The Texas Heart Institute® Journal

Narrative Review

New Biomarkers for Cardiovascular Disease

Stephanie J. Kim[1,2]; Fernanda C. P. Mesquita, PhD[1]; Camila Hochman-Mendez, PhD[1]

[1]Department of Regenerative Medicine Research, The Texas Heart Institute, Houston, Texas
[2]Department of Biosciences, Rice University, Houston, Texas

Abstract

Cardiovascular disease is the leading cause of death and disability worldwide. Early detection and treatment of cardiovascular disease are crucial for patient survival and long-term health. Despite advances in cardiovascular disease biomarkers, the prevalence of cardiovascular disease continues to increase worldwide as the global population ages. To address this problem, novel biomarkers that are more sensitive and specific to cardiovascular diseases must be developed and incorporated into clinical practice. Exosomes are promising biomarkers for cardiovascular disease. These small vesicles are produced and released into body fluids by all cells and carry specific information that can be correlated with disease progression. This article reviews the advantages and limitations of existing biomarkers for cardiovascular disease, such as cardiac troponin and cytokines, and discusses recent evidence suggesting the promise of exosomes as cardiovascular disease biomarkers.

Keywords: Biomarkers; cardiovascular diseases; cytokines; exosomes; troponin

Introduction

Cardiovascular disease (CVD) is the leading global cause of death, resulting in an estimated 18.6 million deaths in 2019.[1] Its most common form, ischemic heart disease, is a prevalent and deadly condition that can lead to acute myocardial infarction (AMI).[2] Only 10.6% of patients survive an out-of-hospital cardiac arrest.[3] After an AMI, patients require screening, preventive care, and coordinated follow-up appointments because of their increased risk of developing heart failure (HF). This comprehensive management may improve patient adherence to treatment guidelines, decrease rehospitalizations, and reduce the chance of developing HF.[4,5]

Biomarkers are critical tools for probing, assessing, and managing cardiovascular risk. In 2001, the National Institute of Health Consortium defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[6] Given the deadly consequences of undiagnosed CVD—including heart attack and sudden cardiac arrest—the American Heart Association in 2009 unprecedentedly established criteria for assessing the usefulness and accuracy of cardiovascular biomarkers.[7]

Two circulating biomarkers—high-sensitivity C-reactive protein and the cardiac troponins (cTns)—appear in the blood when cardiac myocytes undergo necrosis. Testing for high-sensitivity C-reactive protein and cTn plays a crucial role in the diagnosis, risk stratification, and care of patients with CVD.[8] Early diagnosis of CVD can be achieved in the first 2 hours of patient admission through the evaluation of dynamic changes in the concentration of cTns.[9] Although cTns are the gold-standard biomarkers for acute CVD caused by cardiomyocyte necrosis, false-positive results can be problematic because increased cTn levels are observed in nonischemic myocardial injury (eg, myocarditis and cardiotoxicity) and in other conditions with multifactorial injury (eg, congestive HF and pulmonary embolism).[6,10]

Citation: Kim SJ, Mesquita FCP, Hochman-Mendez C. New biomarkers for cardiovascular disease. *Tex Heart Inst J*. 2023;50(5):e238178. doi:10.14503/THIJ-23-8178
Corresponding author: Camila Hochman-Mendez, PhD, 6770 Bertner Ave, MC 1-135, Houston, TX 77030 (cmendez@texasheart.org)

In the past few years, investigators have been searching for alternative biomarkers for CVD that can be quickly detected in the circulatory system and can lead to a more accurate diagnosis. Inflammatory cytokines have shown promise as diagnostic tools in HF, coronary heart disease, and other CVDs, given the strong association between inflammation and CVD, but conflicting results have been reported in studies of cytokine levels in acute CVD. Exosomes have recently emerged as another promising biomarker for CVD. These small vesicles are secreted by all cells and found in many biological fluids. Because they are directly involved in intercellular communication and the transport of several types of cargo, exosomes contain valuable information about the cell of origin and cell-to-cell interactions.[11]

This review discusses the use of traditional and newer biomarkers in CVD diagnosis. A critical analysis of the strengths and limitations of established biomarkers underscores the potential of exosomes as a new category of biomarkers for enhanced risk assessment and diagnosis of CVD.

Key Points

- Current biomarkers for CVD have limitations as diagnostic and prognostic tools.
- Exosomes have the potential to be more efficient and accurate biomarkers for CVD than traditional biomarkers.
- Overcoming current challenges to the clinical use of exosomal biomarkers will involve improving isolation and purification protocols and studying CVD-related miRNAs and proteins.

Abbreviations and Acronyms

AMI	acute myocardial infarction
cTn	cardiac troponin
CVD	cardiovascular disease
EV	extracellular vesicle
HF	heart failure
miRNA	microRNA

Cardiac Biomarkers

Cardiac Troponins

Troponins are proteins that regulate the contraction of muscle cells, including cardiomyocytes. There are 3 isoforms of cTns in cardiomyocytes: cTnI, cTnT, and cTnC. Elevated levels of cTnT and cTnI in the blood indicate myocardial injury.[12] The cTn value is considered abnormal when the blood troponin level exceeds the 99th percentile upper reference limit. This criterion is used to diagnose AMI. Because of its high sensitivity, the troponin immunoassay has become the gold standard for CVD biomarkers.[10]

Despite its advantages and technical efficiency, cTn testing has limitations, including false-positive results resulting from unspecific causes of cTn elevation. Any myocardial damage can cause cTn levels to increase, so a higher concentration of cTn in the blood can be seen even in the absence of AMI.[12] In 1 study, increased levels of the isoform cTnT were not linked to any previously described cause of cTn increase, including AMI, in 30% of cases.[13] Various other conditions can also promote cTn release into the bloodstream, such as fibrin clots, heterophile antibodies, alkaline phosphatase, rheumatoid factor, and cross-reactions of diagnostic antibodies with troponin molecules released from skeletal muscle.[14]

The shortcomings of cTn-based diagnosis of AMI are attributed to the lack of biochemical understanding of the dynamics of the cTnI and cTnT proteins.[15]

The mechanisms of cTn release in non-CVD pathologies are not well understood, and the effect of biological factors such as sex on cTn levels in CVD is an active area of research. Studies have shown that the average concentration of cTn in the blood is lower in female patients than in male patients, resulting in the underdiagnosis of AMI in women.[16] The use of sex-specific parameters for cTn concentration in the diagnosis of AMI may help address this issue.[17]

Cytokines

Patients who survive an MI are at increased risk of developing HF. Because prolonged inflammation caused by AMI can lead to thrombosis, cytokines and chemokines (a type of cytokine associated with cell migration) have shown promise as alternative CVD biomarkers to cTn.[18] Cytokines play a crucial role in the actions and recruitment of immune cells.[19] The most common cytokines associated with cardiac inflammation are tumor necrosis factor α, the interleukins, cardiotropin-1, and various C-C and C-X-C motif chemokine ligands.[20] These small proteins play a role in the interactions between inflammatory cells and cardiomyocytes and are produced when coronary arteries are blocked.[21] As pro-inflammatory cytokines are triggered by increased stress in the left ventricular wall, cytokine levels are directly proportional to HF progression, particularly infarction size and clinical outcome.[21] Studies have shown

that elevated interleukin levels in plasma and serum are associated with adverse outcomes in patients with HF.[22]

Although cytokines can be useful for monitoring CVD progression and predicting prognosis, they are less effective in the diagnosis and prediction of acute CVD. A few cytokines may be strong predictors of a future ischemic stroke, but they are not able to predict future coronary heart disease in asymptomatic patients.[23] Predicting CVD progression with cytokines is also challenging because of the drastic variation in cytokine levels in the different stages of CVD. For example, chemokines such as CCL5 are upregulated in early AMI but are downregulated in coronary heart disease.[24,25]

Moreover, there are no uniform parameters for using cytokines in diagnosing CVD. Detecting cytokines from blood or plasma requires highly sensitive tools that can measure levels in the picogram range.[20] These sensitive tools, such as enzyme-linked immunosorbent assay, will also capture drastic fluctuations in cytokine levels based on biological factors, including age, sex, circadian rhythm, and recent exercise periods. A recent study of circulating inflammatory markers in AMI found that interleukin-6 levels were significantly higher in older patients with AMI than in younger ones, suggesting a worse prognosis with age.[21] These results highlight a correlation between aging of the immune system and cytokine levels, indicating a need for identifying biomarkers that are specific to CVDs in a more consistently measurable manner.

Exosomes

Extracellular vesicles (EVs), found in serum, plasma, and urine, are nanosized vesicles that are released from most cell types by the budding out of the cell membrane. Exosomes are a subtype of EV that are released by all cells and that contain valuable cell-specific proteins and genetic information.[26,27] The information within exosomes reflects the characteristics of the original cell and can therefore be used for diagnostic purposes.[28] Exosomes can be classified by size, shape, density, membrane receptor expression, cargo load, and cell source.[29] Exosomes are involved in crucial intercellular communication, especially in the transfer of cell-derived DNA, RNA, proteins, and lipids between cells.[30] Exosomal cargo can also be used as biomarkers for disease progression, as demonstrated in cancer studies.[31]

RNA-based measurements of exosomal content may provide valuable clinical insight into the onset and progression of diseases.[32] RNA is a versatile biomolecule with numerous essential roles in biological processes and, unlike cytokines, is stable for extended periods of time. In addition to small regulatory RNA molecules such as microRNA (miRNA), circulating RNA, and long noncoding RNA are crucial regulators of gene expression and mediators of human disease, including CVD.[33]

Because brain tissue is usually unavailable for clinical study in cases of brain injury, biomarkers that can be transported across the blood-brain barrier to the circulatory system offer the unique potential for understanding the pathophysiologic state of the brain tissue after injury to the central nervous system.[34] In a case-control study, exosomal miRNA-223 levels were higher in patients with acute ischemic stroke than in patients without stroke, highlighting the potential of exosomal content as a diagnostic tool for non-CVD diseases with ischemic origin.[35]

Exosomes as CVD Biomarkers

Studies have shown that hypoxia significantly affects the exosomal cargo, leading to the secretion of different proteins and miRNA indicative of angiogenesis, growth, and progression of CVD through various signaling pathways.[36] Studying the molecular pathways of CVD progression has helped researchers identify specific exosomal proteins and miRNAs that are associated with certain CVDs (Table I).[10,37-58] For example, levels of miRNA-1, miRNA-133a, and miRNA-499 are higher in the plasma of patients with AMI than in those without AMI.[43] In fact, miRNA-208a was found only in the plasma of patients with AMI and was shown to be a more sensitive and specific biomarker for AMI than conventional cTn testing.[43] Platelet-derived exosomes containing miRNA-21, miRNA-191, miRNA-223, miRNA-320, and miRNA-339 have been linked to the aggregation of platelets, leading to atherosclerosis formation.[48] Exosomal miRNA-486 inhibits the expression of *PTEN,* which codes for a tumor-suppressing enzyme and can help decrease the level of cell death after cardiac ischemia or reperfusion injury.[47,59]

In addition to miRNAs, exosomal proteins and lipids could serve as potential biomarkers for CVD. Inflammatory-cascade proteins, such as serpin C1, serpin G1, CD14, and cystatin C, are elevated in exosomes in stress-induced ischemia, and cystatin C, polygenic immunoglobulin receptor, and complement factor C5a have been linked to acute coronary syndrome. Additionally, serpin F2, serpin G1, CD14, and cystatin C are associated with a higher risk of HF.[57] Exosomal lipid

TABLE I. Cardiovascular Disease-Related Biomarkers

Biomarker	Disease	Function	Reference
cTnI	AMI	Myocardial apoptosis	Chaulin AM[10] (2022)
miRNA-126, miRNA-223, miRNA-320b	AMI	Platelet activation and thrombus formation, endothelial damage, myocardial apoptosis, and fibroblast proliferation	Gidlöf O et al[37] (2013), Garcia A et al[38] (2019), and Hromadka M et al[39] (2021)
miRNA-1, miRNA-21a/b, miRNA-29b	AMI	Myocardial apoptosis, fibroblast proliferation, and cardiac hypertrophy	Grabmaier U et al[40] (2017), Sassi Y et al[41] (2017), and Surina S et al[42] (2021)
miRNA-208a	AMI	Cardiac hypertrophy and electrical conduction	Wang GK et al[43] (2010) and Huang XH et al[44] (2021)
miRNA-499	AMI	Myocardial apoptosis	Zhang L et al[45] (2015) and JFO Sullivan et al[46] (2016)
miRNA-486	AMI	Myocardial apoptosis (protective)	Bei Y et al[47] (2022)
miRNA-223-5p	AMI, atherosclerosis, and heart failure	Cell proliferation, migration, apoptosis, and polarization; cardiomyocyte hypertrophy; and electrical conduction	Zhang MW et al[48] (2020)
miRNA-941	Acute coronary syndrome	Cell proliferation and inflammation	Bai R et al[49] (2017)
miRNA-216a and miRNA-451	Coronary artery disease	Endothelial damage and monocyte recruitment	Lin J et al[50] (2018) and Ghafouri-Fard S et al[51] (2021)
miRNA-223-3p, miRNA-122-5p, miRNA-93-5p	Coronary artery disease	Cell proliferation, migration, and apoptosis; cardiomyocytes hypertrophy; electrical conduction; and cardiomyocytes apoptosis	JFO Sullivan et al[46] (2016), Zhang MW et al[48] (2020), Ghafouri-Fard S et al[51] (2021), and Hosen MR et al (2022)
miRNA-142-3p, miRNA-17-5p, miRNA-126	AMI and coronary artery disease	Inflammation; cardiomyocyte hypertrophy; cell proliferation, migration, and apoptosis	Zhong Z, et al.[53] (2018) Xue S et al[54] (2019)
miRNA-133a	Coronary artery disease	Cell proliferation and differentiation, cardiac hypertrophy, and electrical conduction (arrhythmia)	Li N et al[55] (2018) and Dai R et al[56] (2020)
Serpin G1, serpin F2, cystatin C CD14	Heart failure and acute coronary syndrome	Inflammation, decrease in kidney function, decrease in fibrinolysis, and thrombotic process	Zhang YN et al[57] (2016) and Verbree-Willemsen L et al[58] (2020)

AMI, acute myocardial infarction; CTnI, troponin I, cardiac form; miRNA, microRNA.

levels have been linked to atherosclerosis formation.[60] Furthermore, the actual concentration of exosomes may serve as a possible biomarker for CVD. Recent data indicate that platelet-derived exosomes with coagulation or anticoagulation proteins are associated with the presence and progression or the lack, respectively, of atherosclerosis.[59] Thus, changes in the levels of miRNAs, proteins, and lipids within exosomes as well as changes in overall exosome levels could indicate the presence and progression of CVD.

Using exosomes for early detection allows for a faster diagnosis and more accurate monitoring of CVD conditions (Table I).[10,37-58] Exosomal miRNAs can be detected at early stages of CVD. The protein-based biomarker cTn can be measured 2 to 3 hours after onset of MI at the earliest, and cytokine assays can take several hours to provide results and may not provide specific information regarding the type of cellular damage in the tissue.[59] In contrast, exosomal miRNA can be measured in the plasma in as little as 15 minutes after the onset of MI.[61] By combining the tools of traditional clinical diagnosis with the use of cTn biomarkers and exosomes, clinicians may be able to better predict prognosis and classify patients into distinct subgroups, thus possibly creating personalized therapies.[62,63]

Limitations of Exosomes

Recent studies suggest that the quality of exosomes used in biomarker and therapeutic studies may be compromised by suboptimal storage and processing methods. Current methods of isolation and purification of exosomes, including affinity-based, flow filtration, and centrifugation, are inefficient. Although they are scalable and can be standardized, these common methods are labor intensive, require expensive equipment, and yield inconsistent purity levels.[64] Centrifugation, the current primary method of exosome purification, cannot be used to distinguish between exosomes and other types of EVs because of their similarities in size and overall electrical charge.[65] Furthermore, ultracentrifugation can aggregate vesicles, compressing EVs[66] and making it difficult to isolate exosomes from other EVs. As a result, the purity of exosomes through centrifugation can be low, thus reducing the accuracy of results.[64] Further investigation is needed to understand the impact of EVs on miRNA or protein levels and to develop more effective isolation and purification methods.

Additionally, preparation of plasma for EV analysis can induce cell stress and activate platelets, causing the release of EVs containing biomolecules associated with pathologies. This process can lead to inaccurate measurements of EV-derived miRNAs and proteins, resulting in false diagnoses. Furthermore, the type of anticoagulant used during the collection and storage of plasma can also affect the detectable level of EVs. Platelet activation and hemolysis can easily be triggered by the handling of the sample, leading to further complications in EV analysis.[64]

Further research is needed to determine which exosomal miRNA or protein is the most effective in diagnosing a specific disease. The lack of consistency in the fluctuation of biomolecule levels associated with disease diagnosis is a barrier to the efficiency of using exosomes as biomarkers. Although numerous studies have identified various types of miRs and proteins linked to specific conditions, it is not clear which biomolecules are the most reliable indicators of disease. Analyzing all possible biomolecules is a time-consuming and costly process, so a targeted approach is necessary to identify the most effective biomarker for each condition. It is important to standardize the analysis of exosomal biomolecules and establish a consensus on the most effective biomarkers before exosomes can be routinely used in clinical practice as diagnostic tools.

Future Directions

Developing standardized, efficient methods for exosome isolation and purification will be crucial for improving the accuracy and specificity of exosome-based CVD diagnosis and treatment. Further research into exosome biogenesis, cargo sorting, and secretion mechanisms will be important for advancing our understanding of exosome function in CVD. To identify specific exosomal biomolecules with high diagnostic and therapeutic potential in CVD, large-scale multicenter clinical trials will be necessary. Additionally, new technologies such as microfluidics, high-throughput sequencing, and nanomaterial-based approaches hold promise for addressing these challenges. Although these technologies are currently time consuming, with further advances in the field of molecular biology, exosomes may be used as CVD biomarkers in the near future to help improve patient outcomes. Standardized procedures for exosomal biomarker analysis and evaluation will also facilitate the development of exosome-based clinical assays. This will be an important step toward personalized and effective CVD treatments (Fig. 1).

Conclusion

With continued investment in this research and technology development, exosomes have the potential to revolutionize the diagnosis and treatment of CVD. However, the limitations of exosome isolation and purification as well as the challenge of identifying the most effective exosomal miRNAs and proteins for CVD diagnosis and treatment are significant obstacles to their widespread use. Research in this field will continue to expand our knowledge of CVD pathophysiology and aid in the development of personalized treatments.

Article Information

Published: 17 October 2023

Author Contributions: Stephanie J. Kim, Fernanda C. P. Mesquita, and Camila Hochman-Mendez contributed to conceptualization, formal analysis, investigation, writing—original draft

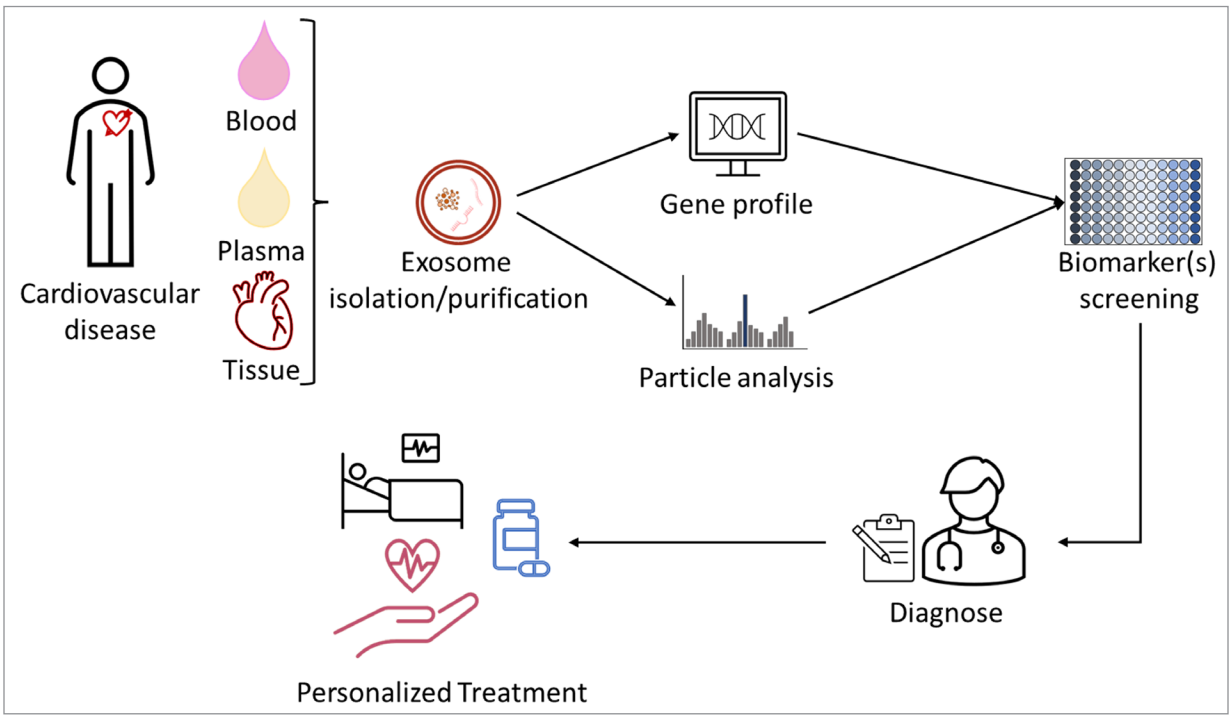

Fig. 1 Potential use of exosomes in the diagnosis and personalized treatment of cardiovascular disease.

preparation, writing—review and editing, and visualization; Fernanda C. P. Mesquita and Camila Hochman-Mendez supervised the work and contributed to project administration; and Camila Hochman-Mendez acquired funding. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Disclosure: The authors declare no conflict of interest.

Funding/Support: There was no funding for this work.

Acknowledgments: The authors thank Kimberly Macellaro, PhD, of the Department of Regenerative Medicine Research, and Rebecca Bartow, PhD, of the Department of Scientific Publications at The Texas Heart Institute for editorial assistance.

References

1. Roth GA, Mensah GA, Johnson CO, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Am Coll Cardiol.* 2020;76(25):2982-3021. doi:10.1016/j.jacc.2020.11.010
2. Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics—2020 update: a report from the American Heart Association. *Circulation.* 2020;141(9):e139-e596. doi:10.1161/CIR.0000000000000757
3. Virani SS, Alonso A, Aparicio HJ, et al. Heart disease and stroke statistics—2021 update: a report from the American Heart Association. *Circulation.* 2021;143(8):e254-e743. doi:10.1161/CIR.0000000000000950
4. Piepoli MF, Corra U, Dendale P, et al. Challenges in secondary prevention after acute myocardial infarction: a call for action. *Eur Heart J Acute Cardiovasc Care.* 2017;6(4):299-310. doi:10.1177/2048872616689773
5. Jenca D, Melenovsky V, Stehlik J, et al. Heart failure after myocardial infarction: incidence and predictors. *ESC Heart Fail.* 2021;8(1):222-237. doi:10.1002/ehf2.13144
6. Dhingra R, Vasan RS. Biomarkers in cardiovascular disease: statistical assessment and section on key novel heart failure biomarkers. *Trends Cardiovasc Med.* 2017;27(2):123-133. doi:10.1016/j.tcm.2016.07.005
7. Hlatky MA, Greenland P, Arnett DK, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation.* 2009;119(17):2408-2416. doi:10.1161/CIRCULATIONAHA.109.192278
8. Wong YK, Tse HF. Circulating biomarkers for cardiovascular disease risk prediction in patients with cardiovascular disease. *Front Cardiovasc Med.* 2021;8:713191. doi:10.3389/fcvm.2021.713191
9. Collet JP, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Rev Esp Cardiol (Engl Ed).* 2021;74(6):544. doi:10.1016/j.rec.2021.05.002
10. Chaulin AM. The importance of cardiac troponin metabolism in the laboratory diagnosis of myocardial infarction (comprehensive review). *Biomed Res Int.* 2022;2022:6454467. doi:10.1155/2022/6454467
11. Morrissey J, Mesquita FCP, Chacon-Alberty L, Hochman-Mendez C. Mining the mesenchymal stromal cell secretome in patients with chronic left ventricular dysfunction. *Cells.* 2022;11(13):2092. doi:10.3390/cells11132092
12. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth universal definition of myocardial infarction (2018).

Circulation. 2018;138(20):e618-e651. doi:10.1161/CIR.0000000000000617
13. Lindner G, Pfortmueller CA, Braun CT, Exadaktylos AK. Non-acute myocardial infarction-related causes of elevated high-sensitive troponin T in the emergency room: a cross-sectional analysis. *Intern Emerg Med.* 2014;9(3):335-339. doi:10.1007/s11739-013-1030-y
14. Chaulin AM. False-positive causes in serum cardiac troponin levels. *J Clin Med Res.* 2022;14(2):80-87. doi:10.14740/jocmr4664
15. Deranek AE, Baldo AP, Lynn ML, Schwartz SD, Tardiff JC. Structure and dynamics of the flexible cardiac troponin t linker domain in a fully reconstituted thin filament. *Biochemistry.* 2022;61(13):1229-1242. doi:10.1021/acs.biochem.2c00091
16. Kimenai DM, Janssen E, Eggers KM, et al. Sex-specific versus overall clinical decision limits for cardiac troponin i and t for the diagnosis of acute myocardial infarction: a systematic review. *Clin Chem.* 2018;64(7):1034-1043. doi:10.1373/clinchem.2018.286781
17. Giannitsis E, Mueller-Hennessen M, Zeller T, et al. Gender-specific reference values for high-sensitivity cardiac troponin T and I in well-phenotyped healthy individuals and validity of high-sensitivity assay designation. *Clin Biochem.* 2020;78:18-24. doi:10.1016/j.clinbiochem.2019.11.013
18. Mitsis A, Kadoglou NPE, Lambadiari V, et al. Prognostic role of inflammatory cytokines and novel adipokines in acute myocardial infarction: an updated and comprehensive review. *Cytokine.* 2022;153:155848. doi:10.1016/j.cyto.2022.155848
19. Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. *Blood.* 2011;118(1):9-18. doi:10.1182/blood-2010-08-265892
20. Ueland T, Gullestad L, Nymo SH, Yndestad A, Aukrust P, Askevold ET. Inflammatory cytokines as biomarkers in heart failure. *Clin Chim Acta.* 2015;443:71-77. doi:10.1016/j.cca.2014.09.001
21. Kumar G, Dey SK, Kundu S. Functional implications of vascular endothelium in regulation of endothelial nitric oxide synthesis to control blood pressure and cardiac functions. *Life Sci.* 2020;259:118377. doi:10.1016/j.lfs.2020.118377
22. Schmitter D, Cotter G, Voors AA. Clinical use of novel biomarkers in heart failure: towards personalized medicine. *Heart Fail Rev.* 2014;19(3):369-381. doi:10.1007/s10741-013-9396-5
23. Canoui-Poitrine F, Luc G, Mallat Z, et al. Systemic chemokine levels, coronary heart disease, and ischemic stroke events: the PRIME study. *Neurology.* 2011;77(12):1165-1173. doi:10.1212/WNL.0b013e31822dc7c8
24. Rothenbacher D, Muller-Scholze S, Herder C, Koenig W, Kolb H. Differential expression of chemokines, risk of stable coronary heart disease, and correlation with established cardiovascular risk markers. *Arterioscler Thromb Vasc Biol.* 2006;26(1):194-199. doi:10.1161/01.ATV.0000191633.52585.14
25. Julian-Villaverde FJ, Serrano-Ponz M, Ramalle-Gomara E, Martinez A, Ochoa-Callejero L. CCL5 levels predict stroke volume growth in acute ischemic stroke and significantly diminish in hemorrhagic stroke patients. *Int J Mol Sci.* 2022;23(17):9967. doi:10.3390/ijms23179967
26. Matsuzaka Y, Yashiro R. Advances in purification, modification, and application of extracellular vesicles for novel clinical treatments. *Membranes (Basel).* 2022;12(12):1244. doi:10.3390/membranes12121244
27. Song M, Bai H, Zhang P, Zhou X, Ying B. Promising applications of human-derived saliva biomarker testing in clinical diagnostics. *Int J Oral Sci.* 2023;15(1):2. doi:10.1038/s41368-022-00209-w
28. Sharma S, Zuniga F, Rice GE, Perrin LC, Hooper JD, Salomon C. Tumor-derived exosomes in ovarian cancer—liquid biopsies for early detection and real-time monitoring of cancer progression. *Oncotarget.* 2017;8(61):104687-104703. doi:10.18632/oncotarget.22191
29. Lo Cicero A, Stahl PD, Raposo G. Extracellular vesicles shuffling intercellular messages: for good or for bad. *Curr Opin Cell Biol.* 2015;35:69-77. doi:10.1016/j.ceb.2015.04.013
30. Xu Y, Hu Y, Xu S, Liu F, Gao Y. Exosomal microRNAs as potential biomarkers and therapeutic agents for acute ischemic stroke: new expectations. *Front Neurol.* 2021;12:747380. doi:10.3389/fneur.2021.747380
31. Yi X, Chen J, Huang D, et al. Current perspectives on clinical use of exosomes as novel biomarkers for cancer diagnosis. *Front Oncol.* 2022;12:966981. doi:10.3389/fonc.2022.966981
32. Narang P, Shah M, Beljanski V. Exosomal RNAs in diagnosis and therapies. *Noncoding RNA Res.* 2022;7(1):7-15. doi:10.1016/j.ncrna.2022.01.001
33. Zhang Z, Salisbury D, Sallam T. Long noncoding RNAs in atherosclerosis: JACC Review Topic of the Week. *J Am Coll Cardiol.* 2018;72(19):2380-2390. doi:10.1016/j.jacc.2018.08.2161
34. Ollen-Bittle N, Roseborough AD, Wang W, Wu JD, Whitehead SN. Mechanisms and biomarker potential of extracellular vesicles in stroke. *Biology (Basel).* 2022;11(8). doi:10.3390/biology11081231
35. Chen Y, Song Y, Huang J, et al. Increased circulating exosomal miRNA-223 is associated with acute ischemic stroke. *Front Neurol.* 2017;8:57. doi:10.3389/fneur.2017.00057
36. Yaghoubi S, Najminejad H, Dabaghian M, et al. How hypoxia regulate exosomes in ischemic diseases and cancer microenvironment? *IUBMB Life.* 2020;72(7):1286-1305. doi:10.1002/iub.2275
37. Gidlöf O, van der Brug M, Ohman J, et al. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. *Blood.* 2013;121(19):3908-3917, S1-S26. doi:10.1182/blood-2012-10-461798
38. Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny JL, Fontana P. Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells. *Thromb Haemost.* 2019;119(2):254-263. doi:10.1055/s-0038-1676802
39. Hromadka M, Motovska Z, Hlinomaz O, et al. MiR-126-3p and MiR-223-3p as biomarkers for prediction of thrombotic risk in patients with acute myocardial infarction and primary angioplasty. *J Pers Med.* 2021;11(6):508. doi:10.3390/jpm11060508
40. Grabmaier U, Clauss S, Gross L, et al. Diagnostic and prognostic value of miR-1 and miR-29b on adverse ventricular remodeling after acute myocardial infarction—the SITAGRAMI-miR analysis. *Int J Cardiol.* 2017;244:30-36. doi:10.1016/j.ijcard.2017.06.054
41. Sassi Y, Avramopoulos P, Ramanujam D, et al. Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. *Nat Commun.* 2017;8(1):1614. doi:10.1038/s41467-017-01737-4
42. Surina S, Fontanella RA, Scisciola L, Marfella R, Paolisso G, Barbieri M. miR-21 in human cardiomyopathies. *Front Cardiovasc Med.* 2021;8:767064. doi:10.3389/fcvm.2021.767064

43. 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
44. Huang XH, Li JL, Li XY, et al. miR-208a in cardiac hypertrophy and remodeling. *Front Cardiovasc Med.* 2021;8:773314. doi:10.3389/fcvm.2021.773314
45. Zhang L, Chen X, Su T, et al. Circulating miR-499 are novel and sensitive biomarker of acute myocardial infarction. *J Thorac Dis.* 2015;7(3):303-308. doi:10.3978/j.issn.2072-1439.2015.02.05
46. JFO Sullivan, Neylon A, McGorrian C, Blake GJ. miRNA-93-5p and other miRNAs as predictors of coronary artery disease and STEMI. *Int J Cardiol.* 2016;224:310-316. doi:10.1016/j.ijcard.2016.09.016
47. Bei Y, Lu D, Bär C, et al. miR-486 attenuates cardiac ischemia/reperfusion injury and mediates the beneficial effect of exercise for myocardial protection. *Mol Ther.* 2022;30(4):1675-1691. doi:10.1016/j.ymthe.2022.01.031
48. Zhang MW, Shen YJ, Shi J, Yu JG. MiR-223-3p in cardiovascular diseases: a biomarker and potential therapeutic target. *Front Cardiovasc Med.* 2021;7:610561. doi:10.3389/fcvm.2020.610561
49. Bai R, Yang Q, Xi R, Li L, Shi D, Chen K. miR-941 as a promising biomarker for acute coronary syndrome. *BMC Cardiovasc Disord.* 2017;17(1):227. doi:10.1186/s12872-017-0653-8
50. Lin J, Jiang J, Zhou R, Li X, Ye J. MicroRNA-451b participates in coronary heart disease by targeting VEGFA. *Open Med (Wars).* 2019;15:1-7. doi:10.1515/med-2020-0001
51. Ghafouri-Fard S, Gholipour M, Taheri M. Role of microRNAs in the pathogenesis of coronary artery disease. *Front Cardiovasc Med.* 2021;8:632392. doi:10.3389/fcvm.2021.632392
52. Hosen MR, Goody PR, Zietzer A, et al. Circulating microRNA-122-5p is associated with a lack of improvement in left ventricular function after transcatheter aortic valve replacement and regulates viability of cardiomyocytes through extracellular vesicles. *Circulation.* 2022;146(24):1836-1854. doi:10.1161/CIRCULATIONAHA.122.060258
53. Zhong Z, Hou J, Zhang Q, et al. Circulating microRNA expression profiling and bioinformatics analysis of dysregulated microRNAs of patients with coronary artery disease. *Medicine (Baltimore).* 2018;97(27):e11428. doi:10.1097/MD.0000000000011428
54. Xue S, Liu D, Zhu W, et al. Circulating MiR-17-5p, MiR-126-5p and MiR-145-3p are novel biomarkers for diagnosis of acute myocardial infarction. *Front Physiol.* 2019;10:123. doi:10.3389/fphys.2019.00123
55. Li N, Zhou H, Tang Q. miR-133: a suppressor of cardiac remodeling? *Front Pharmacol.* 2018;9:903. doi:10.3389/fphar.2018.00903
56. Dai R, Liu Y, Zhou Y, et al. Potential of circulating pro-angiogenic microRNA expressions as biomarkers for rapid angiographic stenotic progression and restenosis risks in coronary artery disease patients underwent percutaneous coronary intervention. *J Clin Lab Anal.* 2020;34(1):e23013. doi:10.1002/jcla.23013
57. Zhang YN, Vernooij F, Ibrahim I, et al. Extracellular vesicle proteins associated with systemic vascular events correlate with heart failure: an observational study in a dyspnoea cohort. *PLoS One.* 2016;11(1):e0148073. doi:10.1371/journal.pone.0148073
58. Verbree-Willemsen L, Zhang YN, Ibrahim I, et al. Extracellular vesicle cystatin C and CD14 are associated with both renal dysfunction and heart failure. *ESC Heart Fail.* 2020;7(5):2240-2249. doi:10.1002/ehf2.12699
59. Chen X, Luo Q. Potential clinical applications of exosomes in the diagnosis, treatment, and prognosis of cardiovascular diseases: a narrative review. *Ann Transl Med.* 2022;10(6):372. doi:10.21037/atm-22-619
60. de Freitas RCC, Hirata RDC, Hirata MH, Aikawa E. Circulating extracellular vesicles as biomarkers and drug delivery vehicles in cardiovascular diseases. *Biomolecules.* 2021;11(3):388. doi:10.3390/biom11030388
61. Ellis BW, Ronan G, Ren X, et al. Human Heart Anoxia and Reperfusion Tissue (HEART) model for the rapid study of exosome bound miRNA expression as biomarkers for myocardial infarction. *Small.* 2022;18(28):e2201330. doi:10.1002/smll.202201330
62. Ahmad T, Fiuzat M, Pencina MJ, et al. Charting a roadmap for heart failure biomarker studies. *JACC Heart Fail.* 2014;2(5):477-488. doi:10.1016/j.jchf.2014.02.005
63. Li J, Cao T, Wei Y, et al. A review of novel cardiac biomarkers in acute or chronic cardiovascular diseases: the role of soluble ST2 (sST2), lipoprotein-associated phospholipase A2 (Lp-PLA2), myeloperoxidase (MPO), and procalcitonin (PCT). *Dis Markers.* 2021;2021:6258865. doi:10.1155/2021/6258865
64. Davidson SM, Boulanger CM, Aikawa E, et al. Methods for the identification and characterization of extracellular vesicles in cardiovascular studies—from exosomes to microvesicles. *Cardiovasc Res.* 2022. doi:10.1093/cvr/cvac031
65. Paolini L, Zendrini A, Di Noto G, et al. Residual matrix from different separation techniques impacts exosome biological activity. *Sci Rep.* 2016;6:23550. doi:10.1038/srep23550
66. Jeppesen DK, Hvam ML, Primdahl-Bengtson B, et al. Comparative analysis of discrete exosome fractions obtained by differential centrifugation. *J Extracell Vesicles.* 2014;3:25011. doi:10.3402/jev.v3.25011