Epigenetic biomarkers in cardiovascular disease

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Abstract: In recent years we have lived a technological revolution that has made it possible to decipher the human genome. However, most diseases, such as cardiovascular diseases (CVDs), are not simply due only to genetic alteration but also to epigenetic events conditioned by environmental inputs and lifestyle. Epigenetics explains the ability to modulate gene expression without altering the genetic sequence. Epigenetic biomarkers are revolutionizing precision medicine and improving the capacity of diagnosing and prognosticating CVDs and predicting the risk of future comorbidities. This review presents a list of the most reliable epigenetic biomarkers (i.e., based on DNA methylation, circulating histones and circulating miRNAs) which will probably improve the diagnostic and prognostic of several CVD conditions, such as coronary artery disease (CAD), hypertrophic cardiomyopathy (HCM), acute myocardial infarction (AMI) and heart failure (HF).

Keywords: Epigenetic biomarkers, microRNAs (miRNAs); DNA methylation; circulating histones and nucleosomes; cardiovascular disease (CVD)

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of human morbidity and mortality in developed countries (1). Despite the considerable progress in the diagnosis and treatment, it is still not only a public health issue but also costs trillions of dollars for the healthcare expenditure worldwide (1). Accurately predicting CVD risk could be especially relevant in humans to reduce short-term risks and future comorbidities. Better biomarkers of risk are needed for the development of personalized CVD prevention approaches. In this sense, the impact of epigenetics in CVD is now emerging as an important linker between genotype and phenotype variability and a key player at different levels from pathophysiology and diagnosis to therapeutics (2). Particularly, an epigenetic biomarker is defined as any epigenetic mark or epigenetic mechanism that (I) predicts the risk of future disease development; (II) defines a disease; (III) predicts the outcome of a disease; (IV) responds to therapy (3,4).

The major epigenetic regulatory mechanisms of mammalian cells include DNA methylation, post-translational histone modifications (PTMs) and RNA-based mechanisms including those controlled by the better characterized small non-coding RNAs (sncRNAs). Particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are recently showing their potential as biomarkers.

DNA methylation is a fairly stable epigenetic modification that consists of the covalent binding of a methyl group to the 5' carbon of cytosine, mainly at sites where cytosine bases are followed by guanine located at CpG islands and CpG shores. The 40% of genes contain CpG-rich islands and up to 70% of all CpG dinucleotides in the genome are methylated. The main function of DNA methylation is to regulate the expression of genes by modifying the accessibility of the transcriptional machinery to DNA (5).

Changes in DNA methylation patterns have been observed in association with CVD (6). Here, it is relevant to indicate that one previous study on peripheral blood leukocytes (PBL) and atrial biopsies collected from patients undergoing coronary bypass surgery reported a high correlation of DNA methylation status between both tissues (7), which supports the utility of DNA methylation in PBL in predicting dysfunction in cardiac cells. In this regard, the methylation status of Long Interspersed Nucleotide Elements (LINE-1) or other repetitive elements such as Alu and juxtacentromeric satellite 2 (Sat2) have been proposed to be used as a biomarker defining risk for cardiovascular events, such as stroke and ischemic heart disease (6,8). Recently, a Swedish team identified disease-specific alterations in DNA methylation, using Illumina Infinum BeadChip technology, in blood samples of a Swedish population of 729 patients affected with hypertension, acute myocardial infarction (AMI), stroke, thrombosis, and cardiac arrhythmias (9). The role of DNA methylation has recently been corroborated by Nakatochi *et al.* after finding the methylation of several genes using a cohort of 192 elderly Japanese case subjects with MI and 192 control subjects (10).

Histones are small (11–22 kDa) nuclear proteins that are essential components of nucleosomes structure in eukaryotic cells since DNA wraps around an octamer of the core histones H2A, H2B, H3, and H4, constructing the fundamental unit of chromatin, the nucleosome (11). Histone proteins have a key role in helping in the regulation of chromatin due to PTMs which control the binding of other proteins called readers, which in turn regulate chromatin function through the remodeling of the chromatin, giving rise to two 3D structures, a relaxed form called euchromatin or a compacted form called heterochromatin.

Recent evidence supports the idea that extracellular histones contribute to human disease because they are cytotoxic for a wide array of human cells and human tissues. Abrams *et al.* (12) demonstrated this by performing several experiments *in vitro* and *in vivo* that showed circulating histones can affect cell membranes after binding to phospholipids, producing the disruption of Ca^{2+} influx, and hence contributing to the release of other intracellular mediators that produce cell death. In fact, circulating nucleosomes were recently associated with disease progression in patients with thrombotic microangiopathies (13). Circulating histones are also found in the blood of healthy individuals at low concentrations, but their levels rise mainly in patients who suffer severe trauma (14), systemic inflammation (15), sepsis or tissue damage (16). It is noteworthy that histone proteins seem to have an interesting cytotoxic potential (16,17), therefore contributing to cardiac injury and dysfunction (18). Alhamdi *et al.*, in a subset of 36 septic patients without previously related cardiac disease, detected high levels of circulating histones, which were significantly associated with new-onset left ventricular dysfunction and arrhythmias. The authors observe that left ventricular dysfunction only predicted adverse outcomes when it was combined with elevated histones or cardiac troponin levels (18). More recently, it has been shown how circulating histones levels predict postoperative clinical outcomes in patients that have undergone heart surgery, therefore serving as a good prognostic indicator for patients after cardiac surgery (19). In addition, PTMs can regulate genes during disease development. Many aspects of the regulatory pathways that control gene expression during cardiac development or disease have been also revealed. For example, the level of H3 and H4 histone acetylation are correlated with atrial natriuretic factor (ANF) expression (7), but these mechanisms are not completely understood.

miRNAs are a class of conserved, short (18–24 nt), non-coding RNAs that have potent capacities to regulate gene expression at the posttranscriptional level in different biological networks. Importantly, miRNAs have demonstrated in recent years the potential to be used as biomarkers (4). The role of miRNAs is attracting significant interest in the area of CVD (20). It has

been established the role of several miRNAs (miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143, miR-208 and the let-7 family) in both normal cardiac maintenance and CVD (21,22). In this regard, a wide array of recent studies is proposing epigenetic biomarkers mainly based on circulating miRNAs as biomarkers of different cardiovascular conditions [e.g., cardiac arrhythmias, coronary artery disease (CAD), AMI, hypertrophic cardiomyopathy (HCM), and heart failure (HF), among others], although some studies are also appearing related with DNA methylation and circulating histones as potential biomarkers.

In the present review, we summarize recent data about epigenetic biomarkers based on DNA methylation, circulating histones and circulating miRNAs in CVDs, and assess the novel opportunities for diagnosis and prognosis of such diseases.

CAD

CAD, also known as coronary heart disease (CHD) (23), is the major cause of death worldwide (24). In CAD, the blood flow to the myocardium is reduced by atherosclerotic plaques (fatty deposits) that narrow the lumen of the coronary arteries (23) and can progress to myocardial ischemia as consequence of thrombosis or acute coronary atherosclerosis, finally leading to angina pectoris or AMI (25,26). According to the American Heart Association (AHA), on the basis of data from National Health and Nutrition Examination Survey (NHANES) from 2011 to 2014, the prevalence of CAD in US adults ≥20 years of age is 6,3% (about 16,5 million people) (27).

Is widely accepted that the development of CAD is determined by genetic bases and environmental factors. The formation of atherosclerotic plaques starts with an endothelial activation followed by an invasion of proinflammatory cells and proliferation and dedifferentiation of smooth muscle cells. Several risk factors, such as hypercholesterolemia, can promote atherosclerosis (28,29). The most common biomarkers in CAD are inflammatory proteins. C-reactive protein (CRP) is the best studied of the inflammatory biomarkers (30). Moreover, PPBP (pro-platelet basic protein) and DEFA1/DEFA3 (α-defensin) are correlated with CAD development (31). Therefore, these biomarkers are occasionally used as biomarkers of CAD.

DNA methylation

A global loss of methylation is related to CHD, in particular, a loss of methylation has been observed in atherosclerosis (32). In fact, the evaluation of the methylation status of LINE-1 due to its high representation in the human genome (55%) and its high methylation levels can be used to evaluate global methylation changes whole genome (33). In atherosclerosis, a decrease in the methylation levels of LINE-1 was reported (27). Another target studied was the hypomethylation of the IL-6 promoter, associated with CHD risk (34). So the analysis of LINE-1 and IL-6 methylation levels can serve as an epigenetic biomarker for diagnosing CHD.

Regarding the analysis of methylation levels of specific genes in atherosclerosis, hypermethylation of atheroprotective estrogen receptor α (ESR1) and estrogen receptor β (ESR2) in vascular smooth muscle cells were described by two independent groups (35,36). In cultured tissues of the aorta and coronary arteries, Zhu and colleagues showed that hypermethylation of the monocarboxylate transporter (MCT3) contributes to the progression of atherosclerosis (37). However, the methylation of these genes has been performed in tissues but not in blood samples. It would be very helpful for clinicians to identify these genes as cell-free DNA in blood samples of patients. This may be helpful for clinicians in order to implement these biomarkers in future feasible diagnostic assays.

Circulating histones and nucleosomes

Previous studies have demonstrated the critical role of nucleosome exposure in the activation of coagulation (38) and thrombus propagation (39). In a study published by van Montfoort *et al.* (40), it was observed that patients with deep venous thrombosis had significantly higher levels of circulating nucleosomes compared to controls. Mean circulating nucleosome levels measured in the cases were 17 U/mL (IQR 9-35) compared to 9 U/mL (IQR 5-17) detected in healthy subjects. It was also observed that patients who had circulating nucleosome levels above 80% of controls had a much higher risk of deep vein thrombosis than patients who had circulating nucleosome levels that were not greater than 80% of controls. Borissof *et al.*, in a prospective, observational and cross-sectional cohort of 282 individuals, suspected CAD demonstrated that extracellular circulating nuclear components, such as chromatin fragments and nucleosomes, were independently associated with CAD, prothrombotic state, and occurrence of adverse cardiac events. In fact, plasma nucleosomes

were positively correlated with plasma concentration of endothelial dysfunction markers and coagulation activation parameters (e.g., thrombin-antithrombin complexes and von Willebrand factor). Finally, Borissof *et al.* concluded that circulating nucleosomes and histones could potentially help in the prediction of cardiovascular risk (41).

miRNAs

Numerous studies have shown a correlation between circulating miRNA levels and diagnostic and prognostic of CAD. *Table 1* shows the most studied miRNA as CAD biomarkers.

Plasma and serum levels of miR-145, miR-155, and miR-17/92a cluster (miR-17, miR-126, miR-92a) related to the inflammatory response-associated cells, smooth muscle, and endothelial, respectively, were highly down-regulated in stable CAD patients compared to healthy controls (42). Thus, the decrease of plasma miR-145 can be useful in predicting CAD and its progression and severity (44).

Fichtlscherer *et al.* studied circulating levels of miRNA that were usually expressed in cells and tissues participating in the development of CAD. This group found that plasma and serum levels of miR-133a and miR-208, from cardiac muscle cells, were up-regulated in stable CAD patients compared to healthy controls. However, the use of miR-133a and 208a biomarkers for CAD remains controversial; this group could not replicate the results of miR-208 in a cohort of independent patients (42). In contrast, Wang *et al.* demonstrated that miR-133a can be a good diagnostic biomarker for CAD; it is important to highlight that the correlation between coronary artery stenosis and this biomarker level was stronger compared to the correlation with cardiac troponin. In addition, the levels of circulating miR-133a have a positive correlation with the severity of coronary stenosis in CHD patients (45). In addition, other research (46) found that circulating miR-208a and miR-370 levels were both significantly up-regulated in CAD patients. Authors showed the combination of both circulating miRNA levels was the best way to determine CAD because every miRNA is involved in different biological pathways underlying CAD. miR-208a is expressed in heart and miR-370 is relevant in lipid metabolism. The up-regulation level of circulating miR-208a can be associated with the severity of CAD according to Zhang and collaborators (47).

Finally, circulating levels of miR-31 were increased in restenosis CAD patients compared to CAD patients without restenosis. Thus, miR-31 could improve the detection of restenosis after cardiac injury (48).

HCM

HCM is characterized as left ventricular hypertrophy in the absence of abnormal loading conditions sufficient to explain the observed degree of hypertrophy. HCM is the most common hereditary heart disease (prevalence of 1:500) with great social impact because it is the leading cause of sudden cardiac death in young individuals (34). The presence of mutations in genes encoding proteins of the cardiac sarcomere (the structural and physiological unit of the heart muscle) is the major cause of this disease. Therefore, these mutations are frequently used as biomarkers of diagnostic. However, in approximately 40% of the patients, it is not possible to identify the causal mutation of this pathology (49). After clinical diagnostic, clinicians perform the follow up of patients by means echocardiography, Holter monitoring, electrocardiogram (ECG), and exercise testing (50).

DNA methylation

Global DNA methylation has been observed in atrium cardiomyocytes, with downregulation of several HCM genes such as *MYH7*, *GATA4*, *MEF2C*, *NFATC1*, *MYH7B*, *TNNI3* and *BNP* (51). Although the methylation of these genes has been postulated as target therapy, there are not evidences that the use of the methylation pattern of these genes can be used as biomarkers for HCM. However, further studies looking for circulating methylated genes focused on these candidates may clarify the potential of these genes being used as biomarkers.

Circulating histones

Greco *et al.* reviewed a list of epigenetic factors related to HCM. In this work, the authors have highlighted overexpression of histone acetyltransferases and majority reduction in deacetylases (52). Moreover, upregulation of H3K9me3-specific demethylase JMJD2A/KDM4A and reduction of its target, H3K9me3, are associated with HCM (53). Although, we did not find any research analyzing circulating H3K9me3 levels, the precedents of the identification of circulating nucleosomes and histones in CVDs (19) and the development of new techniques such as multiple reaction monitoring mass spectrometry (MRM-MS) recently described by our group may further contribute

Table 1 Key circulating miRNAs in coronary artery disease

miRNA	Regulation	Groups	References
miR-155	Down regulated	microRNA profiling:	(42)
		8 healthy volunteers	
		8 patients with stable CHD	
		Confirmation:	
		36 patients with CAD	
		17 healthy volunteers	
		Validation:	
		31 patients with CAD	
		14 controls	
		69 CAD patients	(43)
		32 control	
miR-145	Down regulated	microRNA profiling:	(42)
		8 healthy volunteers	
		8 patients with stable coronary heart disease	
		Confirmation:	
		36 patients with CAD	
		17 healthy volunteers	
		Validation:	
		31 patients with CAD	
		14 controls	
		167 CAD patients	(44)
		28 non CAD patients	
		69 CAD patients	(43)
		32 control	
miR-133a	Up regulated	First cohort:	(45)
		13 AMI patients	
		27 healthy volunteers	
		Second cohort:	
		22 CHD patients	
		8 non-CHD patients	
		Third cohort:	
		154 patients with CHD	
		92 non CHD patients	

Table 1 (*continued*)

Table 1 (*continued*)

miRNA	Regulation	Groups	References
miR-208a	Up regulated	95 CAD patients	(46)
		50 non-CAD control	
		290 CHD patients	(47)
		110 subjects with angiographic exclusion of CHD	
miR-17	Down regulated	MicroRNA profiling:	(42)
		8 healthy volunteers	
		8 patients with stable coronary heart disease	
		Confirmation:	
		36 patients with CAD	
		17 healthy volunteers	
		Validation:	
		31 patients with CAD	
		14 controls	
miR-126	Down regulated	MicroRNA profiling:	(42)
		8 healthy volunteers	
		8 patients with stable CAD	
		Confirmation:	
		36 patients with CAD	
		17 healthy volunteers	
		Validation:	
		31 patients with CAD	
		14 controls	
miR-92a	Down regulated	MicroRNA profiling:	(42)
		8 healthy volunteers	
		8 patients with stable CAD	
		Confirmation:	
		36 patients with CAD	
		17 healthy volunteers	
		Validation:	
		31 patients with CAD	
		14 controls	
miR-370	Up regulated	95 CAD patients	(46)
		50 non-CAD control	

miRNAs, microRNAs; AMI, acute myocardial infarction; CAD, coronary artery disease; CHD, coronary heart disease.

Table 2 Key circulating miRNAs in hypertrophic cardiomyopathy

miRNA	Regulation	Groups	References
miR-29a	Up regulated	41 patients with HCM	(55)
		41 healthy donors	
miR-27a	Up regulated	41 patients with HCM	(55)
		41 healthy donors	
miR-199a-5p	Up regulated	41 patients with HCM	(55)
		41 healthy donors	
miR-323a-3p	Up regulated	25 FRDA patients	(56)
		25 healthy donors	

miRNAs, microRNAs; HCM, hypertrophic cardiomyopathy; FRDA, Friedreich ataxia.

to determining if these histone candidates can serve as diagnostic biomarkers (54).

miRNAs

Very few studies have been done in order to identify circulating miRNA as biomarkers for diagnostic and management of HCM. For example, Roncarati *et al.* studied plasma levels of 21 miRNA by qPCR between 41 HCM patients and 41 control donors. Although 12 of those miRNAs were increased in plasma of HCM patients, only three miRNAs were significantly up-regulated among them: miR-199a-5p, miR-27a, and miR-29a. Importantly, only miR-29a was correlated with myocardial fibrosis (55) (*Table 2*). In another study, Kelly *et al.* found that miR-155 downregulates *AGTR1*, resulting in a reduction of the angiotensin II type 1 receptor. However, a single nucleotide polymorphism (SNP) in the *AGTR1* gene, rs5186 C allele, interrupts complementarity between miR-155 and the regulatory target site of AGTR1, thereby increasing *AGTR1* levels, which may explain an increased degree of cardiac hypertrophy, oxidative stress, and fibrosis in Friedreich ataxia (FRDA) patients (57).

Serial follow-up of apparently healthy members of families with HCM is required, but most important is to follow up with patients diagnosed with HCM. Therapeutic strategies may be designed to modify or prevent phenotypic development rather than treat the symptoms. This is particularly important in, for example, FRDA patients where heart affection is not always evident until cardiac symptoms are clearly observed in advanced phases of myocardiopathy. Clinical guidelines propose that until the ejection fraction falls, you should not begin treatment using either beta blockers or angiotensin-converting-enzyme inhibitors. However, when clinicians have a FRDA patient with depressed ejection fraction, it is usually too late to treat cardiac complications, considering the high probability of sudden death and arrhythmias in these patients (58). In most cases, clinicians wait too long to begin the pharmacological treatment because they have not tools to decide which is the best moment to treat FRDA patients. Therefore, new molecular biomarkers should be very useful to implement clinical decisions regarding the treatment of FRDA patients with beta blockers or ACE inhibitors.

In order to provide new biomarkers to identify cardiac hypertrophy in FRDA, we found that miR-323-3p is a candidate for diagnosing cardiomyopathy in FRDA patients. Using plasma samples from a series of 27 FRDA patients, we found that of the eight FRDA patients with cardiomyopathy, seven (87.5%) showed a fold change above 2.5, and of those not affected by cardiomyopathy, just 41.2% presented a fold change value above 2.5 (P=0.048) (56). Pilbrow *et al.* have described miR-323-3p as a candidate biomarker for CAD in acute coronary syndrome (ACS) patients (59). The use of miR-323a-3p would help clinicians in this regard and could contribute to early diagnosis and prognosis of cardiomyopathy prior to detection using conventional diagnostic procedures of clinical or morphological cardiac tissue manifestations. In fact, to examine the potential use of miR-323a-3p as a biomarker for cardiomyopathy in FRDA cases, we constructed the corresponding receiver operating characteristic (ROC) curve, finding differences in miR-323a-3p fold change in FRDA patients with and without cardiomyopathy. We calculated the optimal cut-off value for the fold change as 2.79. Sensitivity was 88.9% and specificity 62.5%, and the area under the curve (AUC) was 0.75 (P=0.042) (56). Because of the high stability of miRNAs and its dynamic nature, miR-323a-3p could be used as a biomarker for treatment monitoring or HCM follow-up.

AMI

AMI, properly called myocardial injury with myocardial ischemia, is the worst acute syndrome of CAD, affecting around 3% of adults older than 20 years of age (27), and the major cause of morbidity and mortality of the world (1). AMI can be diagnosed when there is evidence of myocardial necrosis with acute myocardial ischemia, and detection of specific characteristics of ECG, elevated values of

certain biomarkers of myocardial necrosis, or specific AMI characteristics observed by imaging (60).

The major causes of AMI are partial or complete coronary artery occlusion caused by vulnerable atherosclerotic plaque rupture and thrombus formation or acute occlusion of a coronary artery by coronary emboli or vasospasm (61,62). The most important biomarker used to diagnose AMI with necrosis is high concentration in blood of circulating cTnI (63), although circulating levels of the isoenzyme creatine kinase (CK)-MB are also frequently determined (60).

An elevated cTnl value can be associated with myocardial injury but does not provide information about an ischemia. For that reason, plasma cTnI concentrations may be elevated in other diseases in an unspecific manner such as septic shock and severe sepsis, chronic kidney disease, atrial fibrillation, and HF (64-67).

In this regard, other specific biomarkers, such as epigenetic biomarkers, could serve to detect AMI with high sensitivity and specificity for early diagnosis. We focus our attention on those epigenetic biomarkers which can be used for diagnostics and prognosis of AMI.

DNA methylation

DNA methylation status of LINE-1 has been also proposed as an epigenetic biomarker in ischemic heart disease and stroke (68). Baccarelli *et al.* obtained DNA from blood samples of 712 elderly patients. The patients were adjusted according to the risk factor, and in total 212 patients had ischemic heart disease and stroke and the remaining 470 patients were free of this condition. The results of this study showed that subjects with ischemic heart disease and stroke had lower methylation levels of LINE-1. Furthermore, when conducting the longitudinal analyses of the results, it was observed that patients who had lower methylation levels of LINE-1 had an increased risk of incident ischemic heart disease, stroke, and mortality (68).

Rask-Andersen *et al.* designed an epigenome-wide association study, consisting of genomic DNA obtained from blood samples of 729 Swedish subjects with CVD history, to identify disease-specific alterations in DNA methylation using Illumina Infinum 450K BeadChip. The cohort of patients with CVD included individuals with hypertension, AMI, stroke, thrombosis and cardiac arrhythmias (9). Rask-Andersen *et al.*, found differential DNA methylation at 211 CpG-sites mapped in 196 individual genes from participants that have experienced an episode of AMI (9). From these 211 CpG sites, at least 42 CpG sites were mapped to genes which are associated with cardiovascular function, myocardial development as well as response to ischemic injury.

Nakatochi *et al.*, using genomic DNA isolated from blood of a cohort of 192 elderly Japanese MI patients and 192 healthy subjects, found that specific methylated DNA sites were significantly associated with MI to nearby SNPs, which previously were proposed to be associated with CVD (10). Particularly, Nakatochi *et al.* identified two differentially methylated sites at cg07786668 in ZFHX3 gene and cg17218495 in SMARCA4 gene that were independently and significantly associated with MI.

Circulating histones and nucleosomes

Atherosclerotic plaque disruption and subsequent intraluminal thrombus formation is the pathological hallmark of ACS, including AMI and ischemic stroke. During the progression of atherosclerosis, circulating cells and cellular components of the vessel wall become more prone to DNA damage, therefore increasing apoptosis and necrosis which contribute to the release of DNA, nucleosomes, histones and histone-DNA complexes (69), sometimes produced via NETosis (process in which neutrophils migrate to the site of tissue damage and extrude decondensed chromatin threads (NETs), which consist of nuclear histones and azurophilic granule proteins such as myeloperoxidase and PMN elastase). Histological studies have reported the presence of NETs within the luminal portion of human atherosclerotic vessels and also in coronary thrombosuction biospecimens obtained post-AMI (70,71). Circulating nucleosome levels in patients with deep venous thrombosis can reflect NET degradation in the thrombus, although they may also derive from other cellular sources within or outside the thrombus (39,72). More recent studies have measured circulating nucleosomes as a marker for NET formation in humans (13). Despite all this data, it is still unknown how nucleosomes contribute to thrombus propagation.

In addition, histones released from necrotic cells accumulate within the myocardium soon after AMI (73), inducing further dose- and time-dependent myocardiocyte toxicity like what occurs in other tissues. Other studies measured DNA levels and circulating nucleosomes in plasma derived from coronary artery blood, correlating them positively with infarct size (74).

In this regard, circulating histones and nucleosomes

could be measured in blood samples in order to evaluate the level of cardiac tissue damage.

miRNAs

Several studies demonstrating the correlation between the level of circulating miRNA and the diagnosis and prognosis of AMI have been done over the last years. *Table 3* shows miRNAs that have been validated as circulating biomarkers for AMI.

After demonstrating that miR-208 is increased in rat blood levels after myocardial injury (89), studies in human have been observed where miR-208a was found in patients with AMI (86). Other analysis of circulating miR-208a and cardiac troponin I (cTnI), which is a commonly used biomarker in AMI, showed that miR-208a was increased in all AMI individuals, but cTnI was only detectable in 85% of patients 4 hours after the initial symptoms manifested (86). Moreover, Corsten *et al.* found that miR-208b plasma levels were increased in a 1,500–1,600 folds in AMI patients than in controls (79).

Circulating miR-499-5p was elevated more than 10-fold in AMI patients compared to healthy controls. miR-499 was also increased in AMI patients by 93% 3 hours after the manifestation of the initial symptoms, while cTnI was increased in 88% of AMI patients, suggesting that miR-499-5p identifies better AMI patients than cTnl. Another study demonstrated that miR-499 plasma levels were higher in AMI patients compared with congestive HF, and their levels increased rapidly within 48h of the last onset of pain and became undetectable before hospital discharge (76). These results point out the relevant role of miR-499-5p for differential diagnosis. Moreover, circulating miR-499-5p levels can be correlated with non-ST elevation AMI better than cTnI in elderly patients. Therefore, miR-499 may help to differentiate AMI from acute congestive HF (87).

In another study by Devaux *et al.*, patients with AMI that showed ST-elevation, had higher levels of miR-208b and miR-499 when compared to patients without ST segment elevation ($P<0.001$) (77).

The level of serum miR-1 was increased after AMI with a peak at 6 h (>200-fold) and returned to basal level 3 days after the disease (83). Circulating miR-1 in AMI patients was elevated compared to patients with CAD without AMI or healthy subjects and no correlation was observed between miR-1 expression and some variables such as age, sex, and arterial blood pressure (80).

Finally, several studies showed that miR-133 levels were increased in both experimental AMI animal models and AMI patients (82). In this regard, miR-1 and miR-133a are increased in AMI patients compared to subjects with unstable angina or another type of acute myocardial syndrome (90).

These facts suggest that miRNAs are better biomarkers for AMI than traditional cTnI. Furthermore, circulating miRNA can serve to differentiate between different types of AMI and also different types of CVDs. However, some variation of circulating miRNA levels is observed in patients when their levels are analyzed over time. For this reason, when analyzing miRNAs, it is highly important to fix the time of sampling or blood collection since AMI onset.

HF

HF is a progressive complex syndrome characterized by a persistent, low-grade, systemic and myocardial inflammation that impairs the ability of the ventricle to fill or eject blood, which correlates with disease severity and prognosis. HF is a major public health problem, with a prevalence of over 23 million worldwide and increased incidence (91). Current guidelines underscore that "it is largely a clinical diagnosis that is based on a careful history and physical examination."

While the cause of this low-grade inflammation is currently unknown, endogenous danger associated molecule-patterns (DAMPs), may be a contributing factor. Among the structures identified as a potential trigger of endogenous inflammation is the nucleosome, a chromatin structure consisting of at least eight histones wrapped by double-stranded DNA (92).

DNA methylation

Movassagh *et al.* demonstrated the differential methylation patterns of three angiogenic genes comparing left ventricular tissue from 8 end-stage HF patients and 6 controls. They observed the promoter hypermethylation of platelet/endothelial cell adhesion molecule (*PCAM1*), hypermethylation within the gene body of Rho GTPase activating protein 24 (*ARHGAP24*) and hypomethylation within the gene body of angiomotin-like 2 (*AMOTL2*) have been found in a series of patients with HF (93,94).

Circulating histones and nucleosomes

A recent study detected increased nucleosome plasmatic concentrations in 179 patients with HF who were clinically

Table 3 Key circulating miRNAs in acute myocardial infarction

miRNA	Regulation	Groups	References
miR-499	Up regulated	67 AMI patients	(75)
		32 controls	
		14 individuals with ACS (9 with AMI)	(76)
		15 individuals with CHF	
		10 controls	
		510 AMI	(77)
		87 controls	
		224 AMI	(78)
		931 non-AMI	
		32 AMI patient	(79)
		36 no cardiac disease	
miR-1	Up regulated	67 AMI patients	
		32 controls	(75)
		93 AMI patients	(80)
		66 healthy patients	
		17 AMI patients	(81)
		25 healthy subjects	
		33 patients with STEMI	(82)
		17 healthy donors	
		31 AMI patients	(83)
		20 control group.	
miR-133	Up regulated	51 AMI subjects	(84)
		28 controls	
		76 AMI cases	(85)
		110 no ami cases	
miR-208a	Up regulated	33 AMI patients	(86)
		33 non-AMI patients	

Table 3 (*continued*)

Table 3 (*continued*)

miRNA	Regulation	Groups	References
miR-133a	Up regulated	First cohort:	(45)
		13 AMI patients	
		27 healthy volunteers	
		Second cohort:	
		22 CHD patients	
		8 non-CHD patients	
		Third cohort:	
		154 patients with CHD	
		92 non CHD patients	
		33 patients with STEMI	(82)
		17 healthy donors	
		67 AMI patients	(75)
		32 controls	
miR-499-5p	Up regulated	92 NSTEMI elderly patients (AMI)	(87)
		81 elderly patients with acute CHF without AMI	
		33 patients with STEMI	(82)
		17 healthy donors	
		319 MI	(88)
		88 non-MI patients	
miR-208b	Up regulated	32 AMI patients	(79)
		36 no cardiac disease	
		319 MI	(88)
		88 non-MI patients	
		510 AMI	(77)
		87 controls	
		67 AMI patients	(75)
		32 controls	
		224 AMI	(78)
		931 non-AMI	

miRNAs, microRNAs; AMI, acute myocardial infarction; ACS, acute coronary syndrome; CHF, congestive heart failure.

stable for 6 months before blood collection. Nucleosome levels were positively correlated with disease severity score and mortality within 4 years of blood sampling (92). These results provide the basis to propose circulating nucleosomes as a predictive marker of mortality of HF affected patients.

On the other hand, Kaneda *et al*. performed ChIP experiments using human left ventricular tissue with retained or damaged function and analyzed by high-throughput pyrosequencing those genes bound to H3K4me3 or H3K9me3, which participate in transduction pathways for cardiac function. The authors identified that trimethylation of histone H3 is markedly affected in cardiomyocytes in association with the development of HF (95). Therefore, it would be interesting to measure the levels of H3K4me3 and H3K9me3 of circulating histones and correlate the levels of these modifications with the risk of HF.

miRNAs

Several studies have revealed that patients with HF have differentially expressed miRNA circulating levels. *Table 4* shows miRNAs that constitute valuable biomarker candidates for HF. The most studied circulating miRNA biomarker for HF is miR-423-5p. Tijsen *et al.* revealed that miR-423-5p, miR-18b, miR-129-5p, miR-1254, miR-675, and miR-622 were up-regulated in HF patients. Among them, miR-423-5p was the most strongly related to the clinical diagnosis of HF. In addition, miR-423-5p allowed the identification of HF patients from healthy controls and other patients diagnosed with dyspnea but not with HF (96). This circulating miRNA was correlated with circulating levels of NT-pro-BNP (pro-brain-natriuretic peptide) which is a common biomarker used for diagnosing HF (96). In another study, it was shown that miR-499 could be a tool to differentiate between acute HF and systolic HF (79). Moreover, circulating miR-499 was only up-regulated in acute HF while remained invariable in diastolic HF (79). Accordingly, these data suggest that both miR-423-5p and miR-499 might be clinical diagnostic biomarkers in HF.

In addition, other circulating miRNA are proven to be prognostic biomarkers of HF. It was reported that circulating levels of miR-16, miR-27a, miR-101 and miR150 improved the prediction of left ventricle contractility six months after suffering for an AMI with other biomarkers, such as NT-pro-BNT, in a multivariable model (100).

In addition to the above-mentioned circulating miRNAs, many other circulating miRNAs have been reported as biomarker candidates for HF, although more studies are required in some of them in order to further validate these candidates as feasible biomarkers for HF.

Future directions

The exciting world of epigenetic biomarkers and their potential clinical application is opening new opportunities in the diagnosis, theragnosis and therapeutic application in CVDs. For example, considering that DNA and histones play essential roles in the pathogenesis of cardiac lesions like, among others, atherosclerosis, arrhythmias, thrombosis and left ventricular dysfunction, strategies aimed at reducing the DNA-histone complex may constitute a viable goal for prevention of cardiac diseases as well as in different heart clinical complications (101).

From a clinical perspective, epigenetic biomarkers may provide valuable tools for diagnosis, prognosis, disease treatment and management and theragnosis, therefore improving precision medicine. However, larger clinical trials are required to establish whether current biomarker candidates offer additional benefit over existing biomarkers of CVDs described in the other chapters of this special issue. Additionally, technological advances such as next-generation sequencing and mass spectrometry are required to enable sensitive, specific, rapid, reliable and reproducible results for the absolute quantification of epigenetic biomarkers in order to facilitate transition into clinical practice.

In this review, we summarized different epigenetic biomarkers as future candidates for the diagnostic of CVDs. Recapitulating the information described in this review, we realize that miRNAs are the most studied biomarkers in CVDs. Conversely, there are only a few studies describing and proposing DNA methylation and histones as candidate biomarkers for CVDs. In this regard, the wide array of diseases in which LINE-1 was reported to be down-methylated make this biomarker excessively nonspecific to be used as a precise biomarker for CVDs. Therefore, it would be interesting to direct the future studies on the identification and validation of circulating DNA methylation levels of specific genes for diagnosing a particular disease condition.

Finally, while there remain a number of challenges to still overcome, clinicians should be aware of the future arrival of this type of new biomarkers into clinical medicine.

Table 4 Key circulating miRNAs in heart failure

miRNA	Regulation	Groups	References
miR-423-5p	Up regulated	Array:	(96)
		12 healthy controls	
		12 HF patients	
		Validation:	
		39 healthy controls	
		20 dyspnea non-HF cases	
		30 dyspnea HF cases	
		30 patients with stable chronic systolic HF	(97)
		30 controls	
		45 heart failure patients caused by dilated cardiomyopathy	(98)
		39 controls	
miR-122	Up regulated	Screening cohort:	(99)
		53 HF patients	
		39 controls	
		Replication cohort:	
		14 HF patients	
		8 controls	
		33 congestive HF	(79)
		34 healthy controls	
miR-622	Up regulated	Screening cohort:	(99)
		53 HF patients	
		39 controls	
		Replication cohort:	
		14 HF patients	
		8 controls	
		Array:	(96)
		12 healthy controls	
		12 HF patients	
		Validation:	
		39 healthy controls	
		20 dyspnea Non-HF cases	
		30 dyspnea HF cases	
miR-499	Up regulated	33 congestive HF	(79)
		34 healthy controls	
		39 diastolic dysfunction patients	
		40 hypertensive or normotensive control subjects	

miRNAs, microRNAs; HF, heart failure.

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References

1. Sanchis-Gomar F, Perez-Quilis C, Leischik R, et al. Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med 2016;4:256.
2. Udali S, Guarini P, Moruzzi S, et al. Cardiovascular epigenetics: from DNA methylation to microRNAs. Mol Aspects Med 2013;34:883-901.
3. García-Giménez JL, Ushijima T, Tollefsbol TO. Epigenetic Biomarkers: New Findings, Perspectives, and Future Directions in Diagnostics. Epigenetic Biomarkers and Diagnostics. Boston: Academic Press; 2016. p. 1-18.
4. Ikeda S, Kong SW, Lu J, et al. Altered microRNA expression in human heart disease. Physiol Genomics 2007;31:367-73.
5. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21.
6. Baccarelli A, Rienstra M, Benjamin EJ. Cardiovascular epigenetics: basic concepts and results from animal and human studies. Circ Cardiovasc Genet 2010;3:567-73.
7. Ma B, Wilker EH, Willis-Owen SA, et al. Predicting DNA methylation level across human tissues. Nucleic Acids Res 2014;42:3515-28.
8. Weisenberger DJ, Campan M, Long TI, et al. Analysis of repetitive element DNA methylation by MethyLight. Nucleic Acids Res 2005;33:6823-36.
9. Rask-Andersen M, Martinsson D, Ahsan M, et al. Epigenome-wide association study reveals differential DNA methylation in individuals with a history of myocardial infarction. Hum Mol Genet 2016;25:4739-48.
10. Nakatochi M, Ichihara S, Yamamoto K, et al. Epigenome-wide association of myocardial infarction with DNA methylation sites at loci related to cardiovascular disease. Clin Epigenetics 2017;9:54.
11. Berger SL. The complex language of chromatin regulation during transcription. Nature 2007;447:407-12.
12. Abrams ST, Zhang N, Manson J, et al. Circulating histones are mediators of trauma-associated lung injury. Am J Respir Crit Care Med 2013;187:160-9.
13. Fuchs TA, Kremer Hovinga JA, Schatzberg D, et al. Circulating DNA and myeloperoxidase indicate disease activity in patients with thrombotic microangiopathies. Blood 2012;120:1157-64.
14. Kutcher ME, Xu J, Vilardi RF, et al. Extracellular histone release in response to traumatic injury: implications for a compensatory role of activated protein C. J Trauma Acute

Care Surg 2012;73:1389-94.
15. Zeerleder S, Stephan F, Emonts M, et al. Circulating nucleosomes and severity of illness in children suffering from meningococcal sepsis treated with protein C. Crit Care Med 2012;40:3224-9.
16. Chen R, Kang R, Fan XG, et al. Release and activity of histone in diseases. Cell Death Dis 2014;5:e1370.
17. Gilthorpe JD, Oozeer F, Nash J, et al. Extracellular histone H1 is neurotoxic and drives a pro-inflammatory response in microglia. F1000Res 2013;2:148.
18. Alhamdi Y, Abrams ST, Cheng Z, et al. Circulating Histones Are Major Mediators of Cardiac Injury in Patients With Sepsis. Crit Care Med 2015;43:2094-103.
19. Gao H, Zhang N, Lu F, et al. Circulating histones for predicting prognosis after cardiac surgery: a prospective study. Interact Cardiovasc Thorac Surg 2016;23:681-7.
20. van Rooij E, Olson EN. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat Rev Drug Discov 2012;11:860-72.
21. Landgraf P, Rusu M, Sheridan R, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129:1401-14.
22. Thum T, Catalucci D, Bauersachs J. MicroRNAs: novel regulators in cardiac development and disease. Cardiovasc Res 2008;79:562-70.
23. Silverthorn DU, Johnson BR, Ober WC, et al. Human physiology : an integrated approach. Pearson,, Boston. 2013.
24. Wang F, Chen C, Wang D. Circulating microRNAs in cardiovascular diseases: from biomarkers to therapeutic targets. Front Med 2014;8:404-18.
25. Fichtlscherer S, Zeiher AM, Dimmeler S. Circulating microRNAs: biomarkers or mediators of cardiovascular diseases? Arterioscler Thromb Vasc Biol 2011;31:2383-90.
26. Creemers EE, Tijsen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circ Res 2012;110:483-95.
27. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017;135:e146-e603.
28. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation 2005;111:363-8.
29. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43.
30. Deodhar SD. C-reactive protein: the best laboratory indicator available for monitoring disease activity. Cleve Clin J Med 1989;56:126-30.
31. Maneerat Y, Prasongsukarn K, Benjathummarak S, et al. PPBP and DEFA1/DEFA3 genes in hyperlipidaemia as feasible synergistic inflammatory biomarkers for coronary heart disease. Lipids Health Dis 2017;16:80.
32. Turunen MP, Aavik E, Yla-Herttuala S. Epigenetics and atherosclerosis. Biochim Biophys Acta 2009;1790:886-91.
33. Yang AS, Estecio MR, Doshi K, et al. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res 2004;32:e38.
34. Zuo HP, Guo YY, Che L, et al. Hypomethylation of Interleukin-6 Promoter is Associated with the Risk of Coronary Heart Disease. Arq Bras Cardiol 2016;107:131-6.
35. Kim J, Kim JY, Song KS, et al. Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and in-vitro vascular senescence. Biochim Biophys Acta 2007;1772:72-80.
36. Post WS, Goldschmidt-Clermont PJ, Wilhide CC, et al. Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. Cardiovasc Res 1999;43:985-91.
37. Zhu S, Goldschmidt-Clermont PJ, Dong C. Inactivation of monocarboxylate transporter MCT3 by DNA methylation in atherosclerosis. Circulation 2005;112:1353-61.
38. Massberg S, Grahl L, von Bruehl ML, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med 2010;16:887-96.
39. von Bruhl ML, Stark K, Steinhart A, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med 2012;209:819-35.
40. van Montfoort ML, Stephan F, Lauw MN, et al. Circulating nucleosomes and neutrophil activation as risk factors for deep vein thrombosis. Arterioscler Thromb Vasc Biol 2013;33:147-51.
41. Borissoff JI, Joosen IA, Versteylen MO, et al. Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. Arterioscler Thromb Vasc Biol 2013;33:2032-40.
42. Fichtlscherer S, De Rosa S, Fox H, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res 2010;107:677-84.
43. Faccini J, Ruidavets JB, Cordelier P, et al. Circulating miR-155, miR-145 and let-7c as diagnostic biomarkers of the coronary artery disease. Sci Rep 2017;7:42916.
44. Gao H, Guddeti RR, Matsuzawa Y, et al. Plasma Levels of microRNA-145 Are Associated with Severity of Coronary

Artery Disease. PLoS One 2015;10:e0123477.
45. Wang F, Long G, Zhao C, et al. Plasma microRNA-133a is a new marker for both acute myocardial infarction and underlying coronary artery stenosis. J Transl Med 2013;11:222.
46. Liu H, Yang N, Fei Z, et al. Analysis of plasma miR-208a and miR-370 expression levels for early diagnosis of coronary artery disease. Biomed Rep 2016;5:332-6.
47. Zhang Y, Li HH, Yang R, et al. Association between circulating microRNA-208a and severity of coronary heart disease. Scand J Clin Lab Invest 2017;77:379-84.
48. Wang J, Yan CH, Li Y, et al. MicroRNA-31 controls phenotypic modulation of human vascular smooth muscle cells by regulating its target gene cellular repressor of E1A-stimulated genes. Exp Cell Res 2013;319:1165-75.
49. Ho CY. Hypertrophic cardiomyopathy. Heart Fail Clin 2010;6:141-59.
50. Maron BJ, Salberg L, Maron BJ. A guide to hypertrophic cardiomyopathy : for patients, families, and interested physicians. Third edition. ed. Chichester, West Sussex, UK: John Wiley & Sons Ltd; 2014.
51. Fang X, Robinson J, Wang-Hu J, et al. cAMP induces hypertrophy and alters DNA methylation in HL-1 cardiomyocytes. Am J Physiol Cell Physiol 2015;309:C425-36.
52. Greco CM, Condorelli G. Epigenetic modifications and noncoding RNAs in cardiac hypertrophy and failure. Nat Rev Cardiol 2015;12:488-97.
53. Zhang QJ, Chen HZ, Wang L, et al. The histone trimethyllysine demethylase JMJD2A promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. J Clin Invest 2011;121:2447-56.
54. Garcia-Gimenez JL, Roma-Mateo C, Carbonell N, et al. A new mass spectrometry-based method for the quantification of histones in plasma from septic shock patients. Sci Rep 2017;7:10643.
55. Roncarati R, Viviani Anselmi C, Losi MA, et al. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2014;63:920-7.
56. Seco-Cervera M, Gonzalez-Rodriguez D, Ibanez-Cabellos JS, et al. Circulating miR-323-3p is a biomarker for cardiomyopathy and an indicator of phenotypic variability in Friedreich's ataxia patients. Sci Rep 2017;7:5237.
57. Kelly M, Bagnall RD, Peverill RE, et al. A polymorphic miR-155 binding site in AGTR1 is associated with cardiac hypertrophy in Friedreich ataxia. J Mol Cell Cardiol 2011;51:848-54.
58. Pousset F, Legrand L, Monin ML, et al. A 22-Year Follow-up Study of Long-term Cardiac Outcome and Predictors of Survival in Friedreich Ataxia. JAMA Neurol 2015;72:1334-41.
59. Pilbrow AP, Cordeddu L, Cameron VA, et al. Circulating miR-323-3p and miR-652: candidate markers for the presence and progression of acute coronary syndromes. Int J Cardiol 2014;176:375-85.
60. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Circulation 2012;126:2020-35.
61. Oerlemans MI, Koudstaal S, Chamuleau SA, et al. Targeting cell death in the reperfused heart: pharmacological approaches for cardioprotection. Int J Cardiol 2013;165:410-22.
62. Oerlemans MI, Liu J, Arslan F, et al. Inhibition of RIP1-dependent necrosis prevents adverse cardiac remodeling after myocardial ischemia-reperfusion in vivo. Basic Res Cardiol 2012;107:270.
63. Jaffe AS, Ravkilde J, Roberts R, et al. It's time for a change to a troponin standard. Circulation 2000;102:1216-20.
64. Abbas NA, John RI, Webb MC, et al. Cardiac troponins and renal function in nondialysis patients with chronic kidney disease. Clin Chem 2005;51:2059-66.
65. Finsterer J, Stollberger C, Krugluger W. Cardiac and noncardiac, particularly neuromuscular, disease with troponin-T positivity. Neth J Med 2007;65:289-95.
66. Giannitsis E, Katus HA. Cardiac troponin level elevations not related to acute coronary syndromes. Nat Rev Cardiol 2013;10:623-34.
67. Rosjo H, Varpula M, Hagve TA, et al. Circulating high sensitivity troponin T in severe sepsis and septic shock: distribution, associated factors, and relation to outcome. Intensive Care Med 2011;37:77-85.
68. Baccarelli A, Wright R, Bollati V, et al. Ischemic heart disease and stroke in relation to blood DNA methylation. Epidemiology 2010;21:819-28.
69. Mahmoudi M, Mercer J, Bennett M. DNA damage and repair in atherosclerosis. Cardiovasc Res 2006;71:259-68.
70. Megens RT, Vijayan S, Lievens D, et al. Presence of luminal neutrophil extracellular traps in atherosclerosis. Thromb Haemost 2012;107:597-8.
71. de Boer OJ, Li X, Teeling P, et al. Neutrophils, neutrophil extracellular traps and interleukin-17 associate with the organisation of thrombi in acute myocardial infarction. Thromb Haemost 2013;109:290-7.
72. Zeerleder S, Zwart B, Wuillemin WA, et al. Elevated

nucleosome levels in systemic inflammation and sepsis. Crit Care Med 2003;31:1947-51.
73. Vogel B, Shinagawa H, Hofmann U, et al. Acute DNase1 treatment improves left ventricular remodeling after myocardial infarction by disruption of free chromatin. Basic Res Cardiol 2015;110:15.
74. Mangold A, Alias S, Scherz T, et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. Circ Res 2015;116:1182-92.
75. Li YQ, Zhang MF, Wen HY, et al. Comparing the diagnostic values of circulating microRNAs and cardiac troponin T in patients with acute myocardial infarction. Clinics (Sao Paulo) 2013;68:75-80.
76. Adachi T, Nakanishi M, Otsuka Y, et al. Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin Chem 2010;56:1183-5.
77. Devaux Y, Vausort M, Goretti E, et al. Use of circulating microRNAs to diagnose acute myocardial infarction. Clin Chem 2012;58:559-67.
78. Devaux Y, Mueller M, Haaf P, et al. Diagnostic and prognostic value of circulating microRNAs in patients with acute chest pain. J Intern Med 2015;277:260-71.
79. Corsten MF, Dennert R, Jochems S, et al. Circulating MicroRNA-208b and MicroRNA-499 reflect myocardial damage in cardiovascular disease. Circ Cardiovasc Genet 2010;3:499-506.
80. Ai J, Zhang R, Li Y, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem Biophys Res Commun 2010;391:73-7.
81. Long G, Wang F, Duan Q, et al. Human circulating microRNA-1 and microRNA-126 as potential novel indicators for acute myocardial infarction. Int J Biol Sci 2012;8:811-8.
82. D'Alessandra Y, Devanna P, Limana F, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur Heart J 2010;31:2765-73.
83. Cheng Y, Tan N, Yang J, et al. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond) 2010;119:87-95.
84. Wang R, Li N, Zhang Y, et al. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. Intern Med 2011;50:1789-95.
85. Peng L, Chun-guang Q, Bei-fang L, et al. Clinical impact of circulating miR-133, miR-1291 and miR-663b in plasma of patients with acute myocardial infarction. Diagn Pathol 2014;9:89.
86. Wang GK, Zhu JQ, Zhang JT, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J 2010;31:659-66.
87. Olivieri F, Antonicelli R, Lorenzi M, et al. Diagnostic potential of circulating miR-499-5p in elderly patients with acute non ST-elevation myocardial infarction. Int J Cardiol 2013;167:531-6.
88. Gidlof O, Smith JG, Miyazu K, et al. Circulating cardio-enriched microRNAs are associated with long-term prognosis following myocardial infarction. BMC Cardiovasc Disord 2013;13:12.
89. Ji X, Takahashi R, Hiura Y, et al. Plasma miR-208 as a biomarker of myocardial injury. Clin Chem 2009;55:1944-9.
90. Widera C, Gupta SK, Lorenzen JM, et al. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. J Mol Cell Cardiol 2011;51:872-5.
91. Roger VL. Epidemiology of myocardial infarction. Med Clin North Am 2007;91:537-52; ix.
92. Nymo SH, Ueland T, Askevold E, et al. Circulating nucleosomes in chronic heart failure. Int J Cardiol 2016;203:742-3.
93. Movassagh M, Choy MK, Knowles DA, et al. Distinct epigenomic features in end-stage failing human hearts. Circulation 2011;124:2411-22.
94. Movassagh M, Choy MK, Goddard M, et al. Differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure. PLoS One 2010;5:e8564.
95. Kaneda R, Takada S, Yamashita Y, et al. Genome-wide histone methylation profile for heart failure. Genes Cells 2009;14:69-77.
96. Tijsen AJ, Creemers EE, Moerland PD, et al. MiR423-5p as a circulating biomarker for heart failure. Circ Res 2010;106:1035-9.
97. Goren Y, Kushnir M, Zafrir B, et al. Serum levels of microRNAs in patients with heart failure. Eur J Heart Fail 2012;14:147-54.
98. Fan KL, Zhang HF, Shen J, et al. Circulating microRNAs levels in Chinese heart failure patients caused by dilated cardiomyopathy. Indian Heart J 2013;65:12-6.
99. Vogel B, Keller A, Frese KS, et al. Multivariate miRNA signatures as biomarkers for non-ischaemic systolic heart failure. Eur Heart J 2013;34:2812-22.
100. Devaux Y, Vausort M, McCann GP, et al. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. PLoS One

2013;8:e70644.
101. Knight JS, Luo W, O'Dell AA, et al. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. Circ Res 2014;114:947-56.

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