

REVIEW

A systematic review of micro-RNAs in aortic stenosis and cardiac fibrosis

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Abstract

Aortic stenosis (AS) is the commonest valve lesion requiring surgery in the Western world. The presence of myocardial fibrosis is associated with mortality even after valve replacement. MicroRNAs could serve as biomarkers of fibrosis and risk stratify patients for earlier intervention. This study aimed to systematically review reports of micro-RNA (miR) associated with fibrosis in AS and identify potential biomarkers. We searched EMBASE, Medline, and Web of Science up to May 2020. Studies that reported on the role of miRs in AS and cardiac fibrosis were included. Study quality was assessed using the Newcastle-Ottawa scale. Of 4230 reports screened, 25 were included. All studies were of low to moderate quality. MiRs were analyzed in myocardial tissue ($n = 10$), aortic valve tissue ($n = 5$), plasma ($n = 5$), and serum ($n = 5$). A total of 365 miRs were reported, of which only a few were reported in more than one paper (3 in the myocardium, 5 in the aortic valve, and 1 in plasma). miR-21 was upregulated in plasma and myocardial tissue. MiR-19b was downregulated in the myocardium. Papers reporting myocardial miR-1 contradicted each other, and miR-133a was associated with increased left ventricular mass regression post-surgery. In the aortic valve, miRs-665, 602 and 939 were downregulated, and miRs-193b and 214 were upregulated. The data on miR in fibrosis in AS is scarce and of low to moderate quality. Further studies are needed to identify novel miRs as biomarkers, especially at an earlier asymptomatic phase of the disease.

Study Highlights**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

The chronic pressure overload exerted on the myocardium by the obstructive valve in aortic stenosis (AS) leads to adverse cardiac remodeling. This includes diffuse interstitial fibrosis, characterized by extracellular space expansion, which is thought to be reversible post-intervention, as well as focal fibrosis which is only partially reversible. Both can be quantified noninvasively using cardiac magnetic

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resonance imaging, are present even in asymptomatic AS and are associated with adverse outcomes and mortality.

WHAT QUESTION DID THIS STUDY ADDRESS?

To elucidate the role of micro-RNAs (miRs) as potential biomarkers of the cardiac fibrosis in AS, studies where miRs were analyzed as biomarkers for fibrosis in AS were systematically reviewed.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This review identified that the information available in existing literature on the role and use of miRs as biomarkers for AS is extremely limited. However, miRs do have great potential to be used as biomarkers due to their cell/tissue specificity, ease of measurement, and accessibility through non-invasive techniques.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The use of miRs as biomarkers in AS could assist in earlier detection of the fibrosis progression in the disease, which in turn could play a role in risk stratification to identify those for early intervention, to optimize the timing of intervention, and allow for better recovery and improved long-term outcomes.

INTRODUCTION

Aortic stenosis (AS) is the narrowing of the aortic valve, caused by thickening and calcification of the cusps. It is the commonest valve lesion requiring surgery in the Western world and affects ~3% of those over 65 years of age.¹ Those with bicuspid valves are at risk of developing AS at an earlier age.² AS is characterized by a long latent, asymptomatic period. The heart undergoes a series of compensatory changes, including left ventricular (LV) hypertrophy, which initially works to reduce wall stress and maintain cardiac output.² However, as the disease progresses, these changes become maladaptive and lead to diastolic dysfunction and interstitial fibrosis, ultimately leading to heart failure if left untreated.³⁻⁵ Diffuse interstitial fibrosis is thought to be reversible and occurs early in the disease and is associated with the expansion of the extracellular volume. Replacement fibrosis is irreversible and is a result of collagen deposition followed by cell death.⁶ Both can be detected and quantified non-invasively using cardiovascular magnetic resonance imaging (MRI).

The only available treatment for AS is surgical or transcatheter aortic valve replacement (AVR), with no medical therapy of proven benefit. AVR is currently recommended once symptoms or LV dysfunction develop.⁷ Focal fibrosis, measured by late gadolinium enhancement imaging on MRI, is found in up to 50% of asymptomatic patients with AS, and progresses rapidly.⁸ It is irreversible up to 1 year post-AVR,⁹ and remains a predictor of mortality even after AVR.¹⁰ This suggests the need for potentially earlier AVR, before irreversible remodeling occurs, and the need for better risk stratification tools, including imaging and blood biomarkers.

Micro-RNAs (miRs) are small noncoding RNAs that regulate gene expression and intracellular signaling by interfering with post-transcriptional gene expression. They are detectable in blood and used as biomarkers in neurology, nephrology, and oncology.¹¹ Previous studies have shown that there is some correlation between changes in miR expression and myocardial fibrosis; additionally, in patients with cardiac diseases, such as AS, increased levels of pro-fibrotic miRs and a decrease in anti-fibrotic miRs have been reported.¹² Levels of miR-21 and miR-29 were associated with diastolic dysfunction, lower ejection fraction, reduced stroke volume index, and are typically upregulated in the fibrotic tissue.¹³ This systematic review aims to synthesize published data on miRs associated with myocardial fibrosis in AS.

METHODS

Protocol and registration

This systematic review was conducted using a predefined protocol in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and is registered in PROSPERO International Prospective Register of Systematic Reviews (<https://www.crd.york.ac.uk/prospero/Identifier:CRD42021241846>).

Eligibility criteria

Studies were included if conducted in humans and reported miR levels in plasma, serum, blood, or myocardial/

aortic valve tissue in patients with AS, with or without cardiac fibrosis. Studies were excluded if the patients were <18 years old, and performed in animals or cell lines. Conference reports where only the abstract was available, protocols and studies examining other cardiovascular diseases, such as heart failure and hypertrophic cardiomyopathy, were also excluded.

Data searches

Electronic searches were carried out using Embase, MEDLINE, and Web of Science to identify potentially eligible studies up to May 2020. The search strategies included terms related to or describing miR and AS with or without myocardial fibrosis. The search terms included aortic valve stenosis OR aortic stenosis AND cardiac fibrosis OR myocardial fibrosis AND microRNA OR miRNA. A full description of the search terms is available in [Figure 1](#).

Study selection

The study titles and abstracts were initially screened for inclusion by author J.O.A. based on the inclusion and exclusion criteria, and a sample was checked, and any uncertainties reviewed by a second reviewer (author A.S.). The full texts for shortlisted studies were retrieved and further assessed for inclusion, and the reasons for exclusion were recorded.

Data extraction

Data were extracted from the included studies by two independent reviewers (authors J.O.A. and R.P.) using a proforma. Extracted information included publication year, the country of the study, the severity of disease, cases and control study population, cases and control sample size, cases and controls age (expressed as mean \pm SD), male and female participants, inclusion and exclusion criteria, sample collected, time of sample collection, sample preparation, miR extraction and processing methods, normalization methods, miR analyzed, differentially expressed miRs, their levels (expressed as mean \pm SD), and the direction of change, and the type of array used. In cases where results were provided only graphically, the software WebPlot Digitizer version 4.5¹⁴ was used to extract the values from the plots. For studies that measured the same outcome in blood and organ tissue, both results were extracted. Furthermore, where different control groups were used, both results

were extracted. Disagreements were resolved by discussion between the reviewers or involvement of a third reviewer (author A.S.).

Quality assessment

Included studies were quality assessed using the Newcastle-Ottawa¹⁵ scale by two reviewers (authors J.O.A. and R.P.). Disagreements were discussed between two reviewers (authors J.O.A. and R.P.). The scores range from one to nine with one being the lowest quality and nine being the highest quality. The papers are judged on selection (i.e., how the case and control groups are selected [maximum of 4 points possible]), comparability (i.e., what kind of control is used [up to 2 points possible]), and exposure (maximum of 3 points possible).

Outcomes of interest

The prespecified primary outcome of this review was to identify miRs associated with myocardial fibrosis in AS.

Data synthesis

The levels of miRs were analyzed as standardized mean difference (SMD). All analyses were conducted using R programming software (version 4.1.1)¹⁶ with the “metafor” package.¹⁷ Independent-sample *t*-test or chi-square test was used to determine if the difference in ages and gender split between case and control were statistically significant and a *p* value of <0.05 was considered significant.

RESULTS

Search results

The search identified 6782 titles (PRISMA diagram, [Figure 1](#)). After removing duplicate reports, 4230 reports were screened, and 4186 were excluded based on titles and abstracts. Forty-four full texts of manuscripts were assessed, out of which 25 met the inclusion criteria, and were used for the quantitative analysis. A summary of the data extracted from these and their full references is presented in [Table S1](#).

Included studies

All 25 included studies examined patients with symptomatic AS that were referred for surgical AVR ([Table S1A](#)).

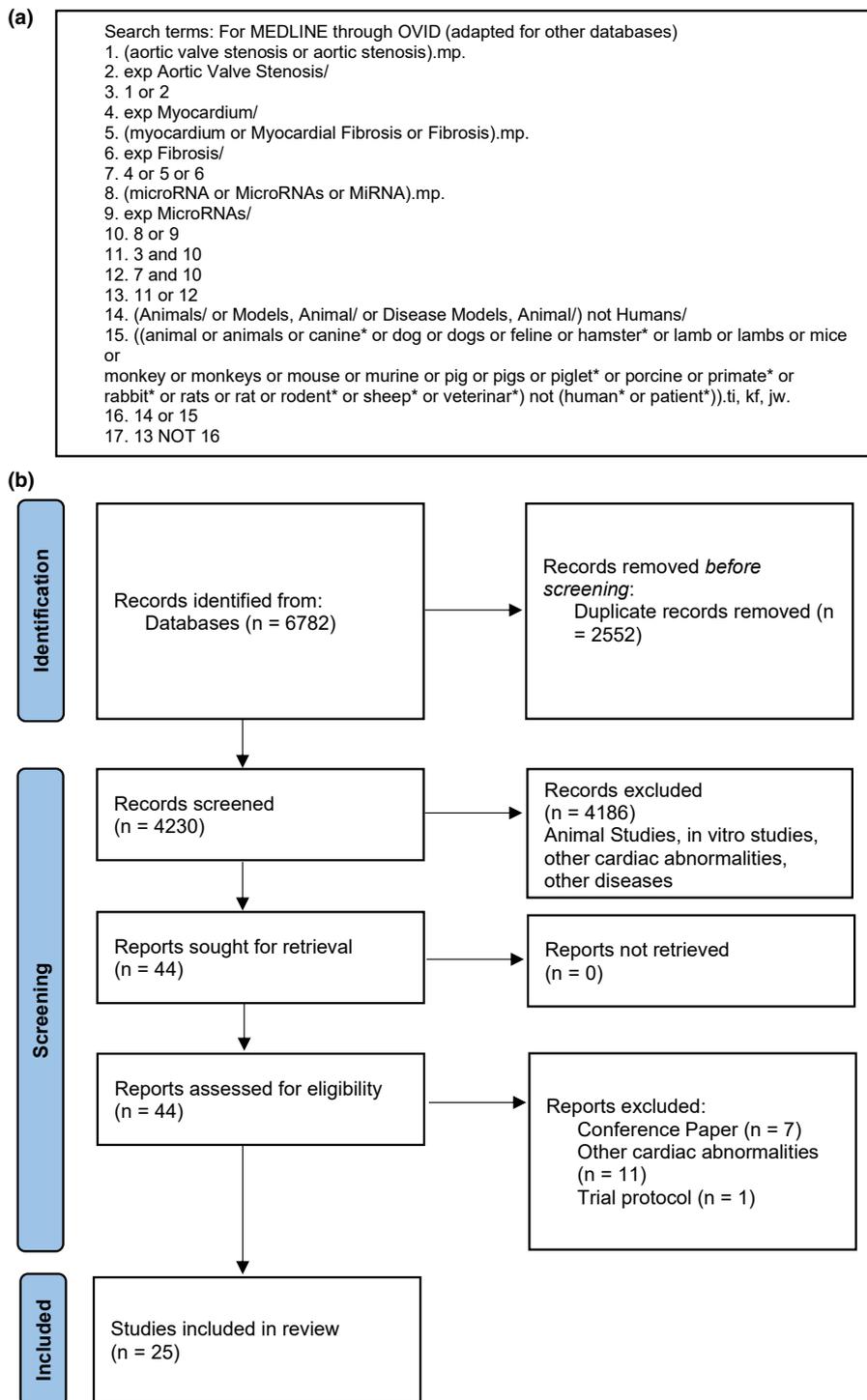


FIGURE 1 Search strategy and PRISMA Diagram of included studies. (a) Terms and strategy for Ovid MEDLINE searches. (b) PRISMA Diagram showing the number of manuscripts identified, screened, and included in the systematic review, with reasons for exclusion. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

Three papers examined AS in tricuspid and bicuspid valves and 22 examined it only in tricuspid valves, furthermore, all the studies included in the review were case-control studies. Twenty-one studies included patients with severe AS, whereas four included moderate to severe AS. Five studies used unselective strategies to assess an unbiased population of miR: hybridization arrays (1 study) and polymerase chain reaction (PCR) arrays (4 studies). Two studies used both hybridization and PCR arrays and two studies used a combination of hybridization arrays and

reverse transcription quantitative polymerase chain reaction (RT-qPCR), where RT-qPCR was used to validate the results obtained from the arrays. Of the studies where unselective strategies were used, only one, ref. 18, deposited their data in a public database (NCBI Gene Expression Omnibus). The rest of the studies (16 studies) prespecified the tested miR (selective assays). Across the studies, the patients with AS were older than the control group, with a mean age of 70.49 ± 7.65 and 61.10 ± 10.23 years, respectively (p value = 0.0003), additionally, there were more

female than male participants in the AS group (46.53%) than in the control group (36.72%; p value = 0.001).

Of the 25 studies, 10 assessed miRs in aortic valve tissue and nine in myocardial tissue. Myocardial samples were obtained from the interventricular septum ($n = 5$) and subepicardial myocardial biopsy from lateral LV wall ($n = 3$). One study did not specify the origin of the myocardial sample. Both aortic valve tissue and myocardial tissue were obtained during surgical AVR. Plasma and serum were assessed in five studies each, and one study used peripheral blood mononuclear cells (PMBCs). All blood samples were obtained before surgery and one was obtained during echocardiography conducted prior to surgery. Three studies used both myocardial tissue and plasma, whereas two studies used myocardial tissue and serum. EDTA was the only anticoagulant reported for plasma, whereas heparin was reported in the study that analyzed PMBCs. The tissue samples obtained during AVR were frozen in liquid nitrogen. Whole blood samples were obtained before surgery ($n = 10$) and were centrifuged to separate the plasma/serum.

Most of the studies ($n = 21$) normalized qRT-PCR results against small nuclear RNA U6 or exogenous cel-miR-39. The use of other controls is listed in Table S1C.

Assessment of methodological quality

The assessment of methodological quality was conducted utilizing the Newcastle-Ottawa scale (Table S2), and no study was found to be without limitations. The most common limitation (52%) was missing data in the comparison between cases and control. In nine out of 25 studies, miRs were stated as analyzed but no data were reported for both the case and control, therefore adequate analysis could not be conducted. Another limitation was the lack of information on the control group ($n = 4$), such as the age or gender of participants or patients' history. Furthermore, the cases were not fully representative of the disease as all studies conducted investigations in symptomatic patients with moderate to severe ($n = 4$) and severe ($n = 21$) AS. Finally, 15 studies used a sample population smaller than 30.

Data synthesis

Ten miRs were measured in plasma, 197 miRs were measured in myocardial tissue, and 158 miRs were measured in aortic valve tissue. For myocardial miRs, 194 miRs were only mentioned in one study, and of the 194 miRs, 153 had no quantifiable data provided. Likewise, nine plasma miRs were only mentioned in a single study, and four papers did not report expression levels. For aortic valve

miRs, 153 miRs were only mentioned in one study, and 144 had no quantifiable data provided.

Figure 2 shows the SMD of the miRs expressed in patients with AS vs. controls, reported in at least two studies. Positive values indicate higher expression or upregulation of miRs in the AS group and negative values indicate lower expressions or downregulation of miRs in the AS group. Due to the small number of studies for each miR, meta-analysis was not conducted. Three myocardial miRs (miR-1, miR-19b, and miR-133a-1) were reported in two studies each. The results showed miR-19b and miR-133a were downregulated in AS, however, the studies where miR-1 was reported showed contradictory results. Five aortic valve miRs were reported in two studies each. MiR-665, miR-602, and miR-939 were downregulated in AS, whereas miR-193b and miR-214 were upregulated. In plasma, only miR-21 was reported in two studies, where it was upregulated. MiR-21 was also found to be upregulated in the myocardium in another study.¹⁹

Overall, the most reported miR was miR-21, which was reported in seven studies. One study measured it in myocardial tissue alone, two in serum, one in plasma, one in myocardial tissue and serum, one in myocardial tissue and plasma, and one in aortic valve tissue (Table S1B). Of these, four studies found miR-21 to be significantly differentially expressed between the AS group and control group.¹⁹⁻²² Of the four, three reported an upregulation of miR-21 in the AS group compared to healthy controls. The only study where a downregulation of miR-21 was recorded compared AS in bicuspid valve and tricuspid valve and found a downregulation of miR-21 in the bicuspid valve group and an upregulation of miR-21 in the tricuspid valve group.²² As a significant difference in miR-21 was only reported in one study in the myocardial tissue, and one study in the aortic valve, these were not included in the analysis.

Furthermore, five studies conducted investigations in both fluids and tissue: three in myocardial tissue and plasma and the other two in myocardial tissue and serum (Table S1B). In two of the studies, miR-21 was found to be upregulated in both plasma and myocardial tissue of the AS cohort. In addition, miR-122 and miR-19b showed downregulation in both myocardial tissue and serum in single studies.

Correlation with imaging

None of the studies used MRI to quantify cardiac remodeling or myocardial fibrosis. Whereas 18 of the studies reported echocardiography assessed AS severity, only four studies correlated miR levels with imaging markers of AS severity or remodeling (LV mass). One study showed

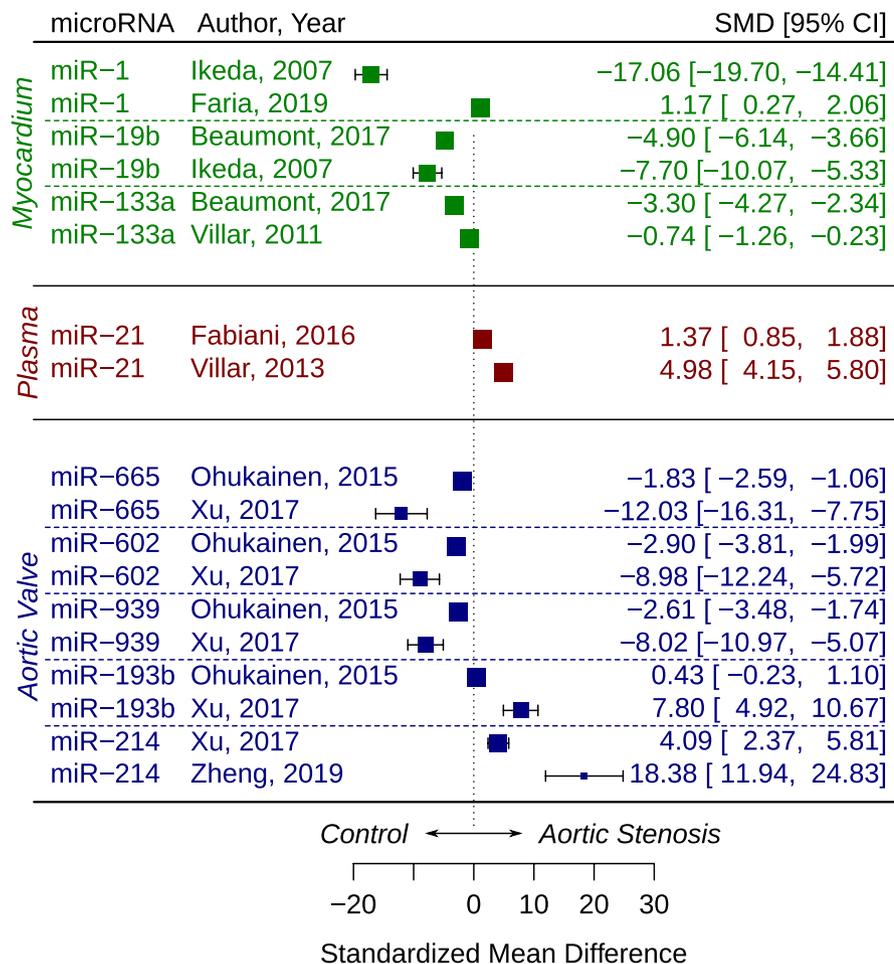


FIGURE 2 Forest plots for the differentially expressed miRs measured in aortic valve tissue, plasma, and myocardial tissue. Only miRs reported in two or more studies are included. Abbreviations: CI, confidence interval; miR, microRNA; SMD, standardized mean difference.

significant correlations of miR-21-5p and miR-382-5p with maximum transvalvular velocity, mean gradient, and LV mass.²³ Two studies showed that patients with normalized LV mass index (LVMI) post-AVR had higher pre-operative expression of miR-133a in the plasma and myocardium, respectively, compared to those who showed residual hypertrophy.^{24,25} Furthermore, miR-125b-5p was shown to be negatively correlated with LV mass and relative wall thickness and miR-4268 positively correlated with LV mass regression.²⁶

DISCUSSION

Our main finding was the overall paucity of data in this field, with the available data being of overall low to moderate quality. A total of 25 studies were included in this review, in which 10 miRs were measured in plasma, 197 miRs in myocardial tissue, and 158 miRs in aortic valve tissue, with the majority of miRs only mentioned in one study, often with insufficient data. Of these, only three miRs in the myocardium and five miRs in the aortic valve tissue were reported in two studies each, with no overlap in the identified miRs in the myocardium and the aortic

valve. MiR-21 was the only one reported in the plasma in two studies. Furthermore, only five studies conducted investigations in both fluids and tissue, of which miR-21 showed upregulation in both plasma and myocardial tissue; and miR-122 and miR-19b showed downregulation in both myocardial tissue and serum. The only miR that was reported to be upregulated in both myocardium and aortic valve was miR-21, but this was only reported in a single study in each tissue source. In addition, higher pre-operative miR-133a in both myocardial and plasma, was associated with post-AVR reduction in LVMI in single studies.

Clinical significance

The most frequently reported miR associated with fibrosis in AS was miR-21. However, it is expressed at relatively high levels in almost all cells and is frequently reported as a biomarker for a range of disorders, including heart disease, cancer, and neurological diseases.²⁷ MiR-21 regulates the ERK-MAP kinase signaling pathway involved in the cardiac fibroblast responsible for cardiac hypertrophy, cardiac remodeling and fibrosis.^{28,29}

MiR-21 was shown to be increased in fibroblasts from a failing heart and the use of antagomir to silence miR-21 *in vivo* showed a reduction in cardiac ERK-MAP kinase activity and an inhibition in interstitial fibrosis.²⁹ Furthermore, Cardin et al.³⁰ showed that in miR-21 knock-out mice, atrial fibrosis was suppressed after induction of myocardial infarction, which further verifies its role in cardiac fibrosis.²⁸ However, the expression of miR-21 also changes in cancer, liver, and kidney disorders, to name a few. The lack of specificity of miR-21 makes it difficult to determine whether the observed changes are solely due to AS or other comorbidities that might be present. Nevertheless, it may be used in predictive models together with other markers of cardiovascular disease. Regrettably, any correlation with other markers was not possible in this study due to the small number of included studies.

Studies reporting miR-1 in the myocardium showed opposite effects. Both studies were performed in cohorts with severe AS, however, comparison was performed with different control subjects; hypertensive without AS³¹ vs. explanted hearts.²⁶ The use of hypertensive subjects as controls might be problematic as hypertrophy and fibrosis occur in response to both hypertension and AS and may therefore influence the results. Santos-Faria's report²⁶ results are further supported by the fact that miR-1 has a protective role in cardiac hypertrophy, where it targets some pro-hypertrophic signaling pathways, such as calcium signaling.³² Alternatively, the discrepancy may be due to very small cohorts (11 and 13 patients).

Studies reporting miR-133a in the myocardium included patients with severe AS. The first study²⁵ assessed the role of miR-133a in the reduction of LVMI and the degree of fibrosis 1 year after AVR, whereas the use of miR-133a among other miRs as a potential biomarker for myocardial fibrosis was assessed in the second study.¹² The results showed upregulation of miR-133a in subjects whose LVMI normalized following surgery than those with persistent hypertrophy,²⁵ which implies that miR-133a plays a protective role in repressing hypertrophy in the cardiac muscle. There was downregulation of miR-133a in those with severe AS before AVR,¹² which further corroborates the theory that downregulation of miR-133a is consistent with increased hypertrophy and fibrosis. These findings are consistent with previous studies that detail miR-133a having a repressing role in hypertrophy and fibrosis.³² This is further backed up by another study included in the review,²⁴ that looked at plasma levels of miR-133a and showed that higher levels were associated with greater LV mass regression after AVR.

There is little known about the role of the other identified myocardial and aortic valve miRs in cardiovascular disease, apart from the fact that they are primarily

used as biomarkers for cancer.^{33–35} No studies included in the review correlated the levels of miR with cardiovascular MRI measures of LV remodeling or fibrosis, and only four studies correlated miR levels with imaging markers of AS severity or remodeling (LV mass) on echocardiography.

Future implications

The studies thus far have mostly proven association, with some demonstrating predictive utility of miRs in AS. Further study could be conducted to determine if the dysregulation of the miRs have a causative effect on the development or progression of fibrosis. This could be achieved using antagomirs and miRs mimics in animal models, to determine if the absence or overexpression of the miRs leads to the development of fibrosis or its progression. In addition, longitudinal clinical studies would be needed to confirm a causative or predictive role, establish their potential use as biomarkers in AS, and correlate them with other established biomarkers of cardiovascular disease.

Limitations of the data

The major limitation of the available data is that all miRs were tested during the symptomatic phase of AS, with many patients already referred for AVR, where the biomarkers are no longer necessary. Because fibrosis measured by late gadolinium enhancement remains a poor prognostic marker even after AVR, biomarkers are needed at an earlier asymptomatic stage of the disease, to better risk-stratify patients who would benefit most from earlier intervention. Another limitation is that none of the identified studies were of high quality. Most studies did not report miR expression levels in the compared groups and only one of the authors deposited their data in public databases. Consequently, meta-analysis could not be performed on the few available data.

Strengths and limitations of the review

This study is the first to our knowledge that has systematically reviewed the role of miRs in cardiac fibrosis and AS. One of the limitations of the current review is we did not contact the authors of the included studies to request for the missing data and further information. This may have provided us more insight and understanding of the results obtained, thereby allowing us to conduct a much more thorough analysis. Second, our search and

inclusion criteria were very specific, potentially limiting the number of included studies. We could have expanded the search criteria to include other remodeling parameters, such as hypertrophy, and other disorders, but this would have introduced bias and was beyond the scope of the review. Finally, we could not test for publication biases because of the small number of studies included.

CONCLUSIONS

In conclusion, our findings indicate that the research into the role of miR in fibrosis associated with AS is of low to moderate quality and missing on conclusive data. Further studies are needed to verify the role of miRs as potential biomarkers in AS, especially at an earlier asymptomatic phase of the disease.

AUTHOR CONTRIBUTIONS

J.O.A., M.J.W., and A.S. wrote the manuscript. M.J.W., A.S., G.M., and G.P.M. conceived the research question. M.J.W. and A.S. designed the research. J.O.A., R.P., and R.A. performed the research. J.O.A. and M.J.W. analyzed the data.

CONFLICT OF INTEREST

All authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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