent of coronary artery disease, and left ventricular function. Stents were placed when residual stenosis $>50\%$ was

present after balloon angioplasty. TIMI flow grade was determined by assessing blood flow in the epicardial arteries. The procedural success was defined as residual stenosis at less than 20% and TIMI flow grade of 2 in the infarct-related artery after PCI. The Gensini score was used to assess the severity of coronary artery stenosis [25].

Quantitative real-time polymerase chain reaction (qRT-PCR)

Plasma miR-181a levels were determined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) [26]. Total RNA was extracted from the plasma using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed on cDNA generated from 50 ng of total RNA using the TaqMan® MicroRNA Assay kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed in triplicate using an ABI Prism 7500 sequence detection system (Applied Biosystems). The amplification reaction was as follows: 94°C for 5 min and 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The cycle number at which the fluorescent signal of the sample rose above the baseline was referred to as the threshold cycle (Ct) and was proportional to the target concentration. Relative gene expression was calculated by comparing the cycle times for each target gene. The target gene Ct values were normalized by subtracting the U6 Ct value, and the relative expression level between groups was calculated according to the $2^{-\Delta\Delta Ct}$ method, as follows: relative gene expression = $2^{-(\Delta Ct_{\text{sample}} - \Delta Ct_{\text{control}})}$ [27]. The primers used were as follows: U6 forward, 5'-GCTTCGGCAGCACATATACTAA-3', U6 reverse, 5'-AACGCTTCACGAATTTGCGT-3'; miR-181a forward, 5'-AACATTCAACGCTGTCTG-3', and miR-181a reverse, 5'-AACTGGTGTCTGGAG-3'.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Continuous data are presented as the mean $\pm$ SD. Categorical variables are presented as counts and percentages. Independent sample *t*-tests and Mann-Whitney U tests were performed to compare groups of continuous variables. Categorical variables were compared by chi-square test. ROC curve analysis and comparison of the derived area under the curve (AUC) were performed to assess the miRNA as a predictor for distinguishing AMI from non-AMI. Correlations between variables were determined by Spearman tests. Statistical significance was set at $P < 0.05$.

Results

Circulating miR-181a levels were increased in patients with AMI

Sixty AMI patients, 60 UA patients, and 60 control subjects were enrolled in this study. The baseline clinical characteristics of the patients and controls are presented in Table 1. The baseline clinical data of the three groups, including age, body mass index, sex, risk factors, total cholesterol, TG, high-density lipoprotein-C, LDL-C, Lp(a), and

Table 1. Baseline Clinical Characteristics of Patients and Controls. Values are mean $\pm$ SD or absolute numbers with relative frequencies. BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein (a); ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers; IQR, interquartile range

Variable	AMI (n = 60)	UA (n = 60)	Control (n = 60)	P value (AMI vs. UA vs. Control)
Male	46 (76.7%)	42 (70.0%)	45 (75.0%)	0.492
BMI (kg/m ²)	24 $\pm$ 2.5	25 $\pm$ 2.6	25 $\pm$ 2.1	0.562
Age (years)	64.1 $\pm$ 10.3	62.1 $\pm$ 11.2	63.2 $\pm$ 12.2	0.634
Risk factors				
Hypertension	26 (43.3%)	24 (40.0%)	27 (45.0%)	0.472
Diabetes mellitus	15 (25.0%)	16 (26.7%)	12 (20.0%)	0.153
Dyslipidemia	29 (48.3%)	26 (43.3%)	28 (46.7%)	0.465
Smoking	38 (63.3%)	40 (66.7%)	39 (65.0%)	0.486
TC (mmol/L)	4.20 $\pm$ 0.90	4.06 $\pm$ 1.0	3.99 $\pm$ 0.85	0.361
TG (mmol/L)	1.53 $\pm$ 0.97	1.48 $\pm$ 0.95	1.42 $\pm$ 0.89	0.456
LDL-C (mmol/L)	2.51 $\pm$ 0.75	2.48 $\pm$ 0.68	2.31 $\pm$ 0.79	0.287
HDL-C (mmol/L)	1.13 $\pm$ 1.01	1.16 $\pm$ 0.95	1.24 $\pm$ 0.89	0.531
Lp(a) (mg/L)	223.42 $\pm$ 130.26	212.56 $\pm$ 126.20	218.65 $\pm$ 128.56	0.486
Peak CK-MB (IU/L)	282.7 $\pm$ 89.6	16.8 $\pm$ 5.7	9.9 $\pm$ 6.4	< 0.001
Peak CK (IU/L)	1642 $\pm$ 903	102 $\pm$ 42	82 $\pm$ 55	< 0.001
cTnI (ng/mL)	21.5 $\pm$ 7.3	0.02 $\pm$ 0.01	0	< 0.001
Medications				
β -blockers	18 (30.0%)	16 (26.7%)	-	0.289
ACEI	12 (20.0%)	10 (16.7%)	-	0.312
ARB	13 (21.7%)	15 (25.0%)	-	0.576
CCB	9 (15.0%)	7 (11.7%)	-	0.259
Nitrates	19 (31.7%)	16 (26.7%)	-	0.350
Statins	38 (63.3%)	42 (70.0%)	-	0.260

Fig. 1. Circulating miR-181a expression levels in the AMI, UA, and control groups. A, Comparison of plasma miR-181a levels in AMI, UA, and control groups. B, Plasma levels of miR-181a in AMI patients at the indicated times after symptom onset. Data are presented as the mean $\pm$ SD; * P < 0.05 compared to the value at 0 h. C, Correlations between plasma miR-181a levels and CK-MB concentrations in AMI patients (r = 0.514, P < 0.05). D, Correlations between plasma miR-181a levels and cTnI concentrations in AMI patients (r = 0.432, P < 0.05). AMI, acute myocardial infarction; UA, unstable angina.

Fig. 2. Pattern of circulating miR-181a expression levels in AMI patients. A, Relative miR-181a levels in AMI patients with 1, 2, or 3 affected branches (n = 1, 2, and 3); The red lines represent the mean values. B, Correlations between plasma miR-181a levels and coronary Gensini scores in AMI patients (r = 0.573, P < 0.05). C, Plasma miR-181a levels in AMI patients at 48 h after PCI were significantly lower than in the before PCI group (P < 0.05). D, Correlations between plasma miR-181a levels and left ventricular ejection fraction (LVEF) in AMI patients (r = -0.489, P < 0.05). AMI, acute myocardial infarction.

medications, did not differ significantly among groups. Blood samples were obtained from AMI patients at the indicated time points (0 h, 6 h, 12 h, 24 h, 3 days, and 7 days) after the onset of AMI. Gender-specific (adjusted OR 1.206, 95% CI 0.86–1.897, P > 0.05) was not significant differences correlated with increase in plasma miR-181a expression levels by the multivariate logistic regression analysis. Plasma miR-181a expression levels detected immediately after admission were higher in the AMI group than in the UA and control groups (Fig. 1A, P < 0.05). As shown in Fig. 1B, the relative plasma miR-181a levels at 6 h, 12 h, and 24 h after the onset of AMI were higher than those at 0 h (P < 0.05); at 3 days and 7 days, the levels were similar to those at 0 h (P > 0.05). Correlation analyses between plasma miR-181a levels and CK-MB and cTnI concentrations are shown in Fig. 1C and Fig. 1D, respectively. The relative miR-181a levels in AMI patients were positively correlated with the serum concentrations of CK-MB (r = 0.514, P < 0.05) and cTnI (r = 0.432, P < 0.05).

Fig. 3. Plasma miR-181a levels as a novel biomarker for the diagnosis of AMI. A, Correlation between plasma miR-181a levels and lipid hydroperoxide concentrations in AMI patients ($r = 0.382$, $P < 0.05$). B, ROC curve analysis of plasma miR-181a to predict AMI. ROC, receiver operating characteristic curve; AMI, acute myocardial infarction; cTnI, cardiac troponin I.

Circulating miR-181a levels in AMI patients undergoing PCI

The door-to-balloon time (D2BT) and symptom-onset-to-door time observed in AMI patients undergoing PCI were 80 min (IQR: 62–112 min) and 120 min (IQR: 80 – 520 min), respectively, and 70.8% of patients had D2BT < 90 min. As shown in Fig. 2A, significantly higher miR-181a levels were detected in AMI patients with two- and three-vessel lesions than in patients with single-vessel lesions ($P < 0.05$). Correlation analysis showed that plasma miR-181a was positively correlated with coronary Gensini score ($r = 0.573$, $P < 0.05$), which is used to evaluate the severity of coronary stenotic lesions (Fig. 2B). Plasma miR-181a levels in AMI patients at 48 h after PCI were significantly lower than in the before PCI group ($P < 0.05$; Fig. 2C). Furthermore, miR-181a levels were negatively correlated with left ventricular ejection fraction by correlation analysis ($r = -0.489$, $P < 0.05$; Fig. 2D).

Circulating miR-181a levels and oxidative stress parameters

We evaluated whether miR-181a levels were correlated with oxidative stress parameters in AMI patients. Correlations analyses showed that miR-181a was positively correlated with lipid hydroperoxide concentrations ($r = 0.382$, $P < 0.05$; Fig. 3A).

Evaluation of plasma miR-181a as novel diagnostic biomarker of AMI

ROC analysis was performed to determine the suitability of miR-181a as a biomarker of AMI. The ROC curves of plasma miR-181a showed separation between the AMI and non-AMI groups, with an AUC of 0.834 (95% CI, 0.756–0.912, $P < 0.05$), compared to cTnI with an AUC of 0.873 (95% CI, 0.810–0.936, $P < 0.05$; Fig. 3B), and cTnT with an AUC of 0.816 (95% CI, 0.735–0.897, $P < 0.05$; Fig. 3B). The ROC curves revealed that the cut-off value of plasma miR-181a differentiating AMI patients from non-AMI subjects was 2.869. The specificity and sensitivity of miR-181a for the diagnosis of AMI were 0.826 and 0.898, respectively. These data indicate that circulating miR-181a can be used as a novel diagnostic biomarker of AMI.

Discussion

Highly sensitive, reliable, early diagnosis of AMI is required for prompt PCI therapy, which may improve the survival rate of patients of AMI. Although it is unlikely that a single biomarker of myocardial ischemic injury will be sufficient to manage AMI diagnosis, a combination of different biomarkers, including circulating miRNAs, may enhance diagnostic sensitivity and specificity [28]. Numerous circulating miRNAs, including miR-208a, miR-126, miR-223-3p, miR-150, miR-19b-3p, miR-21, miR-328, and miR-134, showed potential as biomarkers for cardiovascular diseases [28–34].

The present study indicates that plasma miR-181a is a useful diagnostic biomarker for AMI. In our study, plasma concentrations of miR-181a were significantly elevated at 6 h, 12

h, and 24 h after the onset of symptoms. The relative levels of miR-181a in patients with AMI were positively correlated with the concentrations of CK-MB and cTnI. ROC curve analyses revealed that plasma miR-181a was of significant diagnostic value for AMI (AUC, 0.834; 95% CI, 0.756–0.912, $P < 0.05$). Although the diagnostic value of miR-181a was inferior to that of cTnI (AUC, 0.873; 95% CI, 0.810–0.936, $P < 0.05$), it may be a promising novel biomarker for enhancing diagnostic sensitivity when combined with classical biomarkers.

Previous studies demonstrated that numerous circulating miRNAs are altered in AMI, including the cardiac-specific miRNAs miR-208 and miR-499 [35–40]. However, miRNAs dysregulated in AMI may also be derived from non-myocardium cells that play a vital role in AMI pathophysiology, such as endothelial cells involved in plaque rupture and inflammatory cells that are recruited to the ischemic area [35]. The endothelium-enriched miRNA miR-126 was shown to be useful as a biomarker in AMI, and plasma miR-126 and cTnI expression levels showed the same trends [41]. The inflammation-associated miRNA miR-155 was significantly lower in patients with coronary artery disease than in control subjects [42]. Further studies are needed to determine whether the expression of these miRNAs is correlated with that of miR-181a. Increased reactive oxygen species (ROS) production is involved in adverse cardiac remodeling and development of heart failure after MI. Recently, Magenta *et al* demonstrated that ROS increased cytosolic $[Ca^{2+}]_i$ (Cai) and modulated the expression of miR-181a and miR-1, indicating that both miR-181a and miR-1 affect Cai-dependent intracellular signaling during oxidative stress [43]. *In silico* prediction of target genes by miRanda, miRdb, and TargetScan and subsequent qPCR analysis revealed that miRNAs including miR-208a, miR-133, and miR-181a target genes that primarily regulate cell proliferation and cell death during heart failure [44]. Furthermore, combining plasma miR-181a with other miRNAs in a panel should be established to increase the efficiency of distinguishing patients with AMI and controls.

Symptom onset is associated with the kinetics of plasma miRNA levels in AMI patients, and an advantage of circulating miRs compared to traditional biomarkers may be their early release after symptom onset [45]. Muscle-derived miR-499-5p was shown to be elevated by 1.7-fold as early as 15 min after the induction of coronary artery ligation in mice [35]. In humans, miR-1 and miR-133 were identified to be elevated as early as 156 min after the onset of symptoms and decreased thereafter, whereas miR-499 further increased, achieving maximal levels by approximately 9 h after symptom onset [35]. Because of the variability in symptom onset after AMI in patients, however, these data, including our results, should be considered with caution and further studies are required to monitor the kinetics of miRs release in comparison to CK, CK-MB, and troponins. Moreover, whether specific miRs are released very early after the onset of symptoms within microvesicles or exosomes, indicating the initiating processes of cellular inflammation or apoptosis during myocardial ischemia and injury, must be further examined. In the present study, the D2BT and symptom-onset-to-door time observed in AMI patients undergoing PCI were 80 min (IQR: 62–112 min) and 120 min (IQR: 80–520 min), respectively, and 70.8% of patients showed D2BT < 90 min. Our results demonstrate that plasma miR-181a is elevated at 6 h as well as at 12 h or at 24 h, and decreased at 3 days and 7 days after the onset of symptoms, suggesting that miR-181a is released in a time-dependent manner.

AMI is mainly caused by atherosclerosis, which is considered a chronic inflammatory response to cholesterol accumulation in the arterial wall. In atherosclerotic lesions, DCs, the only professional and most powerful antigen-presenting cells, accumulated in the subendothelium and underwent maturation stimulated by ox-LDL, advanced glycosylation end products, or other antigen components [46]. CD11c⁺ DCs from ApoE^{-/-} mice with hyperlipidemia showed increased inflammatory responses and upregulated miR-181a expression [21]. miR-181a feedback attenuated the ox-LDL-stimulated immune inflammatory response by targeting c-Fos in DCs, suggesting its important diagnostic value for coronary artery disease [21]. miR-181a downregulated IL-6 and TNF- α and inhibited ox-LDL-induced increases in IL-6 and TNF- α in DCs [21]. The present study demonstrated that plasma concentrations of miR-181a were significantly increased after the onset of symptoms in patients with AMI, which may be significantly associated with the immune inflammatory response in atherosclerotic lesions.

cTnI is also expressed in the skeletal muscle and is elevated by skeletal muscle injury during operation, which diminishes the value of cTnI elevation caused by myocardial injury [7]. In addition, the sensitivity and specificity of CK-MB and other conventional markers must be further enhanced, and it is difficult to identify new proteomic markers because of a technical bottleneck [47]. miRNAs are highly stable *in vitro*, can be detected by qRT-PCR, and can be used in remarkably sensitive and specific testing for the early diagnosis of AMI [48]. Our study confirmed that plasma miR-181a was of significant diagnostic value for AMI (AUC, 0.834; 95% CI, 0.756–0.912, $P < 0.05$), similarly to cTnI (AUC, 0.873; 95% CI, 0.810–0.936, $P < 0.05$). Based on the results of the present study, miRNAs may be useful as sensitive, specific circulating biomarkers for the diagnosis of AMI. miR-181a is not correlated with known biochemical indicators of AMI. Therefore, miR-181a levels in the circulation are closely associated with the occurrence of AMI and may be an independent biomarker for the early diagnosis of AMI.

Oxidative stress may play an etiological role in AMI and be useful as a biomarker [49]. When oxidative stress occurs, cells trigger a series of biochemical cascades to alter the redox balance and restore homeostasis following the development of atherosclerotic plaques and subsequent AMI [49]. During AMI, the miRNAs involved in inflammation may be indicators of the oxidative-stress response that maintains cellular homeostasis under ischemic and hypoxic conditions [50]. miR-210 was closely related to oxidative stress regulation, and inhibition of miR-210 led to increased apoptosis and oxidative tissue injury [51]. We measured the plasma levels of miR-181a in patients with AMI at various time points and correlated them with oxidative stress as measured by lipid hydroperoxides. Correlation analyses showed that miR-181a was positively correlated with lipid hydroperoxide concentration ($r = 0.382$, $P < 0.05$). Thus, miR-181a may be an indicator of the adaptive response to reduce oxidative stress in patients with AMI.

The Gensini score indicates the severity of AMI, which facilitates identification of the optimal PCI strategy for patients with AMI [52]. However, calculating the Gensini score is considered difficult and time-consuming. Therefore, it is necessary to identify biomarkers that can accurately reflect the severity of coronary vessel lesions in patients with AMI. We found that plasma miR-181a was positively correlated with the severity of coronary stenosis as measured by Gensini score. These results further indicate that miR-181a release from inflammatory cells reflects the risk of myocardial ischemia and injury. Our results confirmed that plasma miR-181a levels in patients with AMI were significantly lower after PCI compared to the levels before PCI, indicating that miR-181a is an ideal biomarker for predicting myocardial ischemia reperfusion in patients with AMI. Additionally, miR-181a levels were negatively correlated with left ventricular ejection fraction, indicating poor prognosis and an increased risk of heart failure.

In the present study, multivariate logistic regression analysis was performed to adjust for gender-specific differences. Gender-specific differences (adjusted OR 1.206, 95% CI 0.86–1.897, $P > 0.05$) were not significant differences correlated with increased plasma miR-181a expression levels and there were similar trends for kinetics of plasma miR-181a in both male and female patients according to multivariate logistic regression analysis. Gender is an important determinant of cardiovascular risk, and men generally develop cardiovascular disease earlier than women [53]. AMI is the main cause of mortality in men and the incidence increases with age in both genders, but for men the increase is more pronounced [53]. The incidence of coronary artery disease is 3–4-fold higher in men than in women; a male predominance was also found in the incidence of myocardial infarction in industrialized countries, which is consistent with the gender differences determined in our study [54]. In the Framingham cohort, 797 cardiovascular events occurred during 16-year median follow-up among men; 571 events occurred over 19-year median follow-up among women. In the cohorts from China, 254 and 99 events were recorded in men and women, respectively, during the first 8 years of follow-up [55]. There are also gender differences within the spectrum of coronary heart disease and men have higher risk of AMI [56]. The gender gap in coronary artery disease may be influenced by two characteristics of risk factors. First, the prevalence of risk factors for CHD in the population may vary

between men and women. Atherogenic risk factors that are more prevalent in men or protective factors that are more prevalent in women are expected to contribute to a higher incidence of disease in men than in women [57]. Cardiovascular risk factors such as smoking, obesity, and hypertension and higher levels of blood glucose and lipids were more prevalent in men [58]. Second, women may be biologically protected from a particular risk factor compared with men, resulting in the greater susceptibility of men or resistance of women to the same level of the risk factor [57]. The burden of coronary heart disease is consistently higher in men, but gender differences in lifestyle and acquired risk factors do not fully explain this disparity [56]. Sex-specific data focused on cardiovascular disease have increased, but this information is not routinely collected or translated into practice.

These single-center results may not be easily extrapolated. Therefore, additional studies with larger cohorts of patients with AMI and control subjects are needed to validate the diagnostic value of miR-181a as a practical biomarker. Most previous studies have been limited by conducting more broad measurements in very small numbers of individuals. However, Freedman *et al* recently presented comprehensive data demonstrating the broad and consistent detection of numerous classes of circulating miRNAs from diverse genetic backgrounds with a large population including 2763 participants, which presents the largest description of plasma-based miRNAs in a large population [59]. They found that nearly 500 of the most abundant extracellular transcripts such as miRNA and piRNA were expressed in almost all participants [59]. These findings show that diverse classes of circulating miRNAs, including hsa-miR-181a-5p, are consistently present in the plasma from multiple human populations [59]. hsa-miR-181a-5p was expressed in nearly all participants with mean ($\pm$ s.d.) Ct values of 19.39 ± 1.50 and sex (OR 0.96, 95% CI 0.84–1.09, $P = 0.5$) and age (OR 1.08, 95% CI 0.95–1.23, $P = 0.2$) were not significantly correlated with plasma miR-181a expression levels [59]. These results from the diverse populations are consistent with our results in a small single center study in China. Although we assessed plasma miR-181a levels immediately after admission and after PCI, serial measurements of miR-181a may be more useful for evaluating changes in inflammatory status, estimating risk during the follow-up period, and directing in- and out-patient treatments. Further studies are required to clarify the value of miR-181a for prognostic prediction of AMI.

The mechanism by which miR-181a is involved in the regulation of oxidative stress and inflammatory response and associated with infarction is predicted as follows. Oxidative stress, defined as excess ROS production, plays an important role in the pathophysiology of cardiac remodeling including cell death and contractile dysfunction during myocardial infarction. First, Ca^{2+} and ROS signaling interact: Ca^{2+} can increase ROS production, and ROS can significantly affect Ca^{2+} influx into the cell and intracellular Ca^{2+} stores. miR-181a was associated with increased cell susceptibility to oxidative and endoplasmic reticulum stresses via the suppression of ischemia-induced cytosolic $[\text{Ca}^{2+}]$ [43]. Second, miR-181a, which has been reported to play a vital role during the inflammatory response in the cardiovascular system, protects the system from inflammation injury by interfering NF- κ B functioning and cell proliferation during oxidative stress [60]. Finally, miR-181a can attenuate ox-LDL-stimulated immune inflammation responses by targeting c-Fos in DCs and maintain the fate of immune cells by regulating Toll-like receptor signaling in innate immunity [21, 61].

In summary, the present study showed that circulating miR-181a levels in patients with AMI were significantly altered in a time-dependent manner. This indicates the value of plasma miR-181a as a novel biomarker for the diagnosis of AMI. Additional studies are needed to clarify the value of miR-181a for the diagnosis and prognostic prediction of AMI.

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Disclosure Statement

The authors declared no conflicts of interest.

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