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Review

Circulating Extracellular Vesicle-Based Biomarkers: Advances, Clinical Implications and Challenges in Coronary Artery Disease

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Abstract

Coronary artery disease (CAD) is a leading cause of death worldwide, encompassing a broad spectrum of pathological conditions ranging from chronic to acute coronary syndromes. It underlies complex biological mechanisms, among which an emerging role is played by extracellular vesicles (EVs). EVs are non-replicable cell-derived particles enclosed by lipid bilayers acting as mediators of cellular interactions. In the past two decades, there has been a growing interest in EVs as potential diagnostic, prognostic and therapeutic tools in cardiovascular disease. We reviewed the most recent studies on circulating EVs in CAD with a particular focus on their role in biomarker discovery. Our aim was to evaluate the feasibility of translating these findings into routine clinical practice. To this end, we underlie the development and application of integrated *indicators*, referred to as "*Bioscores*", which combine clinical, laboratory, and molecular data to enhance diagnostic and prognostic accuracy. We briefly discuss the opportunity and pitfalls related to the emerging use of Machine Learning (ML) algorithms. Moreover, we highlight that further investigation of mechanistic pathways is required beyond the initially predicted associations generated by in silico studies. Finally, we analyzed the key limitations, challenges, and unmet needs in the field, including small and unrepresentative sample sizes, a lack of external validation, overlapping and often contradictory effects on targeted pathways, difficulties in standardizing EV isolation and characterization methods, as well as concerns regarding affordability and clinical reliability.

Keywords: CAD; ACS; EV; biomarker; *Bioscore*

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1. Introduction

The role of extracellular vesicles (EVs) in cardiovascular disease (CVD) has been extensively reviewed, with recent studies reflecting a growing interest in their diagnostic and prognostic potential [1,2]. In this context, we aim to provide a focused and updated perspective on the contribution of EVs as biomarkers in coronary artery disease (CAD). Specifically, we critically examine the current literature, highlighting both promising findings and the methodological challenges that hinder clinical translation. Our analysis centers on circulating EVs, emphasizing their potential for future clinical applications. While the term "biomarker" is commonly used in the literature, it often lacks a consistent, universally accepted definition. The National Cancer Institute offers a useful framework, defining a biomarker as—"*A characteristic that can be objectively measured and serves as an indicator*

for normal biologic processes, pathogenic processes, state of health or disease, the risk for disease development and/or prognosis, or responsiveness to a particular therapeutic intervention". In line with Aronson et al. [3], we stress the importance of demonstrating mechanistic involvement before designating any molecule as a true biomarker. We therefore recommend applying Bradford Hill's Guidelines [4] to rigorously assess causality. In line with this, and to preserve the integrity of the reviewed studies, we have retained the original terminology used by the authors, while acknowledging that many of the described EV-associated molecules lack confirmed mechanistic validation. Until such evidence is available, we propose referring to these molecules more cautiously as "indicators".

1.1. Coronary Artery Disease (CAD)

Ischemic heart disease (IHD) is the leading cause of mortality worldwide and has kept this record for over 20 years [5]. Ischemic heart disease can present either as an acute coronary syndrome (ACS) or a stable chronic coronary syndrome (CCS) [6,7]. CAD is a common cause of IHD, though it is neither the only cause nor necessarily the most significant one [8]. CAD is often a direct consequence of vascular occlusion due to atherosclerosis [9]. Yet, research has shown a growing prevalence of non-obstructive CAD patients [10]. Throughout this review, some of the referenced studies referred to chronic coronary syndrome (CCS) as stable coronary artery disease (SCAD). While we adhere to the terminology established by the most recent ESC guidelines (i.e., ACS and CCS) [11], we retained the authors' original definitions when discussing their works. CAD diagnosis is based on many different tests, including cardiac markers, but in most cases needs to be confirmed with coronary angiography (CAG) [7]. Coronary angiography is a costly, time-consuming procedure that needs significant expertise [12]. On the other hand, there is no univocal guidance on prognostic stratification of CAD patients [13]. To improve our ability to diagnose and stratify CAD patients in different settings, many different molecules have been studied as biomarkers. They are especially advantageous as their use might help overcome the cost and operator-dependency of angiography [14–16]. Specific molecules carried by EVs, which reflect the physiological and pathological state of their cells of origin, offer valuable insights into the presence of the disease, its progression, and prognosis, thereby performing as biomarkers [17].

1.2. Extracellular Vesicles

Extracellular vesicles (EVs) are nano- to micro-sized particles enclosed by a lipid bilayer membrane. They lack the ability to replicate and are released by all cell types, serving as carriers of bioactive molecules that reflect the state and function of their cells of origin [18]. These particles have been the focus of an increasingly active research field for decades. A search on PubMed using keywords such as "microvesicles," "extracellular vesicles," or "exosomes" yields nearly 60,000 publications from the past 10 years alone, reflecting the rapid growth and expanding interest in this area.

EVs' prominent role in intercellular communication makes them a critical component of many different physiological and pathological pathways [19]. The analysis of their molecular cargo and surface markers offers valuable insights that can be harnessed for diagnostic and prognostic purposes in different clinical settings, including CVD [20]. According to the most prominent position paper regarding EVs, which is the Minimal Information for the Study of Extracellular Vesicles (MISEV 2023), these particles can be classified based on their size or biogenesis. Small EVs, with a diameter smaller than 200 nm, are distinguished from large EVs (diameter > 200 nm). A biogenesis-based approach differentiates exosomes, which originate from the endosomal system, from ectosomes and microvesicles, that are shed directly from the plasma membrane [18].

When working with EVs, particular attention must be given to how these particles are isolated from body fluids and characterized. This implies that isolation and characterization of EVs are critical steps for ensuring the accuracy and consistency of their analyses. Given the frequent inverse correlation between yield and purity, optimizing EV isolation requires a balance between the increase in quantity and the preservation of the sample quality [21–23]. In addition, maintaining this balance depends on both the objectives of the studies and the technical resources available. Ultracentrifugation, size-exclusion chromatography (SEC), polymer-based precipitation, and immunoaffinity capture are the most used procedures for EV isolation. Differences among them mainly lie in their purity, yield, and scalability [24–27]. A trade-off must be made: methods that achieve higher purity typically result in lower yields, while those which prioritize higher yields often do so at the expense of purity [18]. Overall, combining isolation methods might be more effective than using a single technique. However, these sequential approaches, beyond requiring specific expertise, are costly and time-consuming.

Characterization is required to confirm the presence of EVs, quantify them, and assess the extent of non-EV contamination [18]. Characterization requires the assessment of particle size, concentration, morphology, and surface markers by employing nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and Western blotting or flow cytometry for EV-associated proteins [28–30]. Similarly to isolation, for characterization MISEV advocates the employment of at least two orthogonal methods [18]. Once again, combining different methods would be the best approach but demands substantial resources. For these reasons, the lack of standardized protocols for isolation and characterization methods poses a significant challenge, often leading to variability between studies [31]. Detailed information on these specific issues can be found elsewhere [32,33].

2. Multifaceted Role of EVs as a Tool for Biomarker Discovery in CAD

2.1. Short Historical Overview

Over the past two decades, significant advances in understanding the biological and pathophysiological functions of EVs have been achieved. Evidence sustaining the role of EVs in CVD is expanding. It is widely accepted that EVs can exert both detrimental and beneficial effects in cardiovascular disorders [20,34]. In fact, studies have shown that EVs display cardioprotective behavior during both ACS [35] and CAD in general [36], while other studies have highlighted their contribution to ischemic injury and progression of the atherosclerotic process [37]. This reflects EV's heterogeneous functions in intercellular communication across different stages of the disease.

From the early stages of CV research, the pursuit of novel biomarkers for investigative and clinical purposes has driven a thorough examination of EV features. These include their size and concentration [38], surface epitopes [39], non-coding RNAs [40] and, to a lesser extent, their protein cargo [41] under various biological conditions. In the following paragraphs the most significant pioneer studies on potential CAD EV biomarkers will be briefly discussed.

In 2015, Bi S. et al. [42] were among the first to investigate circulating EVs as biomarkers in ACS by analyzing their miRNA content. A significant enrichment of miR-208a was found in ACS patients, while a negative correlation between miR-208a and 1-year survival rate was reported. Similarly, Yang Y et al. [43] proposed AC100865.1, a long non-coding RNA (lncRNA) mainly carried by circulating EVs, as a potential "CoroMarker" in CAD patients.

Since the early 21st century, research efforts have also focused on understanding the impact of changes in circulating EV levels and surface antigens. Sinning et al. [44] reported an independent association between a specific circulating EV subtype (CD31+/Annexin V+) and an increased risk of cardiovascular events in patients with CCS. In a cohort of patients

with high cardiovascular risk profiles, elevated levels of these EVs were linked to a higher incidence of major adverse cardiac and cerebrovascular events (MACCEs), cardiovascular mortality and revascularization over a 6.1-year median follow-up. These findings suggest that CD31+/Annexin V+ EVs may serve as independent prognostic markers beyond traditional risk factors.

Upon closer examination, proteomic research has substantially advanced the understanding of the roles of EVs in CVD. Gidlöf et al. [45] performed a study aimed at evaluating the diagnostic value of circulating EV protein cargo in comparison to total plasma proteins in patients with STEMI (see Section 8).

Ongoing research has continued to expand and refine our knowledge in this field, with several groups contributing significant findings. Accordingly, we have sought to summarize the most pertinent scientific literature in this field.

2.2. Strategies for Identifying EV Cargo: Emerging Role of Computational Analyses

The vast number of potential targets and the complexity of their interactions require the use of advanced processing and computational tools, such as in silico models. In fact, in silico models are applied to high-throughput biological data (i.e., genomic, transcriptomic, proteomic) to determine, and subsequently test, hypotheses related to pathogenetic and pathophysiological processes in medicine. It is worth noting how in silico models rely on bioinformatics for pattern recognition, machine learning (ML), statistical modelling, and database extraction [46].

It should be emphasized that in silico models are computational tools simulating, rather than replacing, in vitro and in vivo models. This implies that computational models must be always validated by experimental data to ensure the robustness of obtained results.

2.3. ML Models for Biomarker Discovery in CVD

ML is rapidly becoming a versatile tool in medical research. Particularly in the CV field. Jin et al. [47] used ML to identify early-warning EV markers in patients at high risk of acute myocardial infarction (AMI). The authors began by identifying heart-derived EVs and proposed that the expression levels of a specific set of genes could serve as early indicators of AMI risk. These candidate biomarkers were then incorporated into a predictive nomogram designed to assess AMI risk in patients with CCS. At a more detailed level, gene set enrichment analysis revealed modulation of oxidative phosphorylation, ribosomal function, and Toll-like receptor (TLR) signaling pathways following AMI—supporting the hypothesis that plasma EVs are involved in these processes during CAD progression. The authors specifically highlighted three differentially expressed genes: Protein Kinase Inhibitor Gamma (PKIG), Oligosaccharyltransferase Complex Subunit 4 (OST4), and Ribosomal Protein L23 (RPL23). PKIG was found to be downregulated in AMI compared to stable coronary artery disease (SCAD) samples, while OST4 and RPL23 were upregulated. Based on these findings, the authors proposed a panel consisting of these three biomarkers for AMI detection.

Zhang et al. [48] employed ML and data from exoRBase to identify hsa_circ_0001360 and hsa_circ_0000038 as potential contributors to atherogenic pathways. Although neither in vitro nor in vivo experiments were conducted to validate these circular RNAs (circRNAs) as biomarkers, the authors constructed a circRNA-miRNA-mRNA regulatory network. This network identified several downstream miRNA and mRNA targets potentially involved in the progression of atherogenesis, underscoring the utility of ML-based approaches as effective screening strategies for EV-derived biomarkers.

Analogously, Huang et al. [49] aimed to identify non-invasive diagnostic biomarkers using surface-enhanced Raman scattering (SERS) to analyze EVs from CAD patients with

varying clinical presentations (stable plaque, NSTEMI, STEMI) and healthy controls. In this context, ML was applied as an innovative approach to address overlapping SERS signals, with support vector machines achieving the best performance in distinguishing CAD patients from controls. However, critical aspects of this approach limit clinical applicability due to the financial burden and, as the authors noted, a lack of mechanistic insights that solely allows us to formulate general hypotheses.

As Babu and Snyder [50] noted, the rise of multi-omics in clinical settings introduces several challenges, including high financial costs (specialized equipment, personnel, data storage), statistical biases from sample heterogeneity, and security/ethical concerns related to patient data. There is also scepticism regarding the interpretability of ML models in clinical contexts, where they are often seen as "black boxes". Nevertheless, studies like the one by Huang et al. [49], which provides a clear explanation of their algorithm, show that clarity is possible. However, a common limitation across many studies is the absence of external validation cohorts, which weakens the reliability and generalizability of the models. Thus, while ML algorithms hold great promise, their success lies in clearly defined research questions, rigorous sample selection, and reproducible methodologies.

2.4. EVs: An Additional Tool to Define Disease Subtypes

The analysis of "big data", including large patient cohorts, advanced monitoring for collecting real-life information, as well as geomobility models and multi-omic data, represents a crucial resource for identifying disease subtypes and matching patients with the most appropriate, individualized therapeutic strategies. While those who prioritize broader cohort analyses may benefit from statistical power and generalizability, they often compromise on the purity and specificity of subtype identification. Conversely, high-resolution molecular studies provide deeper mechanistic insights but may lack scalability. Therefore, a balanced integration of large-scale data and fine-grained molecular profiling is essential to uncover meaningful endotypes and enable precision medicine at the population level [51,52].

A useful and innovative application of ML could be the correlation of EV cargo with a patient's specific endotype and/or phenotype, as means to personalize EV panels based on individual characteristics. A pioneer study in this aspect was conducted by Burrello et al. [53], who developed "EVaging", a ML model that analyzes circulating EV surface markers by age and sex to assess CV risk. The EVaging index correlates with established CV risk factors and shows diagnostic accuracy comparable to CRP and Systematic COronary Risk Evaluation (SCORE risk). Specific markers (e.g., CD31, CD42a, CD44) were linked to worse CV profiles, with stronger performance noted in older individuals, particularly women. The study highlights the potential of ML-driven tools based on EV characterization for personalized CV risk assessment.

We believe that tailored EV profiling may be useful to identify phenotypes at higher risk of cardiovascular disease [54]. In this context, patients with diabetes mellitus (DM) frequently present endothelial dysfunction and are at elevated risk of CVD, especially CAD [55,56]. Likewise, many CAD patients are also diabetic [57]. Clinical management of patients affected by this high-risk overlap is complicated by the atypical or scarcely symptomatic presentation of acute and chronic cardiovascular events [58]. To exemplify how EV cargo correlates with different clinical scenarios, we highlight the strong positive association between EV-associated hsa-let-7c-5a levels and both HbA1c and SYNTAX scores in hyperglycaemic patients with coronary heart disease. This was suggested by Shufang H et al. [59], who proposed circulating EV hsa-let-7c-5a as a non-invasive indicator of the severity of coronary stenosis in patients presenting hyperglycaemia.

In fact, hsa-let-7b-5p levels were significantly elevated in the moderate-severe stenosis SYNTAX subgroup.

Furthermore, changes in EV protein cargo were reported in diabetic individuals compared to healthy subjects [60]. Wang et al. [61] investigated the impact of DM on EV protein expression in patients with AMI, analyzing EV proteins isolated from the coronary serum. The study compared EVs derived from AMI patients with and without DM (AMI + DM-EVs vs. AMI-EVs) and found that AMI-EVs promoted angiogenesis in vitro, whereas AMI + DM-EVs exhibited an anti-angiogenic effect. Mechanistically, reduced levels of angiopoietin-like protein 6 (ANGPTL6) in AMI + DM-EVs appeared to impair angiogenesis by modulating key signaling pathways, including ERK1/2, JNK, and p38 MAPK. These findings suggest that DM may hinder endothelial regeneration via downregulation of EV-associated ANGPTL6. Therefore, utilizing this specific EV cargo to identify the risk of stent restenosis in diabetic patients treated with rapamycin-eluting stents may be relevant for improving risk stratification and guiding clinical decision-making.

In addition to pathogenetic conditions, diagnostic and/or clinical procedures could alter EV cargo, as our group previously demonstrated. Specifically, we have shown that EV mRNA cargo changes in ACS patients before and after PCI [62,63]. Further investigation into EV-miRNA and protein profiles in clinically defined subgroups could help identify specific early disease biomarkers, addressing a critical unmet clinical need.

3. miRNAs and circRNAs for Identifying ACS Among CAD Patients

Several studies have highlighted the diagnostic and therapeutic potential of EV-ncRNAs in CAD patients. Circular RNAs (circRNAs) are involved in a variety of biological functions, ranging from negatively regulating protein activity by acting as molecular sponges to positively influencing gene transcription or serving as protein scaffolds [64]. Particularly, circRNAs have gained interest as CVD biomarkers due to their resistance to exonuclease activity that results in increased molecular stability [65]. Tong et al. [66] showed that an overexpression of circ_0001785 protects endothelial cells from atherogenesis, both in vitro and in ApoE$^{-/-}$ mice, via the miR-513a-5p/TGFBR3 axis. More specifically, this study represents a clear illustration of how circRNA may alter the miRNA function and gene expression in CVD by promoting TGFBR3 expression via the repression (or "sponging") of miR-513a-5p.

Wu et al. [67] explored the role of circ_0005540 as a diagnostic biomarker in CAD patients using two-center cohorts. The authors reported that circ_0005540 was potentially associated with CAD by acting on miR-221 and miR-145. In addition, Liang et al. [68] demonstrated that lncRNA SOCS2 Antisense RNA 1 (SOCS2-AS1) is downregulated in CAD patients. Multivariate logistic regression confirmed SOCS2-AS1's role as independent protective factors even after adjustment for the principal CAD risk factors. Liu X et al. [69] found hsa_circ_0075269 as an effective biomarker for diagnosing CCS, while another study reported that hsa_circ_0001558 [70] was elevated in AMI, particularly STEMI. In a similar manner, Gu et al. [71] reported that lnc_000226 and Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) were elevated in AMI patients. Notably, they observed a positive association between lnc_000226 and Major Adverse Cardiac Events (MACEs) in a 1-year follow-up post-PCI, showing its predictive value. Despite the promising role of EV-derived circRNAs, the mechanisms underlying their protective or harmful effects remain largely unknown. This is mainly due to the complex and heterogeneous nature of their downstream interactions.

Similarly to circRNAs, knowledge about EV miRNAs as CV diagnostic and prognostic biomarkers has grown exponentially [72–74]. Currently, more than 2600 mature human miRNAs have been annotated in the miRbase database, and the number of registered

miRNAs continues to increase [75,76]. This reflects the complexity involved in elucidating the mechanistic pathways that establish miRNAs as potential biomarkers. We aimed to highlight the most recent circulating EV markers potentially serving as diagnostic and/or prognostic tools in patients with CCS and ACS, as we will discuss in the following sections.

4. Acute Coronary Syndrome (ACS)

In this section, we will focus on the most recent publications related to ACS and EV-miRNAs. For earlier studies, please refer to Table 1. The clinical value of the EV-RNA cargo encompasses diagnosis, prognosis, and risk stratification in CVD patients.

Conflicting or opposing roles of miRNAs have been reported in CVD. miR-133a-3p was primarily known for its cardioprotective effects, promoting angiogenesis and inhibiting apoptosis via the AKT pathway [77], reducing hypertrophy by modulating IKKε and pyroptosis [78], limiting fibrosis via the LTBP1-PPP2CA/TGF-β1 pathway [79], and enhancing cardiac function after aerobic exercise through CTGF downregulation [80]. More recently, it was shown to display pro-arrhythmic potential due to downregulation of ion channel regulatory proteins such as PPP2CA and KChIP2, leading to electrical abnormalities [81].

Likewise, in vitro experiments simulating hypoxia/reoxygenation (H/R)-induced myocardial injury have shown that the overexpression of miR-127-3p exerts anti-inflammatory effects by targeting CDKN3 [82]. However, subsequent in vitro and in vivo studies suggest that miR-127-3p may also have detrimental cardiovascular effects. This microRNA, which is upregulated in atherosclerotic carotid plaques, appears to promote the progression of atherosclerosis and plaque instability by enhancing macrophage proliferation and pro-inflammatory M1 polarization through the SCD1/unsaturated fatty acids (UFAs) axis [83].

In a similar manner, circulating EV-miR-186-5p, that was investigated by Ren et al. [84] as a predictor of MACE in patients undergoing PCI, was found elevated in AMI patients and was positively associated with STEMI. However, EV-miR-186-5p levels have been shown to decline after PCI and to continuously decrease over a 24-month follow-up period. The prognostic value of EV-miR-186-5p is supported by findings that stable high levels or a less-than-expected decrease after PCI are associated with an increased risk of MACE. Conversely, Ding J et al. [85] reported that EV miR-186-5p was downregulated in AMI patients not receiving reperfusion therapy compared to hospitalized non-AMI patients. It was demonstrated that miR-186-5p directly inhibits lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in macrophages. Furthermore, treatment of ApoE^{-}/$^{-}$ mice with EVs containing low levels of miR-186-5p, isolated from AMI patients, accelerated the development of atherosclerotic lesions. Therefore, reduced EV miR-186-5p levels promote lipid accumulation and disease progression after AMI, consistent with previous findings that indicate a negative association between miR-186-5p and atherosclerosis progression [86,87]. However, this observation contrasts with other findings suggesting a pro-atherogenic role of macrophage-derived EV miR-186-5p, which promotes vascular smooth muscle cell (VSMC) viability and invasion via the SHIP2-mediated PI3K/AKT/mTOR pathway [88]. This apparent dichotomy undermines the potential of miR-186-5p as a reliable clinical biomarker, highlighting the need to thoroughly investigate the downstream pathways of non-coding RNAs before defining them as diagnostic or prognostic markers.

Liu et al. [89] described the diagnostic potential of circulating EV miR-4516 and miR-203 by demonstrating their elevated expression levels in AMI patients. Secretory frizzled-related protein 1 (SFRP1), a predicted target of both miR-4516 and miR-203, was also found upregulated in plasma of AMI patients. A positive correlation between EV-miR-4516 and SYNTAX scores was established. However, although the study proposed miR-4516 as a potential non-invasive predictor of vascular damage, the lack of

a follow-up and post-PCI clinical data hinders its prognostic significance. Conversely, Zhang et al. [90] confirmed, over a relative long follow-up (median of 49 months), an independent non-linear association between circulating EV-miR-9-5p and PCI mortality in STEMI patients. EV-miR-9-5p was suggested as a key mediator of pro-inflammatory neutrophil N1 polarization in a myocardial I/R model via the activation of JAK2/STAT3 and NF-κB pathways (miR-9-5p/SOCS5/SIRT1 axis). Furthermore, Son et al. [91] demonstrated that some differentially expressed EV miRNAs (DEmiRNAs) can distinguish between chronic total occlusion (CTO) and AMI, among which EV-miR-9-5p and the previously discussed EV-127-3p are included.

Generally, the function of miRNAs is highly context-dependent, varying with the target cell type and microenvironment. Therefore, investigating their expression under more specific conditions may be useful for improving their sensitivity and specificity. To support this possibility, it has been hypothesized that miR-9-5p contributes to increased cardio-metabolic risk in patients with metabolic syndrome by impairing lipid metabolism through downregulation of ABCA1 in peripheral blood mononuclear cells [92]. However, miR-9-5p has also been reported to mitigate endothelial cell dysfunction via the circ_0090231/miR-9-5p/TXNIP axis in oxidized LDL (ox-LDL)-stimulated human umbilical vein endothelial cells (HUVECs) [93], suggesting a potential negative correlation with atherosclerosis progression.

Many recent studies have proposed potential clinical biomarker candidates; however, the authors often fail to provide experimental evidence regarding the downstream pathways of the implicated miRNAs. Advancing these studies by exploring the molecular mechanisms of proposed EV-derived markers—such as miR-208b-3p and miR-143-3p in sudden cardiac death [94], and miR-30a [95] and miR-21-5p/3p [96] in AMI—would be extremely valuable.

Finally, our attention was especially drawn to the work of Chen et al. [97], as we believe they provided a compelling illustration of EV–miRNA findings that may be translated into *Bioscores* applicable in clinical practice. The authors proposed to apply an miRNA EV cargo combined with strain parameters of Real-Time Three-Dimensional Spot Tracking Echocardiography (RT3D-STE) as a detection tool in STEMI patients. A total of 10 DEmiRNAs studied in three different groups (normal individuals, NSTEMI and STEMI patients) showed a strong correlation with strain parameters and lower expression in STEMI compared to NSTEMI patients. Moreover, miR-152-5p and miR-3681-5p were validated, acquiring greater clinical relevance as AMI indicators. In a similar fashion, the previously discussed work of Shufang H et al. [59] may be proposed as a *Bioscore*, since the combination of EV-derived hsa-let-7c-5p and HbA1c levels in hyperglycaemic patients could serve as a proxy for assessing the severity of CAD.

Table 1. Biomarkers: Acute coronary syndromes.

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
protein	GPIIb, VE-cadherin; ceruloplasmin, transthyretin, fibronectin, PLP1	STEMI (n = 35), CCS (n = 32)	Venous, before CAG; Precipitation	STEMI; OHCA	[38]
epitope	CD62P, CD42a, CD41b, CD31, CD40	STEMI (n = 30), SAP (n = 38), CTR (n = 30) *Validation cohort* (n = 80)	Venous; Centrifugation	MI; Diagnosis	[39]

Table 1. *Cont.*

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
miRNA	miR-208a	ACS (n = 500), CTR (n = 200)	Venous; Precipitation	ACS; Diagnosis, Prognosis	[42]
protein	CTRC, SRC, CCL17	STEMI (n = 60), CTR (n = 22); *Validation cohort*: STEMI (n = 8), UA (n = 8), SAP (n = 8)	Venous; Acoustic trapping	MI; Diagnosis	[45]
epitope	N.A.	Stable plaque (n = 15), NSTEMI (n = 15), STEMI (n = 17), CTR (n = 17)	Arterial, during cardiac catheterization; Ultracentrifugation	MI, CAD; Prognosis	[49]
protein	ANGPTL6	MI (n = 20), MI + DM (n = 20), CTR (n = 10)	Arterial (aortic sinus); Ultracentrifugation	MI, DM; Pathophysiology	[61]
circRNA	circ_0001535, circ_0000972, circ_0001558	*Sequencing group*: MI (n = 15), CTR-NCCP (n = 15); *First validation cohort*: MI (n = 20), CTR-NCCP (n = 20); *Second validation cohort*: AMI (n = 85), CTR-NCCP (n = 48)	Venous, fasting; Membrane affinity-based	MI; Diagnosis	[70]
lncRNA	MALAT1 and LNC_000226	MI (n = 90), CTR (n = 88)	Venous; Ultracentrifugation	MI; Diagnosis, Prognosis	[71]
miRNA	miR-1915-3p, miR-4507, miR-3656	MI (n = 6), SCAD (n = 6). *Validation cohort*: MI (n = 30), SCAD (n = 30)	Venous, before heparin treatment; Ultracentrifugation	MI, SCAD; Differential diagnosis	[73]
miRNA	miR-133a-3p	Adult rats	N.A.; Ultracentrifugation	MI; Prognosis	[79]
miRNA	miR-186-5p	MI (n = 150), CTR (n = 50)	Venous; Precipitation	MI; Prognosis	[84]
miRNA	miR-4516, miR-203	MI (n = 62), CTR (n = 31)	Venous, before PCI (AMI); Venous, fasting (CTR); Ultracentrifugation	MI; Diagnosis	[89]
miRNA	miR-9-5p	STEMI (n = 294)	Venous, $\leq$24 h after PCI; Ultracentrifugation	STEMI; PCI mortality, N1 polarization in I/R injury	[90]
miRNA	miR-9-5p, miR-127-3p	*Sequencing group*: CTO (n = 29), MI (n = 24) *Validation cohort*: CTO (n = 35), MI (n = 35), CTR (n = 10)	Arterial (coronary); Precipitation	MI; Differential diagnosis	[91]

Table 1. *Cont.*

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
miRNA	miR-208b-3p, miR-143-3p	ACS-SCD (n = 9), CTR (n = 9); *Validation cohort*: ACS-SCD (n = 30), CTR (n = 30)	Venous, after admission (ACS-SCD); Venous, fasting (CTR); Ultracentrifugation	ACS; SCD prediction	[94]
miRNA	miR-30a	Mice	N.A.; Ultracentrifugation	MI: Pathophysiology	[95]
miRNA	miR-152-5p, miR-3681-5p, miR-193a-5p, miR-193b-5p miR-345-5p, miR-125a-5p, miR-365a-3p, miR-4520-2-3p, miR-193b-3p and miR-5579-5p	*Sequencing group*: STEMI (n = 7), NSTEMI (n = 7), CTR (n = 10);	N.R.; Precipitation	MI (STEMI, NSTEMI); Correlation with echocardiography	[97]
lncRNA protein	NEAT1, miR-204, MMP-9	STEMI (n = 47), UA (n = 24), CTR (n = 27)	Venous, before PCI; Membrane affinity-based	MI; Diagnosis	[98]
miRNA	Differentially expressed miRNAs (n = 18)	MI (n = 55), CAD (n = 26), CTRL (n = 37)	N.R.; Precipitation method	MI, SCAD; Differential diagnosis	[99]
miRNA	miR-301a-3p, miR-374a-5p, miR-423-5p	STEMI RLVR (n = 5), STEMI AVLR (n = 5)	Venous, after PCI; Precipitation	STEMI; ALVR	[100]
miRNA	Differentially expressed miRNAs (n = 77); miR-181a-3p	STEMI (n = 30), CTRL (n = 30); *Validation cohort* (STEMI n = 20, CTRL n = 24)	Venous; Ultracentrifugation	STEMI; ALVR	[101]
N.A.	N.A.	MI (n = 20), CTR (n = 20)	Venous, before CAG; Precipitation	MI; Physiopathology	[102]
epitope	CD29, CD41b, CD42a, CD41-CD61 (GP2IIb/IIIa)	STEMI (n = 42)	N.R.; Immuno-magnetic capture	STEMI; risk stratification	[103]
miRNA	miR-24-3p	STEMI (n = 8), CTR (n = 8);	Venous, before PCI; SEC	MI; Pathophysiology	[104]
miRNA	miR-486-5p	MI (n = 24), CTR (n = 13); Validation cohort: MI (n = 19), CTR (n = 10)	Cardiac (autopsies); Membrane affinity-based	MI; Atherosclerosis severity	[105]
epitope	CD9, CD81, CD90, CD144, CCR4, CCR6, CXCR3	MI (n = 10), CTR (n = 8)	Venous; SEC	MI; Pathophysiology	[106]
lncRNA	LIPCAR	STEMI RLVR (n = 5), STEMI AVLR (n = 5)	Venous; Ultracentrifugation	MI; ALVR	[107]

Table 1. *Cont.*

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
protein	SERPIND1, MASP1, FCN2, AMBP, HLA-C	MI (n = 10), SAP (n = 10), CTR (n = 10)	Venous; SEC	MI; Diagnosis	[108]
protein	F13A1, TSPAN33, YWHAZ, ITGA2B, GP9, GP5, PPIA	*Profiling group*: STEMI (n = 5), NSTEMI (n = 5), UA (n = 5), CTR (n = 5); *Validation: STEMI* (n = 6), NSTEMI (n = 9), CTR (n = 6)	N.R; Precipitation	MI; Diagnosis	[109]

N.A. = Not Apply; N.R. = Not Reported; CTR = control; SCAD = Stable CAD; MI = Myocardial Infarction; STEMI = ST-Elevated Myocardial Infarction; NSTEMI = No ST-Elevated Myocardial Infarction; UA = Unstable angina; SAP = Stable angina pectoris; OHCA = Out-of-Hospital Cardiac Arrest; ACS-SCD = Acute Coronary Syndrome with Sudden Cardiac Death; PCI = Percutaneous Coronary Intervention; CAG = Coronary angiography; SEC = Size Exclusion Chromatography; I/R injury = Ischemia/Reperfusion Injury; CTO = Chronic Total Occlusion.

5. Chronic Coronary Syndrome (CCS)

The past decade has seen some ground-breaking research on EV microRNAs as biomarkers for CCS [110]. For earlier studies, please refer to Table 2.

More recently, a preliminary study by Zhang et al. [111] evaluated eight selected EV miRNAs in patients with SCAD. The results revealed a significant increase in miR-942-5p, miR-149-5p, and miR-32-5p levels in the SCAD group compared to healthy controls, suggesting their potential clinical relevance as disease-associated predictors. Mechanistically, a possible anti-atherogenic role of miR-942-5p has been postulated. MiR-942-5p was reported to protect VSMCs from ox-LDL-induced dysfunction via the circ_0090231/miR-942-5p/PPM1B axis [112] and to attenuate HUVECs ox-LDL-induced injury via both the circ_0003204/miR-942-5p/HDAC9 [113] and the circ_0004104/miR-942-5p/ROCK2 axis [114]. On the other hand, miR-149-5p has been demonstrated to play heterogeneous roles in CVD physiopathology, ranging from anti-inflammatory to anti-fibrogenic effects on murine heart failure models via the HFRL/miR-149-5p/COL22A1 axis [115]. Additionally, miR-149-5p, which is indirectly regulated by hsa_circ_0087352 and MIAT, has been reported to suppress inflammatory responses in abdominal aortic aneurysm and to inhibit the development of necrotic atherosclerotic plaques by downregulating the ERK/NF-κB pathway [116] and the CD47 pathway [117], respectively. Reliably, the anti-atherogenic role of miR-149-5p has been demonstrated in various models, including PDGF-BB-treated VSMCs via the circ_CHFR/miR-149-5p/NRP2 axis [118], PDGF-BB-treated aortic VSMCs through the circDHCR24/miR-149-5p/MMP9 axis [119] and in LDL-induced endothelial injury models in HUVECs involving the circ_0124644/miR-149-5p/PAPP-A axis [120].

As discussed for ACS, on reviewing the latest literature we found CCS indicators that should be further investigated to unravel their mechanistic function in CAD. Interestingly, Han J et al. [121] investigated circulating EV DEmiRNAs in SCAD patients, finding that let-7c-5p and miR-652-3p were significantly downregulated whereas miR-335-3p was upregulated compared to healthy controls, independent of gender. Additionally, miR-335-3p positively correlates with the *Gensini Score*, which we propose could be integrated into a *Bioscore* to improve the prognostic assessment of CAD patients. In fact, these miRNAs were equivalent to or, in some cases, even outperformed traditional diagnostic approaches such as ECG, the Holter/treadmill exercise test and CT angiography.

The results of this study strongly support the prognostic potential of these EV-derived miRNAs in assessing the severity of SCAD. Other similar studies proposed ncRNAs that were differentially expressed according to CAD severity, such as ENST00000560769.1 [122] and miR-382-3p [123]. Still on the topic of atherosclerotic plaques, Kim et al. [105] found post-mortem higher circulating EV miR-486-5p levels in high-grade atherosclerosis subjects compared to low-grade ones.

By contrast, some authors have focused on the pathophysiological correlation between EV DEmiRNAs and the worsening of CAD. This is the case of Han et al. [37], who investigated the role of EV miR-140-3p in worsening atherosclerosis in SCAD patients. The study analyzed the miR-140-3p effect by using human aortic endothelial cells and the ApoE-/- mouse model. Results suggest that ZO-1 and VE-cadherin were suppressed by EV-miR-140-3p, and this was associated with a significant increase in aortic plaques. It is also worth noting that a clinical positive correlation was drawn between EV-miR-140-3p and the Gensini Score.

Lastly, Wang et al. [124] aimed to evaluate whether circulating EV ncRNA could be used to identify patients who have recently experienced coronary artery-related vascular events. Multivariate logistic regression analysis showed that the risk of ACS increased with the decrease in lg (circSCMH1/miR-874), independently of confounding variables such as type 2 DM, male sex, systolic blood pressure, and WBC. The involvement of miR-879 in myocardial necrosis (via Foxo3a/miR-874/caspase-8) [125], murine model I/R injury (via miR-874/JAK2-STAT3) [126], and vascular calcification (via circSmoc1-2/miR-874-3p/Adam19) [127] have been suggested throughout the years. Instead, circSCMH1's role in CAD needs deeper understanding, as this circRNA has been mainly studied in an ischemic stroke context [128–130].

Table 2. Biomarkers: Chronic coronary syndromes.

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
protein	Ubiquitinated adenosine A2A receptor	CAD (*n* = 14), CTR (*n* = 8)	Venous; Precipitation	CAD+/- hyperhomocysteinemia	[36]
miRNA	miR-140-3p	SCAD (*n* = 39), CTR (*n* = 39)	N.R.; Ultracentrifugation	CAD; Physiopathology	[37]
protein	Tenascin-C *	CAD (*n* = 40), CTR (*n* = 20)	N.R.; N.R.;	CAD; Diagnosis	[41]
lncRNA	lncRNA AC100865.1	SCAD (*n* = 201), CTR (*n* = 187)	Arterial (radial); Ultracentrifugation	SCAD; Diagnosis	[43]
epitope	CD31+/Annexin V+	SCAD (*n* = 200)	Arterial (femoral), before cardiac catheterization; N.R.	SCAD; Prognosis	[44]
protein	OST4, PKIG and RPL23	*Database*: SCAD (*n* = 8), MI (*n* = 10); *Validation cohort*: CAD (*n* = 7)	N.R., <48 h admission and prior to CAG; Precipitation	SCAD, MI; Differential diagnosis	[47]
circRNA	circ_0001360, circ_0000038	*Sequencing group*: CAD (*n* = 6), CTR (*n* = 32); *Validation cohort*: CAD (*n* = 10), CTR (*n* = 10)	Venous, fasting; Precipitation	CAD; Diagnosis	[48]

Table 2. *Cont.*

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
epitope	N.A.	CV risk factors (n = 268), Established cardiac disease/Organ damage (n = 138), Acute CV event (n = 8), CTR (n = 132)	Venous; Bead-based immunocapture assay	CV risk profiles; EVaging index	[53]
miRNA	let-7b-5p	hyperglycaemic CAD (n = 8), normoglycemic CAD (n = 8); *Validation cohort*: hyperglycaemic CAD (n = 75), normoglycemic CAD (n = 75)	Venous fasting; Precipitation	CHD with hyperglycaemia	[59]
circRNA	circ_0001785	CAD (n = 31), CTR (n = 24)	N.R.; Ultracentrifugation	CAD; Physiopathology	[66]
circRNA	circ_0005540	*Profiling and internal validation*: CAD (n = 61), CTR (n = 38); *External validation*: CAD (n = 47), CTR (n = 51)	N.R.; Membrane affinity-based	CAD; Diagnosis	[67]
circRNA	SOCS2-AS1	*Sequencing and training group*: CAD (n = 27), CTR (n = 27) *Validation cohort*: CAD (n = 84), mCAS (n = 48), CTR (n = 41)	Venous, fasting+before CAG; Precipitation	CAD; Diagnosis, Prognosis	[68]
circRNA	circ_0075269, circ_0000284	*Sequencing group*: CCS (n = 15), NCCP (n = 15); *Validation cohorts*: CCS (n = 20, 100), NCCP (n = 20, 48);	Venous, after admission; Membrane affinity-based	CCS; Differential diagnosis	[69]
miRNA	miR-21-5p, miR-21-3p	CAD (n = 135), CTR (n = 150)	Venous; Precipitation	CAD; Diagnosis	[96]
miRNA	miR-942-5p, miR-149-5p, miR-32-5p	SCAD (n = 20), CTR (n = 20)	Venous, fasting; Precipitation	SCAD; Diagnosis	[111]
miRNA	let-7c-5p, miR-335-3p, miR-652-3p	SCAD (n = 39), CTR (n = 39)	Venous, fasting; Ultracentrifugation	CAD; Prognosis	[121]
circRNA	ENST00000424615.2, ENST00000560769.1	*Sequencing group*: CCS (n = 15), CTR (n = 15); *Validation cohorts*: CCS (n = 20, 100), CTR (n = 20), 48;	N.R.; Membrane affinity-based	CCS; CAD severity	[122]
miRNA	miR-382-3p	severe CAD, 3-vessel (n = 129), CTR (n = 114)	Arterial (coronary); Precipitation	CAD; Prognosis	[123]
circRNA; miRNA	circSCMH1/ miR-874	SCAD (n = 300), ACS (n = 300), CTR (n = 101)	Venous, fasting; Ultracentrifugation	SCAD, AMI; Carotid plaque stability	[124]
miRNA	miR-30e, miR-92a	CAD (n = 42), CTR (n = 42)	Venous; Precipitation	CAD; Diagnosis	[131]
protein	SC1, CD14, SG1, PLG, CC, SF2	CAD (n = 187), CTR (n = 257)	Venous, before MPI; Magnetic bead-based capture	Stress induced ischemia;	[132]

Table 2. *Cont.*

	EV Cargo or Epitope	Subjects	Blood Sample; EV Isolation Method	Pathological Conditions; Outcomes	Authors
epitope; protein	CD42a+, CD62E+; AXL, CD163, IGFBP7, NEMO, resistin, BAFF, perlecan	CAD, prior MI (*n* = 220)	Venous, fasting; Acoustic trapping	CAD, CFR; Prognosis	[133]
miRNA	miR-382-3p, miR-432-5p, miR-200a-3p, miR-3613-3p; miR-125a-5p, miR-185-5p, miR-151a-3p, miR328-3p	Three-vessel CAD (*n* = 214), no CAD (*n* = 140)	Arterial (coronary); Precipitation	CAD; Prognosis	[134]
miRNA	miR-19a-3p, miR-18a-5p, miR-133a-3p, miR-155-5p, and miR-210-3p	*Sequencing group*: IHD-DM (*n* = 6), CTR (*n* = 6); *Validation cohort*: IHD-DM (*n* = 26), CTR (*n* = 14)	Venous, fasting; Precipitation	IHD in DM; Diagnosis	[135]
epitope	CD41+/CD61+, CD142+, CD31+	CAD (*n* = 26), CTR (*n* = 14)	Venous; N.A.	CAD; Diagnosis	[136]
miRNA	miR-16-2-3p	DM (*n* = 3), DM-CMD (*n* = 3), DM-CAD (*n* = 3)	Venous; Ultracentrifugation	CAD; Differential diagnosis	[137]
miRNA	miR-382-3p, miR-432-5p, miR-200a-3p, mi-3613-3p; miR-125a-5p, miR-185-5p, miR-151a-3p, miR-328-3p	CAD (*n* = 115)	Venous; Precipitation	CAD; Myocardial perfusion	[138]
epitope	CD144+	INOCA-CMD (*n* = 34), INOCA-VSA (*n* = 15), INOCA-mixed endotype (*n* = 24), NCCP (*n* = 23)	Venous, before CAG; N.A.	INOCA; Classification	[139]
APL membrane	Phosphatidylethanolamine, phosphatidylserine	CAD (*n* = 19), ACS (*n* = 24), CTR (*n* = 24), risk-factor CTR (*n* = 23)	Venous; SEC	CAD, ACS; thrombosis	[140]
APL membrane	Phosphatidylthreonine	CAD (*n* = 19), ACS (*n* = 24), CTR (*n* = 24), risk-factor CTR (*n* = 23)	Venous; SEC	CAD, ACS; thrombosis	[141]

* expression assessed through EV RNA level; N.A. = Not Apply; N.R. = Not Reported; CTR = Control; SCAD = Stable CAD; CCS = Chronic Coronary Syndrome; IHD = Ischemic Heart Disease; mCAS = Mild Coronary Stenosis; CAG = coronary angiography; MPI = Myocardial Perfusion Imaging; SEC = Size Exclusion Chromatography; CFR = Coronary Flow Reserve; DM = Diabetes Mellitus; INOCA = Ischemia and Non-Obstructive Coronary Artery disease; CMD = Coronary Microvascular Disfunction; VSA = Vasospastic Angina; NCCP = Non Cardiac Chest Pain; MPI = Myocardial Perfusion Imaging; APL = Aminophospholipid.

6. Ischemia and Non-Obstructive Coronary Artery Disease (INOCA)

Ischemia and Non-Obstructive Coronary Artery Disease (INOCA), a subset of chronic coronary syndromes which accounts for symptomatic patients with no angiographic evidence of relevant myocardial stenosis, has been relatively underexplored in the field of EVs [142]. The pathways underlying INOCA are epicardial vasospasm (VSA) and coronary microvascular dysfunction (CMD), which can exist separately or overlap (CMD-VSA endotype) [143,144]. However, the pathogenesis of INOCA is complex and not yet completely understood [145,146]. In clinical practice, diagnosis of INOCA usually requires functional coronary angiography that shows a preserved fractional flow reserve (FFR) with an increased index of microcirculatory resistance (IMR) or hyperaemic microvascular

resistance (HMR), impaired coronary flow reserve (CFR), or both; in some cases, a positive response to acetylcholine stimuli can be required [147].

Early research suggests that impaired CFR is linked to altered EV profiles. Bryl-Górecka et al. [133] investigated this association in post-MI patients, comparing the circulating EV number and protein cargo between high and low CFR groups. A negative correlation between CFR and both total EV levels and pro-atherogenic protein content was found. Notably and as expected, platelet-derived (CD42+) and endothelial-derived (CD62E+) EVs were significantly elevated in patients with low CFR, indicating that circulating EVs may serve as markers of blood flow impairment and vascular dysfunction. Building on earlier findings, recent studies have further explored EV miRnomics in the context of INOCA. In their observational study, Gąsecka et al. [139] proposed circulating EV profiling as a non-invasive approach to differentiate INOCA endotypes. Specifically, using platelet (CD61+), leukocyte (CD45+), and endothelial-derived (CD62E+, CD144+) EVs, they found that a CD144+ EV/total EV ratio < 0.00016 was able to distinguish INOCA from non-anginal chest pain with moderate sensitivity but limited specificity. Additionally, the mixed INOCA endotype had a significantly lower CD144+ EV ratio compared to other groups.

7. Post-AMI and Cardiac Remodeling

Following AMI, cardiac fibrosis occurs as part of the reparative process. While extracellular matrix (ECM) remodeling is essential for tissue repair, excessive ECM deposition can lead to adverse cardiac remodeling and, thereby, further functional decline [148,149]. Many efforts have been made to answer the question of how EV ncRNA may affect adverse cardiac remodeling. Turkieh et al. [107] provided an example of the potential pro-fibrogenic role of the EV RNA cargo by identifying LIPCAR (Long Intergenic noncoding RNA predicting CARdiac remodeling) in human cardiac samples and in circulating EVs. They showed that EV-LIPCAR was significantly elevated in post-MI patients with left ventricular remodeling (LVR) compared to patients with no LVR. Hence, a positive association between LIPCAR and adverse remodeling might be drawn. Conversely, Senesi et al. [104] demonstrated the potential anti-fibrogenic role of EV RNAs. More precisely, the role of miR-24-3p was assessed by demonstrating its effects in human cardiac samples and in vivo pre-clinical models. Some miR-24-3p target genes involved in the TGFβ1-activated fibrogenic pathway (i.e., FURIN, CCND1, SMAD4) were seen to be differentially expressed in human cardiac fibroblasts (hCF) after MI by using bioinformatic analysis. Thereafter, miR-24-3p's regulatory role against fibrotic process was studied by transfecting hCF with miR-24-3p mimics, inhibiting hCF activation into myofibroblasts and downregulating FURIN, CCND1, SMAD4. Furthermore, a significant decrease in EV miR-24-3p was observed in ischemic conditions using both in vitro and ex vivo models. The robustness of this study is supported by the finding that STEMI patients had lower circulating EV-miR-24-3p levels prior to PCI compared to healthy controls. Taken together, these findings suggest that the reduction in EV miR-24-3p under ischemic conditions may lead to a loss of its anti-fibrotic activity. A second anti-fibrotic miRNA, miR-133a-3p, was identified by Yang et al. [79]. The authors demonstrated that EVs derived from ischemic preconditioning (IPC) in post-MI animals exert cardioprotective effects primarily through miR-133a-3p, which improves cardiac function, reduces inflammation, fibrosis, and apoptosis. The inhibitory effect of miR-133a-3p on TGF-β1 signaling, as well as on its downstream targets LTBP1 and PPP2CA, may underlie its cardioprotective properties.

Limpitikul et al. [150] investigated circulating EV-derived miRNAs in a large cohort of patients following AMI and found that increased levels of miR-378c and decreased levels of miR-223-3p were associated with a reduced post-AMI left ventricular ejection

fraction (LVEF). Interestingly, both miRNAs did not strongly correlate with established post-AMI adverse outcome markers (such as hs-TnI, NT-proBNP os hsCRP), suggesting that our understanding of current post-AMI remodeling is incomplete, possibly leading to inadequate stratification of the post-AMI risk of adverse cardiac remodeling and subsequent allocation of resources.

Finally, an important concept is that the EV cargo fluctuates with disease progression, and its utility as a biomarker may be influenced by the timing of sample collection. This dynamic aspect was illustrated by Eyyupkoca et al. [100]'s prospective study, where EV miRNA profiles in male post-AMI patients at risk of ALVR were investigated. Particularly, ALVR was assessed by cardiac MRI at 2 weeks and 6 months, and miRNA expression was analyzed from blood samples taken on day 1, week 2, and week 6 post-AMI. Dynamic changes regarding various DEmiRNAs were found. In fact, only miR-374a-5p was downregulated at week 2, whereas at week 6 miR-301a-3p was downregulated and miR-423-5p was upregulated. These findings suggest that the functional and structural changes following AMI may correlate with dynamic shifts in EV miRNA profiles, supporting their potential application as early prognostic biomarkers for identifying patients at risk of ALVR.

8. Proteomics

The clinical significance of EVs extends beyond their RNA cargo to include numerous proteins, both inside and expressed on their surface. This implies that EV proteomics may focus on the "EV protein signature". Most studies have focused on either surface proteins with receptor functions or on EV's enclosed protein cargo, which is mainly involved in intercellular and intracellular signaling.

The EV protein corona (PC) warrants independent consideration due to its crucial role in modulating EV behavior. This corona is a dynamic and complex layer of biomolecules that adheres to the EV surface, reflecting the molecular composition of the surrounding microenvironment. PC is typically categorized into a "hard" corona (inner layer with strong binding affinity) and a "soft" corona (outer layer with weaker, reversible interactions) [151]. These layers can significantly alter physicochemical properties of EVs, influence their uptake by immune cells, and reduce both their targeted delivery and biodistribution. The composition of the PC is highly sensitive to even slight changes in biological fluids, making it a valuable proxy for detecting pathological states [152].

Widely studied in nanomedicine, PC is now being explored in other fields [153,154]. For example, Lee et al. [155] developed a diagnostic PC-based array using six nanoparticles (NPs) and plasma from CAD patients, symptomatic non-CAD patients, and healthy controls. Their system distinguished CAD patients from both control groups, demonstrating PC's diagnostic potential. Unfortunately, most studies focus solely on synthetic NPs, with little attention to EVs. Despite growing recognition of PC's diagnostic and functional significance, there is currently a notable gap in the literature exploring the role of EV PC specifically in CVD. Therefore, we believe that extending this research from NPs to EVs could offer valuable insights into the pathophysiological role of EVs in CAD.

Focusing on the EV protein cargo, Vélez et al. [156] compared EV protein profiles between STEMI and SCAD patients, albeit noting methodological limitations in the proteomic analysis (i.e., drug interference with protein analysis, running technical rather than biological replicates due to scarcity of biological material, potential under-representation of hydrophobic proteins, lack of further functional studies). Building on this, Gidlöf et al. [45] performed a more robust study using MI cohorts encompassing STEMI, unstable angina, and stable angina patients. A total of 52 cardiovascular-related proteins were detected in EVs, with 3 of them being specifically dysregulated (i.e., CCL17, CTRC, and SRC). These proteins were linked to a reduced risk of STEMI, independent of age and sex. Furthermore,

to highlight the aggregate value of EV research compared to simply studying circulating proteins, attention should be drawn to the fact that CCL17 had better diagnostic performance in EVs than in plasma. In fact, while most dysregulated proteins in this study appeared in plasma, the EV proteome offered distinct diagnostic value, as levels of SRC, CCL17, and CTRC were significantly reduced in EVs from MI patients but unchanged in plasma. This finding supports the potential of EV-associated proteins, especially SRC, as markers of advanced CAD.

To strengthen this point, we propose Dekker et al. [132]'s study on stress-induced myocardial ischemia (SIMI) in outpatients with chest pain undergoing myocardial perfusion imaging. Patients were stratified by SIMI severity, and protein levels were analyzed both in plasma and in three EV subfractions (LDL-EVs, HDL-EVs, and non-LDL/non-HDL EVs). All targeted proteins—Serpin C1, Serpin G1, Serpin F2, CD14, Cystatin C, and Plasminogen—were detected across EV subfractions. One must consider that significant differences between groups were observed only within EV subfractions but not in plasma, highlighting the added diagnostic value of EV profiling. Moreover, the study suggests that lipoprotein co-isolation does not confound EV analysis and supports EV subfraction profiling as a more sensitive approach for identifying SIMI-related biomarkers.

We recently designed a clinical trial to address the unmet need for tailored interventions in patients with NSTEMI and unstable angina who present high-risk features. The primary objective was to profile circulating EVs isolated from these patients to identify potential predictors for the absence of clinically significant CAD. This approach was aimed to enhance risk stratification, guide clinical decision-making, and reduce unnecessary invasive procedures in these patients. Multivariate analysis identified the circulating EV-associated phospholipid transfer protein and the β-subunit of mitochondrial trifunctional enzyme as potential predictors of patients without critical CAD, while elevated levels of EV miR-130a-3p were associated with a lack of multivessel involvement (Femminò et al. accepted in principle in iScience 2025).

Emphasis should be also made on surface proteins, as they represent a simple method to study EVs coming from specific cellular subtypes. This is the case of the prospective study performed by Zarà et al. [103]. It involved STEMI patients undergoing PCI to examine the association between circulating EVs and the extent of myocardial injury assessed by cardiac MRI. The authors found elevated EV concentrations in patients with anterior STEMI and in those receiving late PCI (>3 h post-symptom onset). In addition, patients with microvascular obstruction exhibited reduced EV size and lower expression of platelet-derived markers (CD41–CD61). Surface marker analysis revealed that, as expected, the predominant sources of post-STEMI EVs were platelets, leukocytes, and endothelial cells. Pathway enrichment analysis further linked these EVs to platelet activation, immune modulation, and intercellular signaling. The findings support previous evidence suggesting that reduced platelet-derived EVs may hinder myocardial repair and contribute to adverse remodeling, highlighting their potential as both biomarkers and therapeutic targets in post-infarction settings. As described for EV ncRNA, proteomics can be integrated into *Bioscores*. The work of McGranaghan et al. [136] suggests using EV profiling to enhance CAD diagnostic accuracy beyond traditional laboratory tools. Their findings showed that integrating clinical data with plasma proteins and EV profiles significantly improves diagnostic performance. Particularly, platelet-derived EVs (CD41+/CD61+) emerged as key contributors. Additionally, the impact of surface proteins as a tool for differential diagnosis may be inferred by observing that EV-CD142+ levels were elevated in CAD patients whereas EV-CD31+ were higher in controls.

In the context of INOCA, Bryl-Górecka et al. [133] focused on proteomic analysis from circulating EVs to explore potential biomarkers associated with CFR. This study, part

of the previously discussed CFR project (See Section 6), analyzed a panel of candidate proteins relevant to CVD. Of particular interest, B cell activating factor (BAFF) demonstrated a strong inverse correlation with CFR. BAFF elevated presence in individuals with low-CFR suggests that it may contribute to microvascular dysfunction and adverse cardiac outcomes, considering BAFF's known role in promoting inflammation, enhancing thrombotic activity, and inducing vascular calcification. This study highlights the diagnostic and prognostic potential of EV-associated proteins in INOCA and suggests that BAFF, particularly, could be a promising biomarker and therapeutic target for identifying and managing patients at risk of microvascular ischemia and chronic cardiac injury.

More generally, Zarà et al. [38] investigated the predictive role of the EV protein cargo in different coronary syndromes. When comparing STEMI and CCS patients, they found that, despite similar troponin content, STEMI-derived EVs showed higher levels of GPIIb and VE-cadherin, but lower acute-phase proteins. Moreover, there were differences within the STEMI group, as EVs from out-of-hospital cardiac arrest patients had elevated GPIIb and PLP1 when compared to uncomplicated STEMI cases. In a similar manner, Zhou et al. [109] investigated differentially expressed proteins (DEPs) in circulating EVs from STEMI, NSTEMI, unstable angina, and healthy controls. They identified several DEPs in ACS patients, three of which (i.e., F13A1, TSPAN33, YWHAZ) were confirmed in STEMI cases and six (i.e., F13A1, TSPAN33, ITGA2B, GP9, GP5, PPIA) in NSTEMI, all downregulated compared to controls. Consistent with these findings, these proteins were linked to platelet activation, thrombosis, inflammation, oxidative stress, and atherogenesis.

A growing area of research has focused on the role of EV surface aminophospholipids (aPL) in coagulation, with implications for CAD and atherosclerosis. Protty et al. [140] analyzed EV-associated aPL in four groups: ACS, SCAD, individuals with CV risk factors, and healthy controls. EV-rich plasma from ACS patients triggered significantly greater thrombin generation, with a similar trend observed in CAD and risk factor groups. A higher EV number and surface area correlated with increased thrombin generation, suggesting a procoagulant role of EV membranes, particularly via exposed phosphatidylserine (PS) and phosphatidylethanolamine (PE). However, after adjustment for the EV count, PS/PE levels normalized, suggesting that the procoagulant effect was primarily driven by the increased number of EVs rather than being directly associated with ACS. Building on these findings, Hajeyah et al. [141] identified phosphatidylthreonine (PT) as another procoagulant aPL enriched in EVs and platelets of CAD patients. Elevated EV-derived PT was linked to enhanced prothrombinase activity. When considered alongside the observation that the overall procoagulant effect correlates more strongly with the EV number than with the presence of ACS itself, these results underscore the potential utility of EV-associated aPLs as biomarkers for thrombotic risk stratification in patients with CAD.

9. Critical Appraisal and Conclusions

In the first part of this review, we provided an overview of EV research focused on identifying their potential clinical applications, specifically emphasizing the role of EVs and their cargo as biomarkers in CAD (Figure 1). Key accuracy metrics can be found in Supplementary Table S1, whereas summaries of the studies are reported in Tables 1 and 2.

Reviewing the literature, we identified several challenges that hinder the introduction of EV biomarkers into routine clinical practice. Prior to the first position statements issued by the International Society for Extracellular Vesicles (ISEV) in 2013, vesicle-related research was marked by extremely low levels of standardization and reproducibility [157]. Although the ISEV has made significant efforts to reduce variability in EV-based studies, the alignment of methods for EV isolation and characterization remains an ongoing objective

rather than an established standard, thereby reflecting the current variability and lack of consensus among studies in the field.

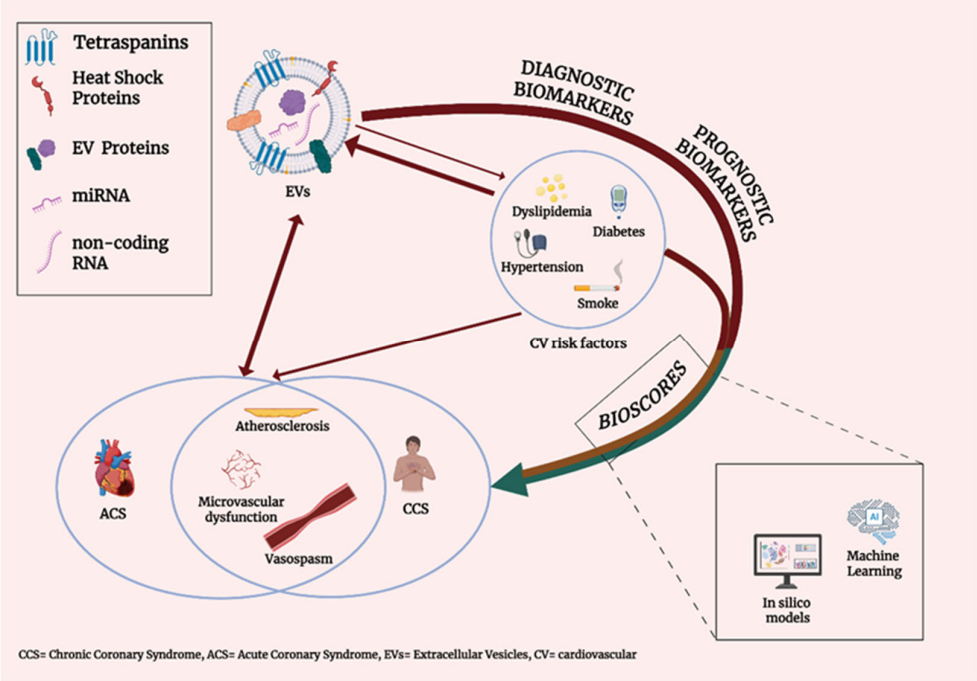

Figure 1. Schematic representation of EV biomarker discovery (*Created with BioRender.com*).

For clinical biomarker discovery and application, isolation methods must balance purity, speed, cost, and scalability. This implies that the choice of isolation method must be carefully tailored to the specific objectives of the study, considering a balance between advantages and limitations. Although some methodology offers high purity yield, we concluded that a quick and accessible technique (i.e., precipitation) must be prioritized in the field of EV biomarker discovery. Alternative approaches to address technical issues of a commonly used isolation methodology are being explored by Gidlöf [45] and Bryl-Górecka [133], who used acoustic trapping to isolate particles in real time. Acoustic trapping shows strong potential for automation, scalability, and clinical reproducibility, but requires further validation before a broad adoption [158].

In the context of complex, and sometimes, opposing EV apparent behavior, the need for integrative multi-omics approaches that combine expression profiling with mechanistic validation is seemingly clear. By integrating these strategies, it becomes possible to better delineate the context-dependent effects of the EV cargo, thereby enabling the identification of precise biomarkers and potential therapeutic targets despite their diverse functions.

Concerning studies evaluating clinical outcomes, metanalyses should be conducted to increase the scientific value, strengthening the level of evidence of circulating EVs as diagnostic and/or prognostic biomarkers. Unfortunately, substantial heterogeneity in study design and methodology limits the ability to plan higher-level evidence studies. First, while some studies isolate EVs from peripheral venous samples, others use intracoronary blood. Secondly, as we previously discussed, the microenvironment highly affects the external layer of EVs' PC, possibly modifying their function. Therefore, CAD patients with different phenotypes may exhibit distinct EV PC profiles. While this heterogeneity is not inherently negative, it limits the generalizability of potential findings across broader patient populations. Thirdly, irrespective of the sample type that was used (e.g., venous blood), blood sampling conditions were various and sometimes not specified (fasting vs. admission time vs. unknown). Although we did not find evidence that supports circadian

variations in the analyzed indicators, we cannot exclude that sampling time and conditions alter either the expression profile or the circulating EV cargo. Fourthly, a common major limitation is the small size and lack of demographic diversity in the studies' cohorts. Fifthly, few results are validated in vitro or in preclinical models after they are putatively described in the studies. The absence of this approach impairs the validation of biomarkers as truly related to the specific disease. Lastly, there is a lack of long-term data, as most prognostic evaluations rely on single EV measurements or short follow-up periods, limiting the understanding of long-term clinical implications. Extended monitoring would provide better insights into biomarker dynamics over time.

We may conclude that EVs show strong potential as biomarkers for CAD and other cardiovascular diseases, but clinical application is hindered by methodological variability and limited validation. Emerging tools, such as improved isolation methods, *Bioscores*, and ML can enhance EV diagnostic value but must be supported by standardized protocols and clinical feasibility. In addition, when considering cost-effectiveness, it is important to acknowledge that although EV analyses may be costly at the onset, their implementation and standardization have the potential to improve cost-efficiency over the long term. Overall, these findings suggest that the current challenges can be effectively overcome through coordinated, multidisciplinary, and translational research efforts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijtm5030039/s1, Table S1 Statistics.

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References

1. Bernáth-Nagy, D.; Kalinyaprak, M.S.; Giannitsis, E.; Ábrahám, P.; Leuschner, F.; Frey, N.; Krohn, J.B. Circulating extracellular vesicles as biomarkers in the diagnosis, prognosis and therapy of cardiovascular diseases. *Front. Cardiovasc. Med.* **2024**, *11*, 1425159. [CrossRef] [PubMed]
2. Wang, Y.; Yuan, Y.; Tang, J. Extracellular Vesicles as Diagnostic Metrics for Cardiovascular Disease: Where We Are and How to Achieve in Clinics. *J. Cardiovasc. Transl. Res.* **2025**. [CrossRef] [PubMed]
3. Aronson, J.K.; Ferner, R.E. Biomarkers—A General Review. *Curr. Protoc. Pharmacol.* **2017**, *76*, 9–23. [CrossRef]
4. Hill, A.B. Section of Occupational Medicine the Environment and Disease: Association or Causation? *Proc. R. Soc. Med.* **1965**, *58*, 295–300.
5. GBD 2021 Adult BMI Collaborators. Global, regional, and national prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: A forecasting study for the Global Burden of Disease Study 2021. *Lancet* **2025**, *405*, 813–838. [CrossRef]
6. Vrints, C.; Andreotti, F.; Koskinas, K.C.; Rossello, X.; Adamo, M.; Ainslie, J.; Banning, A.P.; Budaj, A.; Buechel, R.R.; Chiariello, G.A.; et al. 2024 ESC Guidelines for the management of chronic coronary syndromes. *Eur. Heart J.* **2024**, *45*, 3415–3537. [CrossRef]
7. Byrne, R.A.; Rossello, X.; Coughlan, J.J.; Barbato, E.; Berry, C.; Chieffo, A.; Claeys, M.J.; Dan, G.A.; Dweck, M.R.; Galbraith, M.; et al. 2023 ESC Guidelines for the management of acute coronary syndromes. *Eur. Heart J.* **2023**, *44*, 3720–3826. [CrossRef]
8. Marzilli, M.; Merz, C.N.B.; Boden, W.E.; Bonow, R.O.; Capozza, P.G.; Chilian, W.M.; DeMaria, A.N.; Guarini, G.; Huqi, A.; Morrone, D.; et al. Obstructive Coronary Atherosclerosis and Ischemic Heart Disease: An Elusive Link! *J. Am. Coll. Cardiol.* **2012**, *60*, 951–956. [CrossRef]
9. Attiq, A.; Afzal, S.; Ahmad, W.; Kandeel, M. Hegemony of inflammation in atherosclerosis and coronary artery disease. *Eur. J. Pharmacol.* **2024**, *966*, 176338. [CrossRef]
10. Pepine, C.J. ANOCA/INOCA/MINOCA: Open artery ischemia. *Am. Heart J. Plus Cardiol. Res. Pract.* **2023**, *26*, 100260. [CrossRef]
11. Xie, Y.; Jiang, J.; Wang, J. Management of Chronic Coronary Syndrome: 2024 Update. *JACC Asia* **2025**, *5*, 327–331. [CrossRef]

12. Bass, T.A.; Abbott, J.D.; Mahmud, E.; Parikh, S.A.; Aboulhosn, J.; Ashwath, M.L.; Baranowski, B.; Bergersen, L.; Chaudry, H.I.; Coylewright, M.; et al. 2023 ACC/AHA/SCAI Advanced Training Statement on Interventional Cardiology (Coronary, Peripheral Vascular, and Structural Heart Interventions): A Report of the ACC Competency Management Committee. *J. Am. Coll. Cardiol.* **2023**, *81*, 14. [CrossRef]
13. Zdanyte, M.; Wrazidlo, R.W.; Kaltenbach, S.; Groga-Bada, P.; Gawaz, M.; Geisler, T.; Rath, D. Predicting 1-, 3- and 5-year outcomes in patients with coronary artery disease: A comparison of available risk assessment scores. *Atherosclerosis* **2021**, *318*, 1–7. [CrossRef]
14. Katsioupa, M.; Kourampi, I.; Oikonomou, E.; Tsigkou, V.; Theofilis, P.; Charalambous, G.; Marinos, G.; Gialamas, I.; Zisimos, K.; Anastasiou, A.; et al. Novel Biomarkers and Their Role in the Diagnosis and Prognosis of Acute Coronary Syndrome. *Life* **2023**, *13*, 1992. [CrossRef]
15. Jiang, Y.; Zhao, Y.; Li, Z.; Chen, S.; Fang, F.; Cai, J. Potential roles of microRNAs and long noncoding RNAs as diagnostic, prognostic and therapeutic biomarkers in coronary artery disease. *Int. J. Cardiol.* **2023**, *384*, 90–99. [CrossRef] [PubMed]
16. Yazdani, A.N.; Pletsch, M.; Chorbajian, A.; Zitser, D.; Rai, V. Biomarkers to monitor the prognosis, disease severity, and treatment efficacy in coronary artery disease. *Expert Rev. Cardiovasc. Ther.* **2023**, *21*, 675–692. [CrossRef] [PubMed]
17. Røsand, Ø.; Høydal, M.A. Cardiac exosomes in ischemic heart disease—A narrative review. *Diagnostics* **2021**, *11*, 269. [CrossRef] [PubMed]
18. Welsh, J.A.; Goberdhan, D.C.I.; O'Driscoll, L.; Buzas, E.I.; Blenkiron, C.; Bussolati, B.; Cai, H.; Vizio, D.D.; Driedonks, T.A.P.; Erdbrügger, U.; et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J. Extracell. Vesicles* **2024**, *13*, e12404. [CrossRef]
19. Van Niel, G.; Carter, D.R.F.; Clayton, A.; Lambert, D.W.; Raposo, G.; Vader, P. Challenges and directions in studying cell–cell communication by extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 369–382. [CrossRef]
20. Han, C.; Yang, J.; Sun, J.; Qin, G. Extracellular vesicles in cardiovascular disease: Biological functions and therapeutic implications. *Pharmacol. Ther.* **2022**, *233*, 108025. [CrossRef]
21. Stam, J.; Bartel, S.; Bischoff, R.; Wolters, J.C. Isolation of extracellular vesicles with combined enrichment methods. *J. Chromatogr. B* **2021**, *1169*, 122604. [CrossRef]
22. Altıntaş, Ö.; Saylan, Y. Exploring the Versatility of Exosomes: A Review on Isolation, Characterization, Detection Methods, and Diverse Applications. *Anal. Chem.* **2023**, *95*, 16029–16048. [CrossRef] [PubMed]
23. Dong, L.; Zieren, R.C.; Horie, K.; Kim, C.J.; Mallick, E.; Jing, Y.; Feng, M.; Kuczler, M.D.; Green, J.; Amend, S.R.; et al. Comprehensive evaluation of methods for small extracellular vesicles separation from human plasma, urine and cell culture medium. *J. Extracell. Vesicles* **2020**, *10*, e12044. [CrossRef] [PubMed]
24. Sidhom, K.; Obi, P.O.; Saleem, A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? *Int. J. Mol. Sci.* **2020**, *21*, 6466. [CrossRef] [PubMed]
25. Konoshenko, M.Y.; Lekchnov, E.A.; Bryzgunova, O.E.; Kiseleva, E.; Pyshnaya, I.A.; Laktionov, P.P. Isolation of extracellular vesicles from biological fluids via the aggregation–precipitation approach for downstream mirnas detection. *Diagnostics* **2021**, *11*, 384. [CrossRef]
26. Tiwari, S.; Kumar, V.; Randhawa, S.; Verma, S.K. Preparation and characterization of extracellular vesicles. *Am. J. Reprod. Immunol.* **2021**, *85*, e13367. [CrossRef]
27. Veerman, R.E.; Teeuwen, L.; Czarnewski, P.; Akpinar, G.G.; Sandberg, A.S.; Cao, X.; Pernemalm, M.; Orre, L.M.; Gabrielsson, S.; Eldh, M. Molecular evaluation of five different isolation methods for extracellular vesicles reveals different clinical applicability and subcellular origin. *J. Extracell. Vesicles* **2021**, *10*, e12128. [CrossRef]
28. Comfort, N.; Cai, K.; Bloomquist, T.R.; Strait, M.D.; Ferrante, A.W.; Baccarelli, A.A. Nanoparticle tracking analysis for the quantification and size determination of extracellular vesicles. *J. Vis. Exp.* **2021**, e62447. [CrossRef]
29. Kurian, T.K.; Banik, S.; Gopal, D.; Chakrabarti, S.; Mazumder, N. Elucidating Methods for Isolation and Quantification of Exosomes: A Review. *Mol. Biotechnol.* **2021**, *63*, 249–266. [CrossRef]
30. Welsh, J.A.; Arkesteijn, G.J.A.; Bremer, M.; Cimorelli, M.; Dignat-George, F.; Giebel, B.; Görgens, A.; Hendrix, A.; Kuiper, M.; Lacroix, R.; et al. A compendium of single extracellular vesicle flow cytometry. *J. Extracell. Vesicles* **2023**, *12*, e12299. [CrossRef]
31. Sluijter, J.P.G.; Davidson, S.M.; Boulanger, C.M.; Buzás, E.I.; Kleijn, D.P.V.D.; Engel, F.B.; Giricz, Z.; Hausenloy, D.J.; Kishore, R.; Lecour, S.; et al. Extracellular vesicles in diagnostics and therapy of the ischaemic heart: Position Paper from the Working Group on Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc. Res.* **2018**, *114*, 19–34. [CrossRef]
32. Sahoo, S.; Adamiak, M.; Mathiyalagan, P.; Kenneweg, F.; Kafert-Kasting, S.; Thum, T. Therapeutic and Diagnostic Translation of Extracellular Vesicles in Cardiovascular Diseases: Roadmap to the Clinic. *Circulation* **2021**, *143*, 1426–1449. [CrossRef]
33. Saint-Pol, J.; Culot, M. Minimum information for studies of extracellular vesicles (MISEV) as toolbox for rigorous, reproducible and homogeneous studies on extracellular vesicles. *Toxicol. Vitr.* **2025**, *106*, 106049. [CrossRef]
34. Zheng, D.; Huo, M.; Li, B.; Wang, W.; Piao, H.; Wang, Y.; Zhu, Z.; Li, D.; Wang, T.; Liu, K. The Role of Exosomes and Exosomal MicroRNA in Cardiovascular Disease. *Front. Cell Dev. Biol.* **2021**, *8*, 616161. [CrossRef] [PubMed]

35. Femminò, S.; Penna, C.; Margarita, S.; Comità, S.; Brizzi, M.F.; Pagliaro, P. Extracellular vesicles and cardiovascular system: Biomarkers and Cardioprotective Effectors. *Vasc. Pharmacol.* **2020**, *135*, 106790. [CrossRef] [PubMed]
36. Ruf, J.; Vairo, D.; Paganelli, F.; Guieu, R. Extracellular vesicles with ubiquitinated adenosine A2A receptor in plasma of patients with coronary artery disease. *J. Cell. Mol. Med.* **2019**, *23*, 6805–6811. [CrossRef] [PubMed]
37. Han, J.; Kang, X.; Su, Y.; Wang, J.; Cui, X.; Bian, Y.; Wu, C. Plasma exosomes from patients with coronary artery disease promote atherosclerosis via impairing vascular endothelial junctions. *Sci. Rep.* **2024**, *14*, 29813. [CrossRef]
38. Zarà, M.; Campodonico, J.; Cosentino, N.; Biondi, M.L.; Amadio, P.; Milanesi, G.; Assanelli, E.; Cerri, S.; Biggiogera, M.; Sandrini, L.; et al. Plasma exosome profile in st-elevation myocardial infarction patients with and without out-of-hospital cardiac arrest. *Int. J. Mol. Sci.* **2021**, *22*, 8065. [CrossRef]
39. Burrello, J.; Bolis, S.; Balbi, C.; Burrello, A.; Provasi, E.; Caporali, E.; Gauthier, L.G.; Peirone, A.; D'Ascenzo, F.; Monticone, S.; et al. An extracellular vesicle epitope profile is associated with acute myocardial infarction. *J. Cell. Mol. Med.* **2020**, *24*, 9945–9957. [CrossRef]
40. Li, C.; Ni, Y.Q.; Xu, H.; Xiang, Q.Y.; Zhao, Y.; Zhan, J.K.; He, J.Y.; Li, S.; Liu, Y.S. Roles and mechanisms of exosomal non-coding RNAs in human health and diseases. *Signal Transduct. Target. Ther.* **2021**, *6*, 383. [CrossRef]
41. Gholipour, A.; Shakerian, F.; Zahedmehr, A.; Oveisee, M.; Maleki, M.; Mowla, S.J.; Malakootian, M. Tenascin-C as a noninvasive biomarker of coronary artery disease. *Mol. Biol. Rep.* **2022**, *49*, 9267–9273. [CrossRef]
42. Bi, S.; Wang, C.; Jin, Y.; Lv, Z.; Xing, X.; Lu, Q. Correlation between serum exosome derived miR-208a and acute coronary syndrome. *Int. J. Clin. Exp. Med.* **2015**, *8*, 4275–4280.
43. Yang, Y.; Cai, Y.; Wu, G.; Chen, X.; Liu, Y.; Wang, X.; Yu, J.; Li, C.; Chen, X.; Jose, P.A.; et al. Plasma long non-coding RNA, CoroMarker, a novel biomarker for diagnosis of coronary artery disease. *Clin. Sci.* **2015**, *129*, 675–685. [CrossRef]
44. Sinning, J.M.; Losch, J.; Walenta, K.; Böhm, M.; Nickenig, G.; Werner, N. Circulating CD31 +/Annexin V + microparticles correlate with cardiovascular outcomes. *Eur. Heart J.* **2011**, *32*, 2034–2041. [CrossRef] [PubMed]
45. Gidlöf, O.; Evander, M.; Rezeli, M.; Marko-Varga, G.; Laurell, T.; Erlinge, D. Proteomic profiling of extracellular vesicles reveals additional diagnostic biomarkers for myocardial infarction compared to plasma alone. *Sci. Rep.* **2019**, *9*, 8991. [CrossRef] [PubMed]
46. Barh, D.; Chaitankar, V.; Yiannakopoulou, E.C.; Salawu, E.O.; Chowbina, S.; Ghosh, P.; Azevedo, V. In Silico Models: From Simple Networks to Complex Diseases. In *Animal Biotechnology: Models in Discovery and Translation*; Elsevier Inc.: Amsterdam, The Netherlands, 2013; pp. 385–404; ISBN 978-0-12-416002-6. [CrossRef]
47. Jin, X.; Xu, W.; Wu, Q.; Huang, C.; Song, Y.; Lian, J. Detecting early-warning biomarkers associated with heart-exosome genetic-signature for acute myocardial infarction: A source-tracking study of exosome. *J. Cell. Mol. Med.* **2024**, *28*, e18334. [CrossRef]
48. Zhang, W.; Cui, J.; Li, L.; Zhu, T.; Guo, Z. Identification of Plasma Exosomes hsa_circ_0001360 and hsa_circ_0000038 as Key Biomarkers of Coronary Heart Disease. *Cardiol. Res. Pract.* **2024**, *2024*, 5557143. [CrossRef]
49. Huang, X.; Liu, B.; Guo, S.; Guo, W.; Liao, K.; Hu, G.; Shi, W.; Kuss, M.; Duryee, M.J.; Anderson, D.R.; et al. SERS spectroscopy with machine learning to analyze human plasma derived sEVs for coronary artery disease diagnosis and prognosis. *Bioeng. Transl. Med.* **2023**, *8*, e10420. [CrossRef]
50. Babu, M.; Snyder, M. Multi-omics profiling for health. *Mol. Cell. Proteom.* **2023**, *22*, 100561. [CrossRef]
51. Sethi, Y.; Patel, N.; Kaka, N.; Kaiwan, O.; Kar, J.; Moinuddin, A.; Goel, A.; Chopra, H.; Cavalu, S. Precision Medicine and the future of Cardiovascular Diseases: A Clinically Oriented Comprehensive Review. *J. Clin. Med.* **2023**, *12*, 1799. [CrossRef]
52. Olawade, D.B.; Aderinto, N.; Olatunji, G.; Kokori, E.; David-Olawade, A.C.; Hadi, M. Advancements and applications of Artificial Intelligence in cardiology: Current trends and future prospects. *J. Med. Surg. Public Health* **2024**, *3*, 100109. [CrossRef]
53. Burrello, J.; Goi, J.; Burrello, A.; Vacchi, E.; Rendon-Angel, A.; Lazzarini, E.; Bianco, G.; Limongelli, V.; Vassalli, G.; Cereda, C.W.; et al. Age- and sex-related variations in extracellular vesicle profiling for the assessment of cardiovascular risk: The EVaging index. *Npj Aging* **2024**, *10*, 63. [CrossRef] [PubMed]
54. Cavallari, C.; Figliolini, F.; Tapparo, M.; Cedrino, M.; Trevisan, A.; Positello, L.; Rispoli, P.; Solini, A.; Migliaretti, G.; Camussi, G.; et al. miR-130a and Tgfβ Content in Extracellular Vesicles Derived from the Serum of Subjects at High Cardiovascular Risk Predicts their In-Vivo Angiogenic Potential. *Sci. Rep.* **2020**, *10*, 706. [CrossRef] [PubMed]
55. Einarson, T.R.; Acs, A.; Ludwig, C.; Panton, U.H. Prevalence of cardiovascular disease in type 2 diabetes: A systematic literature review of scientific evidence from across the world in 2007–2017. *Cardiovasc. Diabetol.* **2018**, *17*, 83. [CrossRef]
56. Marx, N.; Federici, M.; Schütt, K.; Müller-Wieland, D.; Ajjan, R.A.; Antunes, M.J.; Christodorescu, R.M.; Crawford, C.; Angelantonio, E.D.; Eliasson, B.; et al. 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes. *Eur. Heart J.* **2023**, *44*, 4043–4140. [CrossRef]
57. Bartnik, M.; Rydén, L.; Ferrari, R.; Malmberg, K.; Pyörälä, K.; Simoons, M.; Standl, E.; Soler-Soler, J.; Öhrvik, J.; Manini, M.; et al. The prevalence of abnormal glucose regulation in patients with coronary artery disease across Europe: The Euro Heart Survey on diabetes and the heart. *Eur. Heart J.* **2004**, *25*, 1880–1890. [CrossRef]

58. Zellweger, M.J. Prognostic significance of silent coronary artery disease in type 2 diabetes. *Herz* **2006**, *31*, 240–245. [CrossRef]
59. Han, S.; Fang, J.; Yu, L.; Li, B.; Hu, Y.; Chen, R.; Li, C.; Zhao, C.; Li, J.; Wang, Y.; et al. Serum-derived exosomal hsa-let-7b-5p as a biomarker for predicting the severity of coronary stenosis in patients with coronary heart disease and hyperglycemia. *Mol. Med. Rep.* **2023**, *28*, 203. [CrossRef]
60. Togliatto, G.; Dentelli, P.; Rosso, A.; Lombardo, G.; Gili, M.; Gallo, S.; Gai, C.; Solini, A.; Camussi, G.; Brizzi, M.F. PDGF-BB carried by endothelial Cell-derived extracellular vesicles reduces vascular smooth muscle cell apoptosis in diabetes. *Diabetes* **2018**, *67*, 704–716. [CrossRef]
61. Wang, W.; Zhao, Y.; Zhu, P.; Jia, X.; Wang, C.; Zhang, Q.; Li, H.; Wang, J.; Hou, Y. Differential Proteomic Profiles of Coronary Serum Exosomes in Acute Myocardial Infarction Patients with or Without Diabetes Mellitus: ANGPTL6 Accelerates Regeneration of Endothelial Cells Treated with Rapamycin via MAPK Pathways. *Cardiovasc. Drugs Ther.* **2024**, *38*, 13–29. [CrossRef]
62. D'Ascenzo, F.; Femminò, S.; Ravera, F.; Angelini, F.; Caccioppo, A.; Franchin, L.; Grosso, A.; Comità, S.; Cavallari, C.; Penna, C.; et al. Extracellular vesicles from patients with Acute Coronary Syndrome impact on ischemia-reperfusion injury. *Pharmacol. Res.* **2021**, *170*, 105715. [CrossRef]
63. Femminò, S.; D'ascenzo, F.; Ravera, F.; Comità, S.; Angelini, F.; Caccioppo, A.; Franchin, L.; Grosso, A.; Thairi, C.; Venturelli, E.; et al. Percutaneous coronary intervention (Pci) reprograms circulating extracellular vesicles from acs patients impairing their cardio-protective properties. *Int. J. Mol. Sci.* **2021**, *22*, 10270. [CrossRef]
64. Ward, Z.; Pearson, J.; Schmeier, S.; Cameron, V.; Pilbrow, A. Insights into circular RNAs: Their biogenesis, detection, and emerging role in cardiovascular disease. *RNA Biol.* **2021**, *18*, 2055–2072. [CrossRef]
65. Wang, Y.; Liu, J.; Ma, J.; Sun, T.; Zhou, Q.; Wang, W.; Wang, G.; Wu, P.; Wang, H.; Jiang, L.; et al. Exosomal circRNAs: Biogenesis, effect and application in human diseases. *Mol. Cancer* **2019**, *18*, 116. [CrossRef] [PubMed]
66. Tong, X.; Dang, X.; Liu, D.; Wang, N.; Li, M.; Han, J.; Zhao, J.; Wang, Y.; Huang, M.; Yang, Y.; et al. Exosome-derived circ_0001785 delays atherogenesis through the ceRNA network mechanism of miR-513a-5p/TGFBR3. *J. Nanobiotechnol.* **2023**, *21*, 362. [CrossRef] [PubMed]
67. Wu, W.P.; Pan, Y.H.; Cai, M.Y.; Cen, J.M.; Chen, C.; Zheng, L.; Liu, X.; Xiong, X.D. Plasma-Derived Exosomal Circular RNA hsa_circ_0005540 as a Novel Diagnostic Biomarker for Coronary Artery Disease. *Dis. Markers* **2020**, *2020*, 1341918. [CrossRef] [PubMed]
68. Liang, C.; Zhang, L.; Lian, X.; Zhu, T.; Zhang, Y.; Gu, N. Circulating Exosomal SOCS2-AS1 Acts as a Novel Biomarker in Predicting the Diagnosis of Coronary Artery Disease. *BioMed Res. Int.* **2020**, *2020*, 9182091. [CrossRef]
69. Liu, X.; Zheng, M.; Han, R.; Yu, Z.; Yuan, W.; Xie, B.; Zhang, Y.; Zhong, J.; Wang, L.; Wang, L.; et al. Circulating Exosomal CircRNAs as Diagnostic Biomarkers for Chronic Coronary Syndrome. *Metabolites* **2023**, *13*, 1066. [CrossRef]
70. Liu, X.; Zhang, Y.; Yuan, W.; Han, R.; Zhong, J.; Yang, X.; Zheng, M.; Xie, B. Exosomal CircRNAs in Circulation Serve as Diagnostic Biomarkers for Acute Myocardial Infarction. *Front. Biosci. Landmark* **2024**, *29*, 149. [CrossRef]
71. Gu, X.; Hou, J.; Weng, R.; Rao, J.; Liu, S. The Diagnosis and Prognosis Value of Circulating Exosomal lncRNA MALAT1 and LNC_000226 in Patients With Acute Myocardial Infarction: An Observational Study. *Immun. Inflamm. Dis.* **2024**, *12*, e70088. [CrossRef]
72. Moreira-Costa, L.; Barros, A.S.; Lourenço, A.P.; Leite-Moreira, A.F.; Nogueira-Ferreira, R.; Thongboonkerd, V.; Vitorino, R. Exosome-derived mediators as potential biomarkers for cardiovascular diseases: A network approach. *Proteomes* **2021**, *9*, 8. [CrossRef]
73. Su, J.; Li, J.; Yu, Q.; Wang, J.; Li, X.; Yang, J.; Xu, J.; Liu, Y.; Xu, Z.; Ji, L.; et al. Exosomal miRNAs as potential biomarkers for acute myocardial infarction. *IUBMB Life* **2020**, *72*, 384–400. [CrossRef]
74. Li, H.; Li, Z.; Fu, Q.; Fu, S.; Xiang, T. Exploring the landscape of exosomes in heart failure: A bibliometric analysis. *Int. J. Surg.* **2025**, *111*, 3356–3372. [CrossRef] [PubMed]
75. Alles, J.; Fehlmann, T.; Fischer, U.; Backes, C.; Galata, V.; Minet, M.; Hart, M.; Abu-Halima, M.; Grässer, F.A.; Lenhof, H.P.; et al. An estimate of the total number of true human miRNAs. *Nucleic Acids Res.* **2019**, *47*, 3353–3364. [CrossRef] [PubMed]
76. Kozomara, A.; Birgaoanu, M.; Griffiths-Jones, S. MiRBase: From microRNA sequences to function. *Nucleic Acids Res.* **2019**, *47*, D155–D162. [CrossRef] [PubMed]
77. Zhu, W.; Sun, L.; Zhao, P.; Liu, Y.; Zhang, J.; Zhang, Y.; Hong, Y.; Zhu, Y.; Lu, Y.; Zhao, W.; et al. Macrophage migration inhibitory factor facilitates the therapeutic efficacy of mesenchymal stem cells derived exosomes in acute myocardial infarction through upregulating miR-133a-3p. *J. Nanobiotechnol.* **2021**, *19*, 61. [CrossRef]
78. Zhu, Y.F.; Wang, R.; Chen, W.; Cao, Y.D.; Li, L.P.; Chen, X. miR-133a-3p attenuates cardiomyocyte hypertrophy through inhibiting pyroptosis activation by targeting IKKε. *Acta Histochem.* **2021**, *123*, 151653. [CrossRef]
79. Yang, N.; Hou, Y.B.; Cui, T.H.; Yu, J.M.; He, S.F.; Zhu, H.J. Ischemic-Preconditioning Induced Serum Exosomal miR-133a-3p Improved Post-Myocardial Infarction Repair via Targeting LTBP1 and PPP2CA. *Int. J. Nanomed.* **2024**, *19*, 9035–9053. [CrossRef]
80. Liu, N.; Zhen, Z.; Xiong, X.; Xue, Y. Aerobic exercise protects MI heart through miR-133a-3p downregulation of connective tissue growth factor. *PLoS ONE* **2024**, *19*, 0296430. [CrossRef]

81. Kuzmin, V.S.; Ivanova, A.D.; Filatova, T.S.; Pustovit, K.B.; Kobylina, A.A.; Atkinson, A.J.; Petkova, M.; Voronkov, Y.I.; Abramochkin, D.V.; Dobrzynski, H. Micro-RNA 133a-3p induces repolarization abnormalities in atrial myocardium and modulates ventricular electrophysiology affecting ICa,L and Ito currents. *Eur. J. Pharmacol.* **2021**, *908*, 174369. [CrossRef]
82. Zhu, S.; Fang, Z. MicroRNA-127-3p Inhibits Cardiomyocyte Inflammation and Apoptosis after Acute Myocardial Infarction via Targeting CDKN3. *Int. Heart J.* **2023**, *64*, 1133–1139. [CrossRef]
83. Liu, Y.; Wu, Y.; Wang, C.; Hu, W.; Zou, S.; Ren, H.; Zuo, Y.; Qu, L. MiR-127-3p enhances macrophagic proliferation via disturbing fatty acid profiles and oxidative phosphorylation in atherosclerosis. *J. Mol. Cell. Cardiol.* **2024**, *193*, 36–52. [CrossRef]
84. Ren, L.; Liu, W.; Chen, S.; Zeng, H. Longitudinal change of serum exosomal miR-186-5p estimates major adverse cardiac events in acute myocardial infarction patients receiving percutaneous coronary intervention. *Front. Cardiovasc. Med.* **2024**, *11*, 1341918. [CrossRef]
85. Ding, J.; Li, H.; Liu, W.; Wang, X.; Feng, Y.; Guan, H.; Chen, Z. miR-186-5p Dysregulation in Serum Exosomes from Patients with AMI Aggravates Atherosclerosis via Targeting LOX-1. *Int. J. Nanomed.* **2022**, *17*, 6301–6316. [CrossRef] [PubMed]
86. Zhang, S.; Zhu, X.; Li, G. E2F1/SNHG7/miR-186-5p/MMP2 axis modulates the proliferation and migration of vascular endothelial cell in atherosclerosis. *Life Sci.* **2020**, *257*, 118013. [CrossRef] [PubMed]
87. Li, S.; Huang, T.; Qin, L.; Yin, L. Circ_0068087 Silencing Ameliorates Oxidized Low-Density Lipoprotein-Induced Dysfunction in Vascular Endothelial Cells Depending on miR-186-5p-Mediated Regulation of Roundabout Guidance Receptor 1. *Front. Cardiovasc. Med.* **2021**, *8*, 650374. [CrossRef] [PubMed]
88. Ren, L.; Chen, S.; Yao, D.; Yan, H. OxLDL-stimulated macrophage exosomes promote proatherogenic vascular smooth muscle cell viability and invasion via delivering miR-186–5p then inactivating SHIP2 mediated PI3K/AKT/mTOR pathway. *Mol. Immunol.* **2022**, *146*, 27–37. [CrossRef]
89. Liu, P.; Wang, S.; Li, K.; Yang, Y.; Man, Y.; Du, F.; Wang, L.; Tian, J.; Su, G. Exosomal microRNA-4516, microRNA-203 and SFRP1 are potential biomarkers of acute myocardial infarction. *Mol. Med. Rep.* **2023**, *27*, 124. [CrossRef]
90. Zhang, Y.; Li, X.; Dai, Y.; Han, Y.; Wei, X.; Wei, G.; Chen, W.; Kong, S.; He, Y.; Liu, H.; et al. Neutrophil N1 polarization induced by cardiomyocyte-derived extracellular vesicle miR-9-5p aggravates myocardial ischemia/reperfusion injury. *J. Nanobiotechnol.* **2024**, *22*, 632. [CrossRef]
91. Son, J.H.; Park, J.K.; Bang, J.H.; Kim, D.; Moon, I.; Kong, M.G.; Park, H.W.; Choi, H.O.; Seo, H.S.; Cho, Y.H.; et al. Exosomal miRNAs Differentiate Chronic Total Occlusion from Acute Myocardial Infarction. *Int. J. Mol. Sci.* **2024**, *25*, 10223. [CrossRef]
92. D'Amore, S.; Härdfeldt, J.; Cariello, M.; Graziano, G.; Copetti, M.; Di Tullio, G.; Piglionica, M.; Scialpi, N.; Sabbà, C.; Palasciano, G.; et al. Identification of miR-9-5p as direct regulator of ABCA1 and HDL-driven reverse cholesterol transport in circulating CD14 + cells of patients with metabolic syndrome. *Cardiovasc. Res.* **2018**, *114*, 1154–1164. [CrossRef]
93. Lei, X.; Yang, Y. Oxidized low-density lipoprotein contributes to injury of endothelial cells via the circ_0090231/miR-9-5p/TXNIP axis. *Cent. Eur. J. Immunol.* **2022**, *47*, 41–57. [CrossRef]
94. Huang, S.; Zhang, J.; Wan, H.; Wang, K.; Wu, J.; Cao, Y.; Hu, L.; Yu, Y.; Sun, H.; Yu, Y.; et al. Plasma extracellular vesicles microRNA-208b-3p and microRNA-143-3p as novel biomarkers for sudden cardiac death prediction in acute coronary syndrome. *Mol. Omics* **2023**, *19*, 262–273. [CrossRef]
95. Li, Y.; Chen, H.; Yang, Y.; Pan, Y.; Yuan, Q.; Liu, Y. Murine exosomal miR-30a aggravates cardiac function after acute myocardial infarction via regulating cell fate of cardiomyocytes and cardiac resident macrophages. *Int. J. Cardiol.* **2024**, *414*, 132395. [CrossRef]
96. Sahebi, R.; Gandomi, F.; Shojaei, M.; Farrokhi, E. Exosomal miRNA-21-5p and miRNA-21-3p as key biomarkers of myocardial infarction. *Health Sci. Rep.* **2024**, *7*, e2228. [CrossRef] [PubMed]
97. Chen, X.; Huang, F.; Liu, Y.; Liu, S.; Tan, G. Exosomal miR-152-5p and miR-3681-5p function as potential biomarkers for ST-segment elevation myocardial infarction. *Clinics* **2022**, *77*, 100038. [CrossRef] [PubMed]
98. Chen, Z.; Yan, Y.; Wu, J.; Qi, C.; Liu, J.; Wang, J. Expression level and diagnostic value of exosomal NEAT1/miR-204/MMP-9 in acute ST-segment elevation myocardial infarction. *IUBMB Life* **2020**, *72*, 2499–2507. [CrossRef]
99. Guo, M.; Li, R.; Yang, L.; Zhu, Q.; Han, M.; Chen, Z.; Ruan, F.; Yuan, Y.; Liu, Z.; Huang, B.; et al. Evaluation of exosomal miRNAs as potential diagnostic biomarkers for acute myocardial infarction using next-generation sequencing. *Ann. Transl. Med.* **2021**, *9*, 219. [CrossRef]
100. Eyyupkoca, F.; Ercan, K.; Kiziltunc, E.; Ugurlu, I.B.; Kocak, A.; Eyerci, N. Determination of microRNAs associated with adverse left ventricular remodeling after myocardial infarction. *Mol. Cell. Biochem.* **2022**, *477*, 781–791. [CrossRef]
101. Guan, R.; Zeng, K.; Zhang, B.; Gao, M.; Li, J.; Jiang, H.; Liu, Y.; Qiang, Y.; Liu, Z.; Li, J.; et al. Plasma Exosome miRNAs Profile in Patients With ST-Segment Elevation Myocardial Infarction. *Front. Cardiovasc. Med.* **2022**, *9*, 848812. [CrossRef]
102. Gong, Z.; Wen, M.; Zhang, W.; Yu, L.; Huang, C.; Xu, Y.; Xia, Z.; Xu, M.; Xu, J.; Liang, Q.; et al. Plasma exosomes induce inflammatory immune response in patients with acute myocardial infarction. *Arch. Physiol. Biochem.* **2023**, *129*, 1168–1176. [CrossRef]

103. Zarà, M.; Baggiano, A.; Amadio, P.; Campodonico, J.; Gili, S.; Annoni, A.; Dona, G.D.; Carerj, M.L.; Cilia, F.; Formenti, A.; et al. Circulating Small Extracellular Vesicles Reflect the Severity of Myocardial Damage in STEMI Patients. *Biomolecules* **2023**, *13*, 1470. [CrossRef] [PubMed]
104. Senesi, G.; Lodrini, A.M.; Mohammed, S.; Mosole, S.; Hjortnaes, J.; Veltrop, R.J.A.; Kubat, B.; Ceresa, D.; Bolis, S.; Raimondi, A.; et al. miR-24-3p secreted as extracellular vesicle cargo by cardiomyocytes inhibits fibrosis in human cardiac microtissues. *Cardiovasc. Res.* **2024**, *121*, 143–156. [CrossRef] [PubMed]
105. Kim, S.-Y.; Lee, S.; Park, J.-T.; Lee, S.-J.; Kim, H.-S. Postmortem-Derived Exosomal MicroRNA 486-5p as Potential Biomarkers for Ischemic Heart Disease Diagnosis. *Int. J. Mol. Sci.* **2024**, *25*, 9619. [CrossRef] [PubMed]
106. Moreno, A.; Alarcón-Zapata, P.; Guzmán-Gútierrez, E.; Radojkovic, C.; Contreras, H.; Nova-Lampeti, E.; Zúñiga, F.A.; Rodriguez-Alvárez, L.; Escudero, C.; Lagos, P.; et al. Changes in the Release of Endothelial Extracellular Vesicles CD144+, CCR6+, and CXCR3+ in Individuals with Acute Myocardial Infarction. *Biomedicines* **2024**, *12*, 2119. [CrossRef]
107. Turkieh, A.; Beseme, O.; Saura, O.; Charrier, H.; Michel, J.B.; Amouyel, P.; Thum, T.; Bauters, C.; Pinet, F. LIPCAR levels in plasma-derived extracellular vesicles is associated with left ventricle remodeling post-myocardial infarction. *J. Transl. Med.* **2024**, *22*, 31. [CrossRef]
108. Xu, S.; Zhai, Y.; Wang, C.; Zhang, Y.; Liu, X.; Jiang, J.; Mi, Y. Proteomics Analysis of Five Potential Plasma-derived Exosomal Biomarkers for Acute Myocardial Infarction. *Curr. Med. Chem.* **2025**, *32*, 4816–4835. [CrossRef]
109. Zhou, J.; Hou, H.T.; Chen, H.X.; Song, Y.; Zhou, X.L.; Zhang, L.L.; Xue, H.M.; Yang, Q.; He, G.W. Plasma Exosomal Proteomics Identifies Differentially Expressed Proteins as Biomarkers for Acute Myocardial Infarction. *Biomolecules* **2025**, *15*, 583. [CrossRef]
110. Dekker, M.; Waissi, F.; Timmerman, N.; Silvis, M.J.M.; Timmers, L.; Kleijn, D.P.V. de Extracellular vesicles in diagnosing chronic coronary syndromes—The bumpy road to clinical implementation. *Int. J. Mol. Sci.* **2020**, *21*, 9128. [CrossRef]
111. Zhang, P.; Liang, T.; Chen, Y.; Wang, X.; Wu, T.; Xie, Z.; Luo, J.; Yu, Y.; Yu, H. Circulating Exosomal miRNAs as Novel Biomarkers for Stable Coronary Artery Disease. *BioMed Res. Int.* **2020**, *2020*, 3593962. [CrossRef]
112. Yang, J.; Li, X.; Zhang, Y.; Che, P.; Qin, W.; Wu, X.; Liu, Y.; Hu, B. Circ_0090231 knockdown protects vascular smooth muscle cells from ox-LDL-induced proliferation, migration and invasion via miR-942-5p/PPM1B axis during atherosclerosis. *Mol. Cell. Biochem.* **2024**, *479*, 2035–2045. [CrossRef]
113. Wan, H.; You, T.; Luo, W. circ_0003204 Regulates Cell Growth, Oxidative Stress, and Inflammation in ox-LDL-Induced Vascular Endothelial Cells via Regulating miR-942-5p/HDAC9 Axis. *Front. Cardiovasc. Med.* **2021**, *8*, 646832. [CrossRef] [PubMed]
114. Zhang, Y.; Wang, S.; Guo, S.; Zhang, X.; Yang, C.; Su, G.; Wan, J. Circ_0004104 participates in the regulation of ox-LDL-induced endothelial cells injury via miR-942-5p/ROCK2 axis. *BMC Cardiovasc. Disord.* **2022**, *22*, 517. [CrossRef] [PubMed]
115. Li, X.; Teng, Y.; Tian, M.; Qiu, H.; Zhao, J.; Gao, Q.; Zhang, Y.; Zhuang, J.; Chen, J. Enhancement of LncRNA-HFRL expression induces cardiomyocyte inflammation, proliferation, and fibrosis via the sequestering of miR-149-5p-mediated collagen 22A inhibition. *Ann. Transl. Med.* **2022**, *10*, 523. [CrossRef] [PubMed]
116. Ma, X.; Xu, J.; Lu, Q.; Feng, X.; Liu, J.; Cui, C.; Song, C. Hsa_circ_0087352 promotes the inflammatory response of macrophages in abdominal aortic aneurysm by adsorbing hsa-miR-149-5p. *Int. Immunopharmacol.* **2022**, *107*, 108691. [CrossRef]
117. Ye, Z.; Yang, S.; Xia, Y.; Hu, R.; Chen, S.; Li, B.; Chen, S.; Luo, X.; Mao, L.; Li, Y.; et al. LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell Death Dis.* **2019**, *10*, 138. [CrossRef]
118. Wang, M.; Li, C.; Cai, T.; Zhang, A.; Cao, J.; Xin, H. Circ_CHFR Promotes Platelet-Derived Growth Factor-BB-Induced Proliferation, Invasion, and Migration in Vascular Smooth Muscle Cells via the miR-149-5p/NRP2 Axis. *Cardiovasc. Pharmacol.* **2021**, *79*, e94–e102. [CrossRef]
119. Peng, W.; Li, T.; Pi, S.; Huang, L.; Liu, Y. Suppression of circular RNA circDHCR24 alleviates aortic smooth muscle cell proliferation and migration by targeting miR-149-5p/MMP9 axis. *Biochem. Biophys. Res. Commun.* **2020**, *529*, 753–759. [CrossRef]
120. Wang, G.; Li, Y.; Liu, Z.; Ma, X.; Li, M.; Lu, Q.; Li, Y.; Lu, Z.; Niu, L.; Fan, Z.; et al. Circular RNA circ_0124644 exacerbates the ox-LDL-induced endothelial injury in human vascular endothelial cells through regulating PAPP-A by acting as a sponge of miR-149-5p. *Mol. Cell. Biochem.* **2020**, *471*, 51–61. [CrossRef]
121. Han, J.; Cui, X.; Yuan, T.; Yang, Z.; Liu, Y.; Ren, Y.; Wu, C.; Bian, Y. Plasma-derived exosomal let-7c-5p, miR-335–3p, and miR-652–3p as potential diagnostic biomarkers for stable coronary artery disease. *Front. Physiol.* **2023**, *14*, 1161612. [CrossRef]
122. Zheng, M.; Han, R.; Yuan, W.; Chi, H.; Zhang, Y.; Sun, K.; Zhong, J.; Liu, X.; Yang, X. Circulating exosomal lncRNAs in patients with chronic coronary syndromes. *Arch. Med. Sci.* **2023**, *19*, 46–56. [CrossRef]
123. Chang, S.N.; Chen, J.J.; Liu, M.T.; Chung, Y.T.; Lin, S.H.; Liu, C.J.; Li, C.; Lin, J.W. Validation of novel exosomal miRNAs identified by next-generation sequencing in coronary artery disease. *J. Formos. Med. Assoc.* **2025**, *in press*. [CrossRef]
124. Wang, J.; Liu, Y.; Tian, P.; Xing, L.; Huang, X.; Fu, C.; Xu, X.; Liu, P. Exosomal circSCMH1/miR-874 ratio in serum to predict carotid and coronary plaque stability. *Front. Cardiovasc. Med.* **2023**, *10*, 1277427. [CrossRef] [PubMed]
125. Wang, K.; Liu, F.; Zhou, L.Y.; Ding, S.L.; Long, B.; Liu, C.Y.; Sun, T.; Fan, Y.Y.; Sun, L.; Li, P.F. MiR-874 regulates myocardial necrosis by targeting caspase-8. *Cell Death Dis.* **2013**, *4*, e709. [CrossRef] [PubMed]

126. Chen, P.J.; Shang, A.Q.; Yang, J.P.; Wang, W.W. microRNA-874 inhibition targeting STAT3 protects the heart from ischemia–reperfusion injury by attenuating cardiomyocyte apoptosis in a mouse model. *J. Cell. Physiol.* **2019**, *234*, 6182–6193. [CrossRef]
127. Ryu, J.; Choe, N.; Kwon, D.H.; Shin, S.; Lim, Y.H.; Yoon, G.; Kim, J.H.; Kim, H.S.; Lee, I.K.; Ahn, Y.; et al. Circular RNA circSmoc1-2 regulates vascular calcification by acting as a miR-874-3p sponge in vascular smooth muscle cells. *Mol. Ther. Nucleic Acids* **2022**, *27*, 645–655. [CrossRef]
128. Li, B.; Xi, W.; Bai, Y.; Liu, X.; Zhang, Y.; Li, L.; Bian, L.; Liu, C.; Tang, Y.; Shen, L.; et al. FTO-dependent m6A modification of Plpp3 in circSCMH1-regulated vascular repair and functional recovery following stroke. *Nat. Commun.* **2023**, *14*, 489. [CrossRef]
129. Yang, L.; Han, B.; Zhang, Z.; Wang, S.; Bai, Y.; Zhang, Y.; Tang, Y.; Du, L.; Xu, L.; Wu, F.; et al. Extracellular vesicle-mediated delivery of circular RNA SCMH1 promotes functional recovery in rodent and nonhuman primate ischemic stroke models. *Circulation* **2020**, *142*, 556–574. [CrossRef]
130. Wang, Y.; Bai, Y.; Cai, Y.; Zhang, Y.; Shen, L.; Xi, W.; Zhou, Z.; Xu, L.; Liu, X.; Han, B.; et al. Circular RNA SCMH1 suppresses KMO expression to inhibit mitophagy and promote functional recovery following stroke. *Theranostics* **2024**, *14*, 7292–7308. [CrossRef]
131. Wang, Z.; Zhang, J.; Zhang, S.; Yan, S.; Wang, Z.; Wang, C.; Zhang, X. MiR-30e and miR-92a are related to atherosclerosis by targeting ABCA1. *Mol. Med. Rep.* **2019**, *19*, 3298–3304. [CrossRef]
132. Dekker, M.; Waissi, F.; van Bennekom, J.; Silvis, M.J.M.; Timmerman, N.; Bank, I.E.M.; Walter, J.E.; Mueller, C.; Schoneveld, A.H.; Schiffelers, R.M.; et al. Plasma extracellular vesicle proteins are associated with stress-induced myocardial ischemia in women presenting with chest pain. *Sci. Rep.* **2020**, *10*, 12257. [CrossRef]
133. Bryl-Górecka, P.; James, K.; Torngren, K.; Haraldsson, I.; Gan, L.M.; Svedlund, S.; Olde, B.; Laurell, T.; Omerovic, E.; Erlinge, D. Microvesicles in plasma reflect coronary flow reserve in patients with cardiovascular disease. *Am. J. Physiol.—Heart Circ. Physiol.* **2021**, *320*, H2147–H2160. [CrossRef]
134. Chang, S.N.; Chen, J.J.; Wu, J.H.; Chung, Y.T.; Chen, J.W.; Chiu, C.H.; Liu, C.J.; Liu, M.T.; Chang, Y.C.; Li, C.; et al. Association between exosomal mirnas and coronary artery disease by next-generation sequencing. *Cells* **2022**, *11*, 98. [CrossRef]
135. Zhang, L.; Zhang, J.; Qin, Z.; Liu, N.; Zhang, Z.; Lu, Y.; Xu, Y.; Zhang, J.; Tang, J. Diagnostic and Predictive Values of Circulating Extracellular Vesicle-Carried microRNAs in Ischemic Heart Disease Patients With Type 2 Diabetes Mellitus. *Front. Cardiovasc. Med.* **2022**, *9*, 813310. [CrossRef]
136. McGranaghan, P.; Pallinger, É.; Fekete, N.; Maurovich-Horvát, P.; Drobni, Z.; Merkely, B.; Menna, L.; Buzás, E.I.; Hegyesi, H. Modeling the Impact of Extracellular Vesicle Cargoes in the Diagnosis of Coronary Artery Disease. *Biomedicines* **2024**, *12*, 2682. [CrossRef]
137. Liu, Y.; Zhong, C.; Chen, S.; Xue, Y.; Wei, Z.; Dong, L.; Kang, L. Circulating exosomal mir-16-2-3p is associated with coronary microvascular dysfunction in diabetes through regulating the fatty acid degradation of endothelial cells. *Cardiovasc. Diabetol.* **2024**, *23*, 60. [CrossRef] [PubMed]
138. Liu, C.J.; Chen, J.; Wu, J.H.; Chung, Y.T.; Chen, J.W.; Liu, M.T.; Chiu, C.H.; Chang, Y.C.; Chang, S.N.; Lin, J.W.; et al. Association of exosomes in patients with compromised myocardial perfusion on functional imaging. *J. Formos. Med. Assoc.* **2024**, *123*, 968–974. [CrossRef] [PubMed]
139. Gąsecka, A.; Szolc, P.; van der Pol, E.; Niewiara, Ł.; Guzik, B.; Kleczyński, P.; Tomaniak, M.; Figura, E.; Zaremba, M.; Grabowski, M.; et al. Endothelial Cell-Derived Extracellular Vesicles Allow to Differentiate Between Various Endotypes of INOCA: A Multicentre, Prospective, Cohort Study. *J. Cardiovasc. Transl. Res.* **2024**, *18*, 305–315. [CrossRef] [PubMed]
140. Protty, M.B.; Tyrrell, V.J.; Allen-Redpath, K.; Soyama, S.; Hajeyah, A.A.; Costa, D.; Choudhury, A.; Mitra, R.; Sharman, A.; Yaqoob, P.; et al. Thrombin Generation Is Associated With Extracellular Vesicle and Leukocyte Lipid Membranes in Atherosclerotic Cardiovascular Disease. *Arterioscler. Thromb. Vasc. Biol.* **2024**, *44*, 2038–2052. [CrossRef]
141. Hajeyah, A.A.; Protty, M.B.; Paul, D.; Costa, D.; Omidvar, N.; Morgan, B.; Iwasaki, Y.; McGill, B.; Jenkins, P.V.; Yousef, Z.; et al. Phosphatidylthreonine is a procoagulant lipid detected in human blood and elevated in coronary artery disease. *J. Lipid Res.* **2024**, *65*, 100484. [CrossRef]
142. Mehta, P.K.; Quesada, O.; Al-Badri, A.; Fleg, J.L.; Volgman, A.S.; Pepine, C.J.; Merz, C.N.B.; Shaw, L.J. Ischemia and no obstructive coronary arteries in patients with stable ischemic heart disease. *Int. J. Cardiol.* **2022**, *348*, 1–8. [CrossRef] [PubMed]
143. Monizzi, G.; Di Lenarda, F.; Gallinoro, E.; Bartorelli, A.L. Myocardial Ischemia: Differentiating between Epicardial Coronary Artery Atherosclerosis, Microvascular Dysfunction and Vasospasm in the Catheterization Laboratory. *J. Clin. Med.* **2024**, *13*, 4172. [CrossRef] [PubMed]
144. Fu, B.; Wei, X.; Lin, Y.; Chen, J.; Yu, D. Pathophysiologic Basis and Diagnostic Approaches for Ischemia With Non-obstructive Coronary Arteries: A Literature Review. *Front. Cardiovasc. Med.* **2022**, *9*, 731059. [CrossRef] [PubMed]
145. Wayne, N.; Singamneni, V.S.; Venkatesh, R.; Cherlin, T.; Verma, S.S.; Guerraty, M.A. Genetic Insights Into Coronary Microvascular Disease. *Microcirculation* **2025**, *32*, e12896. [CrossRef]
146. Mehta, P.K.; Huang, J.; Levit, R.D.; Malas, W.; Waheed, N.; Bairey Merz, C.N. Ischemia and no obstructive coronary arteries (INOCA): A narrative review. *Atherosclerosis* **2022**, *363*, 8–21. [CrossRef]

147. Ford, T.; Zeitz, C.; Spiro, J.; Yong, A.; Layland, J.; Watts, M.; Chan, W.; Girolamo, O.; Marathe, J.A.; Negishi, K.; et al. Functional Coronary Angiography for the Diagnosis of Coronary Vasomotor Disorders. *Heart Lung Circ.* **2025**, *in press*. [CrossRef]
148. Frangogiannis, N.G. The extracellular matrix in myocardial injury, repair, and remodeling. *J. Clin. Investig.* **2017**, *127*, 1600–1612. [CrossRef]
149. Leancă, S.A.; Crișu, D.; Petriș, A.O.; Afrăsânie, I.; Genes, A.; Costache, A.D.; Tesloianu, D.N.; Costache, I.I. Left Ventricular Remodeling after Myocardial Infarction: From Physiopathology to Treatment. *Life* **2022**, *12*, 1111. [CrossRef]
150. Limpitikul, W.B.; Silverman, M.G.; Valkov, N.; Park, J.-G.; Yeri, A.; Garcia, F.C.; Li, G.; Gokulnath, P.; Garcia-Contreras, M.; Alsop, E.; et al. Plasma extracellular vesicle cargo microRNAs are associated with heart failure and cardiovascular death following acute coronary syndrome. *Extracell. Vesicle* **2025**, *5*, 100070. [CrossRef]
151. Mahmoudi, M.; Landry, M.P.; Moore, A.; Coreas, R. The protein corona from nanomedicine to environmental science. *Nat. Rev. Mater.* **2023**, *8*, 422–438. [CrossRef]
152. Heidarzadeh, M.; Zarebkohan, A.; Rahbarghazi, R.; Sokullu, E. Protein corona and exosomes: New challenges and prospects. *Cell Commun. Signal.* **2023**, *21*, 64. [CrossRef]
153. Tabatabaeian Nimavard, R.; Sadeghi, S.A.; Mahmoudi, M.; Zhu, G.; Sun, L. Top-Down Proteomic Profiling of Protein Corona by High-Throughput Capillary Isoelectric Focusing-Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2025**, *36*, 778–786. [CrossRef]
154. Sadeghi, S.A.; Ashkarran, A.A.; Wang, Q.; Zhu, G.; Mahmoudi, M.; Sun, L. Mass Spectrometry-Based Top-Down Proteomics in Nanomedicine: Proteoform-Specific Measurement of Protein Corona. *ACS Nano* **2024**, *18*, 26024–26036. [CrossRef]
155. Lee, G.Y.; Li, A.A.; Moon, I.; Katritsis, D.; Pantos, Y.; Stingo, F.; Fabbrico, D.; Molinaro, R.; Taraballi, F.; Tao, W.; et al. Protein Corona Sensor Array Nanosystem for Detection of Coronary Artery Disease. *Small* **2024**, *20*, 2306168. [CrossRef] [PubMed]
156. Vélez, P.; Parguiña, A.F.; Ocaranza-Sánchez, R.; Grigorian-Shamagian, L.; Rosa, I.; Alonso-Orgaz, S.; de la Cuesta, F.; Guitián, E.; Moreu, J.; Barderas, M.G.; et al. Identification of a circulating microvesicle protein network involved in ST-elevation myocardial infarction. *Thromb. Haemost.* **2014**, *112*, 716–726. [CrossRef]
157. Bazzan, E.; Tinè, M.; Casara, A.; Biondini, D.; Semenzato, U.; Cocconcelli, E.; Balestro, E.; Damin, M.; Radu, C.M.; Turato, G.; et al. Critical review of the evolution of extracellular vesicles' knowledge: From 1946 to today 2021. *Int. J. Mol. Sci.* **2021**, *22*, 6417. [CrossRef]
158. Lee, K.; Shao, H.; Weissleder, R.; Lee, H. Acoustic purification of extracellular microvesicles. *ACS Nano* **2015**, *9*, 2321–2327. [CrossRef]

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