MicroRNAs in acute myocardial infarction: Evident value as novel biomarkers?

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Abstract

Traditional circulating biomarkers play a fundamental role in the diagnosis and prognosis of acute myocardial infarction (AMI). However, they have several limitations. microRNAs (miRs), a class of RNA molecules that do not encode proteins, function directly at the RNA level by inhibiting the translation of messenger RNAs. Due to their significant roles in disease development, they can be used as biomarkers. Accumulating evidence has revealed an attractive role of miRs as biomarkers of AMI and its associated symptoms, including vulnerable atherosclerotic plaques, and their role in disease diagnosis, platelet activation monitoring, and prognostic outcome prediction. This manuscript will highlight the recent updates regarding the involvement of miRs as biomarkers in AMI and emphasize their value in vulnerable atherosclerotic plaque prediction and monitoring of platelet activation. *(Anatol J Cardiol 2018; 19: 140-7)*

Keywords: acute myocardial infarction, microRNAs, biomarkers, atherosclerotic plaques, platelet activation

Introduction

Coronary artery disease (CAD) seriously threatens the health and quality of human life and is the leading cause of death worldwide. Acute myocardial infarction (AMI) is often the first manifestation of CAD. The high incidence and case fatality of AMI is, to a large extent, a consequence of its late diagnosis and lack of highly sensitive and specific markers. Thus, early diagnosis may be particularly important in patients in whom the time lapsed from onset of symptoms is short. In addition, risk stratiŞcation, vulnerable plaque identification, personalized medicine, and prognostic assessment are also important aspects in the management of CAD patients.

Traditional circulating biomarkers, such as cardiac troponin T (cTnT), have facilitated the diagnosis and prognostic assessment of AMI, but have certain limitations. cTnT begins to rise within 3–4 hours after the onset of myocardial injury, eliminating early AMI diagnosis within first 1–2 hours. With the recent clinical application of a highly sensitive cTnT (hs-cTnT) assay, analytical sensitivity has been further improved; however, the specificity is relatively reduced because cTnT can be more easily detected in non-AMI patients, such as those with heart failure or pulmonary embolism (1). Moreover, the therapeutic monitoring of platelet activity and predictive capacity of future cardiovascular mortality is nowadays limited. Owing to these limitations, novel biomarkers, such as molecular and genetic biomarkers, are increasingly being considered for CAD management.

Over recent years, microRNAs (miRs), due to their ideal characteristics, have been studied not only as biomarkers for onset of disease but also for risk prediction of adverse events and platelet activation monitoring in antiplatelet therapy. In this manuscript, we focus on the latest progress achieved in the engagement of circulating miRs as novel biomarkers for AMI, including vulnerable atherosclerotic plaque prediction and platelet activation monitoring (Fig. 1).

Characteristics of miRs as novel biomarkers

miRs are a class of endogenous, single-stranded non-coding RNA molecules with a length of 19–23 nucleotides that play a role in negative post-transcriptional regulation (2). They function by binding to complementary sequences on messenger RNAs and blocking translation of the messenger RNA into protein. miRs not only regulate key functions in the healthy heart but also play a curial role in certain mechanisms of heart disease, such as cardiac hypertrophy, fibrosis, and apoptosis (3). miRs can be released into biological fluids, including blood, from dying cells, such as ne-

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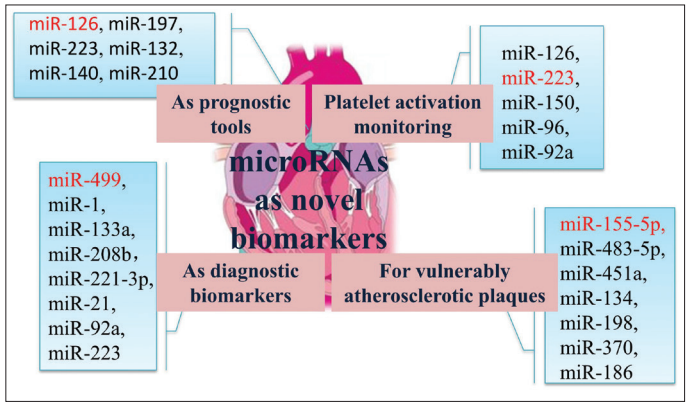

Figure 1. Profile of the circulating miRs as biomarkers for acute myocardial infarction. All known miRs are listed here, and the best known and well understood miRs are presented in red

crotic cardiomyocytes following AMI, or actively secreted from living cells under stimulation. miRs may be integrated into protein complexes, lipids, or high-density lipoproteins or trapped in exosomes, microvesicles, or apoptotic bodies. miRs show a high degree of stability in circulation and withstand conditions such as long-term storage, multiple freeze/thaw cycles, and different pH (4). Furthermore, circulating miRs are stable and can be easily detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays, which increases the potential for their use as biomarkers for diagnosis, prognosis, or in response to cardiovascular therapeutics.

As concluded by Poller et al. (5), miRs meet the following criteria of an ideal biomarker: easily accessible by non-invasive methods, quite stable in body fluids, sensitive to CAD pathology, particularly expressed in pathogenic processes, and reliable indicators before the appearance of clinical symptoms. Some endothelial cell-enriched miRs, such as miR-126, miR-17, and miR-92a, can be secreted into the circulation when vascular injury affected by any form of cellular stress, such as anoxia, lactic acidosis, or dyslipidemia, is observed. In AMI, these events occur earlier than tissue necrosis. The merits of circulating miRs make them novel potential biomarkers for CAD.

As markers for vulnerable atherosclerotic plaques

Atherosclerosis, a major player in the development of CAD, represents a chronic inflammatory disease of the arterial wall initiated by endothelial injury and subendothelial lipoprotein retention, particularly at sites of disturbed blood flow. Cellular effectors and molecular pathways that are implicated in the pathogenesis of atherosclerosis play critical roles in the initiation and progression of atherosclerotic plaques (6). These molecules include inflammatory cytokine, signal transducer and activator of transcription, and mitogen-activated protein kinase. The rupture of coronary atherosclerotic plaques is the culprit of acute coronary syndrome. Thus, early detection of this pathologic phenomenon is critical in the prevention, prognosis, and therapeutic intervention in CAD. Nonetheless, partially due to the lack of an appropriate plaque rupture model, there are still no definitive biomarkers.

miRs are not only regulators of the key signaling pathways involved in lipid homeostasis, endothelial cell inflammation, and induction of atherosclerosis (7) but also emerging biomarkers for atherosclerotic plaques. Wang et al. (8) compared miR expression levels in coronary artery tissues with early atherosclerotic plaques with those in normal coronary artery tissues using microarray analysis. The tissues used in their study were obtained directly from three human coronary artery samples of 18–25-year-old male donors, and the authors identified 101 differentially expressed miRs. Subsequently, qPCR confirmed that miR-221, miR-155, and miR-100 were significantly downregulated in plaques, whereas miR-1273 was significantly upregulated (8). Their study provided identification of miRs involved in atherosclerosis at the tissue level. However, due to sample scarcity, it was difficult to further validate the results.

Another recent study performed by Li et al. (9) aimed to assess the possibility of circulating miRs being biomarkers of acute coronary plaque rupture by virtue of the natural model of percutaneous coronary intervention (PCI)-induced plaque rupture. Stable CAD patients recruited in their study underwent PCI with single stent implantation. Plasma miRs before and after balloon dilatation were compared using a profiling–replication–validation model. Their results demonstrated that miR-155-5p and miR-483-5p were upregulated within 0.5 and 1 hours of plaque rupture and miR-451a was downregulated only 0.5 hours after plaque rupture. Moreover, of the three miRs, miR-483-5p showed the highest discrimination ability in the early identification of plaque rupture at 0.5 and 1 hours. In their study, patients who had only undergone coronary artery angiography were enrolled as negative controls to exclude the effects of other factors (e.g., contrast agent) on circulating miRs. In addition, heparinase was added to the RNA samples to eliminate any effects of heparin on miR measurements. However, the results from this well-designed research study still need to be further verified in larger cohorts.

In addition, miRs have also been proposed as potential biomarkers for vulnerable plaques (10). Hoekstra et al. (11) stated that patients with unstable angina pectoris could be discriminated from stable patients based on the increased peripheral blood mononuclear cell levels of miR-134, miR-198, and miR-370. Their results suggested that the miR signatures can be used to identify patients at risk for acute coronary syndrome (ACS). When the compared group was of patients with non-coronary chest pain (instead of those with stable angina), a different cassette of miRs (miR-132, miR-150, and miR-186) was found to be increased in patients with unstable angina (12). Although studies in this field are relatively limited, miRs have now fundamentally expanded our spectrum of diagnostic options.

As diagnostic biomarkers for disease onset

One of the main aspects explored when investigating miRs as CAD biomarkers is their diagnostic potential in AMI. Four

Table 1. miRs as diagnostic biomarkers for disease onset of AMI

miRs	Patient number	Sample	Biomarker validation	References
miR-208b, -499↑	32 AMI/15 viral myocarditis pt/36 subjects with chest pain	Plasma	Correlated with cTnT	Corsten et al. 2010 (15)
miR-1, -208, -499↑	70 AMI/72 healthy controls	Plasma	–	Liu et al. 2015 (16)
miR-208a↑	19 AMI/20 control	Plasma	Correlation to cTnI/CK-MB; earlier peak	Bialek et al. 2015 (17)
miR-1, 133a/b, 499-5p↑; -122↓	33 AMI/17 healthy control	Plasma	Correlation to cTnI	D'Alessandra et al. 2010 (18)
miR-1, -133a, -208b↑	444 CAD pt, thereof 327 AMI	Plasma	Correlation to hs-cTnT	Widera et al. 2011 (19)
miR-208b, -499↑	510 AMI/87 healthy subjects	Plasma	Correlation to CK/ hs-cTnI; more positive	Devaux et al. 2012 (20)
miR-1, -208a, -499, -21, -146a↑	332 suspected ACS pt, thereof 81 AMI and 25 UA	Serum	Superior to hs-cTnT	Oerlemans et al. 2012 (21)
miR-1, -208b, -499↑	319 MI/88 non MI	Serum	Inferior to cTnT	Gidlöf et al. 2013 (22)
miR-208b, -499, -320a↑	1155 chest pain pt, thereof 224 confirmed AMI	Serum	miR-208b, inferior to cTnT/hs-cTnT	Devaux et al. 2015 (23)
miR-221-3p↑	27 ACS/16 controls	Plasma	Relations with Troponin, GRACE and Syntax score	Coskunpinar et al. 2016 (24)
miR-92a↑	37 AMI/42 stable CAD/35 healthy adults	Endothelial microparticles	Inferior to cTnI	Zhang et al. 2017 (25)
miR-1291, -663b↓	20 AMI/20 control	Total blood	Correlation to cTnI	Meder et al. 2011 (26)
miR-1, -134, -186, -208, -223, -499↑	117 AMI/182 CAD/100 control	Serum	Panel of six miRs superior to predict MI	Li et al. 2013 (27)
miR-1, -21 -133a, -423-5, -499-5p↑	92 non-STEMI/81 acute heart failure pt/99 control	Plasma	miR-499-5p, superior to cTnT/hs-cTnT	Olivieri et al. 2013 (28)
miR-26a, -191↓; -208b↑	87 AMI/87 healthy controls	Plasma	–	Li et al. 2015 (29)
miR-181a↑	60 AMI/60 UA/60 controls	Plasma	Correlated with cTnI/CK-MB	Zhu et al. 2016 (30)
miR-19b-3p, -134-5p, -186-5p↑	18 AMI/20 matched controls	Plasma	Correlation to cTnI	Wang et al. 2016 (31)
miR-21, -361↑; -519e↓	17 AMI/28 control	EDTA plasma	Correlation to cTnI	Wang et al. 2014 (32)
miR-21↑	17 AMI/39 angina pectoris/10 controls	Plasma	Similar to CK, CK-MB and cTnI	Zhang et al. 2016 (33)
miR-1, -21, -29b↑	44 AMI/18 matched controls	Plasma	Correlated with ventricular remodeling	Grabmaier et al. 2017 (34)

ACS - acute coronary syndrome, AMI - acute myocardial infarction, CAD - coronary artery disease, CK - creatine kinase, CK-MB - creatine kinase-MB, cTnI - cardiac troponin I, cTnT - cardiac troponin T, GRACE - global registry of acute coronary events, hs-cTnT - high-sensitive cTnT, pt - patients, STEMI - ST-segment elevation myocardial infarction, Syntax - the synergy between percutaneous coronary intervention with taxus and cardiac surgery, UA - unstable angina

↑ - increased, ↓ - decreased

cardiac-enriched miRs (e.g., miR-499, miR-1, miR-133a, and miR-208b) were consistently found to be rapidly upregulated in plasma after myocardial necrosis in a majority of studies (13-17). For example, D'Alessandra et al. (18) measured circulating levels of miRs in 33 patients with ST-elevation AMI and in 17 healthy subjects. They observed upregulation of miR-1, miR-133a, miR-133b, and miR-499-5p and downregulation of miR-122 and miR-375 in patients with AMI compared with controls. Besides small-scale studies, some large-scale investigations were also performed to assess the ability of miRs to distinguish between patients with AMI and controls. Widera et al. (19) measured miR-1, miR-133a, miR-133b, miR-208a, miR-208b, and miR-499 concentrations in plasma samples obtained from 444 patients with ACS on the day of their admission. miR-1, miR-133a, miR-133b, and miR-208b were independently associated with hs-cTnT levels. Patients with AMI showed higher levels of miR-1, miR-133a, and miR-208b compared with those with unstable angina. However, all six investigated miRs showed a large overlap between patients with

unstable angina and those with AMI. A case-control study of 510 patients with AMI referred for primary mechanical reperfusion and 87 healthy controls was published (20). Circulating levels of cardiac-enriched miR-208b and miR-499 were highly elevated in patients with AMI and almost undetectable in healthy controls. Patients with ST-elevation AMI (n=397) had higher miR concentrations than those with non-ST-elevation AMI (n=113). Both miRs correlated with peak concentrations of the cardiac markers creatine kinase and cTnT, indicating a relationship with the infarct size. miR-499 and hs-cTnT provided comparable diagnostic values to discriminate between patients with AMI and controls, but no incremental diagnostic value of hs-cTnT could be demonstrated.

In addition, the ability of miRs to be used as a diagnostic marker for AMI was investigated in populations of patients presenting with suspected CAD to the emergency department. Oerlemans et al. (21) determined the potential diagnostic value of circulating miRs as novel early biomarkers in 332 patients suspected of having ACS on admission to the emergency department in a prospective single-center study focused on cardiac miRs (miR-1, miR-208a, and miR-499), miR-21 and miR-146a. Levels of all miRs studied were significantly increased in 106 patients diagnosed with ACS, including those with initially negative hs-cTnT or with symptom onset <3 hours prior. miR-1, miR-499, and miR-21 significantly increased the diagnostic value in all patients suspected of having ACS when added to hs-cTnT results. These three miRs were strong predictors of ACS independent of clinical covariates, including patient history and cardiovascular risk factors. Interestingly, the combination of these three miRs resulted in a significantly higher diagnostic value than hs-cTnT alone. In another study conducted by Gidlof et al. (22), circulating miR-1, miR-208b, and miR-499-5p were assessed for discrimination of a clinical diagnosis of AMI in a cohort of 424 patients with suspected ACS. miR levels were higher in patients with AMI and correlated with the left ventricular ejection fraction. However, the discrimination value of miR-208b and miR-499-5p was lower than that of cTnT. The diagnostic value of six miRs (miR-133a, miR-208b, miR-223, miR-320a, miR-451, and miR-499) was also assessed in a cohort of 1155 consecutive patients presenting with acute chest pain to the emergency department, including 224 patients with an adjudicated final diagnosis of AMI (23). miR-208b, miR-499, and miR-320a levels were significantly higher in patients with AMI compared with those with other final diagnoses. miR-208b provided the highest diagnostic accuracy for AMI, with an area under the receiver operating characteristic curve of 0.76. However, in this study, none of the tested miRs outperformed or added diagnostic value to cTnT or hs-cTnT.

Recently, Coskunpinar et al. (24) assessed miR profiling in 27 patients admitted consecutively to the emergency department with acute chest pain and/or dyspnea who were diagnosed with ACS and in 16 non-ACS control subjects. miR-221-3p, a member of the antiangiogenic gene-regulating miR family, was one of the two most dysregulated miRs, with a fold regulation of 3.89. There was a significant positive correlation between miR-221-3p and both troponin and global registry of acute coronary events (GRACE) and Syntax scores. Moreover, miR221-3p was found to be significantly inversely correlated with the left ventricular ejection fraction. miR-221-3p was the most prominent biomarker candidate, with an area under the curve of 0.881. In another recent study conducted by Zhang et al. (25), the diagnostic value of endothelial microparticles and miR-92a as biomarkers in distinguishing patients with AMI from those with CAD was investigated. Plasma samples from 37 patients with AMI, 42 patients with stable CAD, and 35 healthy adults were collected. The number of CD31+/CD42b– endothelial microparticles and the expression of miR-92a were higher in the AMI cohort than in the two other cohorts. Furthermore, evidence showed that there was a positive correlation between the levels of CD31+/CD42b– endothelial microparticles and miR-92a. CD31+/CD42b– endothelial microparticles and miR-92a might have great potential to provide diagnostic value for AMI and may regulate endothelial dysfunction in patients with AMI. However, their diagnostic values were inferior to those of cardiac troponin I. Other non-cardiac-enriched miRs, such as miR-1291 (26), miR-223 (27), miR-423 (28), miR-26a (29), miR-181a (30), miR-134-5p (31), and miR-21 (32-34), have also been proposed as indicators with high sensitivity and speciŞcity for AMI (Table 1).

It is worth noting that most studies have demonstrated that the diagnostic value of miRs did not outperform troponins. This might be explained in part by the fact that the diagnosis of AMI was established with hs-cTnT in clinical investigations. However, studies conducted on patients suspected of having ACS presenting to the emergency department suggested that miR levels were significantly altered even in those who were initially negative for hs-cTnT or with symptom onset <3 hours (20, 21) prior. Thus, circulating miRs hold great potential as novel early biomarkers for the management of patients suspected of having ACS. Because of index heterogeneity, such as specimen types, geographical region, age, sample size, sampling time, comorbidities, medication, and the severity of AMI, the clinical application of miRs for early detection of AMI still requires prospective large-scale studies for further validation. In addition, it is important to note that heparin can inhibit the reverse transcription reaction of miRs. Although the vast majority of earlier studies processed blood samples with EDTA or sodium citrate, researchers did not pay attention to the presence of heparin in the bloodstream because of systemic heparinization before PCI. This issue is of great importance, and it should be taken into consideration in future research.

For platelet activation monitoring

Adequate platelet inhibition is a cornerstone of treatment for CAD. However, the effect of current antiplatelet agents varies considerably due to factors, such as bioavailability and drug-related genetic polymorphisms. Thus, monitoring platelet function and adjusting antiplatelet therapy accordingly could be beneficial for selected patients. Recent routine laboratory platelet function tests include light transmission aggregometry, multiple electrode aggregometry, VerifyNow $P2Y_{12}$ aggregation test, and

vasodilator-stimulated phosphoprotein (VASP) phosphorylation (35). These tests are beneficial to detect high platelet reactivity on clopidogrel treatment and predict the risk of bleeding and thrombotic events but with three serious limitations. First, these platelet function assays are performed ex vivo. Differences in plasma preparation, placement time, pH value, temperature, ion concentration, etc. impact platelet activity *in vitro*. Thus, the results cannot fully reflect the actual platelet status. Second, the above methods mainly detect adenosine diphosphate-induced platelet aggregation. This is accurate in the detection of patient responses to $P2Y_{12}$ receptor antagonists, but it is difficult to accurately reflect responses to aspirin. Third, the method is not well-standardized between laboratories and shows high inter- and intra-individual variability. Due to the abovementioned deficiencies, clinical trials based on conventional platelet function testing to adjust antiplatelet therapy for coronary stenting have failed to obtain positive results. Consequently, there is clear clinical interest in the development of novel biomarkers.

Various studies have analyzed the capacity of circulating miRs to reflect platelet activation. Shi et al. (36) showed that circulating miR-223, an miR with the ability to bind to the 3′ UTR of human $P2Y_{12}$ receptor mRNA, may serve as a novel marker for the assessment of clopidogrel responsiveness in patients with troponin-negative non-ST-elevation ACS. Another study showed that the circulating levels of miR-126-3p, miR-223, and miR-150 were significantly reduced and those of miR-96 were increased after the therapeutic switch from dual antiplatelet treatment with clopidogrel to that with ticagrelor (37).

The correlation between the expression levels of platelet-derived miRs and traditional indicators of platelet activity was also investigated. In a study conducted by Kaudewitz et al. (38), plasma miRs were measured in 125 patients, with a history of ACS, who had undergone detailed assessment of platelet function 30 days after the acute event. Levels of platelet-related miR-126, miR-223, miR-24, and miR-191 were significantly correlated with VASP assay results. For correlation with the VerifyNow $P2Y_{12}$ assay, statistical significance was only noted for miR-126. miR-126 and miR-223 were also strongly correlated with plasma levels of platelet activation markers, including P-selectin, platelet factor 4, and platelet basic protein, in a population-based Bruneck study (n=669). Moreover, a single nucleotide polymorphism, rs4636297 AA genotype, that facilitates processing of pri-miR-126 to mature miR-126, accounted for a rise in circulating platelet activation markers. Inhibition of miR-126 in mice reduced platelet aggregation by directly and indirectly affecting a disintegrin and metalloproteinase domain-containing protein 9 and $P2Y_{12}$ receptor expression. However, there was no correlation between platelet-related miRs and platelet reactive units or VASP in patients with AMI during the perioperative period of PCI. In a recent study conducted by Li et al. (39), the platelet function of 15 patients with ST-elevation AMI was measured by VerifyNow and VASP assays at pre-PCI and 48 hours after PCI. No significant differences among the levels of platelet miRs, including miR-21, miR-126, miR-150, and miR-223, were observed between normal and low responders, as determined by VerifyNow. Similar results were also observed for the VASP assay. There are three possible reasons to explain the contradictory results during PCI. First, the small number of subjects may confound the results. Second, the heparin administered during PCI may affect the RT-qPCR, making the results less reproducible. Third, the release of miRs from platelets after platelet activation is a dynamic process. However, the best detection time is unclear. Inconsistency in the detection time points may lead to discrepancies. Thus, further studies are warranted to clarify these doubts.

Aspirin, another antiplatelet drug, is widely used for treating cardiovascular patients. A recent meta-analysis has revealed that approximately 25% of patients experienced insufficient platelet inhibition due to aspirin resistance and remain at risk of cardiovascular events despite their antiplatelet therapy (40). Binderup et al. (41) demonstrated that aspirin resistance can potentially be identified by determining miR-92a levels in plasma in combination with the platelet distribution width. In particular, 10 of 50 patients with intermittent claudication on daily aspirin therapy were defined as being aspirin resistant, with arachidonic acid-stimulated aggregation test results ≥30 U. The optimal cut-off values to discriminate between aspirin responders and aspirin resistant patients were found to be >11.8 fL for platelet distribution width and >4.5 for the relative expression level of miR-92a. Interestingly, the profiles of circulating miRs derived from platelets can be affected by antiplatelet therapy. Willeit et al. (42) demonstrated that the plasma levels of platelet miRs, such as miR-223, miR-191, miR-126, and miR-150, were decreased on platelet inhibition. Therefore, the effect of antiplatelet therapy involved in the therapeutic strategy of CAD should be taken into account when designing clinical studies in pursuit of novel biomarkers.

In addition, miR levels were different in platelets, platelet microparticles, platelet-rich plasma, platelet-poor plasma, and serum. Platelet-rich plasma showed markedly higher levels of miRs than serum and platelet-poor plasma (42). Thus, careful consideration should be given to the sample preparation procedures for miR analysis. If plasma samples have a high residual platelet content, their miR profile may be more similar to serum samples and reflect predominantly platelet miR content rather than platelet miR release (43). Uniform use of platelet-poor plasma was recommended to avoid confounding of results caused by residual platelets in plasma and to allow comparisons between sample measurements.

As prognostic tools

Predicting the outcome after AMI can offer information about cardiac dysfunction; however, there are still no appropriate prognostic biomarkers recommended for use in clinical practice. B-type natriuretic peptide (BNP), particularly its N-terminal pro-form, is currently considered to be the optimal prognostic tool for AMI. However, BNP lacks public acceptance due to its long half-life and

şuctuating levels in plasma upon AMI. In addition, BNP levels are not only increased in cases of ischemic heart injury but also affected by other confounding factors, such as tachycardia, renal dysfunction, age, obesity, and medication. The release of specific miRs into the circulation may reflect the activation of molecular pathways that have an impact on the clinical outcome after AMI. Adding attractive value to miR markers for risk prediction is of particular interest.

The cardiomyocyte-enriched miRs involved in the AMI diagnosis were also found to have a prognostic value. In a study conducted by Widera et al. (19), miR-133a and miR-208b levels were significantly associated with the risk of death during a 6 month follow-up period, but both miRs lost their independent association with outcome upon further adjustment for hs-cTnT. Gidlof et al. (22) also assessed the association of circulating miR-1, miR-208b, and miR-499-5p for 30-day mortality and diagnosis of heart failure. miRs correlated with left ventricular ejection fraction. Increased levels of miR-208b and miR-499-5p were strongly associated with increased risk of mortality or heart failure within 30 days, but the association was lost when adjusting for cTnT. In a study by Devaux et al. (23) on 1155 patients with acute chest pain, 102 patients died in the course of a 2-year follow-up. Levels of miR-208b were higher in patients who died within 30 days, but the prognostic accuracy was only low to moderate. In addition, none of the tested miRs helped in the prediction of long-term mortality.

The prognostic value of other non-cardiac-enriched miRs was also researched. A recent multicenter prospective study of 1002 patients with ST-elevation AMI suggested that miR-26b-5p, miR-320a, and miR-660-5p are associated with major cardiovascular events within 1 year of follow-up and with an increased risk prediction when added to the GRACE score and a clinical model (44). Zampetaki et al. (45) found that serum miR-126 levels were positively associated with the risk of AMI, whereas miR-197 and miR-223 levels were inversely related to disease risk. These results were obtained by following a cohort of 820 individuals for 10 years. Another study by Yu et al. (46) showed that higher plasma miR-126 levels predicted major adverse cardiac events in 491 patients after PCI during dual antiplatelet therapy. The prognostic potential of miR-197 and miR-223 as predictors of cardiovascular mortality was later corroborated in a cohort of 873 patients with CAD within 4 years of follow-up (47).

miR-132, miR-140, and miR-210 were also associated with cardiovascular death in a cohort of 1112 patients with documented CAD, including 430 patients with ACS and 682 patients with stable angina pectoris (48). Bye et al. (49) recently proposed that a panel of 5 miRs (miR-106a-5p, miR-424-5p, let-7g-5p, miR-144-3p, and miR-660-5p) is able to improve AMI risk prediction in healthy individuals. It is worth noting that miR-328, miR-134, miR-133a, and miR-208b were strongly associated with increased mortality in patients with AMI (19, 50). These results show the promising applicability of miRs as biomarkers in primary and secondary prevention of CAD. In addition, the potential of miRs as novel markers of left ventricular remodeling after AMI was also reported. Grabmaier et al. (34) measured plasma levels of miR-1, miR-21, miR-29b and miR-92a in 44 patients at days 4 and 9 and 6 months after AMI and in 18 matched controls. miR-1 and miR-29b levels significantly correlated with infarct volume changes. Only miR-29b levels were associated with changes to left ventricular end-diastolic volume over time. However, there was no correlation of miR levels with a combined cardiovascular endpoint in long-term follow-ups. Although many miRs have been assessed, their prognostic value for AMI is hampered by contradictory results and there remain no optimal biomarkers for risk stratification and adverse outcome prediction.

Challenges of circulating miRs as biomarkers

Despite studies that reported encouraging results for circulating miRs as novel biomarkers for AMI, there are still some challenges that need to be considered before drawing clinical conclusions. First, most of the sample sizes considered in these studies were limited; thus, the clinical application of miRs for AMI detection still requires long-term and follow-up studies for further validation. Second, standard operational procedures, including the choice of material, sample isolation, detection, processing techniques, and normalization strategies, have not yet been established. In particular, miR levels can vary greatly between the different choices of material (e.g., serum or plasma) and the different isolation procedures (e.g., PCR, microarrays, sequencing, with low correlations between these techniques). Moreover, non-cardiac diseases and phenotypes (e.g., end-stage kidney disease, age, and sex) and intake of medicines (e.g., statins and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker) may influence circulating miR levels. Furthermore, current normalization strategies for reporting miR levels are generally not standardized, ranging from relative normalization to endogenous references (e.g., RNU6, miR-16, and let-7a) or exogenous references (e.g., Caenorhabditis elegans miR-39/54, Quanto EC1/2). Third, current miR screening methods tend to be expensive and time-consuming, limiting their use in clinical practice. Fourth, the lack of speciŞcity of the miRs, proposed as biomarkers, is another limitation. For example, miR-126 has been strongly associated with coronary diseases. However, this miR is also closely linked to heart failure and diabetes, raising questions about its clinical usefulness. Lastly, circulating miRs cannot indicate lesion distribution and severity. Thus, the status of imaging modalities is irreplaceable. Therefore, automatization of work flow is of great importance. Reliable isolation methods, cross-platform accuracy, and standardization are needed to generate robust and reproducible results and to pave the way for the application of miR research in clinical practices.

Conclusions

Increasing evidence has revealed that miRs have great potential as novel diagnostic and prognostic biomarkers for CAD, particularly for AMI. Such biomarkers are clearly needed to help guide clinical decision making in AMI diagnosis and prognosis

and to ease the transition from conventional therapy to personalized and precision medicine. However, there remain certain limitations that need to be overcome before clinical application of these miRs. Circulating miRs have the potential to be promising biomarkers for AMI and miR variability due to technical and analytical factors is expected to be eliminated by accumulation of more data regarding this issue in future studies.

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