Prognostic Value of MicroRNAs in Coronary Artery Diseases: A Meta-Analysis

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Purpose: Coronary artery diseases (CADs) are the leading causes of death in the world. Recent studies have reported that differentially expressed microRNAs (miRNAs) are associated with prognosis or major adverse cardiac events (MACEs) in CAD patients. In a previous meta-analysis, the authors made serious mistakes that we aimed to correct through an updated systematic review and meta-analysis of the prognostic value of altered miRNAs in patients with CADs.

Materials and Methods: We performed a systematic search of MEDLINE (from inception to May 2017) and EMBASE (from inception to May 2017) for English-language publications. Studies of CADs with results on miRNAs that reported survival data or MACEs were included. Data were extracted from each publication independently by two reviewers.

Results: After reviewing 515 articles, a total eight studies were included in this study. We measured pooled hazard ratios (HRs) and 95% confidence intervals (CIs) of miRNA 133a with a fixed-effect model (pooled HR, 2.35; 95% CI, 1.56–3.55). High expression of miRNA 133a, 208b, 126, 197, 223, and 122-5p were associated with high mortality. Additionally, high levels of miRNA 208b, 499-5p, 134, 328, and 34a were related with MACEs.

Conclusion: The present study confirmed that miRNA 133a, which was associated with high mortality in CAD patients, holds prognostic value in CAD. More importantly, this study corrected issues raised against a prior meta-analysis and provides accurate information.

Key Words: Coronary artery disease, microRNA, prognosis, meta-analysis
and more, new strategies based on miRNAs such as diagnostic and therapeutic targets have been developed.8,12,13,16-18 Of these, many researchers have suggested that miRNA or miRNA signatures may be diagnostic and prognostic biomarkers for human diseases.16,17 During a study of the relationship between CADs and miRNAs, we found severe errors in a previous meta-analysis.19 Therefore, we conducted a systematic review and meta-analysis in the present study to better define the prognostic value of various miRNAs in patients with CADs.

**MATERIALS AND METHODS**

**Eligibility and search strategy**

We performed a systematic search of MEDLINE (from inception to May 2017) and EMBASE (from inception to May 2017) for English-language publications using the keywords “coronary artery disease,” “NSTEMI,” “STEMI,” “cardiovascular disease,” “microRNA,” “death,” “prognosis,” and “major adverse cardiovascular events (MACEs).” All searches were limited to human studies. The inclusion criteria were studies of coronary artery diseases that reported the results of miRNA expressions, survival data, and MACEs. Laboratory studies, reviews, letters, comments, and editorial materials were excluded. We also excluded full-text articles that lacked sufficient data for calculating hazard ratios (HRs) or odds ratios (ORs) using Engauge Digitizer (http://digitizer.sourceforge.net). If reported values were much different from calculated values by using Engauge Digitizer, we defined the paper as an inadequate article.

**Quality assessment**

The Newcastle-Ottawa scale was used to evaluate the quality of studies incorporated in this meta-analysis, which was done based on the following three aspects: selection of the study groups, comparability of the groups, and the outcome of interest. The lowest score was 0 and the highest was 9. Studies with a score ≥6 were considered as high quality.20-22 We set 12 months as an adequate follow-up length (Table 1).

**Data extraction and statistical analysis**

Data were extracted from the publications independently by two reviewers, and the following information was recorded: first author, year of publication, country, miRNA expression analyzed, number of patients, and end points. The primary outcome was mortality defined as the time from the initiation of therapy until death from any causes. The secondary endpoint was MACEs defined as cardiac death, heart failure, decreased ejection fraction, or cardiogenic shock.

The effects of miRNA expressions on mortality were assessed using HRs and on MACEs were assessed using ORs. In cases
of mortality, a univariate HR estimate and 95% confidence intervals (CIs) were extracted directly from each study, if provided by the authors. Otherwise, \( p \) values of the log-rank tests, 95% CIs, number of events, and numbers of patients at risk were extracted to estimate the HR indirectly. Survival rates calculated from Kaplan-Meier curves were read using Engauge Digitizer, version 3.0 (http://digitizer.sourceforge.net) to reconstruct the HR estimate and its variance, assuming that patients were censored at a constant rate during follow-up. In case of MACEs, a multivariate OR estimate was extracted directly from each study. However, we could not perform meta-analysis because the variables were different in each of the studies. The HRs and ORs were calculated on the basis of high expression of miRNA, which means HR > 1 and OR > 1 implied poor prognosis and high MACEs for patients. Heterogeneity among studies was assessed using \( \chi^2 \) and \( \Gamma^2 \) statistics. Heterogeneity was considered to be low if \( \Gamma^2 < 25\% \), medium if between 25% and 75%, and high if \( \Gamma^2 > 75\% \). If there was obvious heterogeneity (\( \Gamma^2 > 50\% \)), the random-effects model was used, otherwise the fixed-model was used.\(^9\) Funnel plots were used to assess publication bias.\(^23\) Begg’s test and Egger’s test were also used to identify publication bias, and these tests were performed by using the ‘metafor’ package in R. The forest and funnel plots were depicted using Review Manager (RevMan, version 5.3: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, Copenhagen, Denmark). \( p \) values < 0.05 were considered to be statistically significant. The pooled HR and heterogeneity of miRNA 133a was calculated using Review Manager.

**RESULTS**

**Study characteristics**

The electronic search identified 515 articles. Non-human studies (n=33), non-English articles (n=12), conference abstracts (n=193), and 201 studies that did not meet the inclusion criteria based on their title and abstract were excluded. After reviewing the full text of 76 articles, eight studies were eligible for inclusion in the study (5 articles; prognosis in 1987 patients, 3 articles; MACEs in 792 patients) (Fig. 1). All of the included studies had a prospective design, and reported the prognostic value of six different miRNAs (122-5p, 126, 133a, 197, 208b, 223) in HRs\(^{24-28}\) and five different miRNAs (34a, 134, 208b, 328, 499-5p) in OR.\(^{29-31}\) Included studies were performed recently (2011–2016), and the study characteristics are summarized in Table 2.

**Quality assessment**

The Newcastle-Ottawa scale indicated that the methodological quality of the included studies varied from 6 to 8. Three articles received a score of 6, three articles received a score of 7, and two articles received a score of 8 (Table 1).

**Higher miRNA expression associated with worse prognosis of coronary artery diseases**

To analyze the prognostic value of high expression of miRNAs in CADs, forest plots with mortality and MACEs are depicted in Figs. 2 and 3. High expression of miRNA 133a, 208b, 197, 223, and 122-5p were associated with high mortality (Fig. 2A). The overall HR of all miRNAs for mortality was 1.76 (95% CI 1.40–2.23, \( p < 0.00001 \)) (Fig. 2A). High level of miRNA 208b, 499-
5p, 134, 328, and 34a showed significance with MACEs (overall OR 1.86, 95% CI 1.56−2.21, \( p=0.00001 \)) (Fig. 2B).

We analyzed prognostic significance between mortality and expression of miRNA 133a that were studied by three independent articles. We applied the fixed-effect model on miRNA 133a because we determined that heterogeneity was low through various statistical values (\( \chi^2=0.69, \ p=0.71, I^2=0\% \)) (Fig. 3A). As shown in Fig. 3A, the pooled HR of miRNA 133a for

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\begin{array}{c|c|c|c|c|c|c|c|c}
\text{Study or subgroup} & \text{log(HR)} & \text{SE} & \text{Weight (\%)} & \text{HR} & \text{HR} \\
& & & & \text{IV, fixed, 95\% CI} & \text{Year} \\
\hline
\text{Widera, et al.}^{28} (133a) & 0.9166 & 0.3336 & 39.5 & 2.50 [1.30, 4.81] & 2011 \\
\text{Eitel, et al.}^{25} (133a) & 1.0986 & 0.4467 & 22.0 & 3.00 [1.25, 7.20] & 2012 \\
\text{Ke-Gang, et al.}^{26} (133a) & 0.6523 & 0.3380 & 38.5 & 1.92 [0.99, 3.72] & 2011 \\
\text{Total (95\% CI)} & 100 & 1.76 [1.40, 2.23] \\
\text{Heterogeneity:} & \chi^2=8.12, df=5 (p=0.15); I^2=38\% \\
\text{Test for overall effect:} & Z=6.89 (p<0.00001) \\
\hline
\end{array}
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\[\text{Fig. 2. Systematic summary for prognostic values of miRNAs with CADs. (A) Forest plots for mortality of miRNA expression in patients with CADs. (B) Forest plots for major adverse cardiac events of miRNA expression in patients with CADs. HR, hazard ratio; OR, odds ratio; SE, standard error; CI, confidence interval; miRNA, microRNA; CAD, coronary artery disease.}\]

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\begin{array}{c|c|c|c|c|c|c|c|c}
\text{Study or subgroup} & \text{log(OR)} & \text{SE} & \text{Weight (\%)} & \text{OR} & \text{OR} \\
& & & & \text{IV, fixed, 95\% CI} & \text{Year} \\
\hline
\text{Gidlöf, et al.}^{29} (208b) & 0.5822 & 0.1327 & 45.7 & 1.79 [1.38, 2.32] & 2013 \\
\text{He, et al.}^{30} (328) & 1.9947 & 0.9832 & 0.8 & 7.35 [1.07, 50.49] & 2014 \\
\text{Lv, et al.}^{31} (208b) & 2.8854 & 1.0009 & 0.7 & 17.91 [2.07, 154.95] & 2015 \\
\text{He, et al.}^{30} (134) & 0.8242 & 0.4054 & 4.9 & 2.20 [1.03, 5.05] & 2014 \\
\text{Lv, et al.}^{31} (34a) & 1.4303 & 0.5729 & 2.5 & 4.18 [1.36, 12.85] & 2015 \\
\text{Total (95\% CI)} & 100 & 1.86 [1.56, 2.21] \\
\text{Heterogeneity:} & \chi^2=8.98, df=5 (p=0.11); I^2=44\% \\
\text{Test for overall effect:} & Z=6.89 (p<0.00001) \\
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\end{array}
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\[\text{Fig. 3. Forest plot (A) and funnel plot (B) for mortality of microRNA 133a expression in patients with coronary artery diseases in this meta-analysis. SE, standard error; HR, hazard ratio; CI, confidence interval.}\]
mortality was 2.35 (95% CI 1.56–3.55, p<0.0001), which means high expression of miRNA 133a showed a strong relationship with high mortality in CADs.

Publication bias
The funnel plot in this meta-analysis seemed symmetrical (Fig. 3B). The results of Begg’s and Egger’s test were not significant (Table 3). These results suggested no evidence for publication bias.

DISCUSSION
A systematic review and meta-analysis provides significant information to researchers, such that analysis performed incorrectly can cause serious problems. In a previous meta-analysis, the authors made severe mistakes when they extracted and merged various results. First, the authors changed some ORs in references to HRs, and then the changed HRs that were combined with other HRs from other researches.29-32 Because OR is quite different from HR, they should not be combined in the meta-analysis. Second, they merged HRs from univariate analysis with other HRs from multivariate analysis.19 Univariate analysis is the simplest statistical method because it only considers only one variable, whereas multivariate analysis involves analysis of more than one variable at a time. For that reason, HRs from univariate analysis are not that same as HRs from multivariate analysis, even though they can be calculated using same data.29-31 Time-to-event outcomes are the most important factors in prognostic studies; however, the authors in the previous meta-analysis misused the mean follow-up months from reference.29 For these reason, we corrected critical problems of the previous meta-analysis and updated recent results on the prognostic value of miRNAs in CADs to help scientists interested in miRNA research.

Circulating miRNAs have emerged as potential diagnostic markers in various diseases, including CADs, due to their accessibility by drawing a patient’s blood.24-32 In this study, we found that high levels of circulating miRNA 133a, 208b, 126, 197, 223, and 122-5p in CAD patients were related with high mortality.24-26 In addition, high expression of miRNA 208b, 499-5p, 134, 328, and 34a were associated with MACEs.28-31 Although many miRNAs in this study were found to be associated with prognosis of CADs patients, most of them were identified only by a single report, except miRNA 133a.24-31 Therefore, we have shown through meta-analysis of miRNA 133a that high expression of it is associated with a poor prognosis of CADs.

MiRNAs are known to be important regulators of all major cellular functions, including differentiation, proliferation, apoptosis, and angiogenesis.25-34 Among them, miRNA 133a has been widely reported as a regulator of cardiomyocyte proliferation, and tumor-suppressor.15-30 Moreover, recent studies found that increased levels of circulating miRNA 133a in CADs patients, which had correlation with troponin.13,28,29,40 In this meta-analysis, three articles showed consistent results suggesting that high expression of miRNA 133a is strongly associated with poor prognosis in CAD patients (Fig. 3).25,26,30

Several limitations need to be considered when interpreting the results of the current study, although this study has an advantage because it corrects previous miscalculations.19 Although included studies in this study addressed diverse miRNAs, we could not perform a systematic review of the relationship between miRNA expressions and MACEs because the data were analyzed by multivariate analyses using different variables. Thus, large prospective studies will be required to confirm our findings and would be helpful to developing prognostic markers of CADs.

In summary, despite the limitations, this comprehensive systematic review and meta-analysis reveals that circulating miRNAs, especially miRNA 133a, could be potential prognostic markers of CADs. The most important aspect of this study is that it can prevent problems caused by previously erroneous studies.

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REFERENCES
1. Nabel EG. Cardiovascular disease. N Engl J Med 2003;349:60-72.
2. Roth GA, Forouzanfar MH, Moran AE, Barber R, Nguyen G, Feigin VL, et al. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med 2015;372:1333-41.
3. Roth GA, Nguyen G, Forouzanfar MH, Mokdad AH, Naghavi M, Murray CJ. Estimates of global and regional premature cardiovascular mortality in 2025. Circulation 2015;132:1270-82.
4. Kim YH, Lee SJ, Seo KW, Bae JU, Park SY, Kim EK, et al. PAF enhances MMP-2 production in rat aortic VSMCs via a β-arrestin2-dependent ERK signaling pathway. J Lipid Res 2013;54:2678-86.
5. Kim YH, Bae JU, Lee SJ, Park SY, Kim CD. SIRT1 attenuates PAF-induced MMP-2 production via down-regulation of PAF receptor expression in vascular smooth muscle cells. Vascul Pharmacol
6. Kim YH, Bae JU, Kim IS, Chang CL, Oh SO, Kim CD. SIRT1 prevents pulmonary thrombus formation induced by arachidonic acid via downregulation of PAF receptor expression in platelets. Platelets 2016;27:735–42.

7. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.

8. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509–24.

9. Kim YH, Goh TS, Lee CS, Oh SO, Kim JJ, Jeung SH, et al. Prognostic value of microRNAs in osteosarcoma: a meta-analysis. Onco-target 2017;8:3726–37.

10. Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer 2007;43:1529–44.

11. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res 2010;107:677–84.

12. Latronico MV, Condorelli G. MicroRNAs and cardiac pathology. Nat Rev Cardiol 2009;6:419–29.

13. D’Alessandra Y, Devanna P, Limana F, Straino S, Di Carlo A, Brambilla PG, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur Heart J 2010;31:2765–73.

14. Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 2010;11:354–61.

15. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–8.

16. Christopher AF, Kaur RP, Kaur G, Gupta V, Bansal P. MicroRNA therapeutics: discovering novel targets and developing specific therapy. Perspect Clin Res 2016;7:68–74.

17. Huang W. MicroRNAs: biomarkers, diagnostics, and therapeutics. Methods Mol Biol 2017;1617:57–67.

18. Chan D, Ng LL. Biomarkers in acute myocardial infarction. BMC Med 2010;8:34.

19. Cao W, Guo Q, Zhang T, Zhong D, Yu Q. Prognostic value of microRNAs in acute myocardial infarction: a systematic review and meta-analysis. Int J Cardiol 2015;189:73–80.

20. Shao Y, Geng Y, Gu W, Huang J, Ning Z, Pei H. Prognostic significance of microRNA-375 downregulation in solid tumors: a meta-analysis. Dis Markers 2014;2014:626185.

21. Wang J, Yu M, Guan S, Zhang G, Wang J, Cheng Y. Prognostic significance of microRNA-100 in solid tumors: an updated meta-analysis. Onco Targets Ther 2017;10:493–502.

22. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.

23. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.

24. Cortez-Dias N, Costa MC, Carrilho-Ferreira P, Silva D, Jorge C, Calisto C, et al. Circulating miR-122-5p/miR-133b ratio is a specific early prognostic biomarker in acute myocardial infarction. Circ J 2016;80:2183–91.

25. Eitel I, Adams V, Dieterich P, Fuernau G, de Waha S, et al. Relation of circulating MicroRNA-133a concentrations with myocardial damage and clinical prognosis in ST-elevation myocardial infarction. Am Heart J 2012;164:706–14.

26. Ke-Gang J, Zhi-Wei L, Xin Z, Jing W, Ping S, Xue-Jing H, et al. Evaluating diagnostic and prognostic value of plasma miRNA133a in acute chest pain patients undergoing coronary angiography. Medicine (Baltimore) 2016;95:e3412.

27. Schulte C, Molz S, Appelbaum S, Karakas M, Ojeda E, Lau DM, et al. miRNA-197 and miRNA-223 predict cardiovascular death in a cohort of patients with symptomatic coronary artery disease. PLoS One 2015;10:e0145930.

28. Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, et al. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. J Mol Cell Cardiol 2011;51:872–5.

29. Gidlöf O, Smith JG, Miyazaki K, Gilje P, Spencer A, Blomquist S, et al. Circulating cardio-enriched microRNAs are associated with long-term prognosis following microRNA-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–8.

30. Olivier F, Antonicelli R, Spazzafumo L, Santini G, Rippo MR, Galeazzi R, et al. Admission levels of circulating miR-499-5p and risk of death in elderly patients after acute non-ST elevation myocardial infarction. Int J Cardiol 2014;172:e276–8.

31. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.

32. Kano M, Seki N, Kikkawa N, Fujimura L, Hoshiba I, Akutsu Y, et al. miR-145, miR-133a and miR-133b: tumor-suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma. Int J Cancer 2010;127:2804–14.

33. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Basu-Dubey R, et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 2008;22:3242–54.

34. Vo NK, Dalton RP, Liu N, Olson EN, Goodman RH. Affinity purification of microR-133a with the cardiac transcription factor, Hand2. Proc Natl Acad Sci U S A 2010;107:19231–6.

35. Wu ZS, Wang CQ, Xiang R, Liu X, Ye S, Yang XQ, et al. Loss of miR-133a expression associated with poor survival of breast cancer and restoration of miR-133a expression inhibited breast cancer cell growth and invasion. BMC Cancer 2012;12:51.

36. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–8.

37. Vo NK, Dalton RP, Liu N, Olson EN, Goodman RH. Affinity purification of microRNA-133a with the cardiac transcription factor, Hand2. Proc Natl Acad Sci U S A 2010;107:19231–6.

38. Wu ZS, Wang CQ, Xiang R, Liu X, Ye S, Yang XQ, et al. Loss of miR-133a expression associated with poor survival of breast cancer and restoration of miR-133a expression inhibited breast cancer cell growth and invasion. BMC Cancer 2012;12:51.

39. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, et al. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Eur J Cardiovasc Disord 2013;8:638–44.

40. Devasa V, Vausort M, McCann GP, Kelly D, Collignon O, Ng LL, et al. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. PLoS One 2013;8:e70644.