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Candidate MicroRNAs as Prognostic Biomarkers in Heart Failure: A Systematic Review

Ana Rita Gonçalves de Figueiredo

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To my family.

Acknowledgments

"If you think you are too small to make a difference, try sleeping with a mosquito."

(Dalai Lama)

"The Journey of a thousand miles begins with one step"

(Lao Tzu)

The years of master's degree were the most challenging and accomplished years of my life. I'm very grateful to my family, teachers and friends who always encouraged me and never let me give up. Thank you.

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To my colleagues, specially Isilda for your good mood and positivity.

And finally, to my family, who never let me down, and always encourage me to do better than the day before.

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List of Abbreviations

AF	Atrial Fibrillation
AHF	Acute Heart Failure
ANP	Atrial natriuretic peptide
BMI	Body Mass Index
BNP	B-type natriuretic peptide
BNP-PV	Brain natriuretic peptide from peripheral vein
CHF	Chronic Heart Failure
CK-MB	Creatine kinase, muscle and brain
COACH	Coordinating Study Evaluating Outcomes of Advising and counselling in Heart Failure
CRT	Cardiac-resynchronization Therapy
CV	Cardiovascular
DM II	Type 2 Diabetes Mellitus
DMC	Dilated Cardiomyopathy
eGFR	Estimated Glomerular Filtration Rate
Gamma-GT	Gamma-glutamyl Transferase
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HR	Hazard Ratio
ICM	Ischemic Heart failure
LV	Left Ventricle
MiR	MicroRNA
MiRs	MicroRNAs
NO	Nitric Oxide
NP	Natriuretic Peptides
NT-proBNP	N-terminal proBNP
NYHA	New York Heart Association
OR	Odd Ratio

PROTECT	Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function
RR	Relative Risk
VEGF	Vascular Endothelial Growth Factor

Abstract

Background: Heart failure (HF) is a high prevalent syndrome with significant burden worldwide. BNP and NT-proBNP are the gold standard biomarkers in the management of lure. Although useful in clinical practice, they present limitations as their expression might be influenced by ventricular function, aging, obesity, renal failure and atrial arrhythmias. MicroRNAS have recently emerged as potential diagnostic and prognostic biomarkers given they are related with cell growth, proliferation, differentiation, and metabolism. Increasing research pointed some miRs for their potential as HF biomarkers. However, different study designs, methods and study groups led to inconsistent results.

Methods and Results: We performed a systematic search of available literature on Pubmed and Scopus reporting the prognostic value of on microRNAs in HF, followed by a review of risk of bias, according to Quadas Group Standards. Simultaneously, miRs potential as differential diagnosis and severity biomarkers was also performed. Studies have described circulating miR as potential as diagnostic, prognostic, and severity markers. Mir-622, -519 and -499 were significantly related with HF with reduced ejection fraction (HFrEF), whereas miR-22-3p revealed greater ability as a severity biomarker. Let-7i-5p, miR-223-5p, miR-423-5p, miR-21, miR-1306-5p and miR-122 serum expressions presented a consistent correlation with HF prognosis.

Conclusion: Several miRs were related with HF pathophysiology and demonstrated potential as biomarkers for disease progression. MiRs promising role in HF although unquestionable, requires a deeper and broader understanding about their role and function for future research.

Keywords: heart failure, biomarkers, prognosis, microRNA, circulating microRNA.

1. Introduction

Heart failure (HF) affects 38 million people worldwide, impacting the lives of more than 10% of population over 70 years contributing significantly to hospitalisation rate increase, challenging economic and healthcare systems [1-4]. HF syndrome is characterized by a complex interplay among genetic, neurohormonal, inflammatory and biochemical changes that affect cardiac cells and insterstitium and perpetuate cardiac injury [5-7].

Currently, the natriuretic peptides (NP) are the gold standard serum biomarkers in HF [8, 9]. Atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are essential in the diagnosis and prognosis of HF, and may serve as therapy guide [10]. Prognostic efficiency of BNP and NT-proBNP has been stated in the literature [9]. Indeed, ADHERE study discovered a linear relationship between BNP expression and in-hospital mortality in acute HF (AHF) patients [11]. A meta-analysis of Doust *et al* also found that BNP increase by 100 pg/ml was associated with 35% in the relative risk of death [12]. However, their clinical use has relevant limitations. Ventricular function, aging, obesity, renal failure, atrial arrhythmias may influence clinical interpretation of natriuretic peptides [9].

MiRs are small-non-coding RNAs [13] produced by all cell types which, ultimately, are secreted in blood [14]. The main role of miRs is to regulate the out-put post-transcriptionally proteins and, therefore, they are related with cell growth, proliferation, differentiation, and metabolism [15, 16]. Small non-coding RNAs dysregulation was firstly associated with cancer [17] but, recently, several variations in miRs expression have been implicated in HF [2, 18]. Increasing research highlighted some miRs for their potential as HF biomarkers (miR-423, let-7i-5p, miR-223-5p, miR-1306-5p and miR-22-3p) with inconsistent results. Several articles showed a positive association while others found a negative association or no associations between miRs expression and poor prognosis. These results disparities between miRs and the HF prognosis, hamper a clear assessment of miR biomarker potential, and manifest the need of a systematic research.

In this study, our aim is to identify which miRs are more suitable as biomarkers in HF prognosis by performing a systematic review of the present literature, providing quality evidence for future research.

2. Methods

2.1. Data sources and search strategy

We performed a systematic search of PUBMED and SCOPUS for all studies with prognostic evaluation of microRNAs in HF patients, including all stages of HF and clinical settings.

Publications were limited between 1 January 2010 and 31 July 2020.The search strategy on PUBMED included a MESH search: (("Biomarkers"[Mesh]) OR ("MicroRNAs"[Mesh] OR "Circulating MicroRNA"[Mesh])) AND ("Heart Failure"[Mesh] OR "Heart Failure, Diastolic"[Mesh] OR "Heart Failure, Systolic"[Mesh]), and the filter English was not used. The search strategy for SCOPUS encompassed key-words search: ALL ("microRNAs") OR ("circulating microRNAs") AND ("heart failure") AND (Limit-To (DOCTYPE, "ar")) AND (Limit-To (SUBJAREA, "medi")). Authors were contacted when relevant for missing prognostic performance data. All publications captured were collected in EndNote. After removing duplicates, publications were reviewed by title and abstract to meet the inclusion criteria by one reviewer (R.F.), before full-text screening. Secondly, studies were assessed for risk of bias by R.F., R.A., C.S. PRISMA was used to include all items that should comprise a systematic review [19].Tools recommended when producing a systematic review were adopted (QUADAS-2 [20] and Cochrane Handbook for Systematic Reviews for Diagnostic Test Accuracy [21]).

2.2. Inclusion criteria

Time period. Studies published from 1 January 2010 to 31 July 2020 in English to focus on recent evidence written in English and concerning human participants.
Biomarker types and samples types. Studies focusing on microRNA as individual biomarkers or multiple biomarkers were included. Studies that did not report statistical significance or quantitative prognostic measures (Hazard Ratio (HR), Odd Ratio (OR), or Relative Risk (RR)) of the biomarker's prognostic

performance were excluded. Studies measuring miR as biomarkers in other than serum or plasma samples were excluded. Studies using other method than RT_PCR for miR quantification or studies that used RNU6 as normalizer were excluded.

Study population. Studies in adult subjects with HF diagnosis were included. To minimize the risk of excluding promising early-stage research studies, the inclusion cut-off was studies with >50 human participants in total. There were no criteria for the number of participants per group.

Study types. Studies of any design's cohort, and cross-sectional were included. As recommended by Cochrane Handbook for Systematic Reviews for Diagnostic Test Accuracy, we excluded all case-control design studies [21]. Reviews, systematic-reviews, meta-analysis, books and documents, case reports, comments, news, and editorials were excluded.

2.3. Data collection and quality control

To assess miRs true potential in HF, we analysed miRs expression as: (i) potential discriminator between HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF); (ii) marker for HF severity (including associations between echocardiographic measurements, NYHA class and NP).

Lastly, to evaluate miRs capacity as prognostic biomarkers, three outcomes were established: (i) Cardiovascular hospitalisation (CV hospitalisation) (including the risk of HF patients being admitted or re-admitted into the hospital); (ii) Cardiovascular hospitalisation and/or death (including the risk patients with HF diagnosis being hospitalised/re-hospitalised or death after HF diagnosis) and (iii) all-cause death (including HF patients risk of death, regardless of the cause).

The data collection from included articles comprised: the study design; study population; number of subjects; female and male percentage; mean age ±SD; initial diagnosis; variables included in multivariate models (table 1) and the association measures (HR, OR and RR) for different outcomes and statistical analysis performed (univariate or multivariate).

To assess the studies quality and identify potential bias, we applied QUADAS-2 tool. Considering the risk of bias analysis, study design, patient selection, miR index test and standard reference, studies were scrutinized for each outcome of interest.

3. Results

A total of 52 articles were identified and in 11 of them we performed a prognostic performance evaluation of miR. The included articles have low risk of bias and alike regarding disease and miR being tested. Thus, we classified the selected studies per outcome, as presented in table 1.

3.1. MiRNAs in HFpEF vs HFrEF

Only a few studies analysed miRs as potential discriminators of HF. Authors have hypothesized miR different roles in HFrEF and HFpEF. In fact, Watson *et al* showed that the serum expression of miR-30c, miR-221, miR-328, and miR-375 was able to distinguish HFrEF from HFpEF [22]. Wong *et al* performed the same analysis identifying four miR that can differentiate HF identities (miR-125a-5p, -190a, -550a-5p, and -638) [23]. In HFrEF patients, only miR-499a-5p was found to be positively associated with all-cause mortality and readmission for HF [24].

3.2. MiRNAs and HF severity

Previous literature suggests that specific miR could help in HF severity evaluation. From the articles included in our review, only Klenke *et al* did not find any association [25]. Vogel *et al* concluded that miRNAs expression patterns might correlate with different HF stages or severities. Also, significant correlations between left ventricular (LV) EF and miR-622, miR-520d, miR-519, miR-200b, miR-122, and miR-588 were found [26]. MiR-150-5p expression was significantly correlated with symptoms severity and adverse remodelling degree. Also, miR-150-5p was inversely correlated with NYHA classes (p=0.004) and log NT-proBNP) [27]. MiR-21 and miR-132 expression levels increase along with NYHA grade and consequently with HF severity [24, 28]. On the other hand, negative correlations between miR-145 and cardiac function were found and lower levels of miR-22-3p and Ln_miR-145 (p<0.0001) were associated with HF worsening [24, 29]. Ovchinnikova *et al*, found that AHF is associated with the analysed miR (let-7i-5p, miR-18a-5p, miR-18b-5p, miR-223-3p, miR-301a-3p, miR-423-5p miR-652-3p) by a consistent pattern of decreased miR expression levels with increased HF severity, reaching the conclusion that miR expression levels were

higher at discharge than at admission [30]. In 2017, Vegter *et al* discovered that miRNA levels decreased in parallel with clinical manifestations of atherosclerotic disease, and therefore contributing for HF severity [31].

3.3. Prognosis

3.3.1. Cardiovascular hospitalisation

A total of 5 articles analysed the association between cardiovascular hospitalisation and miR plasma expression [24, 28, 31-33], as shown in table 2. Three articles demonstrated that higher levels of miRs were associated with higher risk of hospitalisation (miR-106-5p, -223-3p, -27a-3p, -16-5p, let-7i-5p, - 1306-5p and -21 with HR of 1.694, 1.478, 1.482, 1.763, 2.058, 1.22 and OR: 1.16, $p<0.05$ respectively, table 2) [28, 31, 34]. Vegter *et al* studied several miR for 18 months and found, after a multivariate analysis, a set of 5 miR that are significantly predictive of CV hospitalisation [31]. Zhang *et al* explored the association between miR-21 level coronary sinus and peripheral venous blood in a follow-up period of 24 months, showing that higher levels of miR-21 in coronary sinus serum was associated with an increased risk of hospitalization (OR:1.160, $p=0.0021$) [35]. By contrast, Masson *et al* showed a negative correlation between CV hospitalisation and miR-132 blood expression in 953 patients over 46.2 months (HR: 0.79, $p=0.01$) [32]. Also, Seronde *et al* demonstrated that miR-423-5p might have a protective role in CV hospitalisation in a test cohort with AHF patient, however, this data was inconclusive using a larger validation cohort (236 patients in test cohort vs. 711 patients in validation cohort), (OR:0.82, $p=0.48$, respectively) [33].

3.3.2. Heart failure rehospitalization and/or death

Four articles provided the value of miRs to predict HF rehospitalisation and/or death (table 3) [24, 31, 32, 34, 36]. Among these, three articles estimated a positive correlation [31, 34, 36] and one achieved a negative correlation [24]. MiR-22-3p was inversely associated with the outcome (HR 0.61, $p=0.001$) [24]. On the other hand, miR 106a-5p, miR-1306-5p, miR-320a, miR-378a-3p, miR-423-5p and miR-1254 were described by included articles as positively correlated with outcome (HR: 1.38; >1.11; 1.10; 1.03; 1.05; and >1.1, respectively) [31, 34, 36].

3.3.3. All-cause death

All-cause death was analysed by 10 articles [25, 28, 30-34, 36-38]. Five articles found a positive correlation between miR expression and outcome [28, 30, 34, 36, 38], four found a negative correlation [25, 32, 33, 37] and two did not find any association between analysed miR and all-cause mortality [31, 32] (table 4).

Vegter *et al,* despite several analysis, did not find any significant miR correlated with all-cause death [31]. MiR-423-5p, miR-192, and miR-30d were individually analysed in HF patients and, after multivariate analysis, a negative correlation was found [25, 33] (table 4). Contrarily, Stojkovic found positive correlations between the outcome and miR-423 and miR-122 in HFrEF patients (HR: 1.15, p=0.021 and 1.27, p=0.001, respectively) [38]. Also, Bayés-genis *et al* completed a multicentred study where a set of miR with positive correlation with outcome (miR-1254, miR-133b, miR-622, miR-208a-3p) were identified (HR: ≥1.19, ≥1.18, ≥1.12 and ≥1.32, p≤0.05, respectively) [36]. Additionally, Ovcchinnikova *et al* using univariate analysis, studied several miRs, in 100 AHF patients, identifying seven miRs (let-7i, -18a-5p, -18b-5p, -301a-5p-423-5p-652-3p) with mortality prediction potential (all HR >1.5, p≤0.05). Also, Vegter *et al* and Van Boven *et al* identified miR-21 and miR-499a as a mortality predictors (relative risk (RR):1.936, p=0.001 and HR: 2.04, p≤0.05, respectively) [34, 35].

4. Discussion

In this systematic review several miRs were identified as potential biomarkers for HF diagnosis, prognosis and disease severity, supporting miR's pivotal role in cardiac physiology.

Several miR were related with HF. **MiR-21, miR-22** and **miR-132** were described as important biomarkers in HF pathophysiology and progression. They are also clinically correlated to New York Heart Association (NYHA) classes, volume status and fluid overload. Although none of the studied miR evidenced better potential as a biomarker, after combining miR expressions with BNP and NT-proBNP, their discriminating power to distinguish HFrEF from HFpEF was prominent, and even better than NP. **MiR-423, let-7i-5p, miR-223-5p, miR-1306-**

5p and **miR-22-3p** were analysed by more than one article and described as potential biomarkers for HF prognosis.

4.1. MicroRNAs in HFpEF vs HFrEF

HFpEF and HFrEF cannot be differentiated on clinical grounds and imaging tests are essential for a correct diagnosis. The use of NP lacks specificity and does not allow a correct differentiation of HF subtypes [22]. Seventeen miRs were described as being able to distinguish HFrEF and HFpEF, whereas several correlated with echocardiographic measurements [26, 27, 29].

MiR-622, -519 and -122 were significantly related with HFrEF. High expression levels of miR-622 and-519 were found in granulocyte cells by Vogel *et al*, reinforcing the inflammatory processes involvement in HF development and progression. MiR-122 is originated in hepatic cells and has been related with HF due to diminished cardiac output, resulting in liver congestion [26, 39, 40]. Nonetheless, only **miR-328, miR-375 and miR-499** expression levels were significantly different between HFrEF and HFpEF [22, 36].

Several articles described that miR expression had a similar but not superior performance than NP. However, when the same analysis combined miR expressions with BNP and NT-proBNP, the discriminating power for distinguish HFrEF from HFpEF was prominent, and even better than NP used alone [22, 23, 34].

4.2. MicroRNAs and NYHA and HF severity

Included studies referred a gradual increase in specific miR levels in more stabilised HF, chronic HF (CHF) and healthy controls, when compared to AHF patients [30, 31, 41]. Indeed, low levels of miRs were associated with increased levels of biochemical markers related to inflammation, angiogenesis and endothelial dysfunction, supporting previous data [31].

Negative correlations between miR-22 with the outcome were described [24]. Scientific works have stated that **miR-22-3p** has a protective role in the myocardium due to its negative regulation of angiotensin II [24, 42]. It is mostly expressed by striated muscle tissues, and is essential in normal cardiac remodelling after environmental stress [24, 41].

In fact, among the included studies, several severity-related miRs were described as upregulated [28, 32] or downregulated [27, 30] regardless of HF aetiology. This suggests that different factors may influence miR serum expression. Volume status variations and fluid overload due to renal impairment and proteinuria (once they are bound to plasma proteins such as albumin, and low filtration rate may lead to the loss of carrier plasma proteins) [32, 41].

4.3. Prognostic role of miR in HF

Recent literature reports miRs ability to independently predict the outcome in HF patients.

Let-7i-5p and miR-223-5p are well known inflammation and fibrosis related miRs [31, 43]. Authors suggested that the Let-7 family might have an active role in HF pathogenesis and it has been related to poor outcomes in dilated cardiomyopathy (DCM) [44, 45]. Interestingly and similarly to miR-22, recent literature refers that let-7i-5p negatively regulates angiotensin II, attenuating cardiac inflammation and fibrosis [43], contradicting previous literature and our findings.

Recently identified for the first time in HF patients, **miR-1306-5p** was considered a possible biomarker by two large cohorts [34, 36]. Its function might be related with numerous processes in the cell, including proliferation, differentiation and cell cycle [46].

Previous literature refers that **miR-208 and miR-499** overexpression, promotes LV hypertrophy, and ultimately HF [47]. Positive correlations with HF were found in miR-208 but not in miR-499, respectively [36].

MiR-122, as previously referred, is a liver-specific miR associated with the risk of metabolic syndrome development and has been related with HF prognosis [39]. Stojkovic *et al* pointed miR-122 to be an independent predictor of all-cause mortality after adjustment to NT-proBNP in HFrEF patients. This data might suggest liver involvement in HF pathophysiology [38].

Several of the included studies analysed **miR-423**-5p. Positive, negative and no association with disease progression were described [30, 31, 33, 34, 38, 48]. MiR-423-5p has been associated with chemotherapy resistance in cancer [49-52] and, recently, with cardiovascular diseases. Literature have suggested a link between miR-423, vascular endothelial growth factor (VEGF) and nitric oxide (NO), concluding that miR-423 may serve as a biomarker of vascular

growth/proliferation or inhibition, depending on the pathological process and target organ [53]. These contrary conclusions could be related with different miR expression in HF patients during an acute phase. In fact, most of the literature describes that miR-423-5p is associated with a poor outcome. Cells and blood are the major source of miR suggesting that they might have a paracrine function that could justify miRs serum dysregulation. This indicates that HF may not be the only source of prognostic information, but it may derive from other damaged organs or cells [54].

Curiously, negative associations were found between miR-423-5p and CV hospitalisation and all-cause death [33], implying a protective role of miR-423. This unexpected result can be explained for different reasons: miR can be diluted and, also, different expression values in collected blood samples can be detected [35]. Indeed, some miR can be undetectable in a large portion of samples [34, 55].

MiR-21 has also been related with cell proliferation, migration and apoptosis processes and it plays an important role in hypertension pathophysiology [56]. Despite its apparent role in heart diseases, only one study clinically referred an association between miR-21 [57] and HF prognosis [33, 35].

4.4. Study Limitations

Analysed studies revealed the need of a common approach for miR quantification, and methods uniformization. A standardisation of methods and an adequate normalisation of miR levels could elucidate about the conclusion's disparities.

MiR quantification method. The small noncoding RNA RNU6 genes are the reference genes most used as normalizers. Nonetheless, RNU is not a miR and, thus, it is not able to reflect the biological and chemical features of miRs among other limitations [58]. Several miR have been described and suggested as normalisers (i.e.miR-16) because of their increased blood expression and uniformity among several samples [58]. Currently, there is no standardised normaliser miR among the scientific community, however, several studies have demonstrated that the use of more than one reference normaliser increases the quantification accuracy. Unfortunately, all included studies in this review only used one miR as normaliser. Proportionally, different miRs were identified as altered in serum along with the study groups, making it impossible to measure

and compare. This implies that using different normalisers may be responsible for distinct miR detection.

Studies population definition criteria. An additional identified limitation was the lack of uniformity regarding to HF definition and functional class criteria among the included studies. Also, study group selection in analysed articles was distinct. Indeed, several groups included numerous definitions such as: hospitalised AHF patients, hospitalised and outpatient CHF patients, unspecified HF, among others.

5. Conclusion

MiR implication in HF is unquestionable. The increasing interest in miR led to the identification of new pathways and mediated mechanisms related to HF and miR potential as a HF biomarker [59]. Thus, there are questions about miR that should be unravelled, such as: how miR are released into circulation and what role they play and in what measure circulating miR can reflect heart tissue expression levels [60].

In the future, improved study designs with analogous miRs and larger cohorts will promote more clinically representative studies. Nevertheless, evidence shows miR potential a prognostic biomarker, as it comprises one of the most resourceful promises for future application in HF.
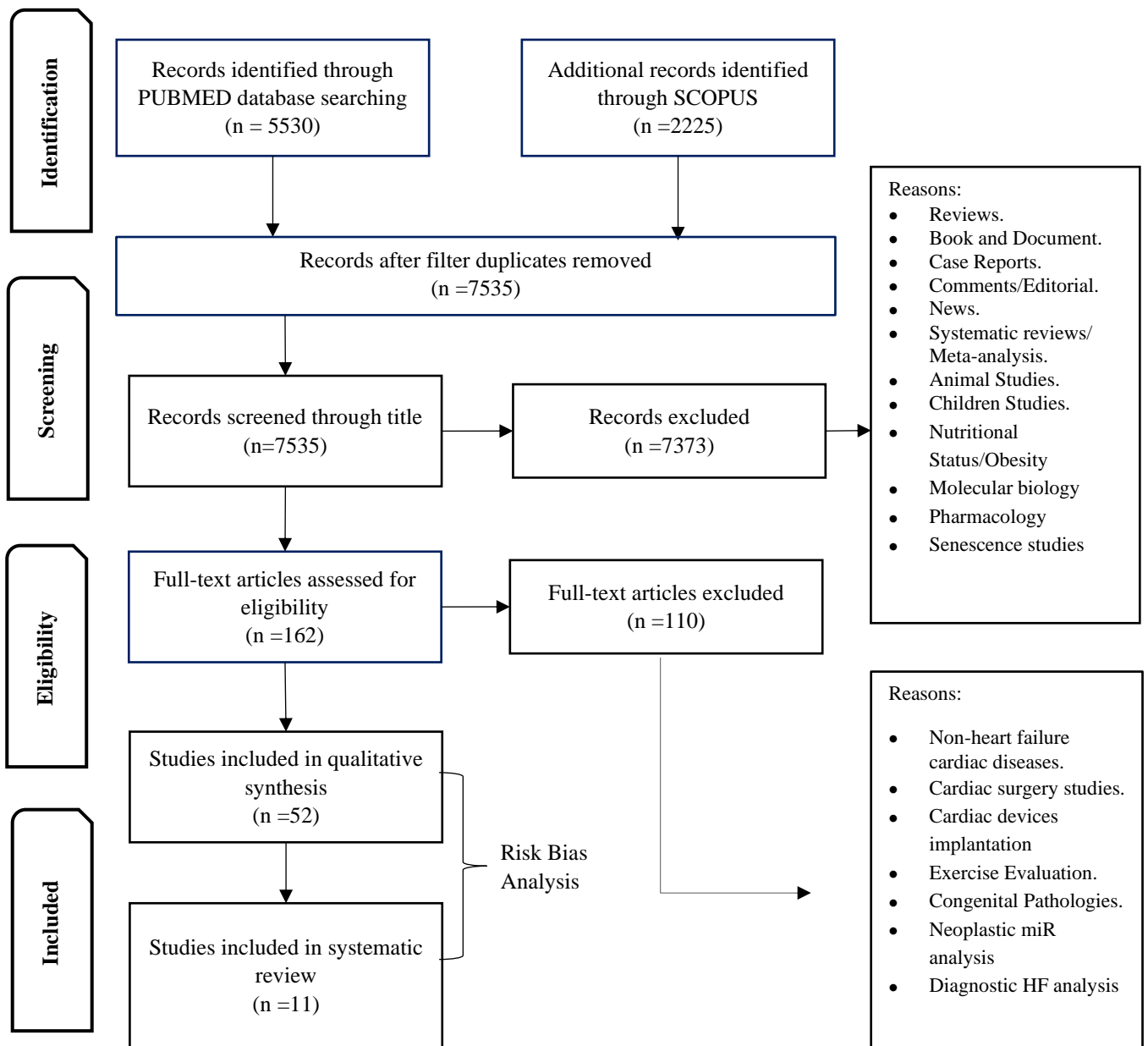
2   # 6. Figures

Figure 1. Flow diagram from study identification to inclusion

7. Tables

Table 1: Overview of articles included in study, organized per outcome								
Authors	**Study design**	**Study Population**	**No. of Subjects**	**Male (%)**	**Age Mean SD (Years)**	**Outcomes**	**Main Findings**	**Variables Included in the Multiple Models**
A. General Population-based Studies *i) Prospective associations – CV hospitalisations*								
Vegter L.E. *et al*, The Netherlands, 2017[31]	Prospective cohort	Subset of random HF patients from COACH	114	66	71.1±10.4	CV hospitalisation	Median follow-up of 18 months; Univariate analysis found miR-106a-5p, miR-223-3p, miR-27a-3p, miR-16-5p, miR-30e-5p and let-7i-5p as significantly predictive. Multivariate analysis resulted in 5 miRNAs significantly predictive (miR-106a-5p, miR-223-3p, miR-27a-3p, miR-16-5p, and let-7i-5p; all HR>1.4; p<0.05).	Age, sex, BNP and eGFR.
Masson S. *et al* Italy 2017[32]	Prospective cohort	GISSI-HF trial	953	80	67.1± 10.76	CV hospitalisation	Median follow-up was 46.2 months, in univariate analysis miR-132 was associated with HF hospitalisation. After adjustment, miR-132 remained associated with outcome (HR 0.79; p=0.001).	Demographic, clinical echocardiographic risk factors, and baseline NT-proBNP concentrations.
Seronde M-F., *et al* France, 2015[33]	Prospective cohorts – test cohort	AHF patients from Leicester hospitals	236	60.6	76 (65.5-84.5)	CV hospitalisation	Multivariate analysis of miR-21, miR-126, miR-423-5p, miR-1 and miR-23; only miR-423-5p was associated with hospital readmission (HR 0.70 [CI 0.53-0.93], p=0.01).	Age, gender, heart rate, systolic and diastolic blood pressure, history of AF and of HF, LVEF, plasma levels of BNP, sodium, creatinine, proteins, and haemoglobin.
Seronde M-F., *et al* France, 2015[33]	Prospective cohorts – validation cohort	AHF patients from Leicester hospitals.	711	64.2	77 (68.6-83)	CV hospitalisation	Multivariate analyses, miR-423-5p was not a significant predictor of 1-year readmission in this cohort.	Age, gender, heart rate, systolic and diastolic blood pressure, history of AF and of HF, LVEF, plasma levels of BNP, sodium, creatinine, proteins, and haemoglobin.

Boven N.v., The Netherlands , 2017[34]	Prospective	TRIUMPH	456	63.4	73 (64-80)	HF hospitalisation	Multivariate analysis evidenced miR-1306-5p as predictor of HF hospitalisation (HR:1.22, p<0.05)	Age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level.
Van Boven N. v, The Netherlands 2017[24]	Prospective	Bio-SHIFT study	263	72	67 ±13	CV hospitalisation	None of the baseline miR values were associated with the secondary endpoint comprising HF hospitalisations.	Age and gender.
Zhang Jianghua. China, 2017[28]	Prospective	Hospitalized HF patients.	120	71.25	59.68 ± 10.24	Re-hospitalisation	Follow-up for 24 months concluded that miR-21 samples from coronary sinus were related with re-hospitalisation (OR 1.160, p=0.0021). In samples from PV miR-21 was not related with the outcome .	EF, BNP-PV, creatinine, CK-MB, alanine aminotransferase, CRT.
ii) Prospective outcomes - Heart failure rehospitalisation and/or death								
Vegter L.E. *et al* , The Netherlands , 2017[31]	Prospective cohort	Subset of patients from COACH	114	66	71.1±10.4	HF hospitalisation and/or death	Only miR-106a-5p was univariate predictive (HR 1.38 CI (1.017-1.882) p=0.039. No significant association was found after adjustment.	
Boven N.v., The Netherlands , 2017[34]	Prospective cohort	TRIUMPH	456	63.4	73 (64-80)	All-cause mortality and readmission for HF	MiR-1306-5p levels were associated with outcome. (HR 1.13 CI [1.03-1.23]). After adjustment, miR-320a, miR-378a-3p and miR-423-5p were positively associated with outcome. MiR-1254 displayed a borderline significant association with all-cause mortality and HF hospitalisation, when adjusted to variables.	Age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level.

Boven N. v, The Netherlands 2017[24]	Prospective	Bio-SHIFT study	263	72	67 ±13	Hospitalisation for management of HF and mortality	The temporal pattern of miR-22-3p was inversely associated with the primary endpoint after adjustment of age and gender (HR per doubling of miR-22-3p level, 0.64; CI 0.47–0.77; p= 0.001). After adjustment, the association of miR-22-3p remained present (HR per doubling of miR-22-3p level at any given time point, 0.61; CI 0.51–0.73; p =0.001).	Age, gender, ICM and NYHA class.
Bayés-Genis A. *et al,* Spain 2017[36]	Prospective cohort	Cohort I (Barcelona)	834	71	68.1 ±12.7	all-cause mortality and HF hospitalisation	MiR-1254 and miR-1306-5p were significantly associated with outcome. (HR of 1.21 [95% CI 1.06–1.39] and a HR of 1.13 [95% CI 1.03–1.25] respectively).	Age, gender, haemoglobin, creatinine and NT-proBNP
Bayés-Genis A. *et al,* Spain 2017[36]	Prospective cohort	Cohort II (Detroit)	1369	58	68.8±12.1	all-cause mortality and HF hospitalisation	MiR-1254 and miR-1306-5p were significantly associated with outcome (HR of 1.14 [95% CI 1.04–1.25] and a HR of 1.11 [95% CI 1.03–1.19] respectively).	Age, gender, haemoglobin, creatinine and NT-proBNP
iii) Prospective outcomes – all cause death								
Vegter L.E. *et al* , The Netherlands , 2017 [31]	Prospective cohort	Subset of randomly selected patients from COACH	114	66	71±10	Mortality	No significant associations were identified for any of the miRNAs with all-cause mortality within 18 months.	Age, sex, BNP and eGFR.
Boven N.v., The Netherlands , 2017[34]	Prospective cohort	TRIUMPH	456	63.4	73 (64-80)	All-cause mortality	Positive associations were found between miR-499a-5p (HR:2.04, CI 0.96-4.43) and miR-1306-5p (HR:1.03, CI 0.90-1.17)	Age, sex, systolic blood pressure, diabetes mellitus, atrial fibrillation, BMI, previous hospitalization for HF during the last 6 months, ischemic HF, baseline eGFR, and baseline NT-proBNP level.

Seronde M-F., *et al* France, 2015[33]	<u>Prospective cohorts – validation cohort</u>	AHF patients from Leicester hospitals	711	64.2	77 (68.6-83)	All-cause mortality	In 20 months of follow-up, miR- 423-5p significantly predicted mortality (OR 0.54 CI [0.36-0.829, p=0.004]). Patients within the lowest quartile of miR-423-5p levels had a higher risk of mortality compared to patients with low levels of miR-423-5p. This association was evident for 2 years.	Age, gender, heart rate, systolic and diastolic blood pressure, history of AF and of HF, LVEF, plasma levels of BNP, sodium, creatinine, proteins, and haemoglobin.
Bayés-Genis A. *et al,* Spain 2017[36]	<u>Prospective cohort</u>	Cohort I (Barcelona)	834	71	68.1 ±12.7	All-cause mortality	MiR-1254, miR-133b, miR-622 and miR-208a-3p were predictive of outcome. (HR of 1.19 CI [1.03-1.38]; HR 1.20 CI [1.06-1.36]; HR 1.18 CI [1.00-1.38]; HR 1.32 CI [1.05-1.42] respectively).	Age, gender, haemoglobin, creatinine and NT-proBNP.
Bayés-Genis A. *et al,* Spain 2017[36]	<u>Prospective cohort</u>	Cohort II (Detroit)	1369	58	68.8±12.1	All-cause mortality	MiR-1254, miR-133b, miR-622, miR-208a-3p were predictive of outcome. (HR of 1.31 CI [1.13-1.52]; HR 1.18 CI [1.03-1.34]; HR 1.12 CI [1.03-1.22]; HR 1.32 CI [1.02-1.34] respectively).	Age, gender, haemoglobin, creatinine and NT-proBNP.
Ovchinnlkova E., *et al* The Netherlands 2015[30]	<u>Prospective cohort</u>	PROTECT trial (AHF patients)	100	50	68.9±11.4	All-cause mortality	An univariate analysis concluded that 7 miRNAs (let-7i-5p, miR-18a-5p, miR-18b-5p, miR-223-3p, miR-301a-3p, miR-423-5p miR-652-3p) were predictive for 180-day mortality. (all HR 1.5, p<0.05).	
Zhang Jianghua. China, 2017[28]	<u>Prospective</u>	Hospitalized HF patients between March 2013 to October 2013	120	71.25	59.68 ± 10.24	All-cause mortality	Samples from PV and coronary sinus were significantly correlated to miR-21. (RR 1.936 and 1.125, p=0.001 respectively).	HF. EF, BNP-PV, creatinine, CRT
Klenke S *et al,* Germany, 2018[25]	<u>Prospective cohort</u>	ICM	91	83.5	56.1±13.9	All-cause mortality	MiR-192 low expression is associated with survival in Univariate analysis (p=0.03) and Multivariate analysis (p=0.014).	NYHA status, EF, and BNP concentration.
Masson S. *et al* Italy 2017[32]	<u>Prospective cohort</u>	GISSI-HF trial	953	80	67.1± 10.76	All-cause mortality	Median follow-up was 46.2 months, in univariate analysis miR-132 was associated with outcome, but not after multivariate analysis (HR 0.95, 95% CI 0.85–1.07 for 1 unit increase in miR-132, *P* = 0.41).	Demographic, clinical echocardiographic risk factors.

Xiao J. *et al* [37]	Prospective cohort	Patients admitted to the cardiac care unit.	95	62.5	61.5±16.5	All-cause mortality	Higher hemoglobin, serum sodium and miR-30d level were associated with a reduced risk of death caused in AHF patients. Patients with higher serum miR-30d levels had significantly lower mortality (P=0.001). Death prediction of miR-30d with OR of 0.610 CI (0.409-0.911) p=0.016.	Heart rate, serum sodium, blood urea nitrogen, haemoglobin, cystatin, uric acid, and serum.
Stojkovic *et al* [38]	Prospective cohort	HFrEF patients	234	81.6	65.1	All-cause Mortality	Circulating miR predictive value was assessed by Cox proportional Hazard regression models. Both miR-122 and mR-423 predicted the outcome with HR per 1-SD of 1.15 (95% CI: 1.02–1.29; p=0.021) and HR per 1-SD 1.27; (95% CI: 1.10–1.46; p = 0.001) respectively.	Age, gender, NYHA classes, DM II, eGFR, BMI, NP-proBNP, right ventricular dysfunction, cholinesterase, gamma-GT, and previous myocardial infarction..

HF: Heart failure; COACH: Coordinating Study Evaluating Outcomes of Advising and counselling in Heart Failure; CV: cardiovascular; AF: atrial fibrillation; DM II : Type 2 Diabetes Mellitus; BMI: body mass Index; AHF: acute heart failure; LVEF: left ventricular ejection fraction; PROTECT: Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function; BNP: b-type natriuretic peptide; eGFR: estimated glomerular filtration rate; NT-proBNP: N-terminal pro-B-type natriuretic peptide; Gamma-GT: gamma-glutamyl transferase; HR: hazard ratio. OR: Odd-ratio; CI: confidence interval; RR: relative risk; CK-MB: creatine kinase, muscle and brain; BNP-PV: brain natriuretic peptide from peripheral vein; CRT: Cardiac-resynchronization Therapy; COACH: Coordinating Study Evaluating Outcomes of Advising and counselling in Heart Failure; ICM: Ischemic Heart failure; Methodological quality of all studies was assessed using the revised and validated version of the Quadas-2.

Table 2: Overview of significant miR – Cardiovascular hospitalisation					
Study	**No of patients**	**Diagnosis**	**Tested miR**	**Significant miRs**	**Statistical analysis**
Outcome: Cardiovascular hospitalisation					
Vegter L.E. *et al*, The Netherlands, 2017[31]	114	HF	let-7i-5p, miR-16-5p, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, miR-223-3p, miR-423-5p, miR-652-3p	miR-106a-5p, miR-223-3p, miR-27a-3p, miR-16-5p, let-7i-5p (+)	Multivariate analysis [p<0.05]
Masson S. *et al* Italy 2017[32]	953	HF	miR-132	miR-132(-)	Multivariate analysis [p=0.001]
Seronde M-F., *et al* France, 2015[33]	236	AHF	miR-423-5p, miR-126, miR-23, miR-21, miR-1	miR-423-5p (-)	Multivariate analysis [p=0.01]
Seronde M-F., *et al* France, 2015[33]	711	AHF	miR-423-5p	--	Multivariate analysis [p=0.48]
Boven N.v., The Netherlands, 2017[34]	456	HF	miR-486-5p, miR-320ª, miR-1254, miR-22-3p, miR-378a-3p, miR-423-5p, miR-345-5p, miR-1306-5p, miR-133a-3p, miR-499a-5p, miR-133b, miR-622, miR-208a-3p	miR-1306-5p (+)	Multivariate analysis [p<0.05]
Van Boven N. v, The Netherlands 2017[24]	263	HF	miR-1254, miR-22-3p, miR-423-5p, miR-486-5p, miR-320ª, miR-345-5p, miR-378a-3p	--	Multivariate analysis [p>0.38]
Zhang Jianghua. China, 2017[28]	80	HF	miR-21	miR-21(+)	Multivariate analysis [p=0.0021]
HF: Heart Failure; AHF: Acute Heart Failure; miR: microRNAs; No: Number (+): positive correlation; (-): negative correlation; (--): no correlation was found					

Table 3: Overview of significant miR – Cardiovascular hospitalisation and/or death					
Study	**No of patients**	**Diagnosis**	**Tested miR**	**Significant miRs**	**Statistical analysis**
Outcome: Cardiovascular hospitalisation and/or death					
Vegter L.E. _et al_, The Netherlands, 2017[31]	114	HF	let-7i-5p, miR-16-5p, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, miR-223-3p, miR-423-5p, miR-652-3p	miR-106a-5p (+)	Univariate analysis [p=0.039]
Boven N.v., The Netherlands, 2017[34]	456	HF	miR-486-5p, miR-320ª, miR-1254, miR-22-3p, miR-378a-3p, miR-423-5p, miR-345-5p, miR-1306-5p, miR-133a-3p, miR-499a-5p, miR-133b, miR-622, miR-208a-3p	miR-1306-5p, miR-320a, miR-378a-3p and miR-423-5p(+)	Multivariate analysis [p<0.05]
Boven N. v, The Netherlands 2017[24]	263	HF	miR-1254, miR-22-3p, miR-423-5p, miR-486-5p, miR-320ª, miR-345-5p, miR-378a-3p	miR-22-3p (-)	Multivariate analysis [p=0.001]
Bayés-Genis A. _et al_, Spain 2017[36]	834	HF	miR-133b, miR-1254, miR-378a-3p, miR-423-5p, miR-320ª, miR-345-5p, miR-22-3p, miR-1306-5p, miR-133a-3p, miR-622, miR-499a-5p, miR-208a-3p	miR-1254, miR-1306-5p (+)	Multivariate analysis [p<0.05]
Bayés-Genis A. _et al_, Spain 2017[36]	1369	HF	miR-133b, miR-1254, miR-378a-3p, miR-423-5p, miR-320ª, miR-345-5p, miR-22-3p, miR-1306-5p, miR-133a-3p, miR-622, miR-499a-5p, miR-208a-3p	miR-1254, miR-1306-5p (+)	Multivariate analysis [p<0.05]
HF: Heart Failure; miR: microRNAs; No: number (+): positive correlation; (-): negative correlation					

Table 4: Overview of significant miR – All-cause death					
Study	**No of patients**	**Diagnosis**	**Tested miR**	**Significant miRs**	**Statistical analysis**
Outcome: All-cause death					
Vegter L.E. *et al*, The Netherlands, 2017 [31]	114	HF	let-7i-5p, miR-16-5p, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, miR-223-3p, miR-423-5p, miR-652-3p	--	Univariate analysis [p>0.05]
Boven N.v., The Netherlands, 2017 [34]	456	HF	miR-486-5p, miR-320ᵃ, miR-1254, miR-22-3p, miR-378a-3p, miR-423-5p, miR-345-5p, miR-1306-5p, miR-133a-3p, miR-499a-5p, miR-133b, miR-622, miR-208a-3p	Mir-1306-5p miR-499ᵃ- (+)	Multivariate analysis [p<0.05]
Seronde M-F., *et al* France, 2015 [33]	711	AHF	miR-423-5p	miR-423-5p (-)	Multivariate analysis [p=0.004]
Bayés-Genis A. *et al*, Spain 2017 [36]	834	HF	miR-133b, miR-1254, miR-378a-3p, miR-423-5p, miR-320ᵃ, miR-345-5p, miR-22-3p, miR-1306-5p, miR-133a-3p, miR-622, miR-499a-5p, miR-208a-3p	miR-1254, miR-133b, miR-622, miR-208a-3p (+)	Multivariate analysis [p<0.05]
Bayés-Genis A. *et al*, Spain 2017 [36]	1369	HF	miR-133b, miR-1254, miR-378a-3p, miR-423-5p, miR-320ᵃ, miR-345-5p, miR-22-3p, miR-1306-5p, miR-133a-3p, miR-622, miR-499a-5p, miR-208a-3p	miR-1254, miR-133b, miR-622, miR-208a-3p (+)	Multivariate analysis [p<0.05]
Ovchinnlkova E., *et al* The Netherlands 2015 [30]	100	AHF	let-7i-5p, miR-16-5p, miR-18b-5p, miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-128, miR-199a-3p, miR-223-3p, miR-301a-3p, miR-423-3p, miR-423-5p, miR-652-3p	let-7i-5p, miR-18a-5p, miR-18b-5p, miR-223-3p, miR-301a-3p, miR-423-5p, miR-652-3p (+)	Univariate analysis [p<0.05]
Zhang Jianghua. China, 2017 [28]	80	HF	miR-21	miR-21 (+)	Multivariate analysis [p=0.001]
Klenke S *et al*, Germany, 2018 [25]	91	HF	miR-192	miR-192 (-)	Multivariate analysis [p=0.014]
Masson S. *et al* Italy 2017 [32]	953	HF	miR-132	--	Multivariate analysis [p=0.41]
Xiao J. *et al* China 2017 [37]	95	HF	miR-30d	miR-30d (-)	Multivariate analysis [p=0.016]
Stojkovic *et al*, Austria, 2020 [38]	234	HFrEF	miR-122, miR-423, miR-126	miR-122, miR-423 (+)	Multivariate analysis [p<0.021]
HF: Heart Failure; AHF: Acute Heart Failure; miR: microRNAs; No: number (+): positive correlation; (-): negative correlation; (--): no correlation was found					

Table 5: Correlations found on included articles per outcome						
	Outcome: Cardiovascular Hospitalisation			*Outcome: HF hospitalisation and/or death*		*Outcome: All-cause death*
miR	Correlation [HR or OR or RR]	*p*	Correlation [HR or OR or RR]	*p*	Correlation [HR or OR or RR]	*p*
miR-423-5p	Negative [[OR: 0.70[(33)] [a]]	0.01	Positive [HR: 1.05[a](34)]	0.05	OR: 0.54[a](33) Positive [HR:1.681[b](30)] Positive [HR: 1.27[a](38)]	0.001
miR-1254	--	--	Positive [HR: 1.14[a](36)] Positive [HR: 1.21[a](36)]	0.05	Positive [HR: 1.19[(36)] Positive [HR: 1.31[a](36)]	0.05
miR-133b	--	--	--	--	Positive [HR: 1.20[(36)] Positive [HR: 1.18[a](36)]	0.05
miR-622	--	--	--	--	Positive [HR: 1.18[a](36)] Positive [HR: 1.12[a](36)]	0.05
miR-208a-3p	--	--	--	--	Positive [HR:1.32[a](36)] Positive [HR:1.32[a](36)]	0.05
let-7i-5p	Positive [HR:2.058[(31)] [a]]	0.002			Positive [HR:1.958[b](30)]	0.007
miR-18a-5p	--	--	--	--	Positive [HR:1.616[b](30)]	0.014
miR-18b-5p	--	--	--	--	Positive [HR:1.851[b](30)]	0.013
miR-223-3p	Positive [HR:1.478[(31)] [a]]	0.039	--	--	Positive [HR:1.557[b](30)]	0.034
miR-301a-3p	--	--	--		Positive [HR:1.782[b](30)]	0.04
miR-652-3p	--	--	--	--	--	--
miR-21	Positive [OR:1.160[(28)][a]]	0.0021			Positive [RR:1.936[(28)]]	0.001
miR-499a					Positive [HR:2.04[a](34)]	0.05
miR-192	--	--	--	--	*(25)	0.014
miR-132	Negative [HR:0.79[(32)] [a]]	0.01	--	--	HR:0.95[a] (32)	0.41
miR-30d	--	--	--	--	Negative [HR: 0.61[(37)][a]]	0.016
miR-122	--	--	--	--	Positive [HR:1.15[a](38)]	0.021
miR-1306-5p	Positive [HR:1.22[(34)]]	0.05	Positive [HR:1.13[a](34)] Positive [HR:1.13[a](36)] Positive [HR:1.11[a](36)]	0.05		
miR-22-3p	--	--	Positive [HR:0.61[a] (24)]	0.001	Positive [HR:1.03[a](34)]	0.05
miR-320a	--	--	Positive [HR:1.10[a](34)]	0.05	--	--
miR-378a-3p	--	--	Positive [HR: 1.03[a](34)]	0.05	--	--
miR-106a-5p	Positive [HR:1.694[(31)] [a]]	0.012	Positive [HR: 1.38[a](31)]	0.039	--	--
miR-27a-3p	Positive [HR:1.482[(31)] [a]]	0.027	--	--	--	--
miR-16-5p	Positive [HR:1.763[(31)] [a]]	0.005	--	--	--	--
(a) Multivariate analysis; OR: Odd Ratio; HR: Hazard ratio; OR: Odd Ratio; RR: Relative Risk; [[b] Univariate analysis; * missing information						

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