Circulating MicroRNA-188, -30a, and -30e as Early Biomarkers for Contrast-Induced Acute Kidney Injury

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Background—Contrast-induced acute kidney injury (CI-AKI) is typically defined by an increase in serum creatinine after intravascular administration of contrast medium. Because creatinine is an unreliable indicator of acute changes in kidney function, we assessed whether circulating microRNAs (miRNAs) could serve as biomarkers for early detection of CI-AKI.

Methods and Results—Using a rat model of CI-AKI, we first evaluated the miRNA profile of rat plasma and kidney. Three miRNA species with >1.5-fold increase in plasma samples of CI-AKI rats, including miRNA-188, miRNA-30a, and miRNA-30e, were selected as candidate miRNAs. Quantitative real-time polymerase chain reaction showed that these candidate miRNAs peaked in concentration around 4 hours after contrast medium exposure and were relatively renal-specific. We compared the plasma levels of these candidate miRNAs in 71 patients who underwent coronary angiography or percutaneous coronary intervention and developed CI-AKI with those of 71 matched controls. The plasma levels of the 3 candidate miRNAs were significantly elevated in the CI-AKI group as compared to the control group. Receiver operating characteristic analysis showed that these miRNAs significantly distinguished patients with CI-AKI from those without CI-AKI. MiRNA composites were highly accurate for CI-AKI prediction, as shown in maximized specificity by treble-positive miRNA composite or maximized Youden index by any-positive miRNA composite. Moreover, the selected miRNAs changes were associated with Mehran Risk Scores.

Conclusions—Plasma levels of candidate miRNAs significantly distinguished patients with CI-AKI from those without CI-AKI. Thus, miRNAs are potential biomarkers for early detection of CI-AKI.  

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Key Words: biomarker • contrast-induced nephropathy • microRNA
Tissue-specific/enriched miRNAs may serve unique functional roles in the normal state and be altered in diseased states. Their expression patterns in plasma, urine, and other body fluids are tightly correlated with various diseases. Circulating miRNAs can be readily detected in plasma and serum in a remarkably stable form. The expression profiles of circulating miRNAs carry immense potential for their use as novel, noninvasive biomarkers in diagnosing and monitoring human diseases, such as cancer and tissue injury.

MiRNAs are also implicated in pathways linked to kidney diseases. Studies have found some miRNAs are upregulated in blood from patients with various kidney diseases. However, the role of circulating miRNAs in CI-AKI has not been examined. We hypothesized that the renal-specific miRNAs might be released into circulation during CI-AKI and could be used to detect kidney injury. Based on the rat model of CI-AKI we previously reported, here we explore the changes in miRNA expression in plasma and kidney during CI-AKI. We identify miRNA-188, -30a, and -30e as the most consistently upregulated miRNA during the early stage following CM exposure. These miRNAs are also specifically expressed in kidney tissue. Further investigation demonstrated that these elevated renal-specific miRNAs in plasma could significantly distinguish patients with CI-AKI from those without CI-AKI. Therefore, our results suggest that circulating miRNAs could serve as potential biomarkers to detect CI-AKI early in the disease process.

Materials and Methods

CI-AKI Rat Model

CI-AKI was induced by the method we previously reported. In brief, male Sprague-Dawley rats (250–300 g) were deprived of water for 3 days and then given furosemide by intramuscular injection at a dose of 10 mg/kg. For the CI-AKI group, a nonionic, low-osmolar CM, Omnipaque (350 mg I/mL; GE Healthcare, Shanghai, China), at a dose of 10 mL/kg was subsequently administered via tail vein over the course of 5 minutes. For the control group, the same amount of normal saline (NS) was given. As reported, an increase in SCr ≥25% over baseline was observed in 83.3% (5/6) of the CI-AKI rats. Histopathological analysis revealed that all CI-AKI rats developed tubular necrosis and medullary congestion, indicating that this method efficiently leads to AKI in the rats. All animal experimental protocols complied with the guidelines on animal care of Shanghai Jiao Tong University.

Population

Consecutive patients who were scheduled for elective coronary angiography (CAG) or percutaneous coronary intervention (PCI) in our center from July 2013 to June 2014 were included in this study. Exclusion criteria were recent CM exposure (within 2 days), AKI from other causes, preexisting dialysis, cardiac shock, missing SCr or CyC values, pregnancy, or breastfeeding. Nonionic, low-osmolality CM and intravenous hydration were used in all patients. The duration and volume of hydration was determined at the discretion of the physicians. N-acetylcysteine was not routinely given. In this cohort, blood samples for miRNA analysis were prospectively collected at baseline and 4 to 6 hours post CM exposures. This time point was prespecified according to the time curve of selected circulating miRNAs in 9 patients receiving primary PCI (see Table S1). SCr and CyC were measured at the time of admission and 24 to 48 hours after the procedure. CI-AKI was defined by an absolute increase in SCr ≥0.3 mg/dL or relative increase in SCr ≥25% or CyC ≥10% over baseline. Estimated glomerular filtration rate (eGFR) was calculated by applying the Modification of Diet in Renal Disease formula as modified by Levey. The Mehran Risk Score was calculated as follows: hypotension (score of 0 for absent or 5 for present), intra-aortic balloon pump (score of 0 for absent or 5 for present), congestive heart failure (score of 0 for absent or 5 for present), age >75 years (if yes, score of 4), diabetes mellitus (score of 0 for absent or 3 for present), eGFR <60 mL/min per 1.73 m² (integer score between 2 and 6 depending on eGFR), preexisting anemia (score of 0 for absent or 3 for present), and CM volume (integer score of 1 for each 100 mL). The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee in Shanghai Ren Ji Hospital. Informed consent was obtained from all the participants before enrollment.

Plasma Collection and Storage

Peripheral blood (5 mL) was collected into ethylenediaminetetraacetic acid–containing tubes (BD Vacutainer, Franklin Lakes, NJ) and processed within 1 hour at room temperature. The plasma was collected by centrifugation at 820 g and 4°C for 10 minutes, transferred into fresh RNase/DNase-free tubes, and centrifuged at 16 000g at 4°C for 10 minutes. The supernatant was transferred to new tubes and stored at −80°C.

RNA Isolation

Total RNA in rat tissue was harvested with TRIzol (Invitrogen, Carlsbad, CA). Total rat and human RNA in plasma was extracted with mirVana following the manufacturer’s protocol (Applied Biosystems, Foster City, CA). Five microliters of synthetic Caenorhabditis elegans miRNA (cel-miRNA-39, 5 fmol/μL, synthesized by Invitrogen) was spiked-in as the
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Statistical Analysis
Continuous variables are represented as the mean±SE or median (with 25th and 75th percentiles). The Student t test or analysis of variance was used to determine the differences between mean values for normally distributed variables. The nonparametric Mann–Whitney U test or the Kruskal–Wallis test was used to determine the differences between median values for non-normally distributed variables. Pearson correlation analysis and Spearman correlation analysis were performed for normally and non-normally distributed variables, respectively. Categorical variables were reported as percentages and were analyzed by either χ² test or Fisher’s exact test, as appropriate. The Bootstrap method with 10 000 bootstrap resamples was used to estimate the mean difference and 95% CI of miRNA levels between 2 groups.  

miRNA Array and Data Analysis
The miRNA profile in rat plasma and kidney was assessed by miRNA microarray analysis using the rat miRNA arrays (Agilent microRNAs microarray v.10.1; Agilent Technologies, Santa Clara, CA) that contains probes for 350 rat miRNAs (Sanger miRbase, release 10.1). The miRNA molecular in total RNA was labeled by miRNA Complete Labeling and Hyb Kit according to the manufacturer’s protocol (Agilent Technologies). Each array slide was hybridized with 100 ng Cy3-labeled RNA using miRNA Complete Labeling and Hyb Kit (Agilent Technologies). After hybridization, slides were washed using the Gene Expression Wash Buffer Kit (Agilent Technologies) and Feature Extraction software 9.5.3 (Agilent Technologies) with default settings. Raw data were normalized by a quantile algorithm using Gene Spring Software 10.0 (Agilent Technologies).

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Analysis
Selected miRNAs were quantified with TaqMan RT-qPCR according to the manufacturer’s protocol (Applied BioSystems). Each reaction was primed using a gene-specific stem-loop primer (Applied BioSystems). The qPCR reactions were carried out by LightCycler® 480 Real-Time PCR System (Roche Applied Science, Penzberg, Germany). Raw data were analyzed with the automatic quantification cycle setting for assigning baseline and quantification for quantification cycle determination. Relative expression of the mature miRNAs was normalized to the internal control small nuclear U6 expression (in tissue) or cel-miRNA-39 (in plasma) and calculated by the 2⁻ΔΔCT method.  

miRNA Spectra in Plasma Sample
One hundred eighty-two miRNAs were detected in the plasma samples. A greater than 1.5-fold change on microarray hybridization intensity was observed in 57 miRNA species (Figure 1B, Table 1). Twenty-two of these species were upregulated in the CI-AKI rats. The miRNA with the greatest increase was miRNA-347, with a 2.8-fold increase.
miRNA Spectra in Kidney Tissues

One hundred sixty-six and 173 miRNAs were detected in the control and CI-AKI kidney samples, respectively. A greater than 1.5-fold change on microarray hybridization intensity was observed in 11 miRNA species (Figure 1B, Table 1). Nine of them were upregulated in the CI-AKI rats. The 2 miRNAs with the greatest increases were miRNA-327, with a 14.3-fold increase, and miRNA-188, with a 3.3-fold increase.

Selection of Candidate Circulating miRNAs as Potential Biomarkers for CI-AKI

Candidate circulating miRNA was defined as human–rat homologous, kidney-specific miRNA with more than 1.5-fold upregulation in plasma of CI-AKI rats and expression in kidney samples. A relatively kidney-specific miRNA was defined as any miRNA with a 2-fold higher expression level in the kidneys than in the other 5 main organs (heart, liver, spleen, lung, and brain). According to this definition, firstly, 22 miRNAs from plasma with more than 1.5-fold upregulation in CI-AKI rats were included. After that, 10 miRNAs without expression in kidney samples were subsequently excluded. Then, 3 miRNAs, including miRNA-347 with the greatest increase in plasma of CI-AKI rats and miRNA-327 with the greatest increase in kidneys of CI-AKI rats, were excluded because they were not expressed in humans. The expression levels of the remaining 9 human–rat homologous miRNAs in 6 human main organs were acquired from a previously reported database of human miRNAs expression profiles. Ultimately, 3 kidney-specific miRNAs were identified. A flow chart of candidate miRNAs selection is depicted in Figure 1C.

Evaluation of Circulating miRNAs as New Biomarkers for CI-AKI in Rats

To confirm the tissue expression patterns of the 3 selected miRNAs, RT-qPCR analysis of miRNA expression in the 6 main organs from rats was performed. As shown in Figure 2A, all 3 miRNAs exhibited high expressed levels in kidneys and lower or barely detectable level in the remaining 5 organs. The 3 miRNAs also displayed a similar expression pattern in rats as
reported in humans. To validate the hybridization results by microarray, RT-qPCR analysis of the 3 selected miRNAs in plasma was performed. The results of RT-qPCR were consistent with those of microarray analyses (Figure 2B).

To further investigate the time curve of selected circulating miRNAs in CI-AKI rats, blood samples were collected at various time points (baseline, 0, 2, 4, 8, 12, and 24 hours post CM administration, n=4 of each time point). RT-qPCR analysis showed that the levels of miRNA-188 did not significantly increase after dehydration, peaked around 4 hours, reached about a 5-fold increase at peak, and decreased at 24 hours post CM exposure (Figure 2C). The levels of miRNA-30a and miRNA-30e in plasma increased after dehydration, peaked around 4 hours, reached about a 15-fold increase, and decreased at 24 hours post CM exposure (Figure 2C).

To further confirm that the increase in plasma miRNA-188, miRNA-30a, and miRNA-30e levels was in response to CI-AKI, but not in the context of CM administration or dehydration, another 16 rats were randomly divided into 4 groups (n=4,
each group): CI-AKI group, dehydration-only (without CM administration) group, CM-exposure-only (without dehydration) group, and control group. Blood samples were drawn at 4 hours post CM exposure. As shown in Figure 2D, compared with the levels in the control group, the levels of the 3 selected circulating miRNAs modestly increased in the dehydration-only group and the CM-exposure-only group, but the extent of the increases in those 2 groups was significantly lower than the increase in the CI-AKI group. This indicated that the increased levels of the 3 selected circulating miRNAs were mainly associated with kidney injury but not dehydration or CM exposure.

Circulating miRNAs as New Early Biomarkers to Detect CI-AKI in Patients Receiving CAG/PCI

From July 2013 to June 2014, 580 consecutive patients receiving elective CAG/PCI in our center fulfilled inclusion and exclusion criteria. The incidence of CI-AKI following CAG/PCI was 23.28% based on our prespecified definition. Of the 135 patients who developed CI-AKI, plasma samples were available in 71 cases. Clinical characteristics of the remaining CI-AKI patients were similar to these 71 cases (Table S2). We performed a case–control study using these 71 cases. Controls were matched by age (<50, 50–70, >70 years), presence of diabetes mellitus (Yes, No), and chronic kidney disease stage (Stage 1: eGFR >90 mL/min per 1.73 m²; Stage 2: eGFR 60–89 mL/min per 1.73 m²; Stage 3: eGFR 30–59 mL/min per 1.73 m²; Stage 4: eGFR 15–29 mL/min per 1.73 m²; Stage 5: eGFR <15 mL/min per 1.73 m²) and were randomly selected from patients who did not develop CI-AKI. A 1:1 ratio of controls to cases was used.

After inclusion of the case controls, the study consisted of 71 CI-AKI cases and 71 controls. Baseline characteristics of these 2 groups of patients are displayed in Table 2. None of these patients required temporary dialysis during hospitalization as a result of AKI. The plasma level of the 3 candidate miRNAs before and post CM exposure were screened by using RT-qPCR. The baseline plasma levels of the 3 candidate miRNAs were low (the average quantification cycle value:
miRNA-188 33.68; miRNA-30a 29.05; miRNA-30e 28.64) and without significant difference across subgroups, including baseline stages of chronic kidney disease (Table S3). The plasma levels of the 3 candidate miRNAs were significantly elevated at 4 to 6 hours postprocedure from baseline in patients with CI-AKI as compared with the control group (mean difference in fold change of miRNAs: miRNA-188, 1.594 [1.087–2.158], P<0.001; miRNA-30a, 2.579 [1.756–3.508], P<0.001; miRNA-30e, 2.283 [1.612–3.038], P<0.001; Figure 3A). The changes in these miRNAs levels in the subgroups categorized by the different CI-AKI diagnostic criteria (SCr or CyC) are presented in Table S4. Logistic regression adjusting for age and eGFR showed significant association of the changes in these miRNAs levels with CI-AKI (Table S5). Correlation analysis indicated that the increases in the 3 candidate miRNAs plasma levels postprocedure were related to the change of SCr or CyC (Figure S1A).

**Discrimination Potential of Circulating miRNAs for CI-AKI**

Receiver operating characteristics analysis was performed to determine the sensitivity and specificity of the 3 miRNAs for CI-AKI prediction. As shown in Figure 3B, the levels of the 3 miRNAs significantly identified patients with CI-AKI from those without CI-AKI (miRNA-188, area under curve 0.784 [0.709–0.858]; miRNA-30a, area under curve 0.802 [0.730–0.874]; miRNA-30e area under curve 0.805 [0.733–0.878]). The cutoff increment values of miRNAs for CI-AKI prediction are presented in Table 3. To obtain the best diagnostic sensitivity and specificity, cutoff point I was used for further analysis. Comparison of these miRNAs in CI-AKI prediction is presented in Table S6.

**Use of miRNA Composites for CI-AKI Prediction**

Treble-positive miRNA composite (fold change: miRNA-188 ≥1.343, miRNA-30a ≥1.405, miRNA-30e ≥1.428) yielded a maximized specificity, up to 97.18%. Any positive miRNA composite (fold change of any miRNA above the cutoff point) yielded a maximized Youden index, up to 0.60. Use of miRNA composites had a high accuracy for CI-AKI prediction.

**Association of Circulating miRNAs With Mehran Risk Score**

The Mehran Risk Score was capable of predicting CI-AKI occurrence and prognosis. Correlation analysis revealed that the change of candidate miRNAs postprocedure was significantly related with the Mehran Risk Score (P<0.05, Figure S1B). The mean Mehran Risk Score and the proportion of patients with a score ≥6 were higher in the group with elevated miRNAs postprocedure indicating CI-AKI (CI-AKImiRNA group) compared with the group that did not have significantly elevated miRNA levels (non-CI-AKImiRNA group); however, no significant difference in Mehran Risk Scores between the CI-AKI group defined by SCr/CyC (CI-AKIScr/CyC) and non-CI-AKIScr/CyC group was found (Figure 4A). Comparing with CI-AKIScr/CyC (+)/CI-AKImiRNA (−) patients, patients with CI-AKImiRNA (+)/CI-AKIScr/CyC (−) had higher Mehran Risk Scores and a higher proportion of patients with Mehran Risk Scores ≥6 (Figure 4B). These results indicated that the 3 candidate miRNAs might be more capable of detecting patients at real danger of CI-AKI than the traditional biomarkers.
Circulating miRNAs carry immense potential as novel, minimally invasive biomarkers in diagnosing human disease.\(^{11}\) In this study, we demonstrated that miRNAs circulating in plasma may be new biomarkers for CI-AKI.

Using a rat model of AKI following nonionic low-osmolar CM infusion, we observed the miRNA expression profiles in plasma and kidneys. Analysis of the microarray hybridization intensity showed 22 miRNA species and 9 miRNA species increased more than 1.5-fold in the plasma and kidneys of CI-AKI rats, respectively. Three human–rat homologous miRNAs, including miRNA-188, miRNA-30a, and miRNA-30e, were ultimately selected as candidate early biomarkers of CI-AKI. The following RT-qPCR examination demonstrated that the plasma levels of these miRNAs were significantly elevated at 4 hours post CM exposure following dehydration. Further investigation in patients undergoing elective CAG and/or PCI

**Table 3.** Discrimination Potential and Cutoff Increment Values of miRNAs for CI-AKI Prediction

| miRNAs      | AUC   | Cutoff Point I \(^*\) | Cutoff Point II \(^†\) |
|-------------|-------|------------------------|------------------------|
| miR-188-5p  | 0.784 | Fold-Change 1.343       | Sensitivity 52.11       |
|             |       |                        | Specificity 92.96       |
|             |       |                        | Youden Index 0.44       |
|             |       |                        | Fold-Change 2.540       |
|             |       |                        | Sensitivity 29.58       |
|             |       |                        | Specificity 100         |
|             |       |                        | Youden Index 0.29       |
| miR-30a-5p  | 0.802 | Fold-Change 1.405       | Sensitivity 56.34       |
|             |       |                        | Specificity 94.37       |
|             |       |                        | Youden Index 0.50       |
|             |       |                        | Fold-Change 2.757       |
|             |       |                        | Sensitivity 38.03       |
|             |       |                        | Specificity 100         |
|             |       |                        | Youden Index 0.37       |
| miR-30e-5p  | 0.805 | Fold-Change 1.428       | Sensitivity 64.79       |
|             |       |                        | Specificity 92.96       |
|             |       |                        | Youden Index 0.57       |
|             |       |                        | Fold-Change 3.005       |
|             |       |                        | Sensitivity 35.21       |
|             |       |                        | Specificity 100         |
|             |       |                        | Youden Index 0.34       |
| Any-positive| 0.818 | Fold-Change 1.428       | Sensitivity 71.83       |
|             |       |                        | Specificity 88.73       |
|             |       |                        | Youden Index 0.60       |
| Treble-positive| 0.818 | Fold-Change 1.428      | Sensitivity 40.85       |
|             |       |                        | Specificity 97.18       |
|             |       |                        | Youden Index 0.37       |

AUC indicates area under the curve; CI-AKI, contrast-induced acute kidney injury; miRNA, microRNA.

\(^*\) Cutoff point I: the best diagnostic sensitivity and specificity by receiver operating characteristic curves.

\(^†\) Cutoff point II: established under 100% specificity.

\(^\dagger\) The fold-change of any 1 of the 3 miRNAs above cutoff point I.

\(^\ddagger\) The fold-change of all 3 miRNAs above cutoff point I.

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indicated that the baseline plasma levels of candidate miRNAs were low and without significant difference among patients. The 3 candidate miRNAs all significantly increased 4 to 6 hours post CM exposure when compared with patients without CI-AKI. Receiver operating characteristics analysis revealed that selected miRNAs identified patients with CI-AKI as opposed to those without CI-AKI. MiRNA composites reached a high accuracy on CI-AKI prediction, as shown in maximized specificity or Youden index. Moreover, circulating miRNA-30a and miRNA-30e levels were also validated as predictors in another CI-AKI cohort study conducted by Gutierrez-Escolano et al.\textsuperscript{22} Taken together, our study indicates that monitoring the circulating levels of miRNA-188, miRNA-30a, and miRNA-30e could aid in early diagnosis of CI-AKI.

Figure 4. Candidate circulating miRNAs were associated with Mehran Risk Scores. A, Comparison of mean Mehran Risk Scores and incidence of Mehran Risk Score $\geq$6 between CI-AKI and non-CI-AKI patients by different CI-AKI criteria. B, Mean Mehran Risk Score and incidence of Mehran Risk Score $\geq$6 in 4 categories of patients. $+$/$+$ indicates patients who met the CI-AKI criteria based both on miRNA and CyC/SCr; $+$/$-$ indicates patients who met the CI-AKI criteria based on miRNA but not CyC/SCr; $-$/$+$ indicates patients who met the CI-AKI criteria based on CyC/SCr but not miRNA; $-$/$-$ indicates patients who did not meet the CI-AKI criteria based both on miRNA and CyC/SCr. Any-positive, the fold-change of any 1 of the 3 selected miRNAs reaching cutoff point I; treble-positive, the fold-change of all 3 selected miRNAs reaching cutoff point I. Error is represented as SEM. **$P<$0.01. ***$P<$0.001. CI-AKI indicates contrast-induced acute kidney injury; CyC/SCr, cystatin C/serum creatinine; miRNA, microRNA.
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The selected miRNAs might be associated with the pathophysiology of CI-AKI. These miRNAs are expressed in renal-specific fashion, indicating that the plasma level of these miRNAs is mainly affected by kidney injury. Only a high dose of CM (3 g I/kg) exposure without dehydration insignificantly impacted the plasma level of these miRNAs, which confirmed that the significant changes in the miRNA plasma levels occurred in the context of kidney injury but not CM exposure. Increased levels of miRNA-188 (up to 3.33-fold increase) were observed in kidney samples from CI-AKI rats. Bioinformatics analysis revealed that miRNA-188 is a key modulator of the MAPK-JNK/p38 pathway. The MAPK-JNK/p38 pathway is the major mechanism of CM-induced tubular renal cell injury, indicating that miRNA-188 might play an important role in the pathophysiology of CI-AKI. MiRNA-30a and miRNA-30e, members of the miRNA-30 family, are functionally related to each other. They play important roles in renal development and maintaining renal function. However, the levels of miRNA-30a and miRNA-30e did not show significant change in kidney samples from CI-AKI rats. Further studies are needed to test the levels of miRNA-30a and miRNA-30e in the outer medulla of kidney and not in the whole kidney, as was done in the current study, which might reveal a significant change in the levels of these 2 miRNAs when kidney injury occurs. Finally, the mechanism causing the change in the levels of the selected miRNAs, the pathophysiological relationship between these miRNAs, and their roles in CI-AKI development remain unknown and require further investigation.

The selected miRNAs might detect CI-AKI in patients at high risk. Current guidelines recommend monitoring SCr post CM exposure to detect CI-AKI. Clinical studies also revealed that CyC is a reliable biomarker of CI-AKI. However, both SCr and CyC are indirect markers of kidney function and unable to timely reflect the renal parenchymal damage during the acute phase of kidney injury. Much evidence demonstrates that the rise of SCr and CyC lags behind renal parenchyma injury, although the levels of SCr and CyC were not checked at 4 to 6 hours post CM exposure in the current study. In a previous study, no significant increase in either SCr or CyC at 5 hours after CAG/PCI compared to baseline was observed. Thus, a major finding in the present study was that changes in the levels of the selected miRNAs identified CI-AKI (4–6 hours post CM exposure) earlier than SCr/CyC. Further analysis showed that the selected miRNAs but not SCr/CyC correlated well with the Mehran Risk Scores, a valid predictor for CI-AKI occurrence and prognosis. The Mehran Risk Score was higher in the patients who had significant changes in the selected miRNA levels but no significant changes in SCr/CyC than in the patients who had no significant changes in miRNAs but positive changes in SCr/CyC, indicating that these select miRNAs might help to identify patients at real danger of CI-AKI and poor prognosis when compared with SCr/CyC.

Study Limitations

This study had several limitations. First, the peak values of these circulating miRNAs occurred at ≈4 hours and then dropped quickly. The narrow time-window for miRNA detection might limit the clinical utility of using these miRNAs as biomarkers for disease monitoring. Second, periprocedure intravenous hydration is the only approach to minimize the occurrence of CI-AKI. Absence of an increase in the levels of these miRNAs does not mean that the patient has no need for periprocedure hydration. Third, as significant kidney injury can exist with minimal change in kidney function, a sensitive definition of CI-AKI using a composite of SCr and CyC was adopted in this study. A portion of patients with a slight change in SCr were placed into the CI-AKI group. Potential bias might be introduced due to the biological averaging. Fourth, the proportion of patients with acute coronary syndrome and volume of CM given in the CI-AKI group was higher than in the controls. Although having demonstrated that the increase of these miRNAs was not in the context of CM administration and the Mehran Risk Score, an algorithm integrating CM volume, well-matched in these 2 groups, potential founders might have still existed. Fifth, baseline hydration status is associated with development of CI-AKI. However, parameters regarding the hydration status, such as left ventricular end-diastolic pressure, were unavailable in this study as factors for propensity matching. Last but not least, this is a single-center, small sample-size study without prognosis information. Larger cohorts are needed to evaluate the diagnostic accuracy of the selected miRNAs in unselected patients receiving CAG/PCI and roles of these miRNAs in predicting prognosis.

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Disclosures

None.
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Table S1. Temporal changes of candidate miRNAs in plasma from 9 patients with acute myocardial infarction receiving emergency PCI.

| Hours after PCI | Fold change from baseline (fold) * |
|----------------|----------------------------------|
|                | miR-188-5p | miR-30a-5p | miR-30e-5p |
| Baseline       | 1          | 1          | 1          |
| 4h             | 4.93±1.83  | 4.67±1.67  | 4.42±1.57  |
| 8h             | 2.85±1.32  | 3.31±1.56  | 3.02±1.68  |
| 12h            | 1.28±0.48  | 2.68±0.96  | 1.78±0.65  |
| 24h            | 1.67±0.45  | 2.22±0.72  | 1.62±0.45  |

* Levels of miRNAs are expressed as fold change relative to the patients’ corresponding baselines. PCI, percutaneous coronary intervention.
Table S2. Clinical characteristics of enrolled CI-AKI cases and remaining CI-AKI cases.

| Age (years) | Enrolled CI-AKI (n=71) | Remaining CI-AKI (n=64) | p-value |
|-------------|------------------------|-------------------------|---------|
| 60.82±1.27  | 64.34±1.29             | 0.054                   |
| Male, n (%) | 45 (63.38)             | 35(54.69)               | 0.305   |
| Current smoker, n (%) | 23 (32.39) | 15(23.44) | 0.248 |
| HBP, n (%)  | 44 (61.97)             | 49(76.56)               | 0.067   |
| DM, n (%)   | 21 (29.58)             | 24(37.50)               | 0.330   |
| Hyperlipidemia, n (%) | 25 (35.21) | 24(37.50) | 0.782 |
| Prior MI, n (%) | 12 (16.90) | 6 (9.38) | 0.217 |
| Prior PCI, n (%) | 20 (28.17) | 10 (15.63) | 0.099 |
| Systolic pressure (mmHg) | 130.24±2.26 | 133.18±1.48 | 0.642 |
| Diastolic pressure (mmHg) | 77.20±1.15 | 79.44±1.32 | 0.738 |
| ACS, n (%)  | 28 (39.44)             | 17 (26.56)              | 0.113   |
| ACEI, n (%) | 37 (52.11)             | 40(62.50)               | 0.223   |
| Statin, n (%) | 64 (90.14) | 63(98.43) | 0.065 |
| CM volume (mL) | 100.99±7.16 | 93.38±5.13 | 0.286 |
| CKD stage   |                        |                         |         |
|   | Mehran Score | Baseline SCr (mg/dL) | Baseline CyC (mg/L) | Baseline eGFR (mL/min/1.73m²) |
|---|--------------|----------------------|---------------------|-------------------------------|
| 1 | 56 (78.87)   | 0.72±0.02            | 0.89±0.05           | 1 (1.41)                     |
| 2 | 13 (18.31)   | 35(54.68)            | 40(62.50)           | 14 (19.72)                   |
| 3 | 2 (2.82)     | 20(31.25)            | 13(20.31)           | 1 (1.41)                     |
|   | 3.90±0.37    | 4.77±0.46            | 0.96±0.16           | 10(15.63)                    |
|   | <6           | 55 (77.46)           | 1.21±0.15           | 1 (1.56)                     |
|   | 6-10         | 0.145               | 0.057               | 0.003                         |
|   | 11-16        | 0.931               | 0.108               | 0.025                         |
| >16| 0.129        | 0.063               | 1.000               | 0.145                         |

CI-AKI, contrast-induced acute kidney injury; HBP, high blood pressure; DM, diabetes mellitus; MI, myocardial infarction; PCI, percutaneous coronary intervention; ACS, acute coronary syndrome; ACEI, angiotensin-converting-enzyme inhibitor; CM, contrast medium; CKD, chronic kidney disease; SCr, serum creatinine; CyC, Cystatin C.
Table S3. Plasma miRNA levels at baseline detected by RT-qPCR.

|                            | Cq value by RT-qPCR |
|---------------------------|---------------------|
|                           | miR-188-5p          | miR-30a-5p          | miR-30e-5p          |
| Total                     | 33.45±1.55          | 29.05±1.40          | 28.64±1.47          |
| CI-AKI                    | 33.60±1.60          | 29.25±1.59          | 28.74±1.60          |
| non CI-AKI                | 33.30±1.49          | 28.85±1.15          | 28.54±1.33          |
| DM                        | 32.87±1.82          | 28.75±1.09          | 28.46±1.32          |
| non DM                    | 33.68±1.37*         | 29.18±1.49          | 28.73±1.52          |
| CKD stage                 |                     |                     |                    |
| 1                         | 33.44±1.53          | 29.04±1.46          | 28.62±1.53          |
| 2                         | 33.73±1.49          | 29.15±1.23          | 28.78±1.27          |
| 3                         | 31.78±1.55          | 28.86±0.38          | 28.34±0.87          |
| Age                       |                     |                     |                    |
| <50                       | 34.34±1.61          | 29.45±1.41          | 29.02±1.55          |
| 50-70                     | 33.31±1.49†         | 28.92±1.46          | 28.61±1.57          |
| >70                       | 33.30±1.52‡         | 29.24±1.12          | 28.74±1.20          |

* P <0.05 vs. DM group. † P <0.05 vs. Age < 50 group. ‡ P <0.05 vs. Age < 50 group.

RT-qPCR, reverse transcription quantitative real-time PCR; CI-AKI, contrast-induced acute kidney injury; DM, diabetes mellitus; CKD, chronic kidney disease; Cq, quantification cycle.
Table S4. The relative change in miRNA levels compared with baseline in subgroups categorized by different CI-AKI criteria.

|                | SCr(-) & CyC(-) | SCr(+) OR CyC(+) | SCr(+) | CyC(+) | SCr(+) & CyC(+) |
|----------------|-----------------|-------------------|--------|--------|-----------------|
| N              | 71              | 71                | 17     | 64     | 10              |
| miRNA-188-5p   | 0.77±0.01       | 2.36±0.03         | 2.52±0.44 | 2.45±0.29 | 3.16±0.52 |
| miRNA-30a-5p   | 0.68±0.01       | 3.26±0.05         | 3.51±0.69 | 3.36±0.48 | 4.28±0.79 |
| miRNA-30e-5p   | 0.74±0.01       | 3.03±0.04         | 3.65±0.87 | 3.13±0.39 | 4.77±1.24 |

Levels of miRNAs are expressed as fold change relative to the patients’ corresponding baselines.

CI-AKI, contrast-induced acute kidney injury; N, number; SCr(+), meeting the SCr-based CI-AKI criteria: an absolute increase in SCr ≥0.3 mg/dL or relative increase in SCr ≥25% over baseline; SCr(-), not meeting the SCr-based CI-AKI criteria; CyC(+), meeting the CyC-based CI-AKI criteria: relative increase in CyC ≥10% over baseline; CyC(-), not meeting the CyC-based CI-AKI criteria.
Table S5. The association of the change of miRNA levels and CI-AKI by logistic regression analysis.

| Variables | miRNA-188-5p | miRNA-30a-5p | miRNA-30e-5p |
|-----------|--------------|--------------|--------------|
|           | OR (95% CI)  | OR (95% CI)  | OR (95% CI)  | \( p \) | \( p \) | \( p \) |
| miRNA*    | 4.238 (2.194-8.185) | 3.931 (2.153-7.178) | 4.261 (2.287-7.940) | <0.001 | <0.001 | <0.001 |
| Age, year | 0.990 (0.951-1.031) | 0.980 (0.939-1.023) | 0.986 (0.944-1.029) | 0.629 | 0.363 | 0.515 |
| eGFR, ml/min/1.73m² | 1.004 (0.988-1.020) | 1.013 (0.997-1.029) | 1.011 (0.995-1.028) | 0.615 | 0.117 | 0.166 |

*, as fold change of miRNA level relative to the patients’ corresponding baselines.

CI-AKI, contrast-induced acute kidney injury; OR, odd ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate.
Table S6. Comparison between the three selected miRNAs in CI-AKI prediction (n=142).

| N (%) | miRNA-188-5p | miRNA-30a-5p | miRNA-30e-5p |
|-------|--------------|--------------|--------------|
| Consistent: | | | |
| 31 (21.83%) | + | + | + |
| 83 (58.45%) | - | - | - |
| Total: 114 (80.28%) | | | |
| Inconsistent: | | | |
| 0 (0.00%) | + | + | - |
| 4 (2.82%) | + | - | + |
| 12 (8.45%) | - | + | + |
| 7 (4.93%) | + | - | - |
| 1 (0.70%) | - | + | - |
| 4 (2.82%) | - | - | + |
| Total: 28 (19.72%) | | | |

CI-AKI, contrast-induced acute kidney injury; N, number; “+,” meeting the miRNA-based CI-AKI criteria; “−,” not meeting the miRNA-based CI-AKI criteria.
Figure S1.
Supplementary Figure Legends

Figure S1. (A) Correlation analysis of fold change of candidate circulating miRNA levels from baseline with the relative increase in Scr and CyC. (B) Correlation analysis of the fold change of candidate circulating miRNA levels with Mehran Risk Scores. r, Pearson correlation coefficient; p, relative computed p-value.