ORIGINAL RESEARCH

Application Value of Combined Detection of miR-208 and miR-92a in Early Diagnosis of Myocardial Infarction

Shenshen Zhi, MS; Guo Liu, MS; Yan Ding; Wenyan Wu; Yuanyuan Zhang; Hai Yao; Jianda Liu; Wei Li, PhD

ABSTRACT

Objective • Myocardial infarction (MI) is a common and serious cardiovascular disease with increasing incidence and mortality rates, making it a major global public health issue. Molecular biology research has shown that the cleavage products miR-208 and miR-92a are microRNAs (miRNAs) associated with myocardial injury. Therefore, this study aims to establish a predictive model and explore the application value of the combined detection of miR-208 and miR-92a in the early diagnosis of MI in microRNA.

Methods • Plasma samples were collected from 231 volunteers divided into 30 healthy and 201 diseased subjects From January 1st, 2021 to December 30th, 2021. Plasma RNA was extracted using a TRIZOL kit, and levels of miR-208 and miR-92a were determined using a real-time polymerase chain reaction (PCR) assay. Subsequently, the logistic regression model, decision tree model analysis, and receiver operating characteristic (ROC) curve were used to evaluate whether miR-208 combined with miR-92a could be used as a biomarker for MI early diagnosis.

Results • In this study, the ROC curve evaluation of the logistic regression model and pruned decision tree model found that age, miR-208, and miR-92a had high early diagnostic accuracy for MI, and the area under the curve (AUC) reached 0.928, showing good predictive value. It was also found that the AUC, optimal threshold, sensitivity, and specificity of age, miR-208, and miR-92a were higher than those of age and miR-208. This indicates that the combination of age, miR-208, and miR-92a has more value in the early diagnosis of MI.

Conclusion • The combined diagnosis of miR-208 and miR-92a is helpful for the early diagnosis of myocardial infarction, which might serve as a new marker of MI benefiting from its early diagnosis. (*Altern Ther Health Med.* 2024;30(5):197-201)

Shenshen Zhi, MS; Yan Ding; Wenyan Wu; Yuanyuan Zhang; Hai Yao; Jianda Liu; Wei Li, PhD; Clinical Laboratory, Chongqing University Central Hospital, School of Medicine, Chongqing University, Chongqing, China; **Guo Liu, MS;** Meigeer Dental, Chongqing, China.

Corresponding author: Wei Li, PhD
E-mail: liwei0111@163.com

INTRODUCTION

Myocardial infarction (MI) is pathologically defined as cardiomyocyte death caused by long-term ischemia in this tissue.[1] The fourth general definition of MI has updated the universal definitions of both myocardial injury and MI. Meanwhile, some researchers have pointed out that the term myocardial injury should be used when the values of cardiac troponin (cTn) are elevated, and at least one value is reported above the 99th percentile upper reference limit (URL); if the cTn value rises or falls, acute myocardial injury is considered.[1] The currently accepted classification of myocardial infarction was first proposed in 2007 when the major cardiology societies recognized five different subtypes of MI.[2,3] Type 1 is classified as spontaneous myocardial infarction which is associated with ischemia because the episode of a coronary event is primary; Type 2 refers to MI secondary to ischemia when oxygen supply is either elevated or declined; Type 3 MI refers to the sudden unexpected cardiac death; The fourth one is the MI associated with percutaneous coronary interventions (PCI) and with stent thrombosis confirmed by angiography or autopsy; And the last type is myocardial infarction with coronary artery bypass graft.[3] Serious myocardial ischemia can lead to acute myocardial infarction, as well as severe complications such as ventricular septal perforation, mitral insufficiency, and ventricular aneurysm. This condition is prevalent in China and worldwide, with rapidly changing symptoms and a high fatality rate of over 50%.[4-6] It is therefore that early diagnosis and treatment are particularly important.[7] For early diagnosis and effective detection of the disease, researchers at home and abroad are searching for sensitive and specific detection indicators for early prediction with long duration.

MI has been recognized as a main cause of death among several diseases in the world, and it is of great clinical significance to accurately assess the symptoms of patients with MI.[8,9] Although multiple methods and techniques are employed for screening and monitoring this disease, to achieve early prevention and diagnosis, hoping to reduce MI incidence and mortality through appropriate disease management, there are few reports on the research progress of effective treatment of myocardial infarction.[10] Therefore, highlight should be made on the research of early diagnosis of myocardial infarction. Related literature has reported that microRNAs (miRNAs) are differently expressed in various diseases and have certain tissue and cell specificities. Changes in miRNAs detected after myocardial infarction can be used as markers of myocardial injury.[11] Similarly, there are some studies have indicated that both miR-208a and miR-92a have the potential as diagnostic and therapeutic targets for MI diseases.[5,12,13] However, Emanuela et al. have also revealed that miR-92a expression was increased in MI patients, but did not provide diagnostic value for MI.[14-16] Consequently, in this study, the TRIZOL kit was used to extract RNA from plasma samples, and real-time PCR was used to detect the expression levels of miR-208 and miR-92a. A logistic regression model, decision tree model analysis, and ROC curve were used to evaluate whether miR-208 combined with miR-92a could be used as a biomarker for early diagnosis of MI.

MATERIALS AND METHODS

Sample information

A total of 231 samples were collected from the Chongqing University Central Hospital. Out of these, 201 samples were collected from patients diagnosed with myocardial infarction (disease group). The disease group consisted of 144 male patients and 57 female patients, with an age range of 23-96 years. In addition, 30 samples were collected from healthy individuals (normal group), including 16 males and 14 females, with an age range of 22-85 years.

Real-time Polymerase Chain Reaction (q-PCR)

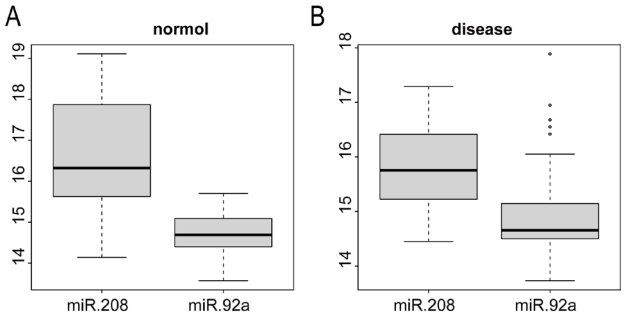
The extraction method of plasma RNA was based on the experimental method of Zhang et al.[17], and the TRIZOL kit (Epicentre) (Beijing, China) (Trizol Kit (T9424, Merck KGaA, Darmstadt, Germany) was used to extract plasma RNA from samples. After RNA extraction from 231 plasma samples, using U6 as the reference gene and 2 target genes (Table 1 for primer information), SYBR GREEN I was adopted and the expression of target genes in the submitted samples was detected. The testing unit is Beijing My Genostics, Inc (Beijing MyGenostics Technology Co., Ltd.). All the samples were classified into healthy group (n = 30) and diseased group (n = 201). Exogenous U6-F and U6-R were added and used as internal references of plasma. The expression differences of miR-208a and miR-92a between groups were compared using the $2^{-\Delta\Delta Ct}$ method. The primer sequences required for q-PCR are shown in Table 1.

Table 1. Primer sequences

Gene sequence number	Primes	Sequence (5'to3')	Annealing temperature (TM)
Internal reference	U6-F	CTCGCTTCGGCAGCACATATACT	60°C
	U6-R	ACGCTTCACGAATTTGCGTGTC	
1	miR-208a-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTGTTCG	
	miR-208a-F	GGCTATTCTGCTCGTTTTTCG	60°C
	miR-208a-R	CAGTGCAGGGTCCGAGGTAT	
2	miR-92a-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTGTCCG	
	miR-92a-F	CATAACGTGAACAGGGCCG	60°C
	miR-92a-R	CAGTGCAGGGTCCGAGGTAT	

Abbreviations: Tm, Melting Temperature. The annealing temperature and Tm were both 60°C, Reverse Transcription (RT).

Figure 1. miR-92a and miR-208 average expression in normal group and diseased group.



Construction and evaluation of logistic regression prediction model

The 231 samples were randomly divided into a training set (159) and a validation set (72) at a 7:3 ratio. The training set samples were used for model construction, while the validation set was used for model evaluation and validation. The Logistic regression prediction model was built using the MASS package in the R language. The receiver operating characteristic (ROC) curve was used to evaluate the logistic regression prediction model. *Pr* (>|z|) < .05 was regarded as statistically significant.

Construction and evaluation of decision tree model

The 231 samples were randomly divided into a training set (159) and a validation set (72) in a ratio of 7:3. The training set samples were used for model construction, while the validation set was used for model evaluation and validation. The decision tree prediction models were built using the part package and party package in the R language. The receiver operating characteristic (ROC) curve was used to evaluate the logistic regression prediction model. *Pr* (>|z|) < .05 was regarded as statistically significant.

RESULTS

miR-208 and miR-92a average expression in plasma samples

We visualized the average expression levels of miR-208 and miR-92a in the plasma of the normal group and the disease group. Figure 1 shows that the average expression of miR-208 is higher than that of miR-92a in both the normal group and the disease group.

Logistic regression model to predict the early diagnostic value of each index for MI

The values of sex, age, miR-208, and miR-92a for the early diagnosis of MI were analyzed. Table 2 shows the results of the logistic regression model, in which age, miR-208, and miR-92a were significant variables for the early diagnosis of MI, while sex was not significant. A logistic regression model was calculated based on the following formula: Y=0.08509593age 1.31409977miR.208 + 1.66957349miR.92a 6.30171680.

The logistic regression model was then evaluated by the ROC curve (age, miR-208, and miR-92a) (Figure 2). ROC curve analysis showed that the AUC was 0.928, and the optimal threshold point was 0.788. That is, when the probability value calculated by logistic regression was greater than 0.788, implying that the subject developed MI, and the sensitivity and specificity under the optimal threshold were 93.2% and 80%, respectively.

The decision tree model predicts the index on the early diagnostic value of MI

A decision tree model was further used to optimize and predict the early diagnostic value of age, miR-208, and miR-92a in MI (Figure 3). The results showed that age was the most important factor, followed by miR-208 and miR-92a. This was consistent with logistic regression analysis, where older age was associated with a greater risk of MI; With the decrease of miR-208 expression level, the risk of MI increases. On the contrary, the higher the expression level of miR-92a, the risk of MI increases.

In the pruned decision tree model, only two variables, age and miR-208, were included, and age was considered as the most important judgment factor (Table 3, Figure 4). The formula of the decision tree model was as follows: Y=0.08633576age 0.89590612 miR.208 + 11.68067994

Subsequently, the pruned decision tree model was evaluated by the ROC curve (age and miR-208) (Figure 5). ROC curve analysis showed that the AUC was 0.907, and the optimal threshold point was 0.750. That is, when the probability value calculated by logistic regression was greater than 0.750, the researched subject was regarded as an MI patient, and the sensitivity and specificity under the optimal threshold were 92.7% and 76.7%, respectively.

DISCUSSION

miRNAs act as vital regulators physiologically mediating the post-transcriptional expression of genes, and they are identified as present in the cytoplasm and some extracellular compartments. Some of these miRNAs are useful not only as biomarkers for cardiac injury detection but also in overcoming limitations of previously adopted strategies and in treating lesions.[18] Therefore, miRNAs have been introduced to be employed as biomarkers for diagnosing a variety of disorders. To achieve an optimal treatment outcome of MI, early diagnosis is critical for this disease, and miRNAs are of special interest as biomarkers of MI because additional identifiers are in need all

Table 2. Logistic regression model analysis

Intercept	Estimate	Std. Error	z value	*Pr* (>|z|)
Sex	0.60	0.60	1.00	.3
Age	0.09	0.02	5.32	<.001
miR-208	-1.29	0.35	-3.68	<.001
miR-92a	1.62	0.60	2.69	.007

Figure 2. Receiver–operator characteristic (ROC) curves of age, miR-208 and miR-92a, AUC is the area under the ROC curve. The maximum value is 1. The point in the upper left corner is the optimal threshold; Specificity and Sensitivity are listed in parentheses.

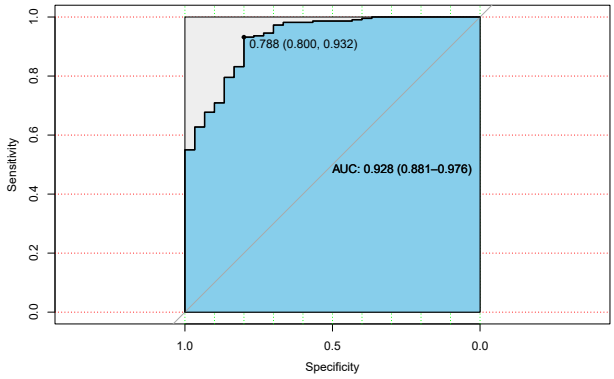


Figure 3. Decision tree model of age, miR-208 and miR-92a.

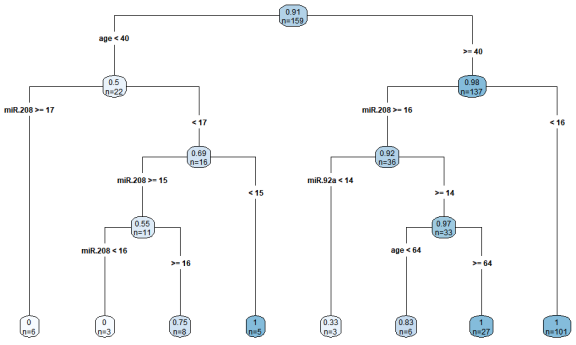


Table 3. Decision tree model

Intercept	Estimate	Std. Error	z value	*Pr*(>|z|)
age	0.08634	0.01562	5.527	<.001
miR-208	-0.89591	0.27076	-3.309	<.001

Figure 4. Analysis of pruned decision tree model.

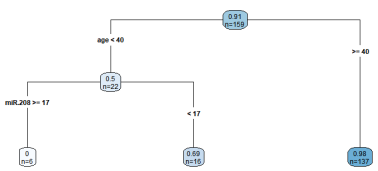
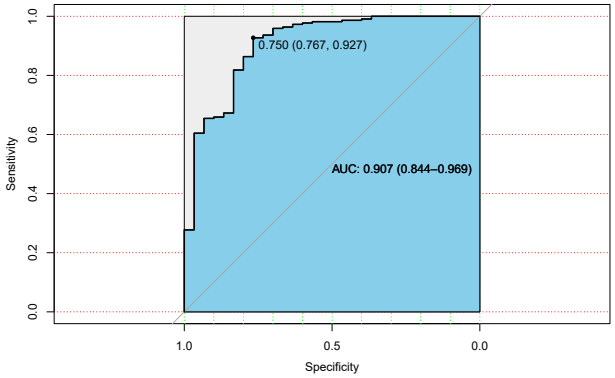


Figure 5. Receiver–operator characteristic (ROC) curves of age and miR-208. AUC is the area under the ROC curve. The maximum value is 1. The point in the upper left corner is the optimal threshold; Specificity and Sensitivity are listed in parentheses.



the time. In recent years, studies on miRNAs as diagnostic biomarkers have been reported by several researchers. Wang et al. conducted relevant studies on whether miR-133 and miR-328 become new biomarkers of acute myocardial infarction. The results showed that miR-133 and miR-328 levels in plasma and whole blood of AMI patients were increased vs. control. These miRNAs are considered to be novel biomarkers of AMI.[19] Through a comparison analysis between healthy adult and fetal hearts, Boštjančič et al. have explored the expression of miR-1, miR-133a, miR-133b, and miR-208 in regulating abnormal MI in humans, and the findings indicated that miR-208 was up-regulated while miR-1 and miR-133a were down-regulated in MI.[20] Similarly, Maarten et al. reported that cardiac injury in different heart-associated diseases initiates cardiomycell-specific miRNA release into circulation (including miR-208b and miR-499), which are revealed poorly sensitive to clinical features of cardiovascular patients. Wu et al. also studied microRNA values during early AMI diagnosis in the elderly, and their results revealed that up-regulation of circulating miR-19b and miR-483-5p could facilitate the early diagnosis of AMI, which is expected to become a new diagnostic marker of AMI for the senior group.[16]

This study assessed the diagnostic values of two selected miRNAs miR-208 and miR-92a in MI patients and the results implied that miR-208 expression level in the plasma was higher than miR-92a expression of both patients and healthy individuals. The ROC curve evaluation of the logistic regression model and pruned decision tree model showed that age, miR-208, and miR-92a had high accuracy in early diagnosis of MI, and the AUC reached 0.928, which indicated a satisfactory predictive value. At the same time, the combination of age, miR-208, and miR-92a is more valuable in the early diagnosis of MI. Similarly, Mohamed et al.[21] have studied the effects of co-peptin and they have identified miR-208 and miR-499 as two novel biomarkers in detecting acute coronary syndrome at an early stage. ROC curve analysis showed that the AUC of copeptin was 0.96 in predicting acute coronary syndrome, miR-499 was 0.97, and miR-208 was 0.97. Interestingly, copeptin combined with miR-208 and miR-499 could markedly increase the AUC value to 0.98 and improve the diagnostic value ($P < .001$). Although the results of this study are not better than other microRNAs and traditional markers reported in previous studies, the study of the combined detection of miR-208 and miR-92a can provide a good reference value for the early diagnosis of advanced MI. This data also extends the previous reports on the predictive value of miR-208 and miR-92a in MI.

In the early diagnostic studies of MI, the subtypes of MI were not distinguished. In the fourth generic definition of MI, changes are found in the concepts of five subtypes. Type 1: emphasizes a cause-and-effect association between plaque destruction and coronary thrombosis; the next type is an imbalance of oxygen demand and supply which is irrelevant to acute coronary thrombosis; the third one is the clarification of the type 3 MI as an essential kind to distinguish sudden cardiac death; and the last two types type 4 and type 5 emphasize the difference between program-associated myocardial injury and MI.[1] Earlier studies reported that when antagonists were administered to block miR-92a in model mice with acute limb ischemia and MI, the results implied that miR-92a blocking contributed to improving angiogenesis and tissue recovery.[22] Similarly, it has also been found that in the blocking of miR-92a using anti-miR modified with locked-in nucleic acids in a porcine MI model, the infarction area was greatly shrunk due to improved angiogenesis and decreased inflammation.[22] Furthermore, as it is also characterized by highly atherosclerotic endothelium specific, treatment of *older* mice with an antagonist of miR-92a indicates the importance of atherosclerotic miR-92a, thus demonstrating that miR-92a has the potential to be a target of atherosclerosis in its diagnosis and management.[13] Furthermore, the results of this study also indicate that the combined diagnosis of miR-208 and miR-92a is helpful for the early diagnosis of MI and can be used as a new marker for the early diagnosis of MI. Future studies on the early diagnosis of MI by miR-208 and miR-92a must distinguish between MI subtypes to explore whether miR-208 and miR-92a are diagnostic markers of different subtypes. This will provide a more valuable theoretical basis for clinical diagnosis. The current study has some limitations, as no external validation of the combined miR-92a and miR-208 diagnostic model has been performed yet. In future studies, a large external validation set from multicenter studies will be used to develop the proposed diagnostic model.

AUTHOR CONTRIBUTION

All authors participated (a) in the experiment design, conception, and data analysis; (b) drafted this manuscript or revised it carefully; and (c) agreed on the final version of the manuscript.

DECLARATION OF COMPETING INTEREST

The authors declared no conflicts of interest concerning this paper.

FUNDING

This work was supported by grants Chongqing Municipal Health and Health Committee & Chongqing Science and Technology Bureau Joint Medical Research Project (2019MSXM054) and the Chongqing Advanced Medical Talents Program for Young and middle-aged People (ZQNYXGDRCGZS2019008).

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included in the article.

REFERENCES

1. Thygesen K, Alpert JS, Jaffe AS, et al; Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth Universal Definition of Myocardial Infarction (2018). *J Am Coll Cardiol*. 2018;72(18):2231-2264. doi:10.1016/j.jacc.2018.08.1038
2. Lippi G, Sanchis-Gomar F, Cervellin G. Chest pain, dyspnea and other symptoms in patients with type 1 and 2 myocardial infarction. A literature review. *Int J Cardiol*. 2016;215:20-22. doi:10.1016/j.ijcard.2016.04.045
3. Thygesen K, Alpert JS, White HD, et al; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. *Eur Heart J*. 2007;28(20):2525-2538. doi:10.1093/eurheartj/ehm355
4. Shah AH, Puri R, Kalra A. Management of cardiogenic shock complicating acute myocardial infarction: A review. *Clin Cardiol*. 2019;42(4):484-493. doi:10.1002/clc.23168
5. Xue W, Xin-Xin W, Tian-liang J. The Value of Serum Mir-208 in the Diagnosis of Hyperacute Myocardial Infarction. *Chinese Journal of Laboratory Diagnosis*. 2022;26(5).
6. Qi LY, Liu HX, Cheng LC, et al. Prognostic Value of the Leuko-Glycemic Index in Acute Myocardial Infarction Patients with or without Diabetes. *Diabetes Metab Syndr Obes*. 2022;15:1725-1736. doi:10.2147/DMSO.S356461
7. Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J*. 2010;31(6):659-666. doi:10.1093/eurheartj/ehq013
8. Liu K, Zhao D, Wang D. LINC00528 regulates myocardial infarction by targeting the miR-143-3p/COX-2 axis. *Bioengineered*. 2020;11(1):11-18. doi:10.1080/21655979.2019.1704535
9. Wang C, Jing Q. Non-coding RNAs as biomarkers for acute myocardial infarction. *Acta Pharmacol Sin*. 2018;39(7):1110-1119. doi:10.1038/aps.2017.205
10. Yao Y, Zhao J, Zhou X, Hu J, Wang Y. Potential role of a three-gene signature in predicting diagnosis in patients with myocardial infarction. *Bioengineered*. 2021;12(1):2734-2749. doi:10.1080/21655979.2021.1938498
11. Ying-Hao P, Jian-Bin G, D.O. Cardiology. Research Progress of miRNA in the Diagnosis of Acute Myocardial Infarction. *Medical Recapitulate*. Published online 2014.
12. Li P, Li SY, Liu M, Ruan JW, Wang ZD, Xie WC. Value of the expression of miR-208, miR-494, miR-499 and miR-1303 in early diagnosis of acute myocardial infarction. *Life Sci*. 2019;232:116547. doi:10.1016/j.lfs.2019.116547
13. de Winther MPJ, Lutgens E. MiR-92a: at the heart of lipid-driven endothelial dysfunction. *Circ Res*. 2014;114(3):399-401. doi:10.1161/CIRCRESAHA.114.303125
14. Bostjancic E, Zidar N, Glavac D. MicroRNA microarray expression profiling in human myocardial infarction. *Dis Markers*. 2009;27(6):255-268. doi:10.1155/2009/641082
15. Deddens JC, Colijn JM, Oerlemans MIFJ, et al. Circulating microRNAs as novel biomarkers for the early diagnosis of acute coronary syndrome. *J Cardiovasc Transl Res*. 2013;6(6):884-898. doi:10.1007/s12265-013-9493-9
16. Li L, Li S, Wu M, et al. Early diagnostic value of circulating microRNAs in patients with suspected acute myocardial infarction. *J Cell Physiol*. 2019;234(8):13649-13658. doi:10.1002/jcp.28045
17. Yue Z, Guang Y, Bian GB. Differential Expression of Circular RNAin the Plasma of Elderly Patients with Heart Failure. *Zhongguo Linchuang Baojian Zazhi*. 2022;25(2).
18. Paiva S, Agbulut O. MiRroring the Multiple Potentials of MicroRNAs in Acute Myocardial Infarction. *Front Cardiovasc Med*. 2017;4:73. doi:10.3389/fcvm.2017.00073
19. Wang R, Li N, Zhang Y, Ran Y, Pu J. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. *Intern Med*. 2011;50(17):1789-1795. doi:10.2169/internalmedicine.50.5129
20. Boštjančič E, Zidar N, Štajer D, Glavač D. MicroRNAs miR-1, miR-133a, miR-133b and miR-208 are dysregulated in human myocardial infarction. *Cardiology*. 2010;115(3):163-169. doi:10.1159/000268088
21. El-Deek S, Meki AR, Hassan A, Gaber M, Mohamed O. Impact of copeptin, miRNA-499 and miRNA-208 as New Biomarkers for Early Detection of Acute Coronary Syndrome. *Eur Heart J*. 2020;41(suppl 2):ehaa946.1387. doi:10.1093/ehjci/ehaa946.1387
22. Bonauer A, Carmona G, Iwasaki M, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science*. 2009;324(5935):1710-1713. doi:10.1126/science.1174381