In this issue: Non-coding RNAs as potential laboratory biomarkers

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Circulating microRNAs as possible biomarkers for coronary artery disease: a narrative review

Tadele Melak, Habtamu Wondifraw Baynes

Department of Clinical Chemistry, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Northwest Ethiopia, Ethiopia

ARTICLE INFO

Corresponding author:
Tadele Melak
Department of Clinical Chemistry
School of Biomedical and Laboratory Sciences
College of Medicine and Health Sciences
University of Gondar
Ethiopia
Phone: +251-921576005
Fax: +251-0581141240
E-mail: 0923tadie@gmail.com

Key words:
circulating, coronary artery disease, microRNA

ABSTRACT

Coronary artery disease is one of the most common cardiovascular diseases in the world. Involvement of microRNAs on the pathogenesis of this disease was reported either in beneficial or detrimental way. Different studies have also speculated that circulating microRNAs can be applied as promising biomarkers for the diagnosis of coronary artery disease. Particularly, microRNA-133a seems to fulfill the criteria of ideal biomarkers due to its role in the diagnosis, severity assessment and in prognosis. The panel of circulating microRNAs has also improved the predictive power of coronary artery disease compared to single microRNAs. In this review, the role of circulating microRNAs for early detection, severity assessment and prognosis of coronary artery disease were reviewed.

INTRODUCTION

Coronary arteries supply blood to the heart muscle and consist of two main arteries: the right and left coronary arteries, and their two branches, the circumflex artery and the left anterior descending artery (1). Analogous to other arteries, normal coronary artery consists of three well-defined layers: the intima, media, and adventitia. These three layers are separated by layers of elastin. Internal elastic lamina separates intima from media and external elastic lamina separates media from the adventitia (2).

Coronary artery disease (CAD) is the leading cause of cardiovascular deaths (CVD) globally (3). In 2020, it is estimated that this disease will account for death of 11.1 million patients globally (4). Someone suffers from coronary disease every 26 seconds, and someone dies from every minute in the USA (5). In Europe, between 1 in 5 and 1 in 7 women die from CAD, and the disease accounts for between 16% and 25% of all deaths in European men (6). Studies suggest that the average age-adjusted incidence rates of CAD per 1,000 person-years are 12.5 for white men, 10.6 for black men and 4.0 for white women (7). The clinical spectrum of CAD ranges from stable angina pectoris (stable CAD) to acute coronary syndromes (ACS) which includes unstable angina (unstable CAD) and myocardial infarction (8). The myocardial infarction (MI) is further classified into ST segment elevated MI (STEMI) and non-ST segment elevated MI (NSTEMI). In terms of arterial occultation STEMI is characterized by a complete occultation of epicardial coronary blood vessel and elevated ST in electrocardiogram whereas NSTEMI is characterized by a sever coronary artery narrowing. However, both of them are accompanied by necrosis of myocardial cell and elevated cardiac biomarkers (9). Only a few previous articles have reviewed the potential of circulating microRNAs (miRNAs) as biomarker on different phases of CAD. Consequently, this review narrates the role of circulating miRNAs in the early detection, diagnosis, severity assessment of CAD as well as restenosis, and their role as a prognosis marker. Furthermore, it describes the pathogenesis, current diagnosis modalities, and limitation of miRNAs as a biomarker of CAD.

PATHOGENESIS OF CORONARY ARTERY DISEASE

The primary pathologic process causing CAD is atherosclerosis of the large and medium sized coronary artery. The increment of cholesterol level which binds with low density lipoprotein and very low-density lipoprotein increases the chance of infiltration of these molecules into the artery wall and leads to oxidation (10). This can initiate migration of smooth muscle cells from the tunica media to intima of the artery (11). Activated smooth muscle cells produce fibrotic extra cellular matrix (ECM), which changes the lipid rich fatty streak into more advanced lesion (12). The ECM forms the fibrous cap that has an important role in maintaining the mechanical stability of the plaque. In addition to calcification, neo-vascularization affects the structure of the plaque. As the plaque size increases, the oxygen from the bloodstream does not reach all areas of the lesion, and the inner section gets hypoxic and these neo-vessels also cause small hemorrhages inside the plaque which subsequently increases its size rapidly (13).

In most cases, ischemia and infarctions are caused by physical disruption of the fibrous cap of the lesion, which allows thrombogenic material to interact with blood cells (14). This contact leads to formation of a thrombosis, which can block the blood flow in the artery. Inflammatory cells also destabilize the plaque by secreting pro-inflammatory cytokines, proteases, coagulation factors and vaso-active molecules. These molecules inhibit the formation of stable fibrous

cap, degrade the collagen in the cap and initiate the formation of the clot (15).

Recently, the discovery of miRNAs involvement on the pathogenesis of CAD reignited the lesson for using them as diagnosis and prognostic marker for cardiovascular disease (CVD). Now, it is accepted that miRNAs are involved either in a beneficial or detrimental way in almost all steps of atherogenesis, including endothelial damage and dysfunction, monocyte-wall invasion and activation, lipoprotein formation, plaque stability, remodeling of the CV system, and platelet and vascular smooth muscle cell function (16).

microRNAs regulate gene expression post transcriptionally by degrading messenger RNA targets and/or by blocking their translation (17, 18). Each miRNA can target multiple mRNAs and regulate ~60% of mammalian protein-coding genes (19). They have diverse functions in the regulation of several key biological and cellular processes including differentiation, proliferation, and apoptosis in cardiovascular system (20).

In recent years, circulating miRNAs have created great interest and have been investigated as a source of novel biomarkers for several human diseases (21-23). They are reported from whole blood, peripheral blood mononuclear cells, platelets, serum, plasma, and other body fluids (24). Regarding, using miRNAs as a biomarker in CAD abundant researches have been undertaken. They revealed that determining the expression level of miRNAs in body fluids have a potential role intended for early detection, diagnosis, severity assessment markers and prognostic indicators.

EXISTING DIAGNOSIS MODALITIES FOR CORONARY ARTERY DISEASE

Currently, common diagnosis of CAD relies on visualization of the anatomic structure of coronary artery and functional assessment of the heart. Coronary angiography is considered as a gold standard method for diagnosis of CAD (25). However, coronary angiography may overestimate or underestimate disease due to the fact that it is influenced by technical factors and complexity of coronary anatomy and plaque configuration (26, 27).

Furthermore, complications from the technique including those related to local anesthesia and use of contrast material, as well as contrast induced nephropathy, infection, local vascular injury, myocardial infarction, stroke, and death are also common (28, 29). Additionally, prevailing of non-flow limiting CAD in women which is undetectable through this technique also compromises its value (30). As a result, the emerging of noninvasive techniques, whether imaging or non-imaging, hold great prospects (31). MicroRNAs in this regard might have potential to skip these bottlenecks.

DETECTION METHODS OF miRNAs

High throughput sequencing, quantitative real time polymerase chain reaction (RT-qPCR) and microarrays are the major quantification methods that are currently being used (32). Sequencing is the best technique for discovering new miRNAs whereas qPCR is the gold standard technique for quantification of miRNAs (33). On the other hand, microarray technique is the best alternative method for genome-wide assays on a larger scale (34). However, quantification of miRNAs, compared to protein, still lacks standardized methods and clear recommendation about which body fluid is appropriate?

SAMPLE PREPARATION AND NORMALIZATION TECHNIQUE OF miRNAs

Selecting the appropriate sample is the basic issue for analyzing miRNAs. MicroRNAs are found intracellularly or can be actively secreted by

cells (35). Even though there is high extracellular RNase activity, miRNAs are stable in extracellular area, due to their packaging in apoptotic bodies, microvesicles (MV), exosomes, lipoproteins (Lp), and special proteins. Previous studies showed that miRNAs are found in blood, urine, breast milk (36), saliva, tears, and other body fluids (37). In this review, blood and its components like plasma, serum, a peripheral blood mononuclear cell (PBMC) were the major sample for miRNAs determination (Table 1).

MicroRNAs can be extracted by different techniques. The most common technique is selected based on the desired purity and amount of miRNA. Some of the extraction methods are TRIzol based, miRNeasy and mirVANA (38). TRIzol based method was the technique used by various studies (Table 1).

Quantification of miRNA expression needs data normalization. The normalizer might be either endogenous or exogenous reference genes. However, there is no consensus on optimal normalization strategy, particularly the choice of reference genes. In terms of the source of the reference, it might be endogenous or exogenous whereas in terms of their nature, it might be miRNAs, synthetic RNA or other genes (39). In this review, the most common exogenous reference gene found in various studies is a miRNA obtained from *C. elegans* which is the cel-miRNA-39. The small non-coding RNA (RNU6) was also the most frequently used non miRNA endogenous reference genes (Table 1). Furthermore, miRNA-156a and miRNA-16 were used as endogenous miRNA normalizer as well.

ROLE OF miRNAs AS POSSIBLE BIOMARKERS FOR CORONARY ARTERY DISEASE

MicroRNAs are small non-coding endogenous RNAs and can regulate different developmental and physiological processes of cardio-vascular system (34). These molecules are also highly valuable biomarkers due to their cell-type specificity, abundance, and stability in most solid and liquid clinical specimens (40). Gustafson *et al.* stated the beneficial aspect of miRNA-guided diagnostics as an increasingly and powerful molecular approach for deriving clinically significant information from patient samples. Li *et al*. also proved that these miRNA molecules can be used as diagnosis, management, and monitoring of numerous diseases. They are also helpful for stratifying the type of CAD patients and even the type of ACS in different groups. For instance, a study done by Ward *et al*. showed that miRNA-25-3p, miRNA-221-3p, and miRNA-374b-5p were highly associated with STEMI, and miRNAs 221-3p and 483-5p were highly correlated to NSTEMI (41).

Early detection of coronary artery disease

Various guidelines (42-44) support to screen individuals having family history of premature CAD and diabetes mellitus (DM). Screening of CAD includes remarkable investigation starting from the easy Framingham risk score screening tool to more complicated and relatively accurate coronary angiography (45). As a result, highly sensitive and specific screening tests with low cost and invasiveness are essential for a better monitoring program of CAD. microRNAs expressed and released from platelet, monocyte, endothelial cells at the initiation stage of CAD may take their share in this regard. Wang *et al.* recommended that circulating levels of miRNA-31 and miRNA-720 have a potential role for early detection of CAD. They proved that these miRNAs can regulate endothelial progenitor cell (EPC) function via the suppression of FAT4 and thromboxane A2 receptor which are expressed in EPC's of CAD patients early (46). Their expressions were remarkably low in CAD patients compared to non-CAD patients.

Table 1 Summary of miRNAs from selected studies in coronary artery disease*

miRNAs	Altera-tion	Study population	Method	Role	Sample type	Reference gene/ miRNA	Extrac-tion	Refer-ence
miR-31 miR-720	Down Down	CAD (n=20) vs. HC (n=15)	qRT-PCR	prognosis, diagnosis	Plasma EPCs	miRNA-16a	TRIzol based	(46)
miR-181a	Down	Obese (n= 21) vs. non-obese (n=125)	Microarray qRT-PCR	diagnosis	monocytes	RNU5G	-	(47)
miR-149 miR-424 miR-765	Down Down Up	CAD (n= 95) vs. HC (n=32)	qRT-PCR	diagnosis	plasma	miRNA-156a	TRIzol based	(49)
miR-765 miR-149	Up Down	SCAD (n= 37) UCAD (n=32) vs. HC (n=20)	Microarray, qRT-PCR	diagnosis	Plasma	miRNA-156a	TRIzol based	(50)
miR-133	Up	AMI (n=13), AP (n=176), vs. HC (n=127)	qRT-PCR	diagnosis	Plasma	RNU6	TRIzol based	(51)
miR-135a miR-147	Up Down	SAP (n= 25) UAP (n= 25) vs controls (n= 20)	qRT-PCR	diagnosis	PBMC	let-7a and miRNA-16	-	(56)
miR-1 miR-126 miR-483 miR-133a	Up Up Up Up	SAP (n= 34) UAP (n=19) vs. non-CAD (n= 20)	qRT-PCR	severity evaluation	Plasma	miRNA-16	TRIzol based	(52)

miR-126 miR-17 miR-92a miR-155 miR-145 miR-133a miR-208a	Down Down Down Down Down Up Up	CAD (n=36) vs. non-CAD (n=17)	qRT-PCR	diagnosis	Serum/ plasma	cel-miRNA-39	TRIzol based	(97)
miR-206 miR-574	Up Up	CAD (n=67) vs. non-CAD (n=67)	Microarray qRT-PCR	diagnosis	Plasma	RNU6	MirVANA	(54)
miR-34a miR-21 miR-23a	Up Up Up	CAD (n=32) non-CAD (n=20)	Microarray qRT-PCR	diagnosis	Plasma	RNU6	TRIzol based	(55)
miR-2861 miR-3135b miR-191	Up Up Up	CAD (n=90), vs. non-CAD (n=70)	Microarray qRT-PCR	Severity assessment	Plasma	cel-miRNA-39	mirVanaTM	(74)
miR-126 miR-199a	Up Up	CAD (n=176)	qRT-PCR	prognostic	Plasma, MVs Exosomes	cel-miR-39	TRIzol based	(75)
miR-197 miR-223	Up Up	(ACS, SAP) (n=873)	qRT-PCR	prognostic	Serum	cel-miRNA-39	TRIzol based	(76)
miR-133a miR-208b	Up Up	ACS (n=444)	qRT-PCR	Prognostic, diagnostic	Plasma	-	-	(77)
miR-208a	Up	CHD (n=290) vs. HC (n=110)	qRT-PCR	Severity	Plasma	RNU6B	TRIzol based	(60)
miR-155	Up	CHD (n=300) vs. HC (n=100)	qRT-PCR	Severity	Serum	RNU6B	TRIzol based	(60)

miR-483 miR-451a miR-155	Up Down Up	SCAD (n=59)	qRT-PCR	Severity	Plasma	cel-miRNA-39	miRNeasy	(68)
miR-486a miR-92a	Up Up	CAD (n=95) SAP (n=30) UAP (n=39) MI (n=26) vs. HC (n=16)	qRT PCR	Severity	Lipo-protein fractions	cel-miRNA-39	miRNeasy	(70)
miR-100 miR-143 miR-145 miR-21	Down Down Down Up	ISR (n=51) non-ISR (n=130) vs HC (n=52)	qRT PCR	Severity	Plasma	RNU6	TRIzol based	(80)
miR-425 miR-93	Up Up	ISR (n=39) vs. non-ISR (n=39)	miRNA PCR array	Severity	Plasma	-	-	(81)
miR-181b miR-155 miR-185	Down Up Up	ISR (n=6) vs non-ISR (N=43)	qRT-PCR	Restenosis	Plasma/ cell culture	miR-24	TRIzol based	(82)

**Abbreviations - ACS: Acute Coronary Syndromes, AP: Angina Pectoris, CAD: Coronary Artery Disease, CHD: Coronary Heart Disease, ISR: In-Stent Restenosis, HC: Health Control, MI: Myocardial Infarction, miR: microRNA, MV: Microvesicles, n: number of participants, qRT-PCR: quantitative Real-Time Polymerase Chain Reaction, SAP: Stable Angina Pectoris, UAP: Unstable Angina Pectoris, RNU: small non-coding RNAs.*

On the other hand, Hulsmans *et al.* showed the down regulation of the monocyte derived three isoforms of miRNA-181 in coronary artery disease. Particularly, miRNA-181-a was associated with CAD even after adjustment for traditional risk factors: obesity and metabolic syndrome (47). They also described that miRNA-181 related with inflammatory toll-like receptor and nuclear factor κB signaling and it may be potential biomarker for early detection of obesity related coronary artery disease. However, expression of miRNA-181 has been observed to be regulated by other toll-like receptor signaling factors that may potentially reduce its specificity of the prediction.

Bialek *et al.* also showed that plasma miRNA-208a is an interesting and promising candidate for a new biomarker released early after onset of myocardial infarction. The peak of miRNA-208a was observed earlier than the traditional biomarkers (cTnI and CK-MB mass). This implies that miRNAs will have an importance as early biomarker role in emergency department than the traditional markers (48).

Differentiate patients with CAD from non-CAD

Diagnostic values, which is commonly expressed in area under the curve (AUC) of the receiver operating characteristics (ROC) in this review, ranges from "bad" classification power (AUC, 0.5-0.6) for some miRNAs to "excellent" for others (AUC, 0.9-1.0).

Various miRNAs have a potential to classify CAD patients from non-CAD. Sayed *et al.* have assayed three plasma miRNAs: miRNA-765, miRNA-149, and miRNA-424 in CAD patients with non-CAD controls. All of them showed promising results to discriminate stable and unstable CAD from controls. ROC-AUC value of down-regulated plasma miRNA-149 classified stable and unstable CAD patients from non-CAD (0.938 and 0.951), respectively. Up-regulated miRNA-765 also distinguished CAD from non-CAD patients (49).

Discriminatory powers of miRNA-149 and miRNA-765 plasma levels were also repeated in other study (50). They classified unstable CAD from the controls with AUC values of 0.972 and 0.977, respectively. Whereas, stable CAD were differentiated from controls with 0.959 and 0.938 AUC values for miRNA-765 and miRNA-149, respectively. With this significant classification power, however, plasma levels of miRNA-765 were significantly correlated with age in all groups. This ultimately affects the characteristics of ideal biomarker. In contrast, plasma levels of miRNA-149 was not statistically significant in this aspect.

Furthermore, Wang *et al.* have revealed that miRNA-133a classified CAD from non-CAD individuals and exceeded the prediction potentials of the demographical data (age, sex, smoke, hypertension, diabetes, hyperlipidemia, etc.) and cardiac troponin I (cTnI) (51). The cTnI, clinical data, and miRNA-133a individually showed AUC value of 0.741, 0.785 and 0.918, respectively. Interestingly, the addition of miRNA-133a to the clinical data and cTnI remarkably increased the AUC values that were 0.942 and 0.925, respectively. Another study also showed that plasma miRNA-133a level was useful for diagnosis of unstable CAD (AUC = 0.906) (52). The combination of other two miRNAs (miRNA-1 and miRNA-126) increased the efficiency of detecting unstable CAD from controls. Moreover, miRNA-1 and miRNA-126 could differentiate both stable and unstable CAD from the controls independently with a potential of ≥ 0.85 value of AUC in the above study. Both of them were up-regulated in CAD patients (53).

Classification of CAD from non-CAD with "satisfactory" power was also reported by Zhou *et al.* through plasma expression of miRNA-206 and miRNA-574-5p (AUC value of 0.607 and 0.699, respectively) (54). Bioinformatics analysis revealed that their potential target gene might be involved in the onset and development of CAD that extend our understanding to validate them for early diagnosis of CAD.

Other studies without ROC curve analysis showed that different miRNAs have statistically significant difference between CAD and non-CAD. Han *et al.* (55) showed that from miRNA-34a, miRNA-21, miRNA-23a, miRNA-30a and miRNA-106b; miRNA-34a and miRNA-21 were significantly higher in the plasma of CAD patients compared to controls, whereas miRNA-23a had reduced expression among CAD patients (all $P<0.01$). The ratio of miRNA-135a to miRNA-147 concentration PBMC had showed 19 fold increment in CAD patients compared with controls. MiRNA/target gene/biological function linkage analysis suggested that the change in PBMC miRNA signature in CAD patients is probably associated with a change in intracellular cadherin/Wnt signaling (56). Dong *et al.* identified a panel of PBMC miRNA (miRNA-24, miRNA-33, miRNA-103a, and miRNA-122) that provided a high diagnostic accuracy of CAD (AUC=0.911, 95% CI 0.880-0.942) (57). Faccin

et al. also showed that a combination of three miRNAs (miRNA-155,-145 and let-7c) revealed a better classification power than the single miRNA alone (58).

Severity assessment of coronary artery disease

The Synergy between percutaneous coronary intervention with Taxus and cardiac surgery (SYNTAX) and gensini score are the two anatomical tools used to assess severity of CAD (59). Various circulating miRNAs have also correlated with the severity of CAD. They are correlated with the level of stenosis, complexity of stenosis and stability of the plaque in CAD. Circulating miRNA-133a expression is one of the miRNA that correlates with the severity of coronary artery stenosis in terms of complexity and level of stenosis.

Quantitative analysis revealed that circulating miRNA-133a level was significantly elevated in CAD patients having stenosis of coronary artery compared to non-coronary heart disease (CHD) patients. It was also moderately correlated with gensini scores and it was a better indicator of severity assessment relative to cTnI (51). However, miRNA-133a couldn't significantly differentiated low level stenosis from non-CHD individuals. Furthermore, in the other studies, miRNA-208a (60), miRNA-155(61) and miRNA-223 (62) were strongly correlated with gensini scores.

Guo *et al.* also tried to correlate plasma level of miRNA-145 with number of diseased vessel, SYNTAX score and stability of the plaques. They found that significantly lower levels of miRNA-145 in patients with three-vessel disease and high SYNTAX score compared with those with one or two-vessel disease and low or intermediate SYNTAX score, respectively. However, the result revealed that the level of miRNAs-145 between patients with one-vessel and two vessel disease, and between low score and intermediate score groups were not significantly different (63). Furthermore, miRNAs-214 tends to correlate with the SYNTAX score (64).

Every year, a large portion of CAD patients experience a sudden cardiac arrest due to unstable plaques rupturing (65). This produce subtotal or total occlusion and leading to ACS. Consequently, noninvasive biomarkers which can identify one of the severe form of CAD is clinically demanding.

In this regard, a study done by *Li X et al.* revealed the expression of miRNAs-122, -140-3p, -720, -2861, and -3149 have been highly elevated in the ACS group compared with the non-ACS groups and have good potential to identify patients. The discriminatory powers of these miRNAs were greater than AUC of 0.8 except for miRNAs-3149, i.e., 0.670. Using panel of miRNAs-122, -2861, and -3149 had a better classification power compared to using it alone (66). Other miRNAs such as: miRNA-106b, miRNA-25, miRNA-92a, miRNA-21, miRNA-590-5p, miRNA-126 and miRNA-451 also classified ACS from non ACS (67). In line with this, a study done by *Li S et al.* showed that combinations of miRNA-483-5p and miRNA-451a can discriminate plaque rapture with an excellent classification power, AUC (0.982; CI: 0.907-0.999). A panel of miRNA-483-5p and miRNA-155-5p had also showed the highest AUC (0.898; CI: 0.790-0.962) (68). In a study done by *Luque et al.* also showed that miRNA-638 was an independent predictor of plaque instability for carotid artery (69).

On the contrary, serum levels of 6 miRNAs including miRNA-92a and miRNA-122 could not differentiate ACS from non-ACS in other study. However, analysis of lipoprotein sub fraction level of miRNA-486 and -92a revealed good distinguishing power of ACS from non ACS (70). Level of high density lipoprotein-2 (HDL-2) miRNA-92a and HDL-3 miRNA-486 could classify

the ACS and non ACS up to an accuracy of 84% with adjustment for age, gender and serum lipids. Coronary bifurcation lesion is also one of the severe forms of CAD. Hence, it is the most challenging lesion in percutaneous coronary intervention (PCI) medicine due to rate of re-stenosis and major adverse cardiac event (71). As a result, determining whether the lesion is bifurcated or not is crucial for effective management of CAD. *Liu et al.* showed that miRNA-30-d was up-regulated and miRNA-1246 down-regulated in bifurcated compared to patients with non-bifurcated lesion (72).

Furthermore, miRNAs have been correlated with the characteristic of different plaques. For instance, more calcified plaque and less calcified plaque have diverse array of clinical outcome and miRNAs which are correlated to the level of calcification may have a potential to assess the severity of CAD. miRNA-21 expression in macrophages of non-calcified coronary artery lesions was significantly higher with an AUC value of 0.655 (73). *Liu et al.* also obtained biomarkers that can classify calcified from non-calcified lesion. Out of 8 miRNAs, further validation of miRNA-2861, miRNA-3135b and miRNA-191-3p showed better classification power (74).

Prognostic markers of coronary artery disease

Though limited information has been reported so far regarding correlation of miRNAs with CAD prognosis, reports indicated that some miRNAs might have a potential. Their ability of involvement in all aspects of CAD progression like vascular performance and cardiac remodeling either in beneficial or detrimental way might make them capable of predicting future consequence of the diseases (16).

To appreciate this, *Jansen et al.* determined plasma and microvesicles (MV) level of 10 miRNAs: miRNA-126, miRNA-222, miRNA-let7d, miRNA-21, miRNA-20a, miRNA-27a, miRNA-92a, miRNA-17, miRNA-130, and miRNA-199a, which are involved in vascular activities. There were no significant association between cardiovascular events and plasma level of the above miRNAs. In contrast, increased expression of miRNA-126 and miRNA-199a in circulating MVs was significantly associated with a lower major adverse CV event rate (75).

Likewise, *Schulte et al.* confirmed in a large cohort that baseline serum levels of miRNA-126 was not a helpful prognostic marker of CAD, even with the adjustment of cases into ACS and stable CAD groups (76). However, Elevated levels of miRNA-197 and miRNA-223 reliably predicted future cardiovascular death. *Widera et al.* also investigated the prognostic value of plasma levels of cardiomyocyte-enriched miRNAs (miRNA-1, miRNA-133a, miRNA-133b, miRNA-208a, miRNA-208b, and miRNA-499) among ACS patients. Out of them, only miRNA-133a and miRNA-208b levels were significantly associated with the risk of death (77).

Association of restenosis with miRNA expression

Restenosis is a common adverse event of endovascular procedure that is characterized by recurrence of narrowing of a blood vessel. If restenosis occurs after stenting, this is called in-stent restenosis (ISR) (78). In general, the threshold value for restenosis is a ≥50% narrowing (79). microRNAs are also associated with the occurrence of restenosis in CAD patients. He *et al*. showed that the reduction of circulating miRNA-143 and miRNA-145 levels were associated with the occurrence of ISR and could serve as novel non-invasive biomarkers for ISR (80). Furthermore, a study done by O'Sullivan *et al.* indicated that miRNA-93-5p independently predicted ISR after adjustment for traditional CAD risk factors (81). Fejes *et al.* examined the role of miRNA-181b, miRNA-185 and miRNA-155 to distinguish ISR patients from non-ISR.

miRNA-181b were downregulated, while both miRNA-185 and miRNA-155 were upregulated in ISR patients compared to non ISR (82). The purpose and implications of various selected miRNAs which have diagnostic and prognostic role for CAD are listed in (Table 1).

PARAMETERS INFLUENCING miRNAs LEVELS

Former studies have proven that heparin administration to the patients prior to blood sampling interferes with result of miRNAs (83,84). Collecting blood samples with heparinized test tube had also mislead miRNA determination (85). *Boileau A et al.* also revealed that endogenous heparin has a great effect on miRNA quantification (86).

Furthermore, anti-platelet therapy has also an effect on miRNAs expression. *Russo et al.* reviewed that platelet-derived miRNAs, like miRNA-92a and miRNA-19b respond to aspirin therapy (87). *Willeitnet al.* also revealed that plasma levels of platelet miRNAs, such as miRNA-223, miRNA-191, and others, that is, miRNA-126 and miRNA-150, were reduced under anti-platelet treatment (88). Therefore, high caution is needed when selecting patients for *in vivo* studies of miRNA quantification with respect to heparin and anti-platelet administration prior to blood sampling. In fact, the addition of heparinase enzyme in the sample reversed the effect of heparin (89, 90).

Quantification of miRNA levels altered in CAD might also be influenced by the intake of medication, such as statins and angiotensin converting enzyme (ACE) inhibitors (91). These findings emphasize the importance of quantifying the drug- and metabolite-based influence on miRNAs in the clinical setting. At the same time the inconsistency of the data reflects the necessity of further studies evaluating pathways of how miRNA levels are influenced in circulating blood. Additionally, it needs to be considered, that levels of biomarkers can also be influenced by the speed of their elimination. *Gidlöf et al*. found cardiac miRNA levels strongly correlating with renal function indicating that the renal function might also influence the plasma levels of miRNAs (92).

The influence of high-altitude hypoxic environments on plasma miRNA profiles has also been observed. *Yan et al.* recently reported that 175 miRNAs differently expressed relative to altitude and their expression level were also correlated with red blood cell counts and hemoglobin values (93). Co-variability of miRNA level with demographic factors was also reported. *Neha Singh et al.* found that miRNA-126-5p and miRNA-92a-3p were co-variables with age and serum creatinine level (94).

LIMITATIONS OF UTILIZING miRNAs AS BIOMARKERS

The major drawback of using miRNAs as biomarkers for clinical diagnosis is their laborious isolation and detection procedures. In addition, the current technology employed to isolate and estimate levels of miRNA requires optimization (95).

Other most significant challenge is their lower tissue and disease specificity because of an apparent expression of miRNAs in different diseased state and tissues. For instance, *Witwer et al.* reviewed that the scenario of miRNA-141 which was increased in pregnant women, prostate cancer and other cancers originated from epithelial, breast, colon and lung (96). Such kind of scenario also exists in CAD.

CONCLUSION AND FUTURE PERSPECTIVE

Circulating miRNAs as blood-based biomarker in CAD is highly promising: for early detection, assessing severity and prognostic indicators. They have potentials of “excellent” to “satisfactory”

power of classifying patients with or without CAD as well as patients with stable CAD or unstable CAD. Furthermore, miRNAs are not specific, a single miRNA can be elevated or reduced in different disease conditions. As a result, developing an algorithm or a panel of tests might have a contribution to increase the specificity of miRNAs. In this review, modeling of panel tests revealed remarkable results for identifying CAD patients and grading of severity of the disease (53, 57, 58, 67, 68). As a result, extensive validation of panels of miRNAs in large cohorts with their physiological role might be an extraordinary finding.

❖❖❖❖❖

Acronyms

ACE: *angiotensin converting enzyme*

ACS*: Acute Coronary Syndrome*

AUC*: Area Under the Curve*

CAD*: Coronary Artery Disease*

CVD*: Cardiovascular Disease*

CHD*: Coronary Heart Disease*

cTnI*: Cardiac Troponin I*

CV*: Cardiovascular*

ECM*: Extra Cellular Matrix*

EPC: *Endothelial Progenitor Cell*

ISR*: In-Stent Restenosis*

Lp*: lipoproteins*

miRNA*: microRNA*

MI*: Myocardial Infarction*

MV*: Microvesicle*

PARS*: Post-Angioplasty Restenosis*

PBMC*: Peripheral Blood Mononuclear Cell*

PCI*: Percutaneous Coronary Intervention*

qRTPCR*: quantitative Real Time Polymerase Chain Reaction*

RNU*: small non-coding RNA*

ROC*: Receiver Operating Characteristics*

STEMI*: ST segment elevated MI*

SYNTAX: *Synergy between percutaneous coronary intervention with Taxus and cardiac surgery*

❖❖❖❖❖

Authors' contributions

TM conceived the idea and wrote the first draft of the review, HWB had contribution on revising the first draft of the review and guiding. All of the authors have amended the final version of the manuscript.

❖❖❖❖❖

REFERENCES

1. Wittlieb Weber CA, Brothers JA. Coronary Artery Anomalies: Current Recognition and Treatment Strategies. Update on Recent Progress. Curr Cardiovasc Risk Rep. 2014;8:395.

2. Waller BF, Orr CM, Slack JD, Pinkerton CA, Van Tassel J, Peters T. Anatomy, histology, and pathology of coronary arteries: a review relevant to new interventional and imaging techniques--Part I. Clin Cardiol. 1992;15(6):451-7.

3. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. Journal of the American College of Cardiology. 2017;70(1):1-25.

4. Mathers CD LD. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:442.

5. Heart Disease and Stroke Statistics-2009 Update. Circulation. 2009;119:e21-e181.

6. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. Diabetes care. 2004;27(2):538-46.

7. Jones DW CL, Folsom AR, Heiss G, Hutchinson RG, Sharrett AR, Szklo M, Taylor HA Jr. Risk factors for coronary heart disease in African Americans: the Atherosclerotic Risk in Communities Study, 1987-1997. Arch Intern Med. 2002;162:2565-71.

8. Ralph E. Spiekerman JTB, Richard W. P. Achor, Jesse E. Edwards. The Spectrum of Coronary Heart Disease in a Community of 30,000 A Clinicopathologic Study. Circulation. 1962;25:57-65.

9. Daga LC, Kaul U, Mansoor A. Approach to STEMI and NSTEMI. The Journal of the Association of Physicians of India. 2011;59 Suppl:19-25.

10. Camejo G H-CE, Wiklund O, Bondjers G. Association of apo B lipoproteins with arterial proteoglycans: pathological significance and molecular basis. . Atherosclerosis. 1998; 139(2):205-22.

11. Mason DP KR, Hasenstab D, Bowen-Pope DF, Seifert RA, Coats S, Hawkins SM & Clowes AW. Matrix metalloproteinase-9 overexpression enhances vascular smooth muscle cell migration and alters remodeling in the injured rat carotid artery. Circulation research. 1999;85(12):1179-85.

12. Raines EW FN. Thematic review series: The immune system and atherogenesis: Cytokines affecting endothelial and smooth muscle cells in vascular disease Journal of lipid research. 2005; 46(6):1081-92.

13. Packard RR LP. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clinical chemistry. 2008;54(1):24-38.

14. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. Circulation 2001;104(3):365-72.

15. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent Advances on the Role of Cytokines in Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31:969-79

16. N. Papageorgiou DT, M. Charakida. Prognostic role of miRNAs in coronary artery disease. Curr Top Med Chem. 2013;13:1540-7.

17. Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nature Reviews Genetics 2012;13,: 271-82.

18. Schmitz U, Lai X, Winter F, Wolkenhauer O, Vera J, Gupta SK. Cooperative gene regulation by microRNA pairs and their identification using a computational workflow. Nucl Acids Res. 2014.

19. Bajan S, Hutvagner G. Regulation of miRNA Processing and miRNA Mediated Gene Repression in Cancer. Microrna. 2014;3(1):10-7.

20. Landskroner-Eiger S, Moneke I, Sessa WC. miRNAs as Modulators of Angiogenesis. Cold Spring Harb Perspect Med 2013;3(2):a006643.

21. Tai-You Ha. MicroRNAs in Human Diseases: From Cancer to Cardiovascular Disease. Immune Netw 2011 11(3):135-54.

22. Galimberti D VC, Fenoglio C, Serpente M, Ghezzi L, Cioffi SM, Arighi A, Fumagalli 1, Scarpini E. Circulating miRNAs as potential biomarkers in Alzheimer's disease. J Alzheimers Dis. 2014;42(4):1261-7.

23. Khoo SK PD, Kang UJ, Resau JH, Berryhill B, Linder J, Forsgren L, Neuman LA, Tan AC. Plasma-based circulating MicroRNA biomarkers for Parkinson's disease. J Parkinsons Dis. 2012;;2(4):321-31.

24. Jessica A. Weber DHB, Shile Zhang, David Y. Huang, Kuo How Huang, Ming Jen Lee, David J. Galas, Kai Wang. The MicroRNA Spectrum in 12 Body Fluids. Clinical Chemistry 2010;56(11):1733-41.

25. Evidence based Practice Center Systematic Review Protocol Noninvasive Testing for Coronary Artery DiseaseDecember 18, 2014.

26. Fuster V. Acute coronary syndromes: the degree and morphology of coronary stenoses. J Am Coll Cardiol. 1999;34(7):1854-6.

27. Topol EJ NS. Our preoccupation with coronary luminology. The dissociation between clinical and angiographic findings in ischemic heart disease. Circulation. 1995;92(8):2333-42.

28. Tavakol M AS, Brener SJ. . Risks and complications of coronary angiography: a comprehensive review. Global journal of health science. 2012;4(1):65-93.

29. Al Adas Z, Lodewyk K, Robinson D, Qureshi S, Kabbani LS, Sullivan B, et al. Contrast-induced nephropathy after peripheral vascular intervention: Long-term renal outcome and risk factors for progressive renal dysfunction. Journal of vascular surgery. 2019;69(3):913-20.

30. Verena Stangl VW, Gert Baumann, Karl Stangl. Current diagnostic concepts to detect coronary artery disease in women. European Heart Journal , . 2008;29:707-17.

31. Sajjadieh A HA, Keivani M, Asoodeh A, Pourmoghaddas M, Sanei H. . Diagnostic performance of 64-row coronary CT angiography in detecting significant stenosis as compared with conventional invasive coronary angiography. ARYA Atherosclerosis. 2013;9(2):157-63.

32. Kang K, Peng X, Luo J, Gou D. Identification of circulating miRNA biomarkers based on global quantitative real-time PCR profiling. Journal of animal science and biotechnology. 2012;3(1):1.

33. Git A, Dvinge H, Salmon-Divon M, Osborne M, Kutter C, Hadfield J, et al. Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. Rna. 2010;16(5):991-1006.

34. Trevino V, Falciani F, Barrera-Saldaña HA. DNA microarrays: a powerful genomic tool for biomedical and clinical research. MOLECULAR MEDICINE-CAMBRIDGE MA THEN NEW YORK-. 2007;13(9/10):527.

35. Boon RA, Vickers KC. Intercellular Transport of MicroRNAs. Arteriosclerosis, Thrombosis, and Vascular Biology. 2013;33(165).

36. Kosaka N, Izumi H, Sekine K, Ochiya T. microRNA as a new immune-regulatory agent in breast milk. Silence. 2010;1(1):7.

37. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010;56(11):1733-41.

38. Gautam A, Kumar R, Dimitrov G, Hoke A, Hammamieh R, Jett M. Identification of extracellular miRNA in archived serum samples by next-generation sequencing from RNA extracted using multiple methods. Molecular biology reports. 2016;43(10):1165-78.

39. Schwarzenbach H, da Silva AM, Calin G, Pantel K. Data Normalization Strategies for MicroRNA Quantification. Clinical chemistry. 2015;61(11):1333-42.

40. Trzybulska D, Vergadi E, Tsatsanis C. miRNA and Other Non-Coding RNAs as Promising Diagnostic Markers. EJIFCC. 2018;29(3):221-6.

41. Ward JA, Esa N, Pidikiti R, Freedman JE, Keaney JF, Tanriverdi K, et al. Circulating Cell and Plasma microRNA Profiles Differ between Non-ST-Segment and ST-Segment-Elevation Myocardial Infarction. Family medicine & medical science research. 2013;2(2):108-.

42. NCEP. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA. 2001;285:2486-97.

43. British Cardiac Society BHA, and British Hypertension Society. Joint British recommendations on prevention of coronary heart disease in clinical practice. Heart 1998;80(suppl 2):S1-29.

44. Wood D DBG, Faergeman O. Prevention of coronary heart disease in clinical practice: recommendations of the second joint task force of European and other societies on coronary prevention. Atherosclerosis. 1998;140:199-270.

45. Guidelines1/2011 SMCP. Screening for Cardiovascular Disease and Risk Factors. 2011.

46. Wang H-W, Huang T-S, Lo H-H, Huang P-H, Lin C-C, Chang S-J, et al. Deficiency of the MicroRNA-31–MicroRNA-720 Pathway in the Plasma and Endothelial Progenitor Cells From Patients With Coronary Artery Disease. Arterioscler Thromb Vasc Biol. 2014;34:857-69.

47. Hulsmans M, Sinnaeve P, Schueren BVd, Mathieu C, Janssens S, Holvoet P. Decreased miR-181a Expression in Monocytes of Obese Patients Is Associated with the Occurrence of Metabolic Syndrome and Coronary Artery Disease. J Clin Endocrinol Metab 2012;97:E1213-E8.

48. Bialek S, Gorko D, Zajkowska A, Koltowski L, Grabowski M, Stachurska A, et al. Release kinetics of circulating miRNA-208a in the early phase of myocardial infarction. Kardiologia polska. 2015;73(8):613-9.

49. Md Sayed AS, K. Xia, F. Li, X. Deng, U. Salma, T. Li, H. Deng. The diagnostic value of circulating microRNAs for middle-aged (40-60-year-old) coronary artery disease patients. Clinics 2015;70(4):257-63.

50. Sheikh MSA, Xia K, Fei Li XD, Salma U, Deng H, Wei L, et al. Circulating miR-765 and miR-149: Potential Non-invasive Diagnostic Biomarkers for Geriatric Coronary Artery Disease Patients. Hindawi Publishing Corporation BioMed Research International 2014;Volume 2015.

51. Wang F, Long G, Zhao C, Li H, Chaugai S, Wang Y, et al. Plasma microRNA-133a is a new marker for both acute myocardial infarction and underlying coronary artery stenosis. Journal of Translational Medicine 2013;11:222.

52. D'Alessandra Y, Carena MC, Spazzafumo L, Martinelli F, Bassetti B, Devanna P, et al. Diagnostic Potential of Plasmatic MicroRNA Signatures in Stable and Unstable Angina. PLoS ONE (): . 2013;8(11):e80345.

53. D'Alessandra Y PG, Capogrossi MC. . MicroRNAs and myocardial infarction Curr Opin Cardiol 2012;27:228-35.

54. Zhou J, Shao G, Chen X, Yang X, Huang X, Peng P, et al. MicroRNA 206 and MicroRNA 574-5p are highly expression in coronary artery disease. Biosci Rep 2015.

55. Han H, Qu G, Han C, Wang Y, Sun T, Li F, et al. MiR-34a, miR-21 and miR-23a as potential biomarkers for coronary artery disease: a pilot microarray study and confirmation in a 32 patient cohort. Experimental & Molecular Medicine 2015;47:e138.

56. Hoekstra M, Lans CACvd, Halvorsen B, Gullestad L, Kuiper J, Aukrust P, et al. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochemical and Biophysical Research Communications 2010;394:792-7.

57. Dong J, Liang YZ, Zhang J, Wu LJ, Wang S, Hua Q, et al. Potential Role of Lipometabolism-Related MicroRNAs in Peripheral Blood Mononuclear Cells as Biomarkers for Coronary Artery Disease. Journal of atherosclerosis and thrombosis. 2017;24(4):430-41.

58. Faccini J, Ruidavets J-B, Cordelier P, Martins F, Maoret J-J, Bongard V, et al. Circulating miR-155, miR-145 and let-7c as diagnostic biomarkers of the coronary artery disease. Scientific Reports. 2017;7:42916.

59. Sinning C, Lillpopp L, Appelbaum S, Ojeda F, Zeller T, Schnabel R, et al. Angiographic score assessment improves cardiovascular risk prediction: the clinical value of SYNTAX and Gensini application. Clinical Research in Cardiology. 2013;102(7):495-503.

60. Zhang Y, Li HH, Yang R, Yang BJ, Gao ZY. Association between circulating microRNA-208a and severity of coronary heart disease. 2017;77(5):379-84.

61. Qiu XK, Ma J. Alteration in microRNA-155 level correspond to severity of coronary heart disease. Scandinavian journal of clinical and laboratory investigation. 2018;78(3):219-23.

62. Guo JF, Zhang Y, Zheng QX, Zhang Y, Zhou HH, Cui LM. Association between elevated plasma microRNA-223 content and severity of coronary heart disease. Scandinavian journal of clinical and laboratory investigation. 2018;78(5):373-8.

63. Gao H, Guddeti RR, Matsuzawa Y, Liu L-P, Li-Xiao, Guo S, et al. Plasma Levels of microRNA-145 Are Associated with Severity of Coronary Artery Disease. PLoS ONE 2015;10(5):e0123477.

64. Lu H-Q, Liang C, He Z-Q, Fan M, Wu Z-G. Circulating miR-214 is associated with the severity of coronary artery disease. Journal of Geriatric Cardiology. 2013;10:34–8.

65. Myerburg RJ IAJ, Mitrani RM. Frequency of sudden cardiac death and profiles of risk. Am J Cardiol 1997;80:10-9.

66. Li X, Yang Y, Wang L, Qiao S, Lu X, Wu Y, et al. Plasma miR-122 and miR-3149 Potentially Novel Biomarkers for Acute Coronary Syndrome. PLoS ONE 2015;10(5):e0125430.

67. Ren J, Zhang J, Xu N, Han G, Geng Q, Song J, et al. Signature of Circulating MicroRNAs as Potential Biomarkers in Vulnerable Coronary Artery Disease. PLoS ONE 2013;8(12):e80738.

68. Li S, Lee C, Song J, Lu C, Liu J, Cui Y, et al. Circulating microRNAs as potential biomarkers for coronary plaque rupture. Oncotarget. 2017;8(29):48145-56.

69. Luque A, Farwati A, Krupinski J, Aran JM. Association between low levels of serum miR-638 and atherosclerotic plaque vulnerability in patients with high-grade carotid stenosis. Journal of neurosurgery. 2018:1-8.

70. Niculescu LS, Simionescu N, Sanda GM, Carnuta MG, Stancu CS, Popescu AC, et al. MiR-486 and miR-92a Identified in Circulating HDL Discriminate between Stable and Vulnerable Coronary Artery Disease Patients. PLoS ONE 2015;10(10):e0140958.

71. C. Frangos SN, N. Piazza Impact of bifurcation lesions on angiographic characteristics and procedural success in primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. Archives of Cardiovascular Diseases. 2011;104(4):234-41.

72. Liu Y, Chen S, Zhang J, Shoujie Shan, Chen L, Wang R, et al. Analysis of Serum MicroRNAs as Potential Biomarker in Coronary Bifurcation Lesion. Disease Markers. 2015;2015.

73. Fan X, EnshiWang, Wang X, Cong X, Chen X. MicroRNA-21 is a unique signature associated with coronary plaque instability in humans by regulating matrix metalloproteinase-9 via reversion-inducing cysteine-rich protein with Kazal motifs. Experimental and Molecular Pathology 2014;96:242-9.

74. Liu W, Ling S, Sun W, Liu T, Li Y, Zhong G, et al. Circulating microRNAs correlated with the level of coronary artery calcification in symptomatic patients. Scientific Reports. 2015;5:16099.

75. Jansen F, Yang X, Proebsting S, Hoelscher M, Przybilla D, Baumann K, et al. MicroRNA Expression in Circulating Microvesicles Predicts Cardiovascular Events in Patients With Coronary Artery Disease. J Am Heart Assoc. 2014;3:e001249.

76. Schulte C, Molz S, Appelbaum S, Karakas M, Ojeda F, Lau DM, et al. miRNA-197 and miRNA-223 Predict Cardiovascular Death in a Cohort of Patients with Symptomatic Coronary Artery Disease. PLoS ONE. 2015;10(12):e0145930.

77. Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, et al. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. Journal of Molecular and Cellular Cardiology. 2011;51 872-5.

78. Bennett MR. In-stent stenosis: pathology and implications for the development of drug eluting stents. Heart (British Cardiac Society). 2003;89(2):218-24.

79. Hamid H, Coltart J. 'Miracle stents'--a future without restenosis. McGill journal of medicine : MJM : an international forum for the advancement of medical sciences by students. 2007;10(2):105-11.

80. He M, Gong Y, Shi J, Pan Z, Zou H, Sun D, et al. Plasma microRNAs as potential noninvasive biomarkers for instent restenosis. PLoS One. 2014;9(11):e112043.

81. O'Sullivan JF, Neylon A, Fahy EF, Yang P, McGorrian C, Blake GJ. MiR-93-5p is a novel predictor of coronary instent restenosis. Heart Asia. 2019;11(1):e011134.

82. Fejes Z, Czimmerer Z, Szuk T, Poliska S, Horvath A, Balogh E, et al. Endothelial cell activation is attenuated by everolimus via transcriptional and posttranscriptional regulatory mechanisms after drug-eluting coronary stenting. PLoS ONE 2018;13(6):e0197890.

83. Kaudewitz D, Lee R, Willeit P, McGregor R, Markus HS, Kiechl S, et al. Impact of intravenous heparin on quantification of circulating microRNAs in patients with coronary artery disease. Thromb Haemost. 2013;110(3):609-15.

84. Boeckel JN TC, Leistner D, Leistner D, Zeiher AM, Fichtlscherer S, Dimmeler S. Heparin selectively affects the quantification of microRNAs in human blood samples. Clin Chem. 2013;59:1125-7.

85. Basso D, Padoan A, Laufer T, Aneloni V, Moz S, Schroers H, et al. Relevance of pre-analytical blood management on the emerging cardiovascular protein biomarkers TWEAK and HMGB1 and on miRNA serum and plasma profiling. Clinical biochemistry. 2017;50(4-5):186-93.

86. Boileau A, Lino Cardenas CL, Lindsay ME, Devaux Y. Endogenous Heparin Interferes with Quantification of MicroRNAs by RT-qPCR. Clin Chem. 2018;64(5):863-5.

87. Russo I, Penna C, Musso T, Popara J, Alloatti G, Cavalot F, et al. Platelets, diabetes and myocardial ischemia/reperfusion injury. Cardiovascular diabetology. 2017;16(1):71-.

88. Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, et al. Circulating microRNAs as novel biomarkers for platelet activation. Circ Res. 2013;112(4):595-600.

89. Li S, Zhang F, Cui Y, Wu M, Lee C, Song J, et al. Modified high-throughput quantification of plasma microRNAs in heparinized patients with coronary artery disease using heparinase. Biochem Biophys Res Commun. 2017;493(1): 556-61.

90. Kondratov K, Kurapeev D, Popov M, Sidorova M, Minasian S, Galagudza M, et al. Heparinase treatment of heparin-contaminated plasma from coronary artery bypass grafting patients enables reliable quantification of microRNAs. Biomolecular detection and quantification. 2016;8:9-14.

91. Weber M BM, Patel RS, Quyyumi AA, Bao G, Searles CD. MicroRNA Expression Profile in CAD Patients and the Impact of ACEI/ARB. Cardiol Res Pract. 2011;2011.

92. Gidlöf O AP, van der Pals J, Götberg M, Erlinge D. Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. Cardiology. 2011;118(4):217-26.

93. Yan Y, Shi Y, Wang C, Guo P, Wang J, Zhang C-Y, et al. Influence of a high-altitude hypoxic environment on human plasma microRNA profiles. Scientific Reports 2015; 5,.

94. Singh N, Heggermont W, Fieuws S, Vanhaecke J, Van-Cleemput J, DeGeest B. Endothelium-enriched microRNAsasdiagnostic biomarkers forcardiacallograftvasculopathy. J HeartLungTransplant 2015;34:1376-84.

95. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, et al. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circulation: Cardiovascular Genetics. 2011; 4(4):446-54.

96. Witwer KW. Circulating microRNA biomarker studies: pitfalls and potential solutions. Clin Chem. 2015;61(1):56-63.