Review Article

Extracellular miRNAs for predicting risk following acute myocardial infarction: a narrative review

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Contributions: (I) Conception and design: Both authors; (II) Administrative support: Both authors; (III) Provision of study materials or patients: Both authors; (IV) Collection and assembly of data: Both authors; (V) Data analysis and interpretation: Both authors; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

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Background and Objective: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide, and survivors are at an increased risk of experiencing adverse left ventricular (LV) remodelling and major adverse cardiovascular events (MACE). As such, the development of non-invasive tools to predict risk following AMI remains a clinical priority. MicroRNAs (miRNAs) are small-noncoding RNAs that regulate gene expression and have come to prominence as a novel class of circulating biomarkers in AMI. In this narrative review, we summarise the current evidence that links circulating miRNAs with parameters of LV remodelling and MACE following AMI, and investigate clinical challenges that must be overcome to facilitate the translation of miRNAs into clinical use.

Methods: A literature search was conducted using PubMed and Web of Science for research articles that investigated circulating miRNAs as prognostic tools following AMI, with an emphasis on articles that examined LV remodelling and MACE as clinical outcome measures. Articles written in English and published prior to April 30th 2022 were included.

Key Content and Findings: A number of novel candidate miRNA biomarkers have been linked to LV remodelling and MACE following AMI. Of these miRNAs, only a handful have been successfully reproduced in independent cohorts and some, but not all, have been identified as equal or superior to current prognostic tools. Measuring miRNAs in combination has proved promising and may be a superior approach when compared to single miRNA analysis. Heterogeneous strategies to measure miRNAs in human biological samples, generally smaller sample sizes and discordance in study design and clinical endpoints are just some of the clinical challenges that must be overcome to strengthen research in this field.

Conclusions: Circulating miRNAs hold promise in patient risk stratification following AMI. Future studies should utilise standardised methodologies, prioritise larger multicentre studies, incorporate miRNA panels over single miRNA analysis and should routinely compare miRNA prognosis to current clinical tools.

Keywords: MicroRNAs (miRNAs); biomarkers; acute myocardial infarction (AMI); left ventricular (LV) remodelling; major adverse cardiovascular events (MACE)

Received: 30 September 2022; Accepted: 29 January 2023; Published online: 22 September 2023.
doi: 10.21037/exrna-22-21
View this article at: https://dx.doi.org/10.21037/exrna-22-21

Introduction

Coronary artery disease (CAD) remains a major health and economic priority worldwide. In 2019, CAD accounted for approximately 50% of all global cardiovascular disease (CVD) mortality and was responsible for an estimated nine million deaths (1). Acute myocardial infarction (AMI) is a common presentation of CAD. While survival following AMI has improved in recent decades due to advancements in revascularization strategies and medical therapies (2-5), population growth and aging has caused the net burden of CVD to remain high (1).

Survivors of AMI are at increased risk of experiencing major adverse cardiovascular events (MACE) when compared to the general population (6,7). Adverse left ventricular (LV) remodelling underpins the development of many cardiac conditions secondary to AMI and describes progressive geometric and functional changes to the myocardium in response to damage (8). Despite modern therapeutic management strategies that include timely revascularization and optimal pharmacotherapy, up to 30% of patients will continue to experience adverse LV remodelling in the months and year following AMI (9,10). This persistence is of significant clinical concern, as patients with LV remodelling are at an increased risk of developing heart failure and poor health outcomes (9,11).

Heart failure is a complex pathophysiology that is associated with impaired ventricular filling and reduced cardiac output (12). The prevalence of heart failure is estimated as 1–2% of the global adult population (13). Importantly, heart failure diagnosis is associated with poor health outcomes in AMI patients (14), and is a common primary or composite endpoint for MACE. In addition to LV remodelling and subsequent heart failure, MACE also commonly comprises unplanned revascularization, reinfarction and all-cause or cardiac-specific mortality, which are all clinical pathologies elevated in patients following AMI (15). Thus, early identification of AMI patients at risk of developing adverse LV remodelling or MACE is of utmost importance for reducing the incidence of morbidity and mortality in this population.

Natriuretic peptides are the gold-standard biomarkers in heart failure diagnosis and management (13,16). Comprised of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and B-type natriuretic peptide (BNP), natriuretic peptides are elevated in response to ventricular wall stretch, dilation and pressure (17,18). While biomarker research has demonstrated that natriuretic peptides have prognostic potential following AMI (19,20), BNP and NT-proBNP are not routinely measured following hospitalisation. An important limitation of measuring natriuretic peptide levels is non-specific elevation in individuals due to differences in clinical features and presentation (21,22). In addition, natriuretic peptides cannot predict damage and are instead elevated as a consequence of irreversible cardiac remodelling processes (17).

More recently, microRNAs (miRNAs) have come to prominence as a novel class of circulating biomarkers that may have some potential at predicting outcomes in patients following AMI (23,24). miRNAs are short non-coding RNAs (~22 nt in length) that are transcribed as primary miRNAs mostly from the intronic regions of genes. These are then processed into precursor miRNAs and transported to the cytoplasm to be cleaved into mature miRNA [reviewed in Zhang *et al.*, in 2020 (23)]. Mature miRNAs regulate the expression of multiple genes by binding to complementary 3' untranslated regions of mRNA, resulting in suppression of translation or degradation of the mRNA (25). They can act as master regulators of cellular networks and have been implicated in the normal physiological functioning of cardiac tissue as well as the pathological processes related to AMI (26), adverse ventricular remodelling (27), and stroke (28). Importantly, miRNAs are also released from cells in the body and are stable in the circulation, due to their encapsulation within membrane-bound particles called extracellular vesicles (EVs) (29) or conjugation to lipoproteins or other protein complexes (e.g., Argonaute-miRNA complexes) (30). Circulating miRNAs can originate from virtually any cell in the body and can be taken up by recipient cells at distant sites where they have functional effects. Because miRNAs are directly involved in the pathogenesis of disease processes and are selectively released from cells (31), they are an ideal class of biomarkers that could potentially outperform traditional protein serum markers. Early research investigating miRNAs in AMI was focused on utility in acute diagnosis, and a recent systematic review and meta-analysis by Zhai *et al.* (26) includes many of the seminal studies associated with this field of research. Findings from these early studies, particularly the identification of an early rise in some miRNAs following AMI onset and the cardiac-specific origin of these miRNAs, became the foundation for researching the prognostic utility of circulating miRNA following AMI, which is the purpose of this review.

This review outlines what is currently known about how miRNA perform as predictors of adverse events following

Table 1 Summary of the search strategy

Items	Specification
Date of search	4th March 2022 to 30th April 2022
Databases and other sources searched	PubMed & Web of Science
Search terms used	(microRNA OR miRNA OR miR) AND ("left ventricular remodel*" OR "LV remodel*)
	(microRNA OR miRNA OR miR) AND ("major adverse cardi* outcome*" OR MACE)
	(microRNA OR miRNA OR miR) AND ("heart failure" OR HF)
	(microRNA OR miRNA OR miR) AND (mortality OR death OR "cardi* death")
Timeframe	Manuscripts published before April 30th 2022
Inclusion and exclusion criteria	Inclusion criteria:
	Primary research manuscript
	Research conducted in human participants
	Examined parameters of LV remodelling and/or MACE outcomes in an ACS context
	Exclusion criteria:
	Published in a non-English language
Selection process	Authors independently selected manuscripts based on the search strategy supplied. Manuscripts identified using the search strategy were further examined for additional references. Manuscripts were selected based on the robustness of their research. The final selection of manuscripts was agreed between authors
Any additional considerations, if applicable	Additional manuscripts were included in this review based on Reviewer suggestions

LV, left ventricular; MACE, major adverse cardiovascular events; ACS, acute coronary syndrome.

AMI (particularly LV remodelling and MACE), the current challenges for implementing miRNA biomarkers clinically, and recommendations for future research in this area. We present this article in accordance with the Narrative Review reporting checklist (available at https://exrna.amegroups.com/article/view/10.21037/exrna-22-21/rc).

Methods

PubMed and Web of Science databases were searched between March and April 2022 for all scientific publications relevant to the scope of this narrative review. Boolean expressions were searched to identify miRNA linked with LV remodelling and MACE outcomes in the context of acute coronary syndrome (ACS). More details regarding the search strategy and inclusion and exclusion criteria are outlined in *Table 1*.

miRNAs for LV remodelling

LV remodelling is a complex and progressive pathology that spans across multiple physiological processes. It is initiated by infarct expansion, which describes the thinning and dilation of the infarcted myocardium (8). Subsequently, non-infarcted myocardium undergoes volume-overload hypertrophy to preserve stroke volume, which increases intra-chamber pressures and results in ventricular chamber enlargement (32). Myocardial fibrosis is also a key mechanism associated with the progression of LV remodelling (33), with excessive and reactive fibrosis causing stiffening of the ventricles and pathological changes to cardiac structure (34). Finally, an incomplete resolution of inflammatory responses to acute cardiac injury and a failure to progress to a reparative phase has more recently been highlighted as a contributor to adverse remodelling (35).

Throughout these processes, miRNA can be released from the multiple cell types involved including cardiomyocytes, fibroblasts, endothelial cells, and immune cells (31). Multiple circulating miRNA have been linked to the development of LV remodelling following AMI (*Table 2*), many of which have also been linked to functional roles in the pathophysiology of LV remodelling. Differential expression of these miRNA

Table 2 miRNAs associated with LV remodelling

miRNA	ACS population	Sample measurement			Imaging measurement			Findings	Ref.
		Source	Timepoint(s)†	Quantification method	Quantification method	Timepoint(s)†	LV remodelling definition		
miR-1	80 STEMI	Plasma	Pre-PCI	RT-qPCR	cMRI	Admission & 6 months post-PCI	ΔLVEDV ≥10%	↑	(36)
	44 AMI	Plasma	Day 9 post-AMI	RT-qPCR	cMRI	Admission & 6 months post-AMI	Δinfarct volume	+ weak correlation	(37)
miR-24	43 patients‡	Plasma	<24 hours post-AMI	RT-qPCR	Echocardiography	<48 hours post-AMI	LVEF values not defined	↓	(38)
miR-29a	43 patients‡	Plasma	<24 hours post-AMI	RT-qPCR	Echocardiography	<48 hours post-AMI	LVEF values not defined	↓	(38)
	12 AMI	Plasma	Day 5 post-AMI	RT-qPCR	Echocardiography	90 days post-AMI	LVEDV	+ strong correlation	(39)
miR-29b	44 AMI	Plasma	Day 9 post-AMI	RT-qPCR	cMRI	Admission & 6 months post-AMI	ΔLVEDV & Δinfarct volume	+ weak correlation	(37)
miR-30a	99 STEMI	[S] Plasma & Serum, [V] Serum	Admission	[S] miRNA PCR panel, [V] ddPCR	Not described	6 months post-AMI	LVEF ≤50%, NT-proBNP ≥150 pg/mL & HF diagnosis	↑	(40)
miR-34a	43 patients‡	Plasma	<24 hours post-AMI	RT-qPCR	Echocardiography	<48 hours post-AMI	LVEF values not defined	↑	(38)
miR-126	43 patients‡	Plasma	<24 hours post-AMI	RT-qPCR	Echocardiography	<48 hours post-AMI	LVEF values not defined	↑	(38)
miR-133a	216 STEMI	Serum	Angiogram	RT-qPCR	cMRI	Admission	IS, MSI & MO	+ relationship with IS – relationship with MSI + relationship with MO	(41)
miR-150	90 STEMI	Plasma	Discharge	[S] Microarray, [V] RT-qPCR	[S] Echocardiography, [V] cMRI	Discharge & 6 months post-AMI	ΔLVEDV >0%	↓	(42)
miR-155	20 STEMI	Serum	Day 5 post-AMI	RT-qPCR	Echocardiography	Day 1 & 6 months post-AMI	ΔLVEDV ≥20%	↑	(43)

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	ACS population	Sample measurement			Imaging measurement			Findings	Ref.
		Source	Timepoint(s)†	Quantification method	Quantification method	Timepoint(s)†	LV remodelling definition		
miR-185	145 STEMI	Serum	Pre-PCI & discharge	[S] Microarray, [V] RT-qPCR	Echocardiography	Admission & 1 month post-AMI	WMSI & LVEF	+ correlation with WMSI – correlation with LVEF	(44)
miR-208b	43 patients‡	Plasma	<24 hours post-AMI	RT-qPCR	Echocardiography	<48 hours post-AMI	LVEF values not defined	↑	(38)
miR-320a	39 STEMI	Serum	Pre-PCI, 6 hours & 1 month post-PCI	Microarray & RT-qPCR	Echocardiography	Admission & 6 months post-PCI	ΔLVEDV ≥20% & LVEF <50%	↑	(45)

†, the sample timepoint associated with LV remodelling; ‡, not all patients were diagnosed with ACS but all received LVEF measurements and were included in subsequent analysis; [S], screening cohort; [V], validation cohort; ↑, increased levels of miRNA in patients with LV remodelling versus no LV remodelling; ↓, decreased levels of miRNA in patients with LV remodelling versus no LV remodelling; Δ, change in measurement between timepoints; <, less than; +, positive relationship; –, negative relationship. miRNA, microRNA; LV, left ventricular; ACS, acute coronary syndrome; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; RT-qPCR, real time-quantitative polymerase chain reaction; cMRI, cardiac magnetic resonance imaging; LVEDV, left ventricular end-diastolic volume; AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; ddPCR, droplet digital PCR; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HF, heart failure; IS, infarct size; MSI, myocardial salvage index; MO, microvascular obstruction; WMSI, wall motion score index.

has been observed at acute (<24 hours: miR-1, miR-24, miR-29a, miR-34a, miR-126, miR-133, miR-185, miR-208b, miR-320) (36,38,44,45), sub-acute (1–10 days: miR-1, miR-29a, miR-29b, miR-150, miR-155, miR-185) (37,39,42-44), and chronic (>1 month: miR-320a) (45) timepoints. These time-points will reflect different stages of the pathophysiological process, with acute time-points potentially more reflective of the extent of initial tissue damage. The timing of miRNA measurement should therefore be considered when assessing the potential of these miRNA as prognostic biomarkers, with preference given to markers that have been identified as independent predictors in multivariate analyses that include current clinical prognostic indicators (which are indicative of initial tissue damage).

There is currently a lack of concordance between studies investigating individual miRNA biomarkers for LV remodelling that is likely due to methodological differences in source material (plasma vs serum), timing of measurement, and chosen endpoints for LV remodelling. However, two miRNAs have been shown in multiple studies to have a significant relationship with parameters of remodelling and are described in more detail below.

miR-1

miR-1 is highly expressed in muscle tissue and is one of the most abundant miRNAs in the adult murine (46), rodent (47) and human (48) heart. Experimental studies using knockdown models have shown an important role for miR-1 in embryonic cardiovascular development and adult heart function (46,49). Additionally, experimental models of MI have demonstrated that circulating miR-1 levels are negligible in healthy rodents and are rapidly upregulated following coronary ligation (47,50). These findings are supported by several clinical studies that have demonstrated elevated circulating miR-1 levels in AMI patients when compared to healthy volunteers (47,50,51). These findings suggest that miR-1 may be upregulated as a consequence of myocardial damage and support its use as a potential prognostic biomarker following AMI. Grabmaier *et al.* (37) has previously demonstrated the relationship between miR-1 and infarct size when measured in 44 patients using cMRI 6-month following AMI. However, the correlation was weak, and the absolute change in left ventricular end-diastolic volume (LVEDV), which is a more common LV remodelling endpoint, was not associated with miR-1 levels. This contrasts with a more recent study conducted by Ma *et al.* (36), where miR-1 was

identified as an independent predictor of LV remodelling when measured 6-month following AMI in 80 ST-segment elevation myocardial infarction (STEMI) patients. While levels were significantly elevated in LV remodelling patients when compared to non-LV remodelling patients, receiver operator characteristic (ROC) curve analysis demonstrated no significant improvement in prognostic utility when compared to peak-logNT-proBNP. However, when added to a multiple logistic regression model including cardiac magnetic resonance imaging (cMRI) parameters and key biochemical measures (peak CK-MB, logNT-proBNP, peak TnT), miR-1 levels significantly increased the predictive power from an area under the curve (AUC) 0.81 [95% confidence interval (CI): 0.71–0.89] to AUC 0.89 (95% CI: 0.80–0.95). These findings suggest that miR-1 measurement alone may not be superior for predicting risk when compared to current clinical tools. However, when used in combination with LV function testing and biochemical measures, it may slightly improve prognostication in patients.

miR-29a

MiR-29a is a non-cardiac specific miRNA that has been linked to cardiac (52), liver (53), and pulmonary fibrosis (54). van Rooij *et al.* (52) has shown that the main source of miR-29a in the heart is cardiac fibroblasts, which are important effector cells in myocardial fibrosis. In this study, downregulation of miR-29a expression in mice using oligonucleotide inhibition was associated with increased collagen synthesis *in vivo* (52). Liu *et al.* (55) showed an opposite effect in which transforming growth factor-beta (TGF-β)-induced collagen expression by cardiac fibroblasts was inhibited by the introduction of a miR-29a mimic *in vitro*. These findings suggest an important role for miR-29a in biological mechanisms associated with fibrosis.

Indeed, a recent study has demonstrated that miR-29a levels are positively correlated with measures of hypertrophy and myocardial fibrosis in hypertrophic cardiomyopathy (HCM) patients (56). This review includes two studies that have linked levels of miR-29a to elements of LV remodelling. Lakhani *et al.* (38) demonstrated that miR-29a levels were decreased in AMI patients with low left ventricular ejection fraction (LVEF) values when compared to healthy participants or patients with normal LVEF values. However, this study did not define what LVEF thresholds were used to determine low versus normal values, so it is difficult to determine what severity of remodelling this miRNA is associated with. In comparison, a further study demonstrated a positive correlation between miR-29a levels and LVEDV measures when recorded 90 days following AMI (39). This creates a complicated picture for both the expression levels of miR-29a following AMI and the relationship of this miRNA with LV remodelling, as these two studies oppose each other.

miRNAs for MACE

MACE is a composite endpoint used to describe adverse outcomes that commonly proceed AMI. There is no gold-standard definition for MACE and instead it encompasses multiple outcome measures that can include: all-cause death, cardiac-specific mortality, unplanned revascularisation, hospitalisation with unstable angina, non-fatal MI, stroke, stent thrombosis and development of heart failure (15). MACE is a popular endpoint in clinical research as it summarises important cardiovascular outcomes that adversely impact patient quality of life. Multiple miRNAs have been linked to short- and long-term MACE outcomes following AMI (*Table 3*), some of which overlap with reported biomarkers for LV remodelling (miR-133a, miR-155, and miR-208b). Similar to LV remodelling studies, the timing of biomarker and outcome measurements vary across studies and a significant number of miRNA are reported in single studies only. However, four miRNA have been reproducibly linked to MACE, including three from the myomiR family (miR-208a, miR208b and miR-499), named as a consequence of their location and co-expression with the myosin gene, in addition to the muscle-enriched miR-133a.

miR-208a

miR-208a is encoded by the α-cardiac muscle myosin heavy chain gene 6 (α-MHC, MYH6) on chromosome X (72). miR-208a is considered a cardiac-specific miRNA, with experimental studies demonstrating exclusive expression in the myocardial tissue of small animals and humans (50,73). A number of experimental studies have demonstrated increased miR-208a levels following myocardial injury (50,74). In rats subjected to coronary artery ligation, Wang *et al.* in 2010 (50) demonstrated a rapid increase in miR-208a levels that peaked 3 hours following ligation and returned to baseline within 24 hours. A similar kinetic profile has been observed in STEMI patients (75). Combined, these findings indicate that miR-208a may be a specific biomarker of myocardial injury. However, despite

Table 3 miRNAs associated with MACE

exRNA	ACS population	Sample measurement			Outcome measurement			Findings	Ref.
		Source	Timepoint(s)†	Quantification method	Timepoint(s)†	Primary endpoint	% of population with primary endpoint		
miR-19a	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-19b	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-21	210 STEMI	Serum	Admission	[S] Illumina sequencing [V] RT-qPCR	2-year post-AMI	Cardiac mortality, hospitalization for HF or non-fatal AMI	70/210 (33.3%)	↓	(58)
	184 AMI	Plasma	Admission	RT-qPCR	30-day post-AMI	All-cause mortality	35/184 (19%)	↓	(59)
miR-26a	210 STEMI	Serum	Admission	[S] Illumina sequencing, [V] RT-qPCR	2-year post-AMI	Cardiac mortality, hospitalization for HF or non-fatal AMI	70/210 (33.3%)	↓	(58)
miR-30d	138 AMI	Plasma	Pre-angiogram	RT-qPCR	1-year post-AMI	Development of HF	46/138 (33.3%)	↑	(60)
miR-132	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-133a	216 STEMI	Serum	Angiogram	RT-qPCR	6-month post-AMI	All-cause mortality, non-fatal AMI, development of congestive HF	33/216 (15.3%)	↑	(41)
	444 ACS	Plasma	Admission	RT-qPCR	6-month post-AMI	All-cause mortality	34/444 (7.7%)	↑	(61)
miR-134	359 AMI	Plasma	Admission	RT-qPCR	176 days (IQR: 121–226)	Cardiac mortality or development of HF (LVEF <40%)	83/359 (23.1%)	↑	(62)
miR-140	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-145	246 STEMI	Serum	Day 5 post-PCI	RT-qPCR-DS	1-year post-AMI	Cardiac mortality or hospitalization for HF	72/246 (29.3%)	↑	(63)
miR-150	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-155	40 AMI	Serum	Admission	[S] miRNA array, [V] RT-qPCR	2-year post-AMI	Cardiac mortality	19/40 (47.5%)	↑	(64)
miR-184	72 AMI	Serum	6, 12, 24 hours & 7, 14 days post-SO	RT-qPCR	1-month post-AMI	Cardiac mortality, non-fatal AMI, development of HF, coronary revascularization	22/72 (30.6%)	↑	(65)
miR-186	92 ACS	Serum	Pre-PCI	RT-qPCR	1-year post-AMI	All-cause mortality, non-fatal AMI, stroke or unplanned coronary revascularisation	31/92 (33.7%)	↑	(66)
	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)

Table 3 (*continued*)

Table 3 (*continued*)

exRNA	ACS population	Sample measurement			Outcome measurement			Findings	Ref.
		Source	Timepoint(s)†	Quantification method	Timepoint(s)†	Primary endpoint	% of population with primary endpoint		
miR-191	210 STEMI	Serum	Admission	[S] Illumina sequencing,[V] RT-qPCR	2-year post-AMI	Cardiac mortality, hospitalization for HF or non-fatal AMI	70/210 (33.3%)	↓	(58)
miR-208a	40 STEMI	Serum	Pre-PCI	RT-qPCR	In-hospital	Insufficient reperfusion (TIMI 0, TIMI 1)	19/40 (47.5%)	↑	(67)
	84 AMI	Serum	Admission	RT-qPCR	6-month post-AMI	All-cause mortality, non-fatal AMI, unplanned coronary revascularization, stroke	17/84 (20.2%)	↑	(68)
miR-208b	407 ACS	Plasma	Admission	RT-qPCR	30-day post-hospitalization	All-cause mortality or development of HF or LVEF <40% or development of cardiogenic shock	74/407 (18.2%)	↑	(69)
	444 ACS	Plasma	Admission	RT-qPCR	6-month	All-cause mortality	34/444 (7.7%)	↑	(61)
	21 AMI	Plasma	Admission	RT-qPCR	6-month post-AMI	All-cause mortality	7/21 (33.3%)	↑	(70)
miR-210	430 ACS	Serum	Admission	RT-qPCR	4-year post-AMI	Cardiac mortality	43/430 (10.0%)‡	↑	(57)
miR-328	359 AMI	Plasma	Admission	RT-qPCR	176 days (IQR: 121–226)	Cardiac mortality, or development of HF (LVEF <40%)	83/359 (23.1%)	↑	(62)
miR-380*	40 AMI	Serum	Admission	[S] miRNA array, [V] RT-qPCR	2-year post-AMI	Cardiac mortality	19/40 (47.5%)	↑	(64)
miR-499	142 NSTEMI	Plasma	Admission	RT-qPCR	1-year & 2-year post-AMI	Cardiac mortality	1-year: 54/142 (38%), 2-year: 45%	↑	(71)
	407 ACS	Plasma	Admission	RT-qPCR	30-day post-hospitalization	All-cause mortality or development of HF or LVEF <40% or development of cardiogenic shock	74/407 (18.2%)	↑	(69)

†, the sample timepoint associated with MACE; ‡, value given for cardiac mortality or non-fatal AMI; [S], screening cohort; [V], validation cohort; ↑, increased levels of miRNA in patients with MACE versus no MACE; ↓, decreased levels of miRNA in patients with MACE versus no MACE; miRNA, microRNA; MACE, major adverse cardiovascular events; ACS, acute coronary syndrome; RT-qPCR, real time-quantitative polymerase chain reaction; AMI, acute myocardial infarction; STEMI, ST-segment elevation myocardial infarction; HF, heart failure; IQR, interquartile rang; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; DS, directly in serum; SO, symptom onset; TIMI, thrombolysis in myocardial infarction; NSTEMI, non-ST elevation myocardial infarction.

multiple animal studies demonstrating this link, limited clinical studies investigating the prognostic utility of miR-208a are available. In a recent study conducted in 84 AMI patients, elevated levels of miR-208a were univariately associated with MACE (68). When ROC curve analysis was assessed, miR-208a had a sensitivity of 49.25% and specificity of 94.12% when a cut-off value of 3.297 was used (68). However, multivariate analysis was not conducted in this study, and no comparisons were made to current risk-stratification biomarkers.

miR-208b

miR-208b belongs to the same family as miR-208a, and is encoded by the β-cardiac muscle myosin heavy chain gene 7 (β-MHC, MYH7) (72). Much like miR-208a, miR-208b is considered a cardiac-specific miRNA (76) and has been extensively researched as a prognostic tool in AMI. Widera *et al.* (61) investigated miR-208b as a prognostic biomarker in 444 ACS patients during a 6-month follow-up period (61). Patients with miR-208b levels above the median had a 2.2-fold higher risk of death when compared to patients with miR-208b levels below the median. However, this association lost significance when adjusted for age, gender and hs-TnT levels (61). A similar finding was observed by Gidlöf *et al.* (69), in which miR-208b levels were univariately associated with MACE at 30-day in 407 ACS patients but lost significance when adjusted for TnT levels. However, when MACE was investigated in AMI patients only (n=319), miR-208b remained a multivariate predictor of the primary endpoint, although prognostic accuracy was similar to that of TnT levels. While important differences exist between these two studies, including MACE definition and follow-up time, these findings suggest that miR-208b may be a more accurate biomarker in AMI patients. Indeed, a more recent study conducted by Alavi-Moghaddam *et al.* in 2018 (70) demonstrated that STEMI patients with miR-208b levels ≥12.38, a value determined using survival analysis, were approximately 5-fold more likely to die within 6-month following infarction when compared to patients with lower levels. While these findings are of interest, it is important to note that this study was a pilot design and was conducted in 21 participants only and were not compared to existing biochemical measurements, such as hs-TnT or natriuretic peptides.

miR-499

miR-499 is a muscle-specific miRNA that is highly expressed by the heart and is encoded by the *MYH7b* gene (50,77). The expression of miR-499 is closely linked to myocardial damage, with rodent (50) and pig (76) models of MI demonstrating rapid upregulation within 1 hour of artery occlusion. Clinical studies reflect these findings, with higher circulating levels of miR-499 observed in AMI patients compared to healthy volunteers (78). As such, miR-499 has been described in the literature as a sensitive biomarker for myocardial damage. Indeed, a recent meta-analysis of 14 studies demonstrated a pooled sensitivity of AUC 0.84 (95% CI: 0.64–0.94) and a pooled specificity of AUC 0.97 (95% CI: 0.90–0.99) to diagnose AMI in patients. In this review, two large clinical studies demonstrated a link between miR-499 levels and short-term and long-term MACE following AMI. Gidlöf *et al.* (69) demonstrated that miR-499 levels were a modest independent predictor of composite MACE 30-day following hospitalisation in AMI patients compared to non-MACE AMI patients [odds ratio (OR) =1.58; 95% CI: 1.17–2.13; P=0.003]. However, when miR-499 levels were compared to TnT, ROC curve analysis demonstrated a similar prognostic performance (AUC: 0.64 versus 0.66). Olivieri *et al.* (71) demonstrated a similar independent relationship between long-term MACE and miR-499 levels in a more niche population of elderly NSTEMI patients. Specifically, this study demonstrated that elevated miR-499 levels were associated with cardiac mortality at 1 year following AMI [hazard ratio (HR) =2.17; 95% CI: 1.20–3.91; P=0.01] and this outperformed TnT, which was not found to discriminate patients in this study. As such, miR-499 appears to be a promising prognostic biomarker that has performed modestly in larger clinical studies.

miR-133a

The miR-133 family comprises three variants that are conserved across the human genome, with miR-133a-1 and miR-133a-2 sharing an identical mature sequence and miR-133b differing by two nucleotides at the 3' terminus (79). Interestingly, miR-133 also belongs to the same transcriptional unit as miR-1, causing these miRNAs to be transcribed together (80). Early animal studies have demonstrated highly specific expression of miR-133/a in skeletal and cardiac muscle

(50,80), with these findings supported by human tissue studies (48,50). miR-133 expression is protective of cardiomyocyte hypertrophy (81,82) and oxidative stress-induced cardiomyocyte apoptosis (83). Additionally, miR-133 expression can modulate cardiac fibroblast ECM activity by post-transcriptional regulation of connective tissue growth factor (84). As such, miR-133 has a key regulatory role in cardiac development and function and is a prognostic biomarker of interest. In an early study by Widera *et al.* (61), miR-133a levels were linked to all-cause mortality at 6 months following an ACS diagnosis. In this study, patients with miR-133a levels in the fourth quartile had a 2.5-fold increased risk of death when compared to patients in the first three quartiles. However, when adjusted for age, gender and hs-TnT levels, this association did not remain significant. A further prospective study demonstrated similar findings in 216 STEMI patients, with increased levels of miR-133a associated with development of all-cause death, non-fatal MI or development of congestive heart failure 6-month following AMI (41). Specifically, this study demonstrated that miR-133a levels above the median were associated with increased mortality and cumulative MACE when compared to patients with levels below the median. However, upon multivariate analysis, miR-133a did not remain an independent predictor (41). As such, these findings suggest some association of elevated miR-133a with prognostic outcomes in patients. However, miR-133a alone may not be a superior biomarker compared to current clinical and biochemical parameters.

Panels of miRNAs

Increasingly, biomarker research is moving away from a single-marker analysis and towards a multi-marker approach that utilises a panel of selected biomarkers that represent different biological pathways to better predict risk in patients (85). Several studies have demonstrated the clinical utility of combining miRNAs to predict LV remodelling and MACE in AMI patients (*Table 4*) which are potentially superior to single-marker analysis.

Creating a molar ratio between two biologically linked miRNAs is a simple but effective statistical approach for combining biomarkers. Cortez-Dias *et al.* (86) compared the prognostic utility of measuring miR-122-5p alone or in a ratio with miR-133b to predict MACE following AMI. Upon univariate Cox regression analysis, miR-122-5p was not associated with MACE, while a higher ratio of miR-122-5p/miR-133b was significantly linked to increased risk of all-cause mortality alone (HR =1.62; 95% CI: 1.22–2.14; P=0.001) or in combination with non-fatal AMI (HR =1.50; 95% CI: 1.21–1.85; P<0.001) and the composite of these endpoints combined with unstable angina, stroke or hospitalization with heart failure (HR =1.44; 95% CI: 1.19–1.74; P<0.001). Hromadka *et al.* (91) also used a molar ratio to investigate the prognostic utility of combining miR-126-3p and miR-223-3p to predict the composite endpoint of cardiovascular death, recurrent AMI and stroke when measured acutely in 598 AMI patients. When ROC curve analysis was conducted, this study did not identify a significant difference in predictive value between miR-126-3p/miR-223-3p and miR-126-3p at 30-day [AUC 0.741 (95% CI: 0.704–0.775) versus AUC 0.686 (95% CI: 0.647–0.723)] or 1-year [AUC 0.642 (95% CI: 0.603–0.681) versus AUC 0.608 (95% CI: 0.567–0.647)] following AMI. These findings demonstrate a complicated picture for the utility of molar ratios in prognostication. Potentially, careful selection of miRNAs with biologically meaningful relationships is an important factor to consider prior to combining miRNAs. Additionally, ratios are inherently flawed by the ability to only combine binary variables.

Multivariate analysis techniques are a common statistical method in the literature, and overcome the limitation of ratios by combining large panels of variables together (92). Lv *et al.* (87) investigated the usefulness of miR-208b and miR-34a in predicting cardiac mortality and development of heart failure 6-month following AMI. This study demonstrated that the combination of these biomarkers using ROC curve [0.777 (95% CI: 0.731–0.819)] was incrementally superior for predicting MACE when compared to miR-208b [0.737 (95% CI: 0.0.689–0.782)] or miR-34a [0.642 (95% CI: 0.590-0.691)] alone. Importantly, when these biomarkers were compared to NT-proBNP, miR-208b and miR-34a combined, but not singularly, demonstrated an improved predictive ability for MACE. In another study, ROC curve analysis was used to predict MACE in 186 STEMI patients (88). When sampled acutely, the combination of miR-26b-5p, miR-660-5p, miR-320a [AUC 0.718 (95% CI: 0.767–0.851)] improved prediction when compared to each biomarker alone. Numerically, all of these miRNAs were comparable or superior for prediction when compared to hs-TNT and pro-BNP.

An alternative multivariate biomarker approach was conducted by Devaux *et al.* (89) for the prediction of impaired wall motion contractility in 150 patients 6-month following AMI. In this study, Akaike Information Criteria (AIC) was utilised to avoid model over-fitting,

Table 4 Single versus combined miRNA for prognosis following AMI

exRNA	Population (n)	Sample measurement		Outcome measurement			Statistical Analysis & Findings			Ref.
		Source	Timepoint(s)†	Timepoint(s)	Primary endpoint	% of population with primary endpoint	Analysis	Single	Combined	
miR-122, miR-133b	142 STEMI	Serum	Pre-angiogram	20.8 months (IQR: 17.7–23.5) post-AMI	All-cause mortality	9/142 (6.3%)	Univariate Cox regression	miR-122-5p only:	HR =1.62 (95% CI: 1.22–2.14; P=0.001)	(86)
					All-cause mortality & non-fatal AMI	15/142 (10.6%)		HR =1.21 (95% CI: 0.85–1.74, P=0.296)	HR =1.50 (95% CI: 1.21–1.85; P<0.001)	
					All-cause mortality, non-fatal AMI, UA, stroke or hospitalization for HF	26/142 (18.3%)		HR =1.35 (95% CI: 0.97–1.88, P=0.153)	HR =1.44 (95% CI: 1.19–1.74; P<0.001)	
								HR =1.08 (95% CI: 0.87–1.33; P=0.494)		
miR-208b, miR-34a	359 AMI	Plasma	Admission	6 months post-AMI	Cardiac mortality or development of HF	83/359 (23.1%)	Multivariate logistic regression (adjusted for age, gender, current smoking, cTnT, NT-proBNP and time for AMI onset)	miR-208b: OR =17.91 (95% CI: 2.07–98.81; P=0.003)	OR =18.73 (95% CI: 1.96–101.23; P<0.001)	(87)
								miR-34a: OR =4.18 (95% CI: 1.36–12.83; P=0.012)		
miR-26b, miR-660, miR-320a	189 STEMI	Plasma	Angiogram	1-year post-AMI	Cardiac mortality & non-fatal AMI	63/189 (33.3%)	Multivariate Cox regression (adjusted for sex and age)	miR-26b-5p: AUC 0.707 (95% CI: 0.68–0.74)	AUC 0.718 (95% CI: 0.68–0.75)	(88)
								miR-660-5p: AUC 0.683 (95% CI: 0.66–0.70)		
								miR-320a: AUC 0.672 (95% CI: 0.65–0.70)		

Table 4 (*continued*)

Table 4 (*continued*)

exRNA	Population (n)	Sample measurement		Outcome measurement			Statistical Analysis & Findings			Ref.
		Source	Timepoint(s)[†]	Timepoint(s)	Primary endpoint	% of population with primary endpoint	Analysis	Single	Combined	
miR-16, miR-27a, miR-101, miR-150	150 AMI	Plasma	Discharge	Discharge & 6 months post-AMI	Impaired LV contractility (WMIS >1.2)	71/150 (47.3%)	Logistic regression analysis using Akaike Information Criteria to determine best fit	miR-16: 188.381, P=0.169 miR-27a: 186.591, P=0.055 miR-101: 189.476, P=0.373 miR-150: 190.261, P=0.931	181.432, P=0.005	(89)
PC1, PC2, PC3	331 AMI	Plasma	2–4 weeks post-AMI	cMRI 2–4 weeks post-AMI & 6 months post-AMI	ΔLV mass, LVESVI, LVEF, LV mass, extracellular volume fraction	N/A	PCA	N/A	PC1 was an independent predictor of ΔLV mass PC2 was an independent predictor of LVESVI, LVEF & LV mass PC3 was an independent predictor of extracellular volume fraction	(90)
miR-126, miR-223	598 AMI	Plasma	Admission	30 days post-AMI & 1-year post-AMI	Cardiovascular death, non-fatal MI or stroke	30 days: 16/598 (2.7%) 1 year: 32/598 (5.4%)	Multivariate logistic regression (adjusted for many baseline characteristics)	30 days: OR =22.83 (95% CI: 1.43–98.01; P=0.022) 1 year: OR =2.39 (95% CI: 1.02–5.61; P=0.045)	30 days: OR =0.15 (95% CI: 0.03–0.76; P=0.022) 1 year: OR =0.41 (95% CI: 0.18–0.92; P=0.032)	(91)

[†], the sample timepoint associated with the outcome measure; Δ, change in measurement between timepoints. miRNA, microRNA; AMI, acute myocardial infarction; STEMI, ST-segment elevation myocardial infarction; IQR, interquartile range; HR, hazard ratio; CI, confidence interval; UA, unstable angina; HF, heart failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OR, odds ratio; AUC, area under the curve; LV, left ventricular; WMSI, wall motion score index; PC, principle component; cMRI, cardiac magnetic resonance imaging; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume index; N/A, not available; PCA, principle component analysis; MI, myocardial infarction.

as this statistical technique accounts for variable input number which can naturally inflate analysis. This study demonstrated that the addition of miR-16, miR-27a, miR-101 and miR-150 to clinical features and NT-proBNP levels resulted in a significantly lower AIC from 188.269 to 181.432 (P=0.005), which indicates an improved model fit. Using net reclassification analysis, this study demonstrated that the combined addition of these four miRNAs could improve classification of patients by 66% when compared to clinical features and NT-proBNP levels alone.

Combined, these studies demonstrate the prognostic benefit of combining multiple biomarkers to risk-stratify AMI patients. However, multivariate statistical approaches assume independence between variables and cannot account for collinearity. This is an important limitation to consider for multi-marker panels, as many miRNAs have a shared biological activity or similar downstream targets that prevent them from being independent factors. As such, emphasis on developing methods to combine miRNAs that account for this shared association is important.

Multidimensional reduction models can overcome collinearity by accounting for statistical correlations between input variables. Principle component analysis (PCA) is one statistical method that reduces complex and multi-dimensional data into fewer components that best describe the original dataset (93). Additionally, scores can be generated to describe an individual's placement on each principle component (PC) (94), and these combined biomarker scores can be correlated with outcome variables. This model has been demonstrated by Danielson *et al.* (90) in a study that investigated the prognostic utility of combining miRNAs to predict LV remodelling 6 months following AMI. Fourteen miRNA targets, that were initially identified using RNA sequencing, were combined into four PC groups that accounted for 61.3% of total variance. When compared to measures of LV function, PC1 was an independent predictor of change in LV mass, PC2 independently predicted parameters of LV size and volume, while PC3 was an independent predictor of extracellular volume fraction. Firstly, this study demonstrates the utility of PCA for combining biomarkers and linking these two primary endpoints while accounting for collinearity. Secondly, this study demonstrates the importance of miRNA selection when designing a panel, as each PC contained a different combination of miRNAs that predicted different parameters of LV remodelling.

Clinical challenges of miRNA biomarkers as prognostic tools

This review has highlighted a number of candidate miRNAs for prognostication following AMI. In total, there are 38 miRNAs included in this review that have been individually linked to LV remodelling or MACE. Of these miRNAs, only 13 were represented across more than one study. Lack of validation and reproducibility represents an important clinical challenge in this field.

Discovered as circulating biomarkers just over a decade ago, miRNAs are still within an infancy stage of research (95). As such, there is an absence of standardised methodologies for sample collection, storage, extraction, detection, and normalisation. At each stage of sample processing, a number of factors can be introduced that alter miRNA expression, and these are well described in reviews by Felekkis and Papaneophytou (96) and Moldovan *et al.* (97). One such example is the choice between plasma or serum as the sample source. Consistently studies have shown discordance in miRNA levels between paired plasma and serum samples (98,99), with a recent study in NSTEMI patients demonstrating an opposite pattern of miR-21 expression with increased levels in serum and decreased levels in plasma (99). While the literature remains undecided on which sample source is superior, there is a lack of consensus in the literature with approximately half of the studies in this review using plasma. Normalization of miRNA expression is also a very important step in result interpretation, and can be undertaken using either exogenous or endogenous controls. Currently, there are no well-established endogenous controls, and these often differ between different disease states and can be affected by sample preparation (96). Alongside creating a standardised methodology for miRNA measurement, reporting on aspects of sample processing that may account for heterogeneity between studies should be encouraged. Storage conditions, sample haemolysis and freeze-thaw cycles are all sources of variation in miRNA expression (96), and including this type of detail in study methodologies will provide transparency and better enable cross-study comparisons.

In addition to methodological challenges, study design considerations are also an important source of discordance in circulating miRNA research; definitions for prognostic endpoints are not well defined in the literature. Indeed,

there is currently no single universal definition to describe LV remodelling (100,101) or MACE (15). In this review, some studies defined LV remodelling based on LVEDV measures (36,39,42,43), others focused on measures of infarct volume, myocardial salvage or parameters of systolic function (37,38,41,89,90), and some studies used a combination of measures to define LV remodelling (40,45). In addition, some studies dichotomised patients based on a predefined endpoint value (36,42,43), while others used continuous measurements for statistical analysis (37,41,44). Imaging techniques were also inconsistent between studies in this review. Four studies employed cMRI, 9 studies used echocardiography, 1 study utilised both imaging methods (42) and 1 study did not disclose the imaging modality used (40).

Similar to LV remodelling, significant heterogeneity exists in the individual outcome measures that are used to define composite MACE endpoints (102), and this is well illustrated in *Table 3*. The syndromes and outcome measures that are encompassed in MACE are described by unique pathophysiological processes. An example of this is sudden cardiac death (SCD), which accounts for approximately 50% of all CAD associated mortality (103). Recently, Silverman *et al.* in 2020 (104) demonstrated that increased levels of miR-150-5p and miR-29a-3p and decreased levels of miR-30a-5p were associated with an increased risk of SCD in CAD patients. Indeed, unfavourable levels of all three increased an individual's risk of SCD by 4.8-fold (95% CI: 1.59–14.51, P=0.006). The pathophysiology of SCD is complex but is associated with dysregulation of cardiac electrophysiology (103), and is a significantly different aetiology to CAD. All three of the miRNA identified by Silverman *et al.* (104) are less specific to cardiac tissue and are more broadly linked to pathways of inflammation and fibrosis. This differs from other studies that have investigated mortality and MACE following AMI, and have more commonly identified myomiR specific miRNAs (e.g., miR-208a, miR-208b and miR-499) as prognostic tools (68,69,71). This illustrates that despite existing in the same all-encompassing clinical endpoint, different disease pathophysiology's may differentially be linked to unique miRNAs.

Alongside endpoint definitions, study size and follow-up time can greatly influence the prognostic utility of miRNAs within a study. Sample size was relatively small for most studies included in this review, with only half comprising a cohort size greater than 100 participants. While this is less concerning for studies where all participants receive an endpoint measurement, such as LV function measures, it can become problematic for studies where only a proportion of the cohort meet the endpoint requirements, such as the development of MACE. Of the cohort-based studies that examined MACE, total case numbers ranged from 7 to 83 participants, which crudely translates to between 10% and 50% of the total cohort size. Under these conditions, small sample sizes can reduce statistical power and can lead to the underestimation of relationships between miRNA and outcome measures. In addition to this, effect sizes of miRNAs are generally small and coefficients of variation can be high which makes replication in small cohorts difficult and sometimes redundant. Follow-up times were also variable for studies included in this review and ranged from in-hospital to 2 years post-AMI. This inequity is problematic when comparing both MACE and LV remodelling endpoints between studies, as both outcome measures worsen overtime. This is particularly prominent for LV remodelling, which is a progressive disease that begins early following AMI and continues beyond 1 year (105). Therefore, studies with shorter follow-up times (less than 6 months) focus on early remodelling and miss long-term remodelling processes. In addition to follow up times for endpoint measurement, the timing of sample collection must be considered when evaluating miRNA biomarkers. Samples collected immediately following AMI may be more reflective of initial tissue damage, whereas samples collected at chronic timepoints may reflect an already significantly advanced disease process. Timing of sample collection is also dependent on practical considerations including timing of patient follow up appointments.

Natriuretic peptides and troponins are critical in the diagnosis of heart failure and AMI, respectively. While these biochemical measures have limitations for prognosis, they are rapid, inexpensive, and routinely measured in a clinical setting. Therefore, any future biomarker tools must be superior to these methods in order to improve clinical practise. Some (42,62,63,71,87,88), but not all (36,61,69), studies included in this review demonstrated a benefit in circulating miRNAs when compared to these traditional tools. In general, combined biomarker studies were more consistently associated with superior prediction of future risk when compared to single biomarker studies. This was elegantly shown by Lv *et al.* (87), who demonstrated that miR-208b and miR-34a combined, but not alone, were superior at predicting LV remodelling when compared to NT-proBNP.

Future perspectives and recommendations

Circulating miRNAs are a promising tool for predicting future risk in AMI patients. However, for miRNA biomarkers to be moved towards the clinic, the following recommendations are suggested for future research.

(I) Standardisation of methodologies and reporting for extracellular miRNA isolation, measurement and study design is a priority for all future research in this field. Currently, it remains difficult to discern between miRNAs that are ineffective at predicting adverse outcomes following AMI and miRNAs that are clinically useful but vary between studies due to methodological issues. There are resources available from groups such as the Extracellular RNA Communication Consortium (exrna.org) (106,107) and the International Society for Extracellular Vesicles (108) that provide frameworks for technical aspects of circulating miRNA research and reporting standards.

(II) Larger trials, preferably multicentre studies, should be prioritised to establish whether candidate miRNA biomarkers are superior to current clinical tools in predicting MACE and LV remodelling outcomes. Endpoints, outcomes, and measurement tools (e.g., imaging modalities) should be clearly defined.

(III) Future studies should prioritise the measurement of miRNA panels over single biomarker analysis. The measurement of multiple biomarkers in combination has proved promising and may more accurately capture complex pathophysiological processes associated with the development adverse outcomes.

(IV) A comparison between current clinical biomarkers, such as troponins and natriuretic peptides, and novel miRNA candidates should be examined within all future studies. This provides an opportunity to assess if the miRNA candidates correlate with measures of myocardial damage and if they can be additive to current biomarkers commonly used to facilitate clinical decision-making.

Conclusions

miRNAs are a promising tool for predicting the development of MACE and LV remodelling following AMI that could be additive to current clinical practice. However, there has been inconsistency between studies to date that have identified candidate predictive and prognostic miRNA biomarkers. Standardisation of methodology and robust validation of candidate biomarkers in larger multicentre trials are required to establish miRNAs as future prognostic tools for the clinic.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Chenyu Zhang) for the series "Extracellular RNAs and Human Health" published in *ExRNA*. The article has undergone external peer review.

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://exrna.amegroups.com/article/view/10.21037/exrna-22-21/rc

Peer Review File: Available at https://exrna.amegroups.com/article/view/10.21037/exrna-22-21/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://exrna.amegroups.com/article/view/10.21037/exrna-22-21/coif). The series "Extracellular RNAs and Human Health" was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/exrna-22-21
Cite this article as: Brunton-O'Sullivan MM, Danielson KM. Extracellular miRNAs for predicting risk following acute myocardial infarction: a narrative review. ExRNA 2023;5:3.