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# Kinetics Analysis of Circulating MicroRNAs Unveils Markers of Failed Myocardial Reperfusion

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**BACKGROUND:** Failed myocardial reperfusion occurs in approximately 50% of patients with ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (PPCI). It manifests as microvascular obstruction (MVO) on cardiac magnetic resonance (CMR) imaging. Although prognostically important, MVO is not routinely screened for. Our aim was to investigate the kinetics of circulating short noncoding ribonucleic acids [microRNAs (miRNAs)] following PPCI and their association with MVO in STEMI patients.

**METHODS:** Screening of 2083 miRNAs in plasma from STEMI patients with (n = 6) and without (n = 6) MVO was performed by next-generation sequencing. Two candidate miRNAs were selected and quantified at 13 time points within 3 h postreperfusion in 20 STEMI patients by reverse transcription and quantitative PCR. Subsequently, these 2 miRNAs were measured in a "validation" STEMI cohort (n = 50) that had CMR imaging performed at baseline and 3 months post-PPCI to evaluate their association with MVO.

**RESULTS:** miR-1 and miR-133b were rapidly released following PPCI in a monophasic or biphasic pattern. Both miRNAs were enriched in circulating microparticles. A second miR-1 peak (90–180 min postreperfusion) seemed to be associated with a higher index of microvascular resistance. In addition, miR-1 and miR-133b levels at 90 min post-PPCI were approximately 3-fold ($P = 0.001$) and 4.4-fold ($P = 0.008$) higher in patients with MVO, respectively. Finally, miR-1 was significantly increased in a subgroup of patients with worse left ventricular (LV) functional recovery 3 months post-PPCI.

**CONCLUSIONS:** miR-1 and miR-133b levels increase within 3 h of PPCI. They are positively associated with MVO and worse LV functional recovery post-PPCI.


Primary percutaneous coronary intervention (PPCI)[4] has substantially reduced mortality following ST-elevation myocardial infarction (STEMI) *(1, 2)*. Nonetheless, approximately 40% and 28% of patients who survive a STEMI develop adverse left ventricular (LV) remodeling and heart failure *(3, 4)*, respectively. This may partially result from suboptimal myocardial reperfusion despite successful stenting of the epicardial vessel. A complex interaction between factors associated with ischemia (e.g., endothelial cell protrusion, subsarcolemmal blebs), reperfusion (e.g., neutrophil and platelet aggregates, injury to the endothelial glycocalyx), and atherothrombotic microembolization has been implicated in the pathophysiology of failed myocardial reperfusion *(5, 6)*. Failed myocardial reperfusion can be detected by cardiac magnetic resonance (CMR) imaging as a hypoenhanced area within the hyperenhanced infarct core, termed microvascular obstruction (MVO) *(7)*. MVO is strongly associated with worse clinical outcome in STEMI patients *(8)*. Although MVO occurs in up to 50% of patients treated with PPCI *(9)*, it usually passes undetected because of lack of sensitivity or availability of current diagnostic methods. Therefore, identification of novel affordable approaches for detection of cardiac injury associated with MVO is warranted.

MicroRNAs (miRNAs) are short noncoding ribonucleic acids of approximately 18 to 22 nucleotides in length that regulate gene expression at the posttranscriptional level *(10)*. MiRNAs can be released into the extra-

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[4] Nonstandard abbreviations: PPCI, primary percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; LV, left ventricular; CMR, cardiac magnetic resonance; MVO, microvascular obstruction; miRNA, microRNA; TIMI, Thrombolysis in Myocardial Infarction; MP, microparticle; NGS, next-generation sequencing; RT-qPCR, reverse transcription and quantitative PCR; IMR, index of microvascular resistance; IS, infarct size; LVEF, left ventricular ejection fraction; IMH, intramyocardial hemorrhage.

cellular space in response to cellular activation, stress, or injury *(11)*. Consequently, their role as disease markers has been the focus of intense investigation during the past decade. To date, their potential role for STEMI diagnosis has been reported by several studies, which have shown deregulated miRNA levels early after symptom onset *(12)*. However, circulating miRNA kinetics following PPCI are still poorly understood. Exploratory studies suggest that the most significant changes in miRNA levels seem to occur within 2 to 3 h post-PPCI *(13, 14)*, but no detailed miRNA kinetics study in this time frame has been performed thus far. The aims of this study were to investigate the kinetics of circulating miRNAs in the initial 3 h post-PPCI and whether postreperfusion miRNA levels are associated with failed myocardial reperfusion and cardiac damage as assessed by CMR.

## Materials and Methods

### PATIENT COHORTS AND BLOOD SAMPLING

To study circulating miRNA kinetics following PPCI, a cohort of 20 STEMI patients (derivation cohort) was recruited at the Freeman Hospital (Newcastle upon Tyne, UK) between January and October 2017. The study was approved by the local ethics committee (REC reference 16/NE/0405), and written consent was obtained. A second cohort of 50 STEMI patients (validation cohort) was used to validate candidate miRNAs and to assess their correlation with CMR parameters. This cohort comprised participants of the CAPRI trial (EudraCT number 2014–002628–29; REC reference NE/14/1070). In both STEMI cohorts, blood samples were collected at the start of the PPCI procedure (prereperfusion) and at multiple time points within 3 h following coronary reperfusion. Only patients who achieved Thrombolysis in Myocardial Infarction (TIMI) flow 2 or 3 post-PPCI were included. To gain insight on the effect of coronary artery perfusion status on miRNA kinetics, (*a*) one patient undergoing transcoronary ablation of septal hypertrophy, hence with optimal coronary perfusion at the time of cardiomyocyte injury induction, (*b*) one STEMI patient with TIMI flow 3 pre-PPCI, and (*c*) one STEMI patient with TIMI flow 0 post-PPCI were also included. For details, see the Data Supplement that accompanies the online version of this article.

### STANDARD AND PLATELET-POOR PLASMA ISOLATION

Standard plasma was obtained from all blood samples for miRNA quantification, whereas platelet-poor plasma was obtained for circulating microparticle (MP) isolation, as previously recommended *(15)*. For detailed protocols, see the online Data Supplement.

### MP ISOLATION AND QUANTIFICATION

To gain insight on candidate miRNA transport in plasma, circulating MPs were isolated from fresh platelet-poor plasma samples collected before and at 30 min and 90 min post-PPCI from the derivation STEMI cohort (n = 10). MP quantification was performed by flow cytometry using Megamix-Plus SSC calibration beads (BioCytex) and TruCount™ tubes (BD Biosciences) in a BD FACS Canto II cytometer. For details, see the online Data Supplement.

### RNA ISOLATION

Total RNA was isolated from 200 μL of plasma and freshly isolated MP using the miRNeasy serum/plasma kit (Qiagen). See protocol in the online Data Supplement.

### miRNA NEXT-GENERATION SEQUENCING AND REVERSE TRANSCRIPTION AND QUANTITATIVE PCR SCREENING

To identify candidate miRNA markers of MVO, a 2-step miRNA screening approach was used. First, the HTG EdgeSeq miRNA Whole Transcriptome Assay (miRNA WTA; HTG Molecular) was used to quantify 2083 human miRNA transcripts using next-generation sequencing (NGS) in plasma collected before and at 90 min post-PPCI from STEMI patients with (n = 6) and without (n = 6) MVO. Subsequently, screening of 179 circulating miRNAs was performed with human serum/plasma focused miRNA PCR panels (Exiqon) in STEMI patients with MVO at 30 min post-PPCI (n = 5) and healthy controls (n = 6). For detailed protocols, see the online Data Supplement.

### REVERSE TRANSCRIPTION AND QUANTITATIVE PCR

Following screening, the top 2 miRNA candidates were quantified in the derivation and validation cohorts by reverse transcription and quantitative PCR (RT-qPCR) using stem-loop specific TaqMan microRNA assays (Applied Biosystems). To circumvent the inhibitory effect of heparin on RT-qPCR, RNA samples were incubated with 0.3 U of heparinase I from *Flavobacterium heparinum* (Sigma-Aldrich) for 1 h at room temperature before reverse transcription, as previously described *(16)*. For protocol details, see the online Data Supplement.

### INDEX OF MICROVASCULAR RESISTANCE

The index of microvascular resistance (IMR) was invasively determined in 14 STEMI patients immediately after stent deployment in the infarct-related artery using a combined temperature and pressure coronary wire sensor (Certus, St. Jude Medical). Hyperemia was induced by intravenous adenosine infusion at 140 μg/kg/min. IMR was determined as the distal coronary pressure multiplied by the mean transit time of 3 consecutive bolus

injections of room temperature saline (3 mL) during maximal coronary hyperemia.

#### CMR IMAGING

CMR imaging was performed in patients from the validation cohort at 1 to 7 days post-PPCI (baseline CMR) and at 3 months post-PPCI (follow-up CMR) with a Siemens Avanto 1.5-Telsa MRI scanner using a phased array body coil combined with a spine coil. Gadobutrol (Gadovist, Bayer Schering Pharma AG) was administered intravenously at a dose of 0.1 mmol/kg, and after 10 min, short axis end-diastolic late gadolinium enhancement (LGE) images were obtained. Imaging analysis was carried out using the cvi42 software (Circle Cardiovascular Imaging). CMR was used to determine infarct size (IS), MVO, left ventricular ejection fraction (LVEF), and end systolic and diastolic volumes. More information is available in the online Data Supplement.

#### STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSS software version 22.0 (IBM). Data normality was assessed using the Shapiro–Wilk test. Gaussian-distributed data were analyzed using parametric tests, and nongaussian data were analyzed using nonparametric tests. Correlations between variables were analyzed with the Spearman correlation test. Data are presented as mean (SD) or median and interquartile ranges as appropriate. All $P$ values are 2-sided, and $P < 0.05$ was considered statistically significant.

## Results

#### PATIENT CHARACTERISTICS

Baseline characteristics of the derivation cohort are displayed in Table 1 of the online Data Supplement. Baseline characteristics of the validation cohort are summarized in Table 2 of the online Data Supplement. In summary, patients with MVO were more likely to have an anterior infarct ($P = 0.009$) and higher 12-h post-PPCI high-sensitivity cardiac troponin T levels ($P < 0.001$). Otherwise, no differences in baseline characteristics were observed between patients with and without MVO (see Table 2 in the online Data Supplement).

#### SELECTION OF CANDIDATE miRNA MARKERS OF FAILED MYOCARDIAL REPERFUSION

Screening of 179 miRNAs revealed a cluster of 7 miRNAs with increasing expression across the initial 90-min postreperfusion in STEMI patients (n = 3) (Fig. 1A). Subsequently, miRNA NGS and miRNA screening by RT-qPCR identified a group of 17 and 20 miRNAs with expression at least 2-fold higher in STEMI patients with MVO compared with patients without MVO and healthy controls, respectively (Fig. 1, B and C). The 3 most highly expressed miRNAs in patients with MVO by both NGS (miR-133a, miR-133b, and miR-378a) and RT-qPCR screening (miR-1, miR-133a, and miR-133b) are known to be muscle-enriched. Considering these findings and previous reports from the literature, miR-1 and miR-133b were selected for further validation.

#### miR-1 AND miR-133b RELEASE FOLLOWING MYOCARDIAL REPERFUSION

Before reperfusion, median miR-1 levels were approximately 4-fold higher in STEMI patients (n = 18) compared with healthy controls (n = 6). Following reperfusion, gradually increasing median miR-1 levels were observed until a first peak [49 (9.8–169.3)-fold] was achieved at 30 min post-PPCI followed by a second peak [53.1 (8.6–123.1)-fold] at 90 min post-PPCI, returning to comparable levels of prereperfusion at 24 h post-PPCI (Fig. 2A). A very similar pattern was observed for miR-133b, with the presence of 2 distinct peaks at 30 min [32.6 (10.3–125)-fold] and 90 min [28.8 (9.5–106)-fold] post-PPCI (Fig. 2B). When kinetics curves were analyzed at an individual level, it was possible to observe that some patients had a more prominent initial peak (n = 7), whereas others had both an early and later peak (n = 10, data not shown). Indeed, by grouping these patients, 2 distinct miR-1 and miR-133b kinetics patterns emerged: (*a*) monophasic, with an initial peak at 20 to 40 min postreperfusion, with decreasing levels thereafter; (*b*) biphasic, with a first peak at 20 to 40 min post-PPCI followed by even higher levels at 90 to 180 min post-PPCI (Fig. 2, C and D).

In terms of miRNA transport in plasma, significantly high median circulating MP concentrations were observed in the aorta (1400 MP/$\mu$L; $P < 0.008$) and culprit coronary artery (1385 MP/$\mu$L; $P = 0.024$) before reperfusion in STEMI patients compared with controls (526 MP/$\mu$L). This was followed by a rapid decrease in MP concentrations as early as 5 min and at 180 min postreperfusion (Fig. 3A). Despite the decrease in circulating MP concentration post-PPCI, miR-1 and miR-133b expression in MP isolated from STEMI patients before and at 30 and 90 min post-PPCI remained significantly higher than in controls ($P < 0.05$). Before reperfusion, no difference in miR-1 and miR-133b expression was observed between plasma and MP from the same STEMI patients (Fig. 3, B and C). Following reperfusion, miR-1 expression in MPs was significantly lower than in plasma, but no difference in miR-133b expression between these 2 compartments was observed (Fig. 3, B and C).

#### PRESENCE OF A SECOND miR-1 PEAK SEEMS TO BE ASSOCIATED WITH HIGHER IMR

When patients were divided according to IMR tertiles, miR-1 kinetics seemed to differ among IMR tertile groups, especially regarding the magnitude and timing of peak miRNA expression (Fig. 4). All IMR tertile groups pre-

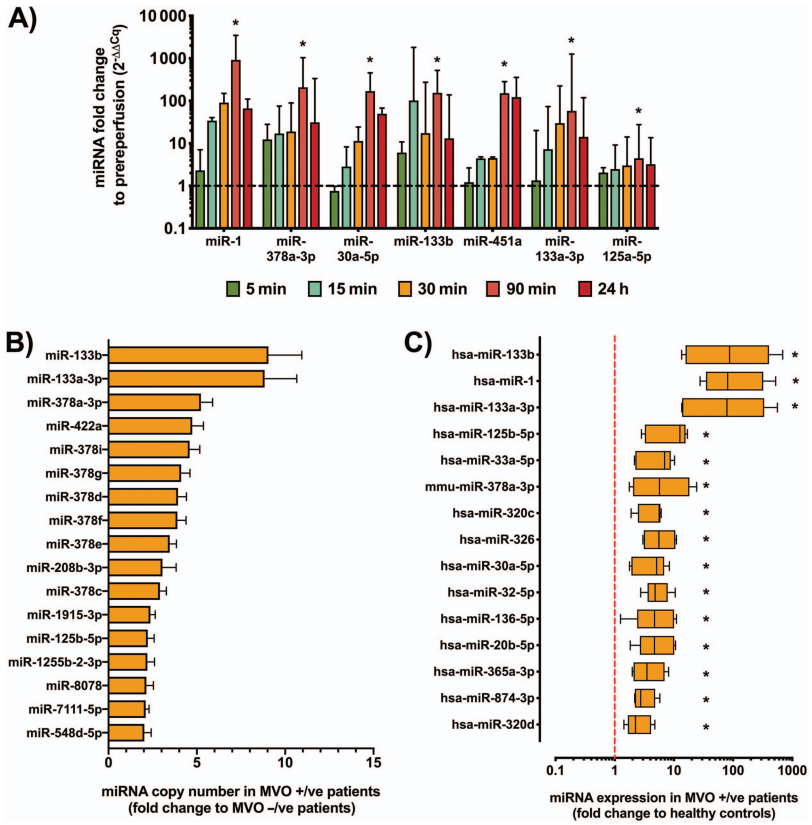


**Fig. 1. miRNA screening for selection of candidate markers of failed myocardial reperfusion.**

(A), Screening of 179 miRNAs using PCR panels revealed a cluster of 7 miRNAs with progressive increase in expression across the initial 90 min postreperfusion in STEMI patients (n = 3). (B), Group of 17 miRNAs presenting copy number at least 2-fold higher in patients with MVO compared with patients without MVO at 90 min postreperfusion (MVO +/ve n = 6; MVO −/ve n = 6) after screening of 2083 miRNAs. (C), The 15 most highly expressed miRNAs after screening of 179 miRNAs in 30 min postreperfusion samples from STEMI patients with MVO (n = 5). In both screenings, samples from age-matched patients with stable coronary artery disease were used as controls (n = 4). *$P < 0.05$, Kruskal–Wallis test with the Dunn correction for multiple comparisons (A) and Mann–Whitney *U* test (B).

sented an initial miR-1 peak, which occurred at 30 min post-PPCI for the highest tertile, at 40 min for the middle tertile, and at 60 min for the lowest tertile group (Fig. 4). As for a second peak, this was absent in the first tertile group, which presented a monophasic kinetics pattern (Fig. 4). A second miR-1 peak was observed at 90 min post-PPCI in the middle tertile group and at 180 min post-PPCI for the highest tertile group (Fig. 4). Regarding miR-133b kinetics, all patient groups presented biphasic kinetics; however, association with IMR values was not observed (see Fig. 3 in the online Data Supplement).

### HIGHER POST-PPCI miR-1 AND miR-133b LEVELS ARE ASSOCIATED WITH THE OCCURRENCE OF MVO AND LARGER INFARCT SIZE

Baseline CMR imaging was performed at an average of 3.1 (±1.7) days post-PPCI in all patients, with no difference in time of CMR acquisition between MVO groups ($P = 0.603$). The "follow-up" CMR imaging was performed in 45 of the 48 patients in the validation cohort at 3 months post-PPCI. In patients with MVO, miR-1 and miR-133b levels were approximately 4.3-fold ($P = 0.006$) and 2.3-fold ($P = 0.048$) higher at 30 min post-PPCI compared with patients without MVO, respectively (Fig. 5). Similarly, 90-min postreperfusion levels of these miRNAs were also significantly increased in patients with MVO (miR-1: 3-fold higher in MVO positive vs MVO negative, $P = 0.001$; miR-133b: 4.4-fold higher in MVO positive vs MVO negative, $P = 0.008$) (Fig. 5). In addition, circulating levels of miR-1 and miR-133b at 30 and 90 min postreperfusion positively correlated with baseline and 3-month infarct size (see Table 3 in the online Data Supplement).

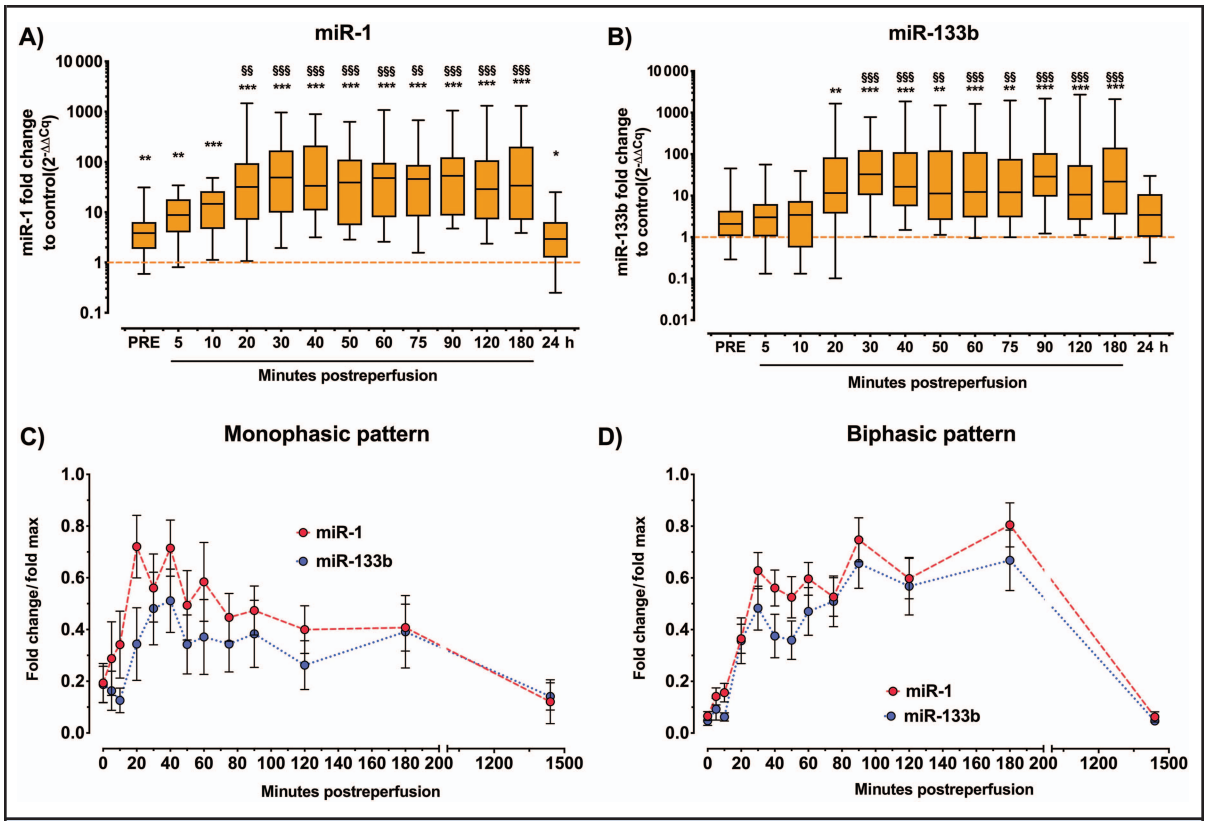


**Fig. 2. miR-1 and miR-133b plasmatic kinetics following myocardial reperfusion in STEMI patients.**

(A), miR-1 expression was higher than in healthy controls at all time points and increased in relation to prereperfusion levels between 20 min and 180 min post-PPCI. (B), Kinetics analysis revealed that 20 min up to 180 min post-PCI, miR-133b levels were increased in relation to controls. Also, 20 min up to 180 min postreperfusion miR-133b expression was higher than prereperfusion levels. (C), Monophasic miR-1 and miR-133b kinetics pattern. Data presented as mean and SE of the ratio fold change/fold max for each time point, n = 7. (D), Biphasic miR-1 and miR-133b kinetics pattern. Data presented as mean and SE of the ratio fold change/fold max for each time point, n = 10. (A, B), Data presented as median (central line in boxes), interquartile range (limits of the boxes), and range (error bars). Dashed lines represent reference control levels. n, STEMI = 18; n, controls = 6. Differences between STEMI patients and control miRNA levels were determined by Kruskal–Wallis test with the Dunn correction for multiple comparisons. Differences in miRNA expression among time points in STEMI patients were determined by Friedman test with Bonferroni post hoc correction for multiple comparisons. *$P < 0.05$ vs controls; **$P < 0.01$ vs controls; ***$P < 0.001$ vs controls; §§$P < 0.01$ vs prereperfusion; §§§$P < 0.001$ vs prereperfusion.

### miR-1 IS INCREASED IN A SUBGROUP OF PATIENTS WITH WORSE 3-MONTH LV FUNCTION RECOVERY

When patients were divided according to baseline IS tertiles, those in the highest tertile group (IS > 13.3%; n = 16) had no improvement in LVEF after 3 months of PPCI as opposed to a significant increase in LVEF observed in the lower baseline IS tertile groups (see Fig. 4 in the online Data Supplement). Median miR-1 levels at 90 min post-PPCI were 3.3-fold and 2.7-fold higher in the highest baseline IS tertile compared with the lowest ($P = 0.013$) and middle ($P = 0.031$) tertile groups, respectively (Fig. 6B). Although miR-1 levels at 30 min and miR-133b at 90 min were also significantly increased in the highest IS tertile group in relation to the lowest tertile, they were not significantly raised compared with the middle tertile (Fig. 6, A and D). There was no difference in miR-133b levels at 30 min post-PPCI across baseline IS tertile groups (Fig. 6C).

## Discussion

The main findings of this study are that (*a*) miR-1 and miR-133b are quickly released into the circulation in a monophasic or biphasic pattern following successful coronary artery recanalization (TIMI flow 2 or 3) in STEMI patients; (*b*) the presence of a second miR-1 peak seems to be associated with higher IMR; (*c*) higher miR-1 and miR-133b levels post-PPCI are associated with MVO

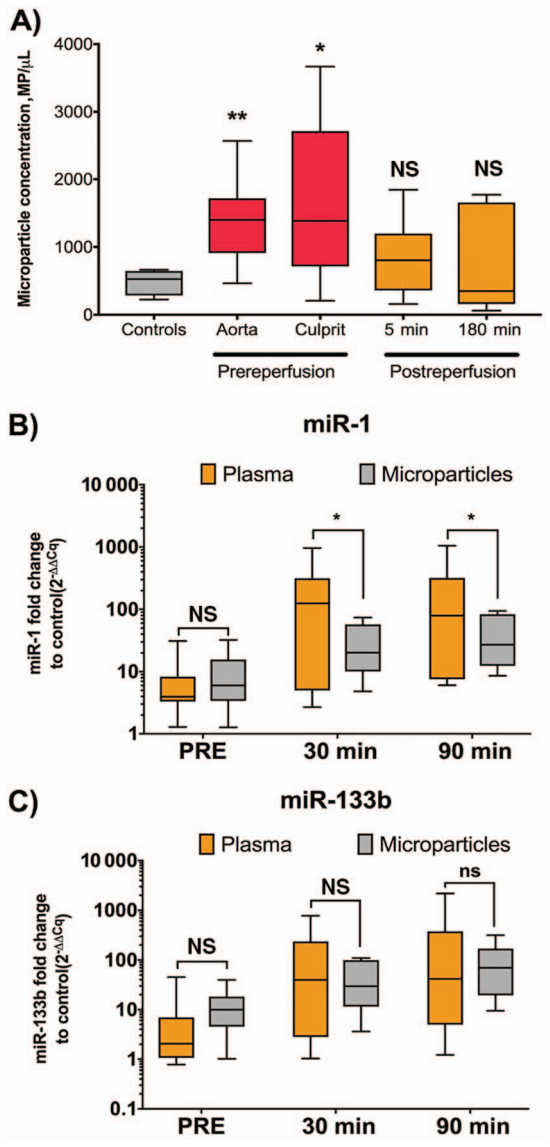


**Fig. 3. miR-1 and miR-133b expression in circulating MPs isolated from STEMI cohort 1 patients.**

(A), MP concentration in STEMI patients before and after coronary reperfusion. MP concentration is significantly raised in the aorta and culprit coronary artery of STEMI patients compared with controls. *$P < 0.05$ vs controls; **$P < 0.01$ vs controls; NS, nonsignificant. Kruskal–Wallis test with the Dunn correction for multiple comparisons. (B), Comparative miR-1 expression between plasma and MPs isolated from the same STEMI patients before and at 30 min and 90 min postreperfusion. *$P < 0.05$, Wilcoxon matched-pairs signed rank test. (C), Comparative miR-133b expression between plasma and MPs isolated from the same STEMI patients before and at 30 min and 90 min postreperfusion. *$P <$ 0.05, Wilcoxon matched-pairs signed rank test. n, STEMI = 10; n, controls = 3.

and positively correlate with acute and 3-month IS; and (*d*) increased miR-1 levels are associated with worse 3-month LV functional recovery.

PPCI successfully restores normal or near-normal coronary blood flow in >90% of STEMI patients *(17)*. Despite reestablishment of coronary artery perfusion, impairment in microvascular flow occurs in approximately 50% of reperfused STEMI patients *(9)*. This results in a failure of myocardial reperfusion, which can effectively hinder the benefits of coronary reperfusion therapy *(18)*. Recent advancements in CMR protocols revealed that MVO substantially overlaps with areas of intramyocardial hemorrhage (IMH) and collectively represents regions of myocardial tissue with vascular damage and erythrocyte extravasation, instead of microvascular occlusion *(19, 20)*. Therefore, the current understanding is that failed myocardial reperfusion initially manifests as MVO in the core infarct zone followed by severe microvascular injury and IMH in 40% of the cases *(20)*. Failed myocardial reperfusion can be detected by invasive and noninvasive methods. Electrocardiographic ST segment resolution and angiographic myocardial blush grade score lack sensitivity and reproducibility as routine tests *(21)*. CMR imaging, the gold-standard technique for MVO detection, has prognostic importance *(22, 23)*. However, CMR is not feasible as a routine investigation because of high costs, contraindications *(24)*, and lack of capacity. More recently, assessment of coronary microcirculatory function with the IMR has been shown to reliably inform about severe microvascular pathology, IMH, LV remodeling *(25)*, and mortality after STEMI *(26)*. Nevertheless, although safe in principle, IMR is a costly invasive procedure that adds in the time of exposure to radiation and is not available in all centers. Therefore, identification of early affordable markers of myocardial damage associated with failed myocardial reperfusion could help in risk stratification and clinical management optimization of STEMI patients.

In this study, miR-1 and miR-133b were identified as the top 2 most highly expressed candidates in STEMI patients with MVO after screening of 2083 and 179 miRNAs by NGS and RT-qPCR, respectively. miR-1 is encoded by 2 loci (miR-1–1 and miR-1–2) in the intron 2 of an uncharacterized gene (*MIR1–1HG*) on the chromosome 20 and in the intron 12 of the E3 ubiquitin-protein ligase MIB1 on the chromosome 18 *(27)*, respectively. The miR-133b gene is located in the chromosome 6 *(28)*. In normal human tissues, miR-1 and miR-133b are highly expressed in cardiac and skeletal muscle as well as the thyroid, with less significant expression in the breast, esophagus, prostate, bladder, and testicles *(29, 30)*. miR-1 is fundamental for cardiac development and has been shown to regulate cardiac repolarization and hypertrophy *(27, 31)*. Similarly, miR-133b is involved in many stages of myocyte development, cell fate

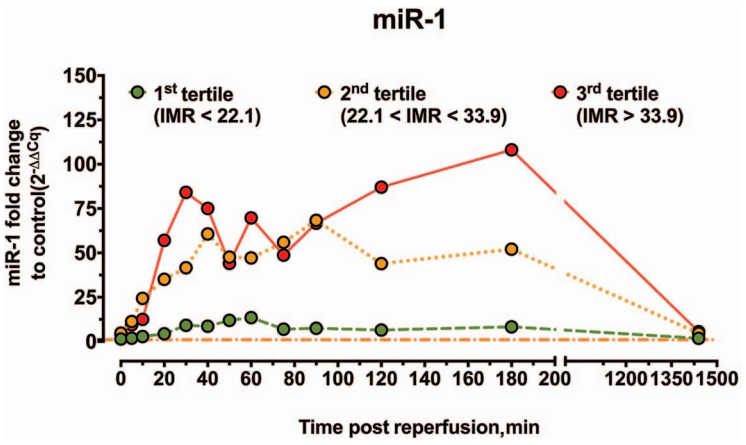


**Fig. 4. miR-1 kinetics according to IMR tertiles.**
Data presented as median (dots). n = 14; n, first tertile = 4; n, second tertile = 5; n, third tertile = 5.

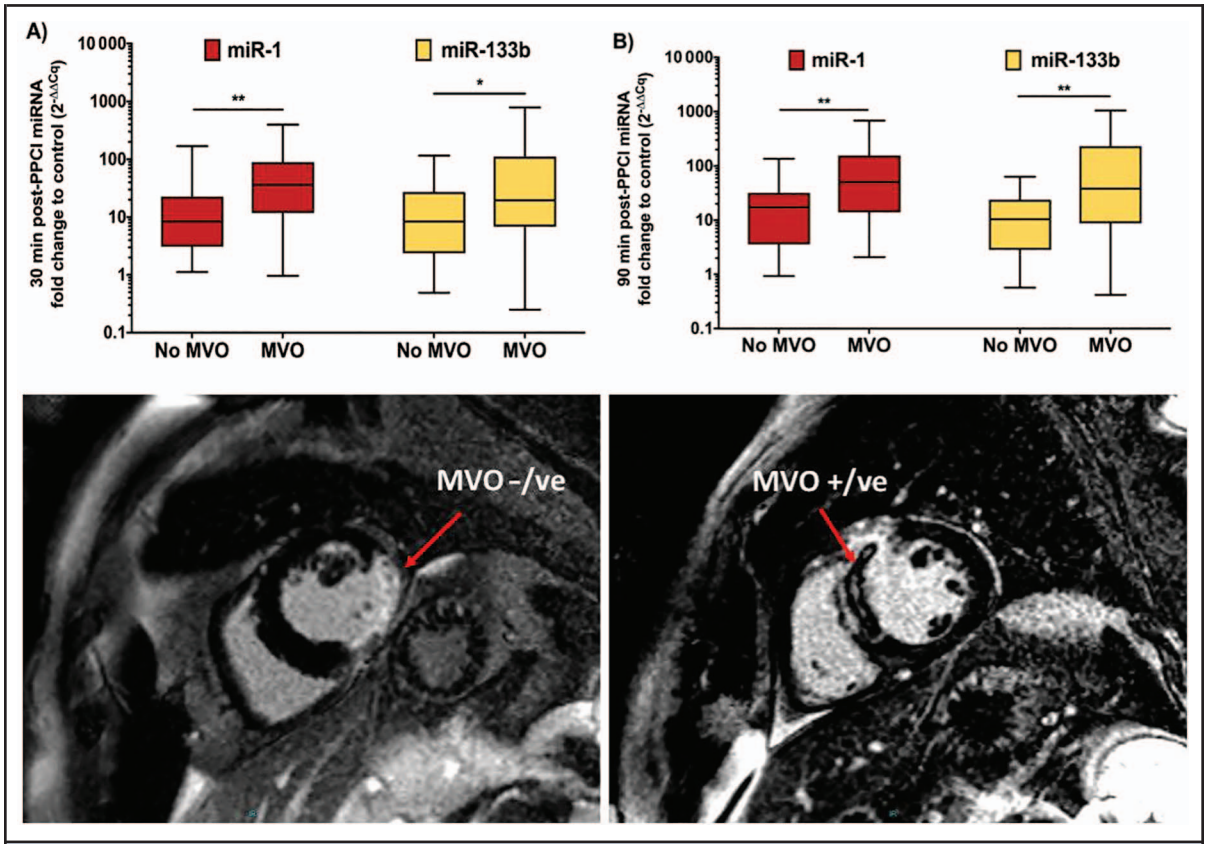


**Fig. 5. Postreperfusion miR-1 and miR-133b levels are increased in patients with MVO.**
(A), 30 min postreperfusion miR-1 and miR-133b levels in patients with (n = 19) and without (n = 29) MVO determined by the baseline MRI; (B), 90 min postreperfusion miR-1 and miR-133b levels in patients with and without MVO. Lower panels depict examples of CMR imaging showing infarct cores with and without MVO. Differences between MVO (n = 19) and no MVO (n = 29) patients were determined by Mann–Whitney *U* test. **P* < 0.05; ***P* < 0.01.

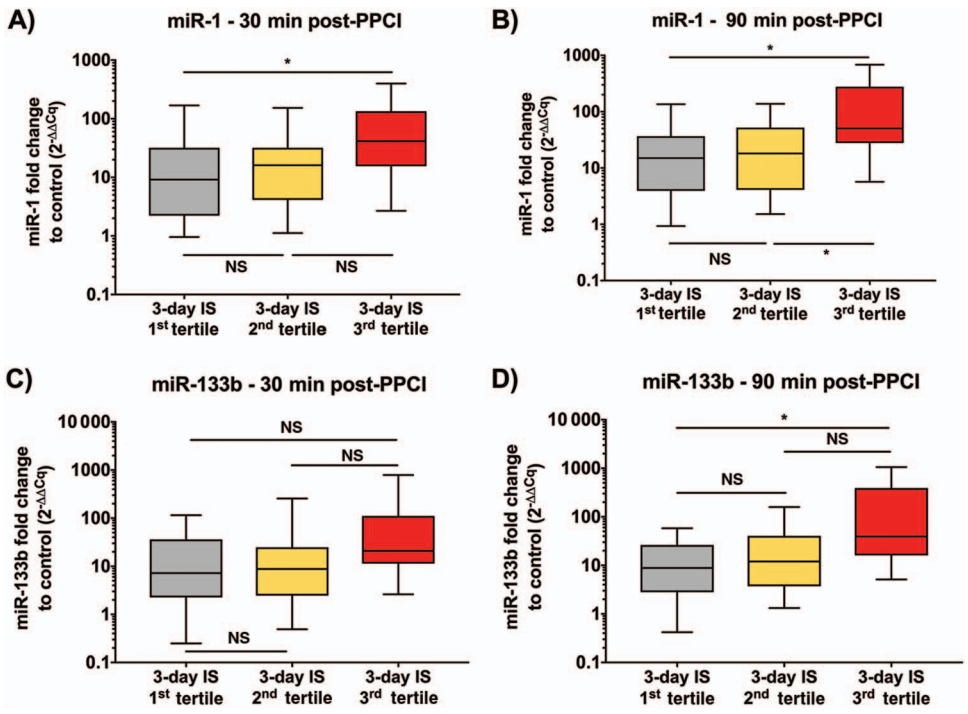


**Fig. 6. Postreperfusion miR-1 and miR-133b levels according to baseline IS tertiles.**

(A), miR-1 levels at 30 min post-PPCI. Significantly higher miR-1 measurements were observed in the highest IS tertile compared with the lowest tertile. (B), miR-1 levels at 90 min post-PCI were significantly increased in the highest tertile compared with the 2 lower tertile groups. (C), No differences in miR-133b levels at 30 min post-PPCI among baseline IS tertile groups were observed. (D), miR-133b levels at 90 min post-PCI were significantly increased in the highest IS tertile compared with the lowest tertile. Differences between groups were determined by Kruskal–Wallis test with the Dunn corrections for multiple comparisons. *$P < 0.05$; NS, nonsignificant. n (first tertile) = 16; n (second tertile) = 16; n (third tertile) = 13.

determination, apoptosis, and gene program regulation in cardiomyopathies *(32)*. Now, our meticulous study of circulating miR-1 and miR-133b kinetics in patients who achieved TIMI 2 or 3 flow post-PPCI reveals for the first time that these miRNAs quickly increase in the circulation in the initial 3 h post-PPCI following a monophasic or biphasic pattern with peaks at 30 min and 90 min.

In addition, we hypothesize that, at least for miR-1, the occurrence of a second peak at 90 min post-PPCI may reflect a different myocardial injury process as that of the first peak at 30 min. Considering that (*a*) cardiac-enriched miRNAs are released almost immediately after cardiomyocyte injury under optimal coronary perfusion *(33)*, (*b*) all STEMI patients presented an "early" peak, and (*c*) the occurrence of the "late" peak was associated with a surrogate marker of failed myocardial reperfusion (i.e., high IMR) *(25)*, this study provides a hypothesis-generating concept that the early miR-1 peak might reflect ischemia-related myocardial injury, whereas the late miR-1 peak might be associated with cardiac injury owing to failed myocardial reperfusion in STEMI patients who achieved TIMI 2 or 3 flow after PPCI. The patterns of association between miR-1 and miR-133b kinetics and IMR may be explained by the distinct mechanism of release of these miRNAs after reperfusion, which might reflect different cellular injury processes, as miR-133b was predominantly transported within MPs whereas miR-1 was not. Interestingly, very similar results were described by Deddens et al. *(34)* in a porcine model of myocardial ischemia and reperfusion, in which miR-1 and miR-133b were raised in plasma and circulating MP at 60 min postreperfusion, but only miR-133b expression was enriched in MP in relation to plasma.

Further validating the association between miR-1 and miR-133b levels and failed myocardial reperfusion, this study is the first to show higher post-PPCI levels of such miRNAs in patients with MVO detected by CMR. Previously, Eitel et al. reported that occurrence of MVO was significantly higher in a group of STEMI patients with admission miR-133a concentration equal to or greater than the median *(35)*. Considering the strong

association between MVO and IS *(36–38)*, it is not surprising that miR-1 and miR-133b postreperfusion levels correlated with the extent of cardiac damage both in the acute (3-day) and the convalescent (3-month) phases of STEMI. Furthermore, miR-1 levels were specifically increased in a subgroup of patients with worse LV functional recovery at 3 months post-PPCI. There is discrepancy in the literature regarding the association between increased cardiac-enriched miRNAs and reduced LVEF in patients with acute myocardial infarction or heart failure *(35, 39, 40)*. The studies that showed no association, however, performed miRNA quantification at 24 to 72 h postadmission or at patient discharge *(40)*, which are late time points considering miR-1 and miR-133a kinetics described herein. This highlights the importance of understanding the release kinetics of cardiac-enriched miRNAs following coronary reperfusion so that optimal time points for miRNA quantification and their correlation with clinical parameters can be identified. Our findings suggest a potential role for cardiac-enriched miRNAs, especially miR-1, in the detection of MVO and monitoring of therapeutic strategies targeting failed myocardial reperfusion.

This study has some limitations, such as (*a*) it is a single-center study; (*b*) the CMR protocol did not include parameters such as IMH and area at risk; (*c*) small sample size; (*d*) lack of simultaneous IMR and CMR measurement in the same patients; and (*e*) in this setting, it was not possible to perform characterization and isolation of cardiomyocyte-derived MPs owing to the lack of reliable, specific surface markers.

In conclusion, this study presented the most detailed description of cardiac-enriched miRNA (miR-1 and miR-133b) release kinetics in the initial 3 h following PPCI. This analysis, coupled with invasive measurement of microvascular function and CMR imaging, revealed that miR-1 and miR-133b might be useful to understand different forms of cardiac injury associated with failed myocardial reperfusion. Finally, our study provided evidence for a potential clinical role of circulating miR-1 and miR-133b as early surrogate markers of failed myocardial reperfusion as well as cardiac function after PPCI in STEMI patients.

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