Review Article

Prospective and therapeutic screening value of non-coding RNA as biomarkers in cardiovascular disease

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Abstract: Non-coding RNA (ncRNA) is a class of genetic, epigenetic and translational regulators, containing short and long transcripts with intriguing abilities for use as biomarkers due to their superordinate role in disease development. In the past five years many of these have been investigated in cardiovascular diseases (CVD), mainly myocardial infarction (MI) and heart failure. To extend this view, we summarize the existing data about ncRNA as biomarker in the whole entity of CVDs by literature-based review and comparison of the identified candidates. The myomirs miRNA-1, -133a/b, -208a, -499 with well-defined cellular functions have proven equal to classic protein biomarkers for disease detection in MI. Other microRNAs (miRNAs) were reproducibly found to correlate with disease, disease severity and outcome in heart failure, stroke, coronary artery disease (CAD) and aortic aneurysm. An additional utilization has been discovered for therapeutic monitoring. The function of long non-coding transcripts is only about to be unraveled, yet shows great potential for outcome prediction. ncRNA biomarkers have a distinct role if no alternative test is available or has is performing poorly. With increasing mechanistic understanding, circulating miRNA and long non-coding transcripts will provide useful disease information with high predictive power.

Keywords: Non-coding RNA (ncRNA); microRNA (miRNA); long ncRNA (lncRNA); biomarker; cardiovascular disease (CVD)

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Introduction

According to data from the World Health Organization, an estimated 17.5 million people die annually from cardiovascular diseases (CVD), representing 31% of all global deaths. The vast majority dies from stroke and coronary artery disease (CAD).

Atherosclerosis impairs vascular functional integrity, and causes serious morbidity long before these "combined endpoints". One quarter of the Western population suffers from some form of CAD or the consequences of stroke (1,2). This is compounded by aneurysm, peripheral arterial occlusive disease (PAOD), vascular dementia, pulmonary arterial hypertension (PAH), and venous disease. Treatment strategies are focused on prevention by controlling risk factors and early states in the epidemiologic—and sepcific diagnosis and treatment in the individualized setting, as well as specific diagnosis and treatment in the individualized setting.

For the latter, disease biomarkers are of utmost utility and importance, for instance, cardiac troponin T/I (TnT/I) for the diagnosis of myocardial infarction (MI), and NT-proBNP (BNP) for the diagnosis and monitoring of heart failure due to left ventricular remodeling (LVM) (3). A biomarker is a measurable and quantifiable biological parameter, and might as such be used for screening, identifying, categorizing, monitoring or predicting disease, risk and therapy (4).

Classic protein biomarkers, however, are only available and clinically useful for a limited number of diseases. Their utility is compromised by inter- and intra-individual heterogeneity of diseases, specific genetics and proteomics, as well as the influence of lifestyle (5). In contrast, due to their generalized role in pathologic conditions, non-coding RNAs (ncRNAs) have great potential for future biomarker approaches.

Non-coding RNA (ncRNA)

Over the last few years, it has been established that only 3% of the human genome codes for protein genes. Approximately 80% of the genome is, however, transcribed regularly (6). RNA and genomic deep sequencing have revealed that the ever growing number of non-coding transcripts by far exceeds protein-coding mRNA. This is believed to shape complexity in species, since the proportion of ncRNA increases with higher rank in evolutionary development (7,8).

The first microRNA (miRNA) was described in 1993, and since then, approximately 1,800 of these 16–22 nucleotide (nt) long transcripts have been annotated, with great uncertainty about the final number (9,10). Some of them have been well investigated in various diseases, and the first candidates have now entered clinical trials, both for diagnostic and therapeutic applications (11). They constitute the vast majority of small ncRNA (<200 nt), whereas long ncRNA (lncRNA) includes transcripts >200 nt. This number is believed to be around 9,000 (6). miRNAs act mainly at the post-transcriptional level by regulating mRNA decay or inhibition.

LncRNAs multiply the challenge to the central dogma of nuclear DNA-transcription and subsequent cytoplasmic mRNA-translation via multiple mechanisms. These include histone modification, transcript regulation, alternate splicing, mRNA fragmentation, endo-sponge activity and direct protein interaction (*Figure 1*). miRNAs are most often located in the promoter regions of distinct genes, either singly or in clusters. One molecule can have hundreds of (often functionally related) mRNA targets, thus miRNAs constitute dense regulatory networks for approximately two thirds of all genes (12). LncRNAs have a more heterogeneous distribution in the genome, with nested and overlapping, sense and antisense transcripts (13). Although their structure is not as evolutionary conserved as that for miRNAs, their function within the regulatory network is (14,15).

Additionally, there is growing evidence suggesting numerous interactions between the different classes of RNAs due to complementary binding sites. miRNAs might thus directly control action and transcription of lncRNAs, whereas these in return might smother miRNA effects, i.e., by endo-sponge-activity (16,17). These closely interwoven relationships emphasize the likely involvement of a network, rather than a single gene, when investigating ncRNA changes in disease (18). A genome-wide shift of corresponding miRNA-mRNA expressions in CVD patients was demonstrated in the Framingham population, and a fast dynamically regulated transcriptome of the myocardium, after mechanical support in a small patient cohort, showed significant changes in lncRNA expression (19-21). Whereas the mechanistic role of ncRNA in CVD has been reviewed extensively before, their potential as novel biomarkers in CVDs appears as an intriguing topic, especially since different expression levels might represent different stages of a disease.

Circulating non-coding RNA (ncRNA)

For use as classic biomarkers, ncRNAs must be easily accessible by routine diagnostic methods, suggesting extracellular circulating candidates as valuable targets. Their abundance in plasma, urine, saliva and cerebrospinal fluid (CSF) suggests a specific role in inter-cellular signaling, rather than just an intracellular function. Their high stability in body fluids compared to mRNA or genomic DNA is due to smaller size, and compartmentalization into exosomes, microparticles, apoptotic bodies, lipoprotein and protein complexes [*Figure 1*; reviewed in detail by (18)].

Blood is the most promising compartment for biomarker investigations in the context of CVD, due to its close relationship with the affected tissues, easy accessibility and the possibility of testing multiple targets with only one probe. There have been confounding reports with inconsistent results for various miRNAs regarding CVD for other body fluids; urine analysis might have a role for investigating kidney disease (22-24). Most studies report analysis from “serum”, “plasma” or “whole blood”, with, unfortunately, heterogeneous definitions of these terms. Only very few studies address the cellular and acellular fractions, and their respective role, in ncRNA transport in circulation (*Table S1*) (25).

Nevertheless, the use of circulating ncRNA as biomarkers is persuasive, especially in the context of signature and network analysis for disease detection and

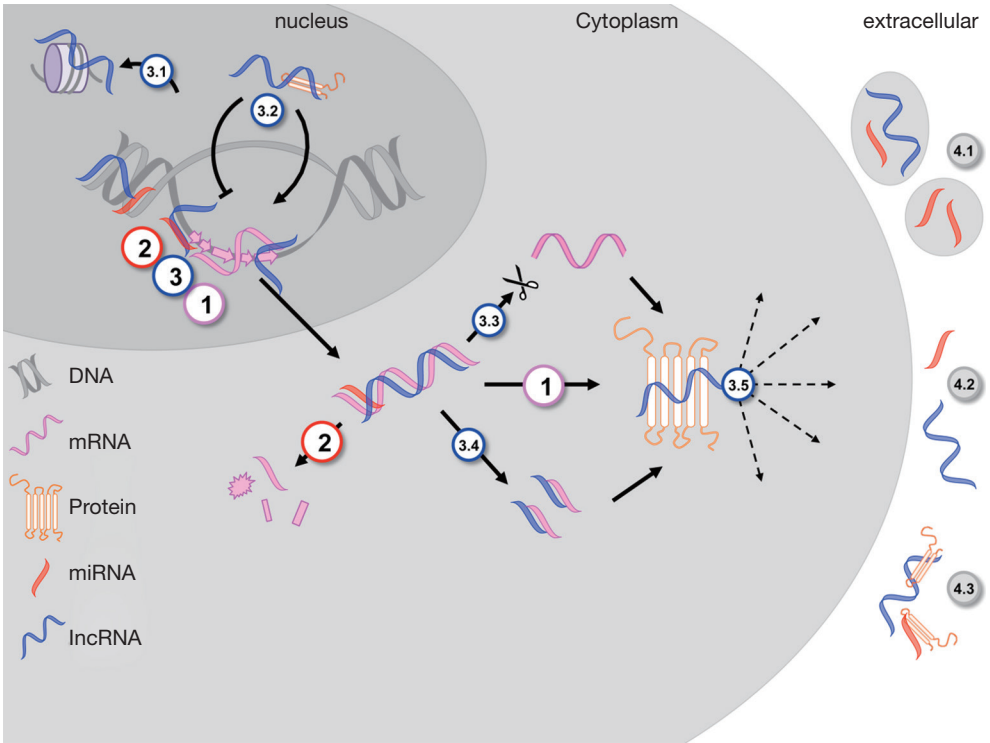

Figure 1 ncRNA in distinct cellular compartments: [1] transcribed mRNA from unwound exonic DNA in the nucleus is translated into proteins in the cytoplasm, which subsequently exert different functions on various localizations within and outside the cell. miRNAs [2], frequently transcribed within the promoter region of genes, alter this chain of events by post-transcriptional tacking of the polyA-tail and thus mRNA decay. lncRNAs are transcribed within various regions of the genome, in sense and antisense direction. Their numerous interactions include nuclear histone modification (3.1) and mRNA transcriptory regulation by binding specific proteins (3.2). Cytoplasmic effects are alternative splicing (3.3), mRNA fragmentation and endo-sponge activity (3.4) as well as direct interaction with proteins, changing their function and localization (3.5). Extracellularly, ncRNAs are found freely circulating (4.2), grouped in microvesicles and microparticles (4.1) or bound to distinct proteins or lipoproteins (4.3). ncRNA, non-coding RNA; miRNA, microRNA; lncRNA, long ncRNA.

progression. Therefore, our aim here is to summarize and evaluate the existing evidence for the analytic and predictive potential of different ncRNAs in CVD.

microRNA (miRNA) in cardiovascular diseases (CVDs)

A large number of miRNAs has been studied in the context of acute MI and its concluding remodeling processes within the heart. Nevertheless, a variety of other CVDs, both on the arterial and the venous side, have followed.

We therefore performed a complete literature review on the terms "miRNA", "ncRNA" and "lncRNA" in combination with "biomarker" and the respective CVDs discussed below. The results from over 100 studies are shown in detail in the supplementary material (*Table S1*). From these, miRNAs reported more than once in two independent studies, and all lncRNAs are reviewed in detail in this manuscript (*Tables 1*,*2*).

Myocardial infarction (MI)

Since 2010, more than 30 studies have assessed the use of miRNAs as biomarkers for acute MI diagnosis, and their predictive values for post-MI outcomes, in a combined total of over 3,500 patients. Unfortunately, most of them were identified using a retrospective approach, with however, fairly large and often well diagnosed cohorts. Protein biomarkers, based on cellular decay, such as TnI/T and creatine kinase MB (CK-MB), have been very well established in clinical use, so the evaluation versus this current standard is of utmost importance.

Table 1 miRNA in CVD

Disease	Regulation	miRNA	Studies/patients	Biomarker validation
Myocardial infarction	↑	miR-1, -133a, -134, -186, -208 a/b, -499	34, thereof 6 screening studies; N=3,621 myocardial infarction; N=1,489 control patients	Correlation to protein biomarkers: CK/myoglobin/ hs-TnI/BNP; correlation with severity score
	↓	–		
	↑↓	miR-663b, -126, -223, -380*, -19a, -150, -320a/b		
Heart failure	↑	miR-21, -27a, -29a, -155, -210	28, thereof 10 screening studies; N=1,011 HF, N=456 ICM, N=371 AS, N=131 DCM; N=648 control	Correlation to NT-proBNP; correlation to NYHA
	↓	miR-1, -142, -150		
	↑↓	miR-133a/b, -146a, -423		
Coronary artery disease	↑	miR-21, -133a/b, -199a	10, thereof 3 screening studies; N=125 unstable AP, N=735 stable CAD; N=208 control patients	Correlation with severity score
	↓	miR-145, -155		
	↑↓	miR-92a, -126		
Aortic aneurysm	↑	–	4, thereof 3 screening studies; N=140 AAA/TAA; N=92 control patients	–
	↓	miR-15a, -21, -29a, -124a, -143, -145, -155, -223		
	↑↓	–		
Paod	↑	–	4, thereof 2 screening studies; N=189 PAOD; N=214 control patients	–
	↓	let-7e		
	↑↓	miR-15a/b, -16, -27b		
Stroke	↑	miR-21, -151a	9, thereof 3 screening studies; N=956 stroke; N=473 control patients	Correlation to traditional risk factors; correlation to diffusion MRI
	↓	miR-126		
	↑↓	miR-16, -30a, -106b, -320d		

The table lists all miRNAs that have been reported in more than two independent studies and shows the reported direction of regulation (↑, up; ↓, down). ↑↓ implies that results from two or more studies have been heterogenous. The number of studies includes those focusing on preselected miRNAs and those with array based screening. The validation column shows the reported test against established biomarkers. The table is a synopsis of *Table S1*. miRNA, microRNA; CVD, cardiovascular diseases; HF, heart failure; CK, creatine kinase; TnI, troponin I; ICM, ischemic cardiomyopathy; AS, aortic stenosis; DCM, dilated cardiomyopathy; AP, angina pectoris; CAD, coronary artery disease; AAA, abdominal aortic; TAA, thoracic aortic; PAOD, peripheral arterial occlusive disease; MRI, magnetic resonance imaging; NYHA, New York Heart Association.

Table 2 lncRNAs in CVD

Disease	lncRNA	Number	Probe	Patient number	Validation	Reference
Myocardial infarction	HIF1A-AS2 ↑, ANRIL ↓, KCNQ1OT1 ↑, MIAT ↓ (STEMI), MALAT1 ↑	5	PAX blood + PBMC	274 STEMI/140 NSTEMI/86 control	Correlation to TnI	Vausort 2014
Heart failure	LIPCAR ↑	Screen: 33,045; valid: 7	Plasma	Screening: 15/15 high/low LVM; validation: 87 ICM w/139 ICM w/o LVM	Associated with future cardiovascular death	Kumarswamy 2014
Thoracic aortic aneurysm	HIF1A-AS1 ↑	–	Serum	50 TAA/50 control	–	Zhao 2014
Coronary artery disease	CoroMarker ↑	5	EV + PBMC	221 CAD/187 control	Expression analysis vs. a variety of other cardiovascular diseases	Yang 2015

The table shows the name of the lncRNA, the number of candidates investigated in the study, sample type and the patient cohorts as well as validation *vs.* commonly used biomarkers. lncRNA, long ncRNA; CVD, cardiovascular diseases; HIF1A-AS2, hypoxia inducible factor 1A antisense RNA 2; ANRIL, cyclin-dependent kinase inhibitor 2B antisense RNA 1; KCNQ1OT1, potassium voltage-gated channel, KQT-like subfamily, member 1 opposite strand/antisense transcript 1; MIAT, myocardial infarction-associated transcript; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; PBMC, peripheral blood mononuclear cell; TnI, troponin I; LIPCAR, mitochondrial long noncoding RNA uc022bqs.1; LVM, left ventricular remodeling; ICM, ischemic cardiomyopathy; HIF1A-AS1, hypoxia inducible factor 1A antisense RNA 1; TAA, thoracic aortic; EV, extracellular vesicle; CAD, coronary artery disease.

Most studies were focused on the group miRNA-1, -133a/b, -208a, -499. These so-called *myomirs* are heart specific, due to their regulatory interaction with the transcripts of different cardiac muscle myosin chains, similar to CK-MB (*Table 1*) (26). Changes in the circulatory levels of these miRNAs specifically represent heart tissue. A small number of screening studies did not lead to further validated MI specific candidates, and only miRNA-134 and miRNA-186 were detected in more than two separate studies (27-29). While there appears to be no heart specific context for miRNA-186, miRNA-134 has been suggested as a promoter of cardiac progenitor cells *in vitro* (30). All studies report higher abundance of those candidates in blood of MI patients, however receiver-operator curve (ROC) analysis of the predictive power of miRNA changes did not outperform classic TnI/T or CK-MB in most studies, and was shown to be inferior in the biggest cohort with 1,155 prospective patients with acute onset of chest pain (*Table S1*) (31). In addition, this cohort attributed a great predictive power for MI lifetime risk to circulating miRs-126, -223 and -197 after ten years follow-up (32).

Release kinetics have been closely studied by Liebetrau *et al.* in an experimental setting, revealing significant elevation after 15 minutes and a peak at 85 minutes for miRNA-1 and -133, in close correlation with TnI/T, but without additional benefit. Other studies showed earlier peaks for miRNA-133a/b, -208 and -499 (33-36). Similar results were also demonstrated for heart specific enzymes and miRNAs in marathon runners, before and after aerobic exercise (37). miRNA-499 levels may, however, be predictive of one-year mortality after MI, whereas miRNA-208a levels may be predictive of 30-day mortality (31,38). Additionally, miRNA-133a, -499 levels were shown to correlate with CAD severity, based on percentage and number of occluded coronary vessels (Gensini score) (39,40).

In summary, these results demonstrate very well the utility of circulating miRNAs as specific biomarkers for mechanistically well-defined candidates. The *myomir*s, however, are currently not superior to protein biomarkers for diagnosing MI. Their predictive power for specific "after-effects" requires further evaluation and research.

Coronary artery disease (CAD)

For discriminating unstable angina pectoris (AP) and stable CAD, higher levels of miRNA-21, -133a/b, -199a, and lower levels of -145, -155 were suggested (*Table 1)*. Until now, only a small number of 125 patients with unstable AP, combined in ten studies, have been analyzed, requiring confirmation in larger cohorts for more reliable findings when facing clinical decision making.

Most studies failed to show discriminatory power for either a single or a subset of circulating miRNAs *(Table S1*). However, lower levels of miRNA-145 and -155 showed an inverse correlation with CAD severity scores (Gensini and SYNTAX), and thus can add to the above-mentioned miRNA-133a, -499 correlations with vessel calcification (41,42). Interestingly, miRNA-155 was shown to be down-regulated in the plasma and tissue of AAA patients, and up-regulated in the plasma and tissue of heart failure patients (43-45). *In vitro* studies suggest a cell-specific pro-atherogenic effect under adaptive angiogenesis (46). Proliferative effects of miRNA-21, -199a, and anti-proliferative effects of miRNA-145, add to the possible concerted hyperplastic ability of these candidates (47).

All current studies are unable to provide sufficient statistical power to detect unstable AP in a clinically useful setting. The suggested candidate miRNAs have, however, a high potential to achieve this in larger trials, based on their experimentally proven role in atherosclerosis.

Heart failure and remodeling

Heart failure has been extensively studied for the involvement of miRNAs, and the first pre-clinical trials on therapeutic applications come from this field. Their use as biomarkers was addressed in over 25 studies. However, these often lacked sufficient power due to the inclusion of heterogeneous clinical phenotypes, such as ischemic, obstructive and dilative cardiomyopathy in relation to aortic stenosis (AS), congenital or post-MI origin (*Table 1*).

High circulating levels of miRNA-21, -27a, -29a, -155, -210, and low levels of miRNA-1, -142, -150, singly or in different combinations, could be demonstrated to correlate with the established protein biomarker for HF and BNP (*Table S1*). While miRNA-1, -21, -29a, -155, -210 have more or less defined roles from coherent *in vitro* and tissue studies, little is known about the other candidates (48,49).

Identification of HF in dyspneic patients could not be achieved by circulating miRNAs. Some candidate levels did however correlate well with disease severity indicated by NYHA stage (50-52). A lower expression of miRNA-150 was consistently associated with worse left ventricular function (53,54). Confounding evidence exists for the cardiac miRNA-133a, which was found at higher or lower expression levels in two independent cohorts of

246 and 64 patients. In both studies, its expression levels were associated with a worse outcome for LVM (55,56). In addition, subgroup analysis for AS indicated higher circulating levels for pro-fibrotic miRNA-21 (57,58).

In summary, the very well-studied influence of miRNAs on cardiac remodeling is currently poorly reflected by their applicability as biomarkers for disease, or severity predictors in heart failure.

Stroke

Although the most frequent outcome of CVD, only few studies have investigated miRNA biomarkers in stroke (*Table S1*). Among them, no prospective cohorts were studied, and only patients with the diagnosis of stroke by traditional means were included.

Leung *et al.* suggested a signature to discriminate between hemorrhagic and ischemic stroke, which was not, however, reproducible by others (59). Comparative analysis of blood and CSF has been confounding, suggesting a distinct role for the blood brain barrier in circulating miRNA shuttling (24,60). Coherent reports revealed up-regulation of miRNA-21 and -151a in patients with ischemic stroke (*Table 1*) (24,61,62). miRNA-21 elevation has also been shown in patients with carotid artery disease, and therefore been attributed with a predictive power for cerebrovascular events (63). The combination with miRNA-151a might be of interest, since it is encoded in the *PTK2* gene, which is eventually triggered in response to neuronal damage (64).

Currently, no correlation for circulating miRNA in the detection or outcome after stroke exists.

Aneurysm disease

Aneurysm disease is mostly investigated in the setting of thoracic aortic, abdominal aortic (TAA/AAA), or intracranial aneurysms (ICA), via array-based candidate screening in small patient cohorts.

No coherent results from two studies of ICA were reported (*Table S1*). A subset of miRNA-15a, -21, -29a, -124a, -143, -145, -155, -223, however, showed down-regulation in AAA serum (*Table 1*) (45,65). Among them, miRNA-21 and -145 had a lower expression in AAA and TAA, despite the different embryologic background of these distinct parts of the aorta (66). Parallel tissue analysis revealed that miRNA-29, -124a, -155, and -223 were also repressed at the cellular level, whereas miRNA-21 expression was enhanced when compared to non-aneurysmatic controls (67). Additionally, a distinct role has been attributed to the miRNA-29 in AAA development (68). Apart from miR-21, detailed mechanistic studies are currently missing (67).

Future biomarkers on this subject should address a correlation with aneurysm size and rupture rate as eventual predictors of expansion and fatal outcome, as well as indicating patients prone to aneurysm development not only at aortic locations. This is of special interest, since there is currently no biomarker available for this clinically most-relevant purpose.

Peripheral arterial disease

Four independent studies on PAOD and critical limb ischemia (CLI) have not been able to identify an miRNA signature specific for the distinct clinical problems associated with this disease. These include stenosis-rate, localization, re-stenosis and ischemia. Only let-7e was reported to be lower in expression in the serum of PAOD patients in two studies (*Table 1*) (69,70). Despite its eventual role as a regulator of angiogenesis at the level of endothelial cells, little is known about its involvement in CVD (71).

Venous disease

Venous disease has been very sparsely addressed in two studies profiling patients after venous thromboembolism, and after pulmonary embolism (*Table S1*) (72,73). Unfortunately no matching candidates were found. Zhang *et al.*, however, identified an up-regulation of miRNA-210, a known promoter of cell survival, in the plasma of eight patients with radiographic cerebral AV-malformation, thereby providing a link between the venous to the arterial side, in which miRNA-210 is involved in various conditions (*Table S1*) (74).

Diabetic vascular manifestations

In the context of diabetes, miRNAs are most often studied for disease identification (75). Despite the manifold heterogeneous aspects and complications of the disease, a few studies have focused on vascular manifestations other than diabetic retinopathy.

Peng *et al.* identified a correlation between urine miRNA-29a and albuminuria in a total of 83 type II diabetic patients. Pro-fibrotic miRNA-29b correlated inversely with carotid

intima-media thickness, further suggesting a kidney-independent clearance of these miRNAs (68,76). Neointimal hyperplasia after coronary artery stenting, and its response to therapy after oral pioglitazone, an insulin sensitizer, was found to correlate with serum levels of miRNA-24 in 72 diabetic patients (77). This short ncRNA is a regulator of cytokine synthesis in macrophages, and migration in aortic smooth muscle cells (78). Finally Caporali *et al.* showed in 11 diabetic CLI patients, a concordant elevation in the serum and tissue levels of miRNA-503, a transcript with anti-proliferative effects, but with no known functional role in the field of CVD (79).

Other CVD

Besides these more or less frequent CVDs, a few pilot studies have been performed on more rare diseases, or special settings of pathologies (*Table S1*). In a study of 37 children with Kawasaki's disease, among them 50% with coronary artery aneurysm, no significantly altered miRNA could be identified (80). Dong *et al.* reported a specific signature of three miRNAs that distinguished vascular dementia from Alzheimer's disease (81). Two studies investigated serum from a total of 187 PAH patients, and suggested low miRNA-150 and high miRNA-23a correlated with survival and cardiac index respectively (82,83). The 28-day survival in critically ill patients with acute kidney injury was reported to be another predictive ability for miRNA-210 (84). Ferreira *et al.* could validate the cardiac *myomirs* to be elevated in Chagas disease associated cardiomyopathy (85).

Long non-coding RNA (lncRNA) in CVD

The number of lncRNAs studied in CVD is still very limited. Eight different transcripts have been studied as potential biomarkers in four studies with promising results (*Table 2*).

Higher levels of LIPCAR in plasma from HF patients following ICM were independently associated with an elevated risk for future cardiovascular death, and predictive for LVM (86). This effect was also reported for ANRIL, KCNQ1OT1, MIAT, and MALAT1 in a cohort of 414 MI patients (87). HIF1a-AS2, KCNQ1OT1, and MALAT1 were higher, ANRIL was lower, in patients with acute MI compared to healthy volunteers, and HIF1a-AS2 levels varied based on time of presentation after onset of chest pain. Additionally, ANRIL, KCNQ1OT1, MIAT, and MALAT1 had good predictive power to distinguish between ST-elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI). CAD could be distinguished with high sensitivity from other CVD in the serum of 221 CAD patients, compared to 187 controls, by higher plasma levels of CoroMarker. This is a novel transcript without any determinable annotation (88). Finally, HIF1a-AS1 serum levels were significantly increased in 50 patients with TAA (89).

Little is known about the specific functions of these transcripts within the heart or vascular tissue. MIAT expression has been linked to genetic susceptibility for MI, and might act as a competing endogenous RNA for various targets (90,91). ANRIL was shown to be highly expressed in atherosclerotic plaques, and might be a "fine-tuner" within the inflammatory NF-κB pathway, by acting as an antisense regulator to the CDKN2B-CDKN2A gene cluster at the 9p21 locus (92,93). KCNQ1OT1 is an antisense transcript to KCNQ1, an epigenetic regulator of many targets, known from its pathogenic role in Beckwith-Wiedemann syndrome (94). HIF1a-AS1 and -AS2 are also antisense transcripts modulating HIF1A, a pro-angiogenic and anti-apoptotic gene up-regulated in many, if not all, CVDs (89).

ncRNA for therapy monitoring

Therapy monitoring via ncRNA in CVD has so far only been reported in pilot studies, but nevertheless has great potential for future investigations.

Three studies have investigated the predictive role of miRNAs for rejection and graft failure after heart transplantation. Duong Van Huyen *et al.* screened a total of 113 heart transplant patients, and correlated serum and biopsy specimen miRNA levels from 30 subjects suffering from graft rejection. Four candidates were differentially expressed in blood and tissue coherently, and suggested as early markers for graft rejections, with special interest towards miRNA-155 up-regulation, which is also found in the failing heart (*Table 1*) (95). Wang *et al.* reported that preselected miRNA-133a/b, -208a levels in a very small set of seven patients were superior to TnI in predicting early graft failure in association with MI (96). Another seven candidates were found to be up-regulated in the same setting by Sukma *et al.*, with no overlap with the previous studies or heart-specific miRNAs (97).

Willeit *et al.* discovered miRNAs with dose- and substance-specific expression during anti-platelet therapy, suggestive of a potential role for monitoring drugs that affect platelet function (98). Positive thiazolidinedione

treatment response in type II diabetics was indicated by a lower expression of miRNA-320a, known to be elevated in hyperglycemic individuals (99,100).

Limitations in circulating ncRNA

All investigations reported so far have weaknesses in study design and applied methods, emphasizing a need for large multicenter trial cohort studies with, ideally, a screening and a validation cohort.

Only very few studies screened for multiple ncRNAs, due to high costs of array based investigations, but rather focused on preselected candidates from previous tissue profiling evaluations *(Tables 1*,*S1*). Cellular and extracellular signatures, however, cannot be expected to match completely, due to tissue-, cell-, and compartment-specific expression levels, and differing functions (45,65,95). Several other factors have been shown to influence ncRNA serum levels, some of which have great importance when investigating CVD. In particular, anti-platelet medication, heparin and statin treatment might influence circulating miRNA levels and release kinetics (101,102). In patients with end-stage kidney disease and eventual dialysis, the validity of circulating miRNAs is controversial, and warrants further research (56,103). From a more general perspective, age, sex, and smoking have been identified as confounders of circulating miRNA and microparticle distribution (104-106).

Especially for acute events, the timing and site of blood collection remains important, since the circulating transcriptome changes rapidly, and their levels might be altered during the passage from the arterial to the venous side (33,107,108). Furthermore, eventual heterogeneity of expression among different ethnic groups has to be taken into account (109,110). A major shortcoming in miRNA biomarker research is the difficulty of standardization for endogenous controls, which differs tremendously among all reported studies (111). Whether or not these concerns are also valid for circulating lncRNAs remains to be elucidated.

Therefore, discovery and evaluation of a more generalized biomarker in the complex regulatory network of disease, apart from generic markers of cellular damage and apoptosis (e.g., TnI or transaminase levels) requires elaborate study preparation, execution, and analysis.

Conclusions

In the emerging field of circulating ncRNA as biomarkers in CVD, the most persuasive results have come from plasma studies in MI and HF. In these conditions, tissue-specific signatures of miRNA expression levels in particular have proven equal to protein biomarkers in their great potential for predictive clinical use. The main reason for this is the well-advanced mechanistic understanding of certain ncRNAs, and how they are regulated (and regulating) under physiologic as well as pathologic conditions.

Probably the greatest clinical need for ncRNA biomarkers can be found in disease settings for which currently no alternatives are available. Disease development, outcome prediction and treatment response are such areas, especially when complicated pathologies require stratification of complex and cost-worthy treatment strategies. In addition to the sheer information, whether a patient has an acute MI or not, a disease-specific signature of ncRNAs could provide distinct information about localization and lesion area, the number of obstructed vessels, and early and/or late ischemia-related mortality. For this purpose, future studies require the necessary power, patient characteristics, uniform disease definitions, and ideally, parallel tissue expression detection for mechanistic purposes. If all these criteria are thoughtfully taken into account, the ncRNAs certainly have the ability to optimize biomarker applications in a time of evolving personalized medicine.

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Footnote

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Supplementary

Table S1 Comprehensive view of miRNA in CVD

Disease	ncRNA	Number[#]	Probe	Patient number	Biomarker validation	Reference
Myocardial infarction	miRNA-1↑	1	Plasma	93 MI/66 control	No correlation to TnI/CK-MB	Ai 2010
	miRNA-1↑; -133a↑; -499↑; -208a↑	4	Plasma	33 MI/30 control	–	Wang 2010
	miRNA-1↑; -133a↑; -133b↑; -499↑; -122↓	7	Plasma	33 MI/17 control	Correlation to TnI	D'Alessandra 2010
	miRNA-499↑	1	EDTA plasma	14 MI/15 CHF/10 control	–	Adachi 2010
	miRNA-1291↓; -663b↓	866	PAX blood	20 MI/20 control	Correlation to TnI	Meder 2011
	miRNA-126↑; -223↓; -197↓	19	Microparticles	820 pt (Bruneck cohort)	–	Zampetaki 2012
	miRNA-133a↑	1	Serum	126 MI	Correlation with cMRI markers; no independent outcome prediction	Eitel 2012
	miRNA-155↑; -380*↑	667	Serum	14 MI: 7 death/7 no event	–	Matsumoto 2012
	miRNA-30a↑; -195↑; let-7b↓	3	Plasma	18 MI/30 control	–	Long 2012
	miRNA-1↑; -126↓	2	Plasma	17 MI/25 control	Correlation to TnI	Long 2012
	miRNA-636↓; -7-1*↓; -380*↓; -1254↓; -455↓; -566↓; -1291↓	-	PAX blood	18 MI/21 control	–	Vogel 2013
	miRNA-1↑; -208b↑; -499↑	3	Serum 2 sites	319 MI/88 non MI	Inferior to TnT	Gidlöf 2013
	miRNA-133a↑	1	Serum	13 MI/176 AP/127 control	Correlation to TnI; mir-133a correlates to CAD severity (Gensini score)	Wang 2013
	miRNA-208a↑; -1↑; -133a↑	4	Serum	21 TASH procedure	Different release kinetics compared to hs-TnT	Liebetrau 2013
	miRNA-1↑; -134↑; -186↑; -208↑; -223↑; -499↑	-	Serum	117 MI/182 CAD/100 control	Panel of six mirnas superior to predict MI	Li 2013
	miRNA-1↑; -133a↑; -208b↑; -499↑	4	EDTA plasma	67 MI/32 control	Inferior to TnT	Li 2013
	miRNA-19a↑; -1↑	2	–	156 MI/145 control	Superior to CK/CK-MB/MYO/hs-TnI/BNP	Wei 2014
	miRNA-19a↓; -19b↓; -132↓; -140↓; -150↓; -186↑; -210;	667	EDTA serum	105 MI/141 control	Inferior to TnI Panel of mir-132/-150/-186 has high discriminatory power for MI	Zeller 2014
	miRNA-21↑; -361↑; -519e↓	3	EDTA plasma	17 MI/28 control	Correlation to TnI	Wang 2014
	miRNA-1↑	1	EDTA plasma	56 MI/28 control	Inferior to TnI	Li 2014
	miRNA-497↑	1	Serum	27 MI/31 control	Superior to TnI	Li 2014
	miRNA-486↑; -150↑; -126↓; -26a↓; -191↓	270	Serum	39 MI/39 control	–	Hsu 2014
	miRNA-133↑; -1291↑; -663b↑	3	Plasma	76 MI/110 control	–	Peng2014
	miRNA-328↑; -134↑	2	EDTA plasma	359 MI/30 control	Inferior to hs-TnT	He 2014
	miRNA-320b↓; -125b↓	77	EDTA serum	178 MI/198 control	No correlation to TnI/CK-MB	Huang 2014
	miRNA-499↑	2	–	54 MI: death/88 MI: survival	Superior to TnT in discriminating MI survival	Olivieri 2014
	miRNA-323↑, -652↑; -27b↑	375	EDTA plasma	235 MI/100 control	–	Pilbrow 2014
	miRNA-499↑	3	–	30 on-/off-pump CABG pt, 120 pt prospective cohort	Correlation to TnI; peak significantly earlier (3 vs. 6 h)	Yao 2014
	miRNA-208a↑;	1	Plasma	19 MI/20 control	Correlation to TnI/CK-MB; earlier peak	Bialek 2015
	miRNA-208b↑; -499↑; -320a↑	6	Serum	1155 chest pain pt, thereof 224 confirmed MI	Inferior to TnT/hs-TnT Mir-208b correlates with early death (30 d) post MI	Devaux 2015
	miRNA-499↑	1	–	53 MI/30 control	Correlation to TnI/CK-MB; correlation to CAD severity (Gensini score)	Chen 2015
	miRNA-499↑	1	–	142 MI/85 CAD/100 control	Correlation to TnI/CK-MB; earlier peak	Zhang 2015
	miRNA-133a/b↑; -499↑	3	Serum	98 MI/23 control	Correlation to TnI; earlier peak	Ji 2015
Coronary artery disease	miRNA-126↓; -17↓; -92a↓; -155↓; -133a↑; -208a↑;	-	EDTA plasma	42 sCAD/25 control	–	Fichtlscherer 2010
	miRNA-1↑; -122↑; -126↑; -133a↑; -133b↑; -199a↑; -337↑; -433↑; -485↑	358	EDTA plasma	19 uAP/34 sCAD/20 control	No discrimination between uAP and scad;Mirna-1/-133/-126 identify uAP; mirna-1/-126/-485 identify sCAD	D'Alessandra 2013
	miRNA-106b↑; -25↑; -92a↑; -21↑; -590↑; -126*↑; -451↑	754	EDTA plasma	58 uAP/31 sCAD/37 control	Good discrimination of MIR panel for uAP vs. sCAD and control	Ren 2013
	miRNA-155↓	1	PBMC + serum	56 CAD/54 control	Inverse correlation with CAD severity (Gensini score)	Zhu 2014
	miRNA-126↑; -199a↑;	10	Arterial plasma	178 CAD	Mirna-126 and mirna-199a predict CV events	Jansen 2014
	miRNA-21↑; -100↓; -143↓; -145↓	-	EDTA plasma	51 ISR/130 non-ISR/52 con	Good discrimination for in-stent-re-stenosis	He 2014
	miRNA-145↓	1	EDTA plasma	167 CAD	Mirna-145 level correlates with CAD severity (SYNTAX score)	Gao 2015
	miRNA-30d↑; -1246↓	2042	EDTA serum	105 bifurcation lesion CAD/105 nonbifurcation CAD	–	Liu 2015
	miRNA-423↑	4	Serum + PF	16 uAP/17 sCAD	–	Miyamoto 2015
	miRNA-765↑; -149↓	2	EDTA plasma	32 uAP/37 sCAD/20 control	Good discrimination for CAD, no discrimination for uAP vs. sCAD	Ali Sheikh 2015
Heart failure/cardiomyopathy	miRNA-423↑; -129↑; -675↑; -18b↑; -1254↑;	600	Citrate plasma	42 HF/51 control	Mirna-423 levels correlate to BNP	TiJsen 2010
	miRNA-16↑; -27a↑; -101↓; -150↓	4	EDTA plasma	150 ICM (74 <40% LVEF)	Combination with BNP improves low LVEF prediction	Devaux 2013
	miRNA-133a↑	1	EDTA plasma	74 AS (40 hypertrophy)	Mirna-133a levels correlate LV hypertrophy reversibility	Garcia 2013
	miRNA-558↓; -122*↑; -520d↑	883	PAX blood	53 HFrEF/39 control	Mirna levels correlate with NYHA class	Vogel 2013
	miRNA-548c↓; -548i↓	948	PBMC	44 DCM/48 control	–	Gupta 2013
	miRNA-150↓	2,549	EDTA plasma	60 ICM (30 LV function low)	Superior to NT-proBNP to predict LV remodelling	Devaux 2013
	miRNA-210↑	1	Plasma	8 NYHA II/5 NYHA III/IV/6 control	No correlation to NT-proBNP	Endo 2013
	miRNA-103↓; -142↓; -30b↓; -342↓;	17	Plasma	44 HF (22 HFrEF)/32 COPD/59 "breathless"/15 control	Any MIR inferior to NT-proBNP and hsTnT	Ellis 2013
	miRNA-454↓; -500↓; -142↓; -1246↑	–	Buffy coat	13 HF/10 DCM/8 control	Mirna-454 and -500 levels inversely correlate with NT-proBNP	Nair 2013
	miRNA-21↑	1	Plasma + tissue	75 AS/25 control	–	Villar 2013
	miRNA-210↑; -30a↑	40	Serum	22 HF/18 control/9 fetal control	Correlation to BNP	Zhao 2013
	miRNA-423↑	5	EDTA plasma	45 DCM/39 control	Correlation to BNP	Fan 2013
	miRNA-133a↑; -423↑;	2	Plasma	246 ICM	Inferior to BNP to predict LV-remodelling or -dysfunction	Bauters 2013
	miRNA-29b↓ (AF); -29b↓ (HF); -29b↓ (AF + HF)	4	–	16 HF + AF/32 HF/17 AF/30 control	–	Dawson 2013
	miRNA-146a↑	1	Plasma	38 peripartum CM/30 HF/23 control	Significant discrimination for peripartum CM from HF	Halkein 2013
	miRNA-133a↓	1	Plasma	64 ESRD (40 LVH)/18 control	Inverse correlation with LVH; stable before/after HD	Wen 2014
	miRNA-208b↑; -208a↑; -499↑; -1↑; -133b↑;	–	Serum	24 HF/13 control	Good correlation with BNP and TnI	Akat 2014
	miRNA-1202↑; -483↑	1,113	Serum + tissue	19 HF (7 good LVAD response)	Mirna-483 inverse correlation with BNP; mirna-1202 correlates with ΔBNP for good/bad response to LVAD	MorleySmith 2014
	miRNA-27a↑; -199a↑; -26a↑; -145↑; -133a↑; -143↑; -126↑; -29a↑; -155↑; -21↑	21	Serum	41 HCM/41 control	Mirna-27a, -29a, and -199a correlate with echocardiographic hypertrophy	Roncarati 2014
	miRNA-210↑	1	EDTA serum	57 AS/10 control	Correlation to BNP	Røsjø 2014
	miRNA-1↓; -21↑	3	Serum	35 NYHAII/III/26 NYHA IV	Inverse correlation mirna-1 with NT-proBNP	Sygitowicz 2015
	miRNA-19a↑	8	Serum	32 DCM/9 control	Correlation with BNP and camp	Miao 2015
	miRNA-182↑	15,644	EDTA serum	20 NYHA II/22 NYHA III/IV/15 control	Superior to NT-proBNP; superior to CRP	Cakmak 2015
	miRNA-423↓	5	Serum	294 "breathless" (236 NYHA IV)/44 HF	–	Seronde 2015
	miRNA-22↑; -24↓; -382↓; -451↑; -21↑;	756	–	24 AS/27 control/94 AS + 101 control (w o w/o CAD)	Failure to reproduce in validation cohort	Coffey 2015
	miRNA-135b↑; -155↑; -190↑; -422a↑; -489↑; -590↑; -601↑; -1290↑	756	Myocardial biopsies	17 CVB3-PERS/36 CVB3-ELIM/6 control	–	Kuehl 2015
	miRNA-146a↓; -221↓; -328↓; -375↓; -30c↓	745	Serum	90 HFpEF/90 HFrEF/90 control	Increase of HF prediction by combination of BNP with either miRNA; miRNA-signature can distinguish between r/pEF	Watson 2015
	miRNA-29a↑ in HOCM; miRNA-29c↑ in AS	8	Serum	23 HNCM/28 HOCM/47 AS/22 control	–	Derda 2015
stroke	various dysregulated miRNAs	–	Whole blood	19 stroke/5 control	–	Tan 2009
	miRNA-210↑	1	EDTA plasma	112 stroke/60 control	–	Zeng 2011
	miRNA-30a↓; -126↓	3	-	197 stroke/50 control	–	Long 2013
	miRNA-21↑; miRNA-221↓	3	EDTA serum	167 stroke/157 control	Correlation to traditional risk factors	Tsai 2013
	miRNA-124↑; -16↓	2	EDTA plasma	74 stroke/19 hemorrhage	Discrimination between ischemic and hemorrhagic stroke	Leung 2014
	CSF: let-7c↑; miRNA-221↑; blood: miRNA-151a↑; -140↑; -18b↓	378	CSF + Whole blood	10 stroke/10 control	–	Sorensen 2014
	miRNA-106b↑; -4306↑; -320e↓; -320d↓;	1,347	EDTA plasma	136 stroke/116 control	Correlation to diffusion-weighted MRI	Wang 2014
	47 miRNAs↑; 58 miRNAs↓	102	Whole blood	169 stroke/24 control	–	Sepramaniam 2014
	CSF: let-7e↑; miRNA-338↑; blood: let-7e↑; miRNA-338↑;	2	CSF + serum	72 stroke/51 control	–	Peng 2015
	miRNA-17↑, -21↑, -106a↑, -126↑, -200b↑	5	Whole blood	–	–	Kim 2015
Aortic aneurysm	miRNA-29b↓; -124a↓; -155↓; -223↓	756	Plasma + tissue	23 AAA/12 control	–	Kin 2012
	miRNA-21↓ (TAV); -29a↓ (TAV); -133a↑ (BAV/TAV); -143↓ (TAV); -145↓ (BAV)	6	Plasma + tissue	TAA: 21 BAV/21 TAV/; 10 control	Correlation to MMP8/TIMP1/TIMP3/TIMP4	Ikonomidis 2013
	let-7e↓; miRNA-15a↓; -196b↓; -411↑	754	Whole blood	15 AAA/10 control	–	Stather 2015
	miRNA-191↑; -45↑; -1281↑	1,105	EDTA serum	60 AAA/60 control	–	Zhang 2015
Peripheral arterial occlusive disease	miRNA-130a↑; -27b↑; -210↑	–	Serum + tissue	104 PAOD/105 control	Mirna-130a and -27b correlate with Fontaine Stage	Li 2011
	Let-7e↓; miRNA-15b↓; -16↓; -20b↓; -25↓; -26b↓; -27b↓; -28↓; -126↓; -195↓; -335↓; -363↓; -720↑; -1274↑	754	PAX blood	25 PAOD/26 control	–	Stather 2013
	miRNA-15a↑; -16↑	12	Citrate plasma	20 PAOD/43 control	Correlation to disease progress and restenosis after 1 year	Spinetti 2013
	Let-7e↓; miRNA-15a↓; -196b↓; -411↑	12	Whole blood	40 PAOD/40 control	–	Stather 2015
Cranial aneurysm	Various dysregulated miRNAs	1,205	EDTA serum	151 aneurysms/27 control/17 complicated neurysms/21 ruptured aneruysms	–	Jin 2013
	miRNA-16↑; -25↑	–	EDTA plasma	20 aneurysms/20 control/20 ruptured aneurysms/93 aneurysm (second cohort)	–	Li 2014
PAH	miRNA-150↓	86	EDTA plasma	175 PAH/x control	Independent survival predictor in an eight item multivariate analysis	Rhodes 2013
	miRNA-23a↑	700	PAX blood	12 PAH/10 control	Correlation with cardiac index and higher PAP	Sarrion 2015
Carotid disease	miRNA-21↑	3	EDTA plasma	66 plaque/157 control	–	Tsai 2013
VTE	miRNA-10b↑; -320a↑; -320b↑; -424↑; -423↑; -103a↓; -191↓; -301a↓; -199b↓	742	EDTA plasma	20 VTE/20 control	–	Starikova 2015
Pulmonary embolism	miRNA-134↑	667	EDTA plasma	32 pulmonary embolism/32 control/22 dyspnea	–	Xiao 2011
Vascular dementia	miRNA-93↑; -146a↑; -143↓	–	EDTA serum	127 Alzheimer disease/30 vascular dementia	miRNA signature discriminates vascular dementia from others	Dong 2015
Endothelial dysfunction	miRNA-125a↓; -342↓; -365b↑	84	–	60 obese children	Correlation with early endothelial dysfunction in time to peak post-occlusive reperfusion assay	Khalyfa 2015
Kawasaki disease	–	650	Serum	18 KD with coronary dis/19 KD w/o coronary dis	–	Rowley 2015
Pulmonary AV malformation	miRNA-210↑	756	EDTA plasma	8 PAVM/7 control	Correlation to CT-angiography positivity	Zhang 2013
Diabetic vascular manifestation	miRNA-29a↑	3	Urin	83 T2DM (42 albuminuria)	miRNA-29a correlates with albuminuria; miRNA-29b correlates with carotid intima-media thickness	Peng 2013
	miRNA-503↑	–	Serum + tissue	11 T2DM/11 control (tissue from leg amputation/biobpsy)	–	Caporali 2011
	miRNA-24↑	5	Serum	72 T2DM w/o pioglitazone	Inverse correlation with neointimal hyperplasia after coronary stenting	Hong 2015

The table lists all miRNAs that have been reported as potential use for biomarker in CVD and their direction of regulation in disease as compared to the respective control (↑ up; ↓ down). Additionally, the number of miRNAs investigated in the study ([#]) and the compartment used to analyse is listed. miRNA, microRNA; CVD, cardiovascular diseases; ncRNA, non-coding RNA; MI, myocardial infarction; TnI, troponin I; CK-MB, creatine kinase MB; CHF, congestive heart failure; CABG, coronary arterial bypass graft; pt, patients; MRI, magnetic resonance imaging; CAD, coronary artery disease; ISR, in-stent-restenosis; TASH, transcoronar ablation of septum hypertrophy; MYO, myoglobin; uAP, unstable angina pectoris; HF, heart failure; ICM, ischemic cardiomyopathy; AF, atrial fibrillation; LVH, left ventricle hypertrophy; AS, aortic stenosis; HFrEF, heart failure with reduced ejection fraction; COPD, chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; NYHA, New York Heart Association; CSF, cerebrospinal fluid; AAA, abdominal aortic; BAV/TAV, bicuspid/tricuspid aortic valve; PAOD, peripheral arterial occlusive disease; PAH, pulmonary arterial hypertension; VTE, venous thromboembolism; KD, Kawasaki disease; T2DM, type II diabetes.