Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2024): 2.2
CiteScore® (2024): 5.2
www.biomolbiomed.com | blog.bjbms.org

META-ANALYSIS

Nugrahani et al: miRNA-21 as heart failure biomarker

Diagnostic and prognostic value of circulating microRNA-21 in heart failure: A systematic review and meta-analysis

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DOI: https://doi.org/10.17305/bb.2025.13164

ABSTRACT

Heart failure (HF) remains a leading cause of global mortality, underscoring the urgent need for reliable, minimally invasive biomarkers to facilitate early diagnosis and risk stratification. MicroRNA-21 (miR-21) has been implicated in cardiac fibrosis, hypertrophy, and the progression of HF; however, its clinical utility remains uncertain. This study presents a systematic review and diagnostic test accuracy (DTA) meta-analysis aimed at assessing the diagnostic and prognostic performance of circulating miR-21 in HF. We estimated pooled sensitivity, specificity, and area under the curve (AUC) for the DTA analysis, and synthesized hazard ratios (HRs) with 95% confidence intervals (CIs) for prognostic outcomes. Additionally, univariate meta-regression was conducted to explore demographic and clinical moderators. Our analysis included fourteen studies with a total of 1,327 participants. Results demonstrated that circulating miR-21 levels were significantly elevated in HF patients compared to controls (fold change 1.61; 95% CI 1.46–1.78; $p < 0.001$). The diagnostic accuracy was notably high, with a sensitivity of 0.94 (95% CI 82.0–98.0), specificity of 0.90 (95% CI 79.0–96.0), and AUC of 0.97 (95% CI 96.0–98.0). Elevated levels of miR-21 were associated with an increased risk of worsening HF severity (HR 1.84; 95% CI 1.14–2.97; p=0.01) and HF-related cardiovascular death (HR 2.00; 95% CI 1.30–3.03; p=0.001). However, no significant association was found with HF-related hospitalization (HR 0.97; 95% CI 0.61–1.52; p=0.88). Variability in sample type and differing clinical thresholds contributed to heterogeneity across studies. These findings support the potential of circulating miR-21 as a diagnostic and prognostic biomarker for HF. Nevertheless, further research with standardized sample sizes and clinical thresholds is necessary to establish robust evidence for its clinical application.

Keywords: Circulating miRNA, micro-RNA, miRNA-21, heart failure, diagnostic value.

INTRODUCTION

Heart failure (HF) is a complex global health challenge which responsible for over 56 million patients globally (1) . As the leading cause of morbidity and mortality worldwide, the 5-year prognosis of HF remains dismal at less than 45% (2). While HF is often a consequence of a myocardial infarction (MI), currently neither of clinical risk scoring nor biomarkers allow for early detection of HF post-MI. Factors such as ventricular function, aging, obesity, renal failure, and atrial arrhythmias can affect the clinical interpretation of Natriutic Peptide (NP) (3). As accurate detection of HF in a timely manner can facilitate timely interventions and improve patient outcomes, there is a pressing need for reliable, minimally invasive biomarkers to improve the diagnosis and prognosis of HF. Therefore, novel strategies should be developed to improve risk stratification to prevent long-term health disaster (4).

MicroRNAs (miRNAs) are non-coding, single-stranded RNAs approximately 22 nucleotides in length that modulate gene expression post-transcriptionally by inhibiting translation or promoting the degradation of target mRNAs (5). Among the identified miRNA, miRNA-21 is one of the most extensively studied in cardiovascular diseases. It has been reported to be linked in key pathological pathways of HF development, including cardiac remodeling, apoptosis, and hypoxic signaling (6) . Elevated miRNA-21 levels have been reported to increase the major adverse cardiovascular event (MACE) in diabetic patient (7) , and found to be significantly elevated in elderly patients with non-ST elevated myocardial infarction (NSTEMI), suggesting its role in cardiac fibrosis (7) . Notably, miRNA-21 has reported to distinguish between Heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) ; and correlates with echocardiographic parameters (8), hence suggest its potential as a biomarker in HF.

In the context of HF, circulating miRNA-21 originates predominantly from cardiomyocytes, cardiac fibroblasts, and vascular endothelial cells exposed to mechanical stress, ischemia, or inflammatory stimuli (9) . Under these pathological conditions, miRNA-21 is actively secreted into the bloodstream via exosomes and microvesicles or bound to RNA-binding proteins such as Argonaute-2, which protect it from enzymatic degradation. The increased circulating miRNA-21 levels mirror its

intracellular upregulation within the failing myocardium, where it modulates fibroblast activation, extracellular matrix remodeling, and cardiomyocyte apoptosis through pathways such as PTEN/AKT and TGF-β/Smad. The presence of miRNA-21 in the circulation represents both a consequence of myocardial stress and a potential intercellular signaling mechanism, linking myocardial pathology with measurable molecular changes in plasma or serum (10,11).

Despite the existing evidence, the clinical utility of circulating miRNA-21 diagnosing and predicting the prognosis of HF remains unclear. Hence, this study aims to address the diagnostic accuracy and prognostic value of miRNA-21 for HF incidence, hospitalization, and mortality through systematic review and meta-analysis, including meta-regression to explore factors influencing its performance.

MATERIAL AND METHODS

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guideline (12) . This study was adhered to PROSPERO under the number of CRD42024576851 on August 24, 2024.

Search strategy and data sources

A comprehensive search strategy was developed to pool relevant articles from MEDLINE, Web of Science, EMBASE, Scopus, and gray literature up to August 2024. Manual searches of the references were looked through to ensure detailed inclusion. The search was performed independently by three authors (ASDN, WW, RNR) using the query formulation of “microRNA-21” OR “miRNA-21” AND “heart failure” and their synonyms and was detailed using Boolean operators. Searches were restricted to English language and human subjects. Full search strategy term used in each database is available in **Supplementary Material 1**.

Study selection and eligibility criteria

Included studies for this meta-analysis are those that: a) include individuals diagnosed with heart failure based on clinical manifestations or echocardiography according to established guideline, b) measure miRNA-21 levels during an incidence of HF using standardized methods such as qPCR, miRNA sequencing, or real-time PCR, c) using

serum or plasma sample for circulating miRNA-21, and d) are observational, prospective, or retrospective studies, along with DTA studies. Studies providing the information on diagnostic values such as sensitivity, specificity, or area under curve (AUC) along with their 95% confidence intervals (CIs) or provide correlation values between for miRNA-21 and HF were included in diagnostic meta-analysis. Included studies for prognostic meta-analysis are those studies providing the data for prognostic endpoints (New York Heart Association (NYHA) functional class, hospitalization, and HF-related mortality).

Studies lacking sufficient data for analysis, duplicate studies, reviews, case reports, or conducted in non-human subjects were all excluded. Study selection and screening were performed by three authors independently (ASDN, WW, RNR). Any disagreements were settled through discussion with the referee (HS, CDK).

Data extraction

The following details were obtained from each study: author, publication year, study design, country, sample size, diagnostic values (specificity, sensitivity, AUC), predictive values (HF incidence, hospitalization, NYHA class, CV deaths), and cut-off values were extracted. Extracted information was pooled in Microsoft Excel 2021. The comprehensive search and data extraction processes were carried out independently by three authors (ASDN, WW, RNR) to assess study eligibility. Any disagreements were resolved through discussion.

Quality assessment

The risk of bias was investigated for diagnostic test studies using the Quality Assessment of Diagnostic Accuracy Studies 2 Revised (QUADAS-2) tool, while the Quality in Prognosis Studies (QUIPS) tool was used for prognostic studies (13). The QUADAS-2 tool assessed four phases in DTA studies, including patient selection, index test, reference standard, and flow with timing. On the other hand, the QUIPS tool evaluates six domains: study participation, attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis with reporting. The QUIPS tool categorizes the risk of bias as low (green), moderate (yellow), or high (red).

Statistical analysis

To synthesize the result, several analyses were performed in this study. First, bivariate random-effects model meta-analysis for DTA studies was conducted to pool the outcome of sensitivity, specificity, and AUC and their 95% confidence intervals (CIs) from each study. If not available, the sensitivity will be calculated as the proportion of true positives (TP) out of the total number of individuals who have the disease (TP plus false negatives (FN)). In contrast, specificity will be calculated as the proportion of true negatives (TN) out of the total number of individuals who do not have the condition (TN plus false positives (FP)). Summary receiver operating characteristics (SROC) models and plots will be employed to visualize the pooled sensitivity and specificity data. For studies providing different threshold for individual analysis, the threshold effect was quantified by performing a Spearman correlation analysis between logit (sensitivity) and logit (1-specificity).

Meta-analyses of prognostic studies pooled hazard ratios (HRs) with 95% CIs for three endpoints: (1) NYHA class progression, (2) HF-related hospitalization, and (3) HF-related cardiovascular death. Prespecified subgroup analyses explored heterogeneity by acute versus chronic HF, baseline severity (stage/NYHA), and quantification methods used. Between-study heterogeneity was assessed with Cochran's Q and I^2. We applied a coherent decision rule: fixed-effect inverse-variance models when $I^2 < 50\%$ and between-study variance was negligible, and random-effects models (restricted maximum likelihood with Hartung–Knapp adjustment) when $I^2 \geq 50\%$ or τ^2 was non-zero. Sensitivity analyses (leave-one-out and exclusion of abstracts) tested robustness; small-study effects were examined with Egger's test and, when indicated, bias-adjusted estimates were derived using trim-and-fill (14). All analyses were performed in Stata BE version 19 (College Station, Texas).

Meta-regression and sensitivity analysis

If the study heterogeneity is considered moderate (50-75%) or high (>75%), a univariate random-effects meta-regression analysis will be conducted to assess the source of heterogeneity. Sensitivity analysis was performed using the "leave-one-out" method to ensure the robustness of findings by omitting a single study at a time.

Publication bias

Two different methods for publication bias in this meta-analysis were analyzed to assess different analyses. Publication bias for diagnostic test meta-analysis was measured using Deeks' funnel plot (15). A statistically significant asymmetry value, represented by less than a 0.10 *p-value* for the slope coefficient, was considered indicative of publication bias. Additionally, Egger's test was quantified to identify publication bias or small-study effects for prognostic meta-analysis, with less than 0.05 *p-value* was considered significant.

RESULTS

Study selection

A total of 607 study records were identified from four scientific databases. After removing 517 duplicate records, 90 studies were screened based on title and abstract. Following abstract and title screening, 59 articles were excluded due to irrelevant topic. The remaining 31 records were sought for retrieval. During this eligibility assessment, 17 studies were excluded due to irrelevant outcomes (n=8), irrelevant specimen (n=3), non-specific populations (n=2), the use of non-human subjects (n=2), and unsuitable study designs (n=2). Ultimately, 14 studies were included in this meta-analysis. Of these, six studies, seven datasets were analyzed for diagnostic accuracy, five for prognostic value, and eleven of them for expression patterns, with certain studies contributing to multiple analyses due to overlap outcomes. Detail of the study selection process is at **Figure 1.**

Characteristics of the included studies

Fourteen studies were ultimately included in this study (4,16–28) . The included studies were conducted in Asia, Europe, and America. The included studies included various types of HF, including acute decompensated heart failure (ADHF) and chronic heart failure (CHF) with both systolic and diastolic dysfunctions. Studies measuring circulating miRNA-21 from both plasma and serum were included. Measurement methods for miRNA-21 included in this study predominantly involved RT-PCR, RT-qPCR, microarray analyses, and next generation sequencing. Several studies reported specific cut-off values for biomarkers, often utilizing the Youden

Index to determine sensitivity and specificity, with some showing high diagnostic accuracy. Characteristics of the included studies are available in **Table 1.**

Diagnostic studies were assessed using QUADAS-2 while prognostic studies were evaluated using QUIPS for quality assessment (13) . The quality of the included studies is summarized in **Supplementary Material 2.**

Diagnostic values of miRNA-21 in HF

Six diagnostic test accuracy studies, with seven datasets, (n=507 participants) were included. One study provided two outcomes from different samples, which are from peripheral vein (PV) and coronary sinus (CS) (26). Pooled estimates from six studies, seven datasets (Zhang 2017 CS and PV reported separately), showed that circulating miR-21 detected heart failure with sensitivity 0.94 (95% CI 82.0–98.0) and specificity 0.90 (95% CI 79.0–96.0). Heterogeneity was high for both sensitivity (I^2=85.0%) and specificity (I^2=82.4%). The summary ROC analysis yielded an AUC of 0.97 (95% CI 0.95–0.97). Deeks' funnel plot showed a non-significant slope (p=0.60), suggesting no significant publication bias.

Our study explored three clinically plausible scenarios using Fagan's nomogram to illustrate a range of pre-test probability settings. For a pre-test probability of 25%, the application of Fagan's nomogram indicated that a positive test result would raise the post-test probability to 76%, while a negative result would decrease it to 3%. Additionally, in scenarios where the initial pre-test probability was set at 10%, a positive test increased the post-test probability to 51% and negative result would decrease to 1%. Furthermore, when the pre-test probability was 50%, a positive result elevated the post-test probability to 90% and negative result would decrease to 6%. These findings collectively underscore the potential utility of miRNA-21 in supporting the diagnosis of heart failure across a variety of clinical contexts. Further details regarding the selection and rationale for these pre-test probabilities are provided in Supplementary Material 5.

Details of the forest plot for specificity and sensitivity result is available in **Figure 2-3.** **Supplementary Material 3** provided the total of TP/FP/TN/FN counts for each study.

However, there is a significant threshold effect as noted using Spearman's rho = −0.78 with the p-value of 0.045 detected in our analysis, suggesting that heterogeneity in our meta-analysis is at least partly due to differences in test positivity thresholds across studies.

Upregulated miRNA-21 levels in heart failure patients

We included the pooled analysis of eleven studies evaluating differentially expressed miRNA-21 levels in HF subjects compared to non-HF **(Figure 4).** The pooled analysis showed that circulating miR-21 was significantly upregulated in HF compared with controls (fold change 1.61; 95% CI 1.46–1.78; $p < 0.001$). Between-study heterogeneity was low (I^2=47.2%), so a fixed-effect model was used. To explore potential sources of heterogeneity, meta-regression was performed and will be explained in next section (**Table 2).**

Subgroup analysis

Subgroup analyses were performed across clinical onset (acute or chronic heart failure), LVEF category (reduced vs preserved), sample type, quantification method, and study continent. The pooled effect size was significantly differed across onset and continents. The pooled effect size differed significantly by clinical onset ($p = 0.01$), studies including only ADHF patients showed a 1.92-fold increase, those including only CHF patients showed a 1.43-fold increase, and studies including both ADHF and CHF patients showed a 2.09-fold increase. Effect sizes also differed significantly by continent ($p < 0.01$), with studies conducted in Europe demonstrating a 2.07-fold increase, Asia 1.33-fold increase, and America a 1.90-fold increase. No other interaction tests reached statistical significance ($p > 0.05$) (**Figure 5**).

Association between miRNA-21 in NYHA class

The association between upregulated miRNA-21 levels and HF severity was assessed by evaluating its association with the NYHA class category. The fixed-effects meta-analysis of three studies showed that patients with higher miRNA-21 levels had an 84% higher risk of having NYHA class ≥ 2 compared with those with lower miRNA-21 levels (HR: 1.84, 95% CI: 1.14–2.97, $p = 0.01$) **(Figure 6A).** Heterogeneity was found to be low ($I^2 = 0\%$), hence fixed model analysis was utilized.

Prognostic value of miRNA-21 in HF hospitalization

Three studies evaluated miR-21 and HF-related hospitalization **(Figure 6B).** The random-effects meta-analysis showed no significant association (HR 0.97; 95% CI 0.61–1.52; p=0.88). Heterogeneity was moderate (I^2=50%), therefore a random-effects model was used. Sensitivity analyses indicated that no single study materially influenced the pooled effect.

Prognostic value of miRNA-21 in HF-related cardiovascular death

Two studies assessed HF-related CV death. The pooled estimate indicated that higher miR-21 levels were associated with a two-fold greater risk (HR 2.00; 95% CI 1.30–3.03; p=0.001) **(Figure 6C).** Heterogeneity was negligible (I^2=0%), with both studies showing a positive association; results were consistent under a fixed-effect model.

Meta-regression analysis

Meta-regression was performed to analyze the potential sources of heterogeneity in the pooled estimates of expression and diagnostic studies meta-analysis. Meta-regression assessed the impact of study location by continent, sample type, heart failure subtype (HF with reduced ejection fraction vs preserved ejection fraction (HFrEF vs HfpEF), miRNA quantification method, and acute vs chronic disease status.

None of the variables reached statistical significance in expression studies, indicating that these factors did not significantly explain heterogeneity between studies as observed. Differences in sample type (plasma vs. serum), heart failure subtype, or quantification method, including RT-qPCR, RT-PCR, microarray, and next-generation sequencing, were not associated with significant changes in the effect estimates.

However, for meta-regression analysis for diagnostic studies revealed the significant influence of sample type in the analysis (plasma/serum), with p<0.01 and p=0.02 for sensitivity and specificity, respectively. Detailed statistical analysis for meta-regression for expression studies is available **Table 2,** while meta-regression for diagnostic accuracy studies is available at **Table 3.**

Publication bias

Publication bias was assessed using Deeks' funnel plot for the DTA meta-analysis, and Egger's test ± trim-and-fill for the expression studies meta-analysis. For the DTA meta-analysis, Deeks' funnel plot showed no evidence of asymmetry ($p = 0.60$). In the expression studies meta-analysis, no significant publication bias or small-study effects were detected, as indicated by Egger's test ($p = 0.80$) and the visually inspected trim-and-fill funnel plot **(Supplementary Material 4).** The bias estimate (intercept = 0.11, SE = 0.46) was not statistically significant, further indicating no evidence of small-study effects.

Sensitivity analysis

Sensitivity analysis assessed the robustness of the meta-analysis results by systematically excluding one research at a time. This approach was used to discover if any single study or combination of studies substantially impacted the overall findings. As demonstrated in **Supplementary Material 5**, excluding one study had no significant impact on the overall results, explaining the conclusion are robust even when individual studies are omitted.

DISCUSSION

Our findings suggest that miRNA-21 showed a pooled sensitivity of 94% and specificity of 90%, as evidenced by the area under the SROC curve (AUC) of 0.97 to diagnose HF. Fagan's nomogram confirms potential clinical utility, indicating that the positive post-test probability of diagnosing HF increases substantially. On the prognostic side, the upregulation of miRNA-21 was significantly associated with the severity and outcomes of HF. Higher miRNA-21 levels are linked to a higher New York Heart Association (NYHA) class, with an 84% increased risk of being classified into the NYHA class above 2. This finding was consistent across studies with low heterogeneity, emphasizing miRNA-21's role in reflecting disease severity. Additionally, miRNA-21 levels were predictive of HF-related cardiovascular death, with a two-fold increase in risk, further highlighting its prognostic value. However, no significant association was found between miRNA-21 levels and HF-related hospitalization, which may reflect limitations in available data or heterogeneity in hospitalization criteria across studies.

Initially studied in tumor growth, miRNA-21 is now recognized for its role in maintaining cardiovascular homeostasis. In HF, it is known to promote cardiac fibrosis by activating the ERK-MAPK pathway via sprout homolog 1 (SPRY1) suppression and modulating Ang II-induced cardiac fibrosis by inhibiting phosphatase and tensin homolog (PTEN) and SMAD family member 7 (SMAD7) (29). The other strand of miRNA-21, miRNA-21-3p, has also been noted in cardiac hypertrophy progression to HF. Increased miRNA-21-3p expression during HF in humans can induce cardiomyocyte hypertrophy via exosome communication between cardiac fibroblasts and cardiomyocytes (30).

Overall, miRNA-21 is upregulated during the progression of cardiac hypertrophy to HF, reducing cardiomyocyte size and mediating cardiac fibrosis, underscoring its significant role in HF pathogenesis (28). This finding also supported by Pan et al., which linked miRNA-21 in key remodeling and fibrosis process by regulating connective tissue growth factor (CTGF). It is recognized to be activated by hypoxia and inflammation, influencing the survival of cardiomyocytes (31). Understanding the precise, cell-specific functions and targets of miRNA-21 is critical for its potential clinical application in treating cardiovascular diseases.

According to the pooled outcomes, the changes in miRNA-21 levels are closely associated with HF, particularly in predicting its incidence, NYHA functional class progression, hospitalization rate, and cardiovascular death. Aligned with previous studies, circulating miRNA-21 levels in patients with stable or acute decompensated HF, differ significantly from those in control subjects, supporting their utilization as biomarkers in diagnosing HF. However, it is noteworthy that the role of miRNA-21 differs between acute and chronic HF. In acute phases, miRNA-21 is typically upregulated, contributing to the immediate response to cardiac injury. It promotes tissue repair and reduces infarct size. However, as the disease progresses to a chronic state, the expression of miRNA-21 tends to return to baseline, reflecting a stabilization phase where the acute repair mechanisms are less active (32).

Previous study by Ding et al., (2020) reported a 0.944 AUC of miRNA-21 in diagnosing HF, with a sensitivity of 89.7 and specificity of 82.8. This result can be obtained as miRNA-21 is highly stable in blood and thus can regulate sequence-

specific gene expression, allowing them to be unaffected by hemolysis, age, or gender in HF diagnosis (28). Studies have shown that miRNA-21 modulates critical signaling pathways, such as ERK-MAPK, crucial in developing cardiac hypertrophy and fibrosis. The ability miRNA-21 to influence critical processes in HF pathophysiology supports its use as a diagnostic tool, identifying patients at risk for HF and guiding therapeutic interventions (33,34) . Previous studies demonstrated that miRNA-21 levels correlate significantly with the severity of HF, offering a non-invasive method for early HF detection and monitoring disease progression (26).

Moreover, circulating miRNA levels correlate with well-established prognostic clinical parameters, such as Brain Natriutic Peptide (BNP) or N-terminal prohormone BNP (NT-proBNP) and left ventricular ejection fraction (LVEF) (27). This reinforces the potential of miRNA-21 as a biomarker for HF progression and highlights the complexity of its role in different aspects of heart failure pathophysiology. The ongoing research underscores the need for further studies to fully understand the multifaceted roles of miRNA-21 and its potential implications in HF management (32) . BNP and its inactive fragment NT-proBNP remain the current gold-standard biomarkers for diagnosing and monitoring HF, reflecting myocardial wall stress and hemodynamic overload. However, these peptides can be affected by factors such as age, renal function, obesity, and atrial fibrillation, which may limit their diagnostic accuracy in specific patient populations (35) . In contrast, miRNA-21 reflects molecular and structural remodeling processes rather than hemodynamic changes, participating directly in the regulation of fibrosis, inflammation, and cardiomyocyte survival. Several studies have shown that circulating miRNA-21 levels correlate with BNP and NT-proBNP yet may also detect subclinical myocardial remodeling even when natriuretic peptide levels are normal. A recent study demonstrated that miRNA-21-5p levels were significantly elevated in patients with HFrEF and correlated moderately with NT-proBNP, suggesting that miRNA-21 may complement, rather than replace, traditional biomarkers in comprehensive HF assessment. The pooled result of included studies also documented that the diagnostic accuracy of miRNA-21 is notably higher in diabetic patients. Hyperglycemia, insulin resistance, and the associated oxidative stress and inflammation in diabetes enhance the expression and effects of miRNA-21. Analysis of the GSE4745 microarray dataset found that high glucose conditions cause down-regulation of Hk2 miRNA and increased expression of

regulatory miRNAs, including miRNA-21, in rats, indicating miRNA-21's significant role in the disease's development (36) . Hence, the presence of diabetes, in combination with upregulated miRNA-21, can worsen HF outcomes in diabetic patients (37).

Despite advancements, the rising incidence of heart failure HF highlights the need for effective diagnostic and treatment options, such as blood biomarkers. While many biomarkers have been identified, most lack the required sensitivity and specificity to reliably detect the progression of HF severity. Hence, miRNA-21 shows a potential because the pooled results of the included studies demonstrated good sensitivity and specificity (38) . The ability of miRNA-21 to influence critical processes in HF pathophysiology supports its use as a diagnostic tool, capable of identifying patients at risk for HF and guiding therapeutic interventions. It is also indicated that miRNA-21 are involved in inflammation and immune-related pathways, suggesting its role in regulating inflammation by modulating genes critical to inflammatory responses, which is a key factor in HF to initiate myocardial remodeling and injury. Moreover, its stress-responsive characteristics, pro-fibrotic miRNA central to cardiac fibroblast function and particularly enriched in HF patients' fibroblasts (39) . In vivo, inhibition of miRNA-21 prevents pressure overload-induced cardiac interstitial fibrosis and cardiac dysfunction. Pathological hypertrophy and fibrosis in myocardial cells, which lead to increased left ventricular filling pressures and eventually HF syndrome, are the main mechanisms of HFpEF (38).

In clinical practice, miRNA-21 could serve as a non-invasive biomarker for early detection, prognosis, and monitoring of HF progression. Its integration into routine clinical assays potentially allow patient risk stratification, thereby facilitating personalized treatment. Additionally, miRNAs have been reported to differentiate between HFrEF and HFpEF and show correlations with echocardiographic measurements, which could address the limitations of NT-proBNP (8,26). As the field advances, clinical trials will be essential to determine the safety, efficacy, and long-term outcomes of anti-miRNA-21 therapy in humans. However, the cost and accessibility of miRNA-based diagnostics and therapeutics must be considered, especially in resource-limited settings. In addition, no standardized clinical threshold for circulating miRNA-21 has yet been established, and variations in assay techniques

and normalization methods limit comparability across studies. Future work should prioritize the development of standardized quantification protocols and clinically validated cut-off values to ensure reproducible interpretation and facilitate the integration of miRNA-21 into daily practice. As our understanding of miRNA-21 in HF evolves, its integration into clinical practice can potentially transform the management and outcomes of this complex and prevalent condition.

CONCLUSION

Circulating miRNA-21, as a minimally invasive biomarker, demonstrates strong diagnostic and prognostic potential for heart failure detection and monitoring. Elevated miRNA-21 levels are consistently associated with HF occurrence, NYHA class progression, and cardiovascular mortality. These results support its integration as a biomarker for early diagnosis and patient risk stratification, aligning with precision medicine approaches in HF management. However, standardized analytical thresholds and validated cut-off points for miRNA-21 quantification remain to be established. Therefore, large, prospective, multicentre studies are required to confirm its predictive power, particularly for HF-related hospitalization, and to define its practical utility in clinical workflows. Collectively, this meta-analysis reinforces miRNA-21 as a potential biomarker for the early detection, prognostic assessment, and personalized management of heart failure.

GAMER statement: The authors declare that no generative AI tools were used in the writing, editing, figure/table preparation, or reference management of this manuscript.

Conflicts of interest: Authors declare no conflicts of interest.

Funding: Authors received no specific funding for this work.

Data availability: The dataset used in this study is available within this manuscript and supplementary information can be requested from the corresponding author on request.

Submitted: August 21, 2025

Accepted: November 26, 2025

Published online: December 24, 2025

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TABLES AND FIGURES WITH LEGENDS

Table 1. Characteristics of the included studies

Study, year	Country	Study design	Acute/ chronic HF	LVEF	No. patients with HF	No. control	Assay methods
Al-Hayali et al., 2019 (16)	Turkey	Case-control	ADHF	HFrEF	45	45	RT-PCR
Ben-Zvi et al., 2020 (17)	Israel	Case-control	ADHF	HFrEF	39	21	RT-qPCR
Cakmak et al., 2015 (18)	Turkey	Prospective Cohort	CHF	HFrEF	42	15	Microarray
Davydova et al., 2020 † (19)	Russia	Prospective Cohort	CHF	HFpEF	180	60	RT-PCR
Ding et al., 2020 (28)	China	Case-control	All HF	all	62	62	RT-PCR
Galluzzo et al., 2021 (20)	Italy	Case-control	CHF	HFrEF	30	36	RT-PCR
Goren et al., 2012 (21)	Israel	Case-control	CHF	HFrEF	30	30	RT-qPCR
Kan et al., 2019 (22)	China	Case-control	CHF	HFpEF	60	35	RT-PCR
Marketou et al., 2024 (23)	Greece	Prospective cohort	ADHF	HFpEF	56	94	RT-qPCR
Meiri et al., 2020 (24)	Israel	Prospective cohort	CHF	HFrEF	8	10	RT-qPCR
Rincón et al.,	Spain	Prospective	CHF	all	43	268	RT-qPCR

2022 (4)		cohort					
Schneider et al., 2018 (25)	Brazil	Prospective cohort	ADHF	HFrEF	48	17	RT-qPCR
Sygitowicz et al., 2015 (27)	Poland	Prospective cohort	Both	HFrEF	35	26	RT-qPCR
Zhang et al., 2017 (26)	China	Prospective Cohort	ADHF	HFrEF	80	40	RT-qPCR

† Abstract only. Abbreviations: HF: Heart failure; LVEF: Left ventricular ejection fraction; ADHF: Acute decompensated heart failure; CHF: Chronic heart failure; HFrEF: Heart failure with reduced ejection fraction; HFpEF: Heart failure with preserved ejection fraction; RT-PCR: Reverse transcription polymerase chain reaction; RT-qPCR: Reverse transcription quantitative polymerase chain reaction.

Table 2. Meta-regression analysis from the pooled studies

Category	Variable	Coefficient	Std. Err.	z	P>|z|	95% Conf. Interval (Lower)	95% Conf. Interval (Upper)
Onset	ADHF	0.32	0.46	0.71	0.48	-0.57	1.22
	CHF	-0.01	0.42	-0.02	0.98	-0.84	0.82
LVEF	HFpEF	0.72	1.56	0.46	0.65	-2.35	3.79
	HFrEF	-0.20	0.52	-0.38	0.71	-1.22	0.83
Specimen	Plasma	Ref.					
	serum	0.36	0.39	0.93	0.35	-0.40	1.13
Quantification Method	RT-qPCR	Ref.					
	RT-PCR	0.66	1.58	0.42	0.68	-2.43	3.75
	microarray	0.59	1.75	0.34	0.74	-2.84	4.02
Continent	America	Ref.					
	Asia	-0.41	0.85	-0.48	0.63	-2.08	1.26
	Europe	-0.14	1.28	-0.11	0.91	-2.65	2.37
Constant	cons	-0.15	2.04	-0.07	0.94	-4.15	3.85

Abbreviations: ADHF: Acute decompensated heart failure; CHF: Chronic heart failure; LVEF: Left ventricular ejection fraction; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; RT-qPCR: Reverse transcription quantitative polymerase chain reaction; RT-PCR: Reverse transcription polymerase chain reaction.

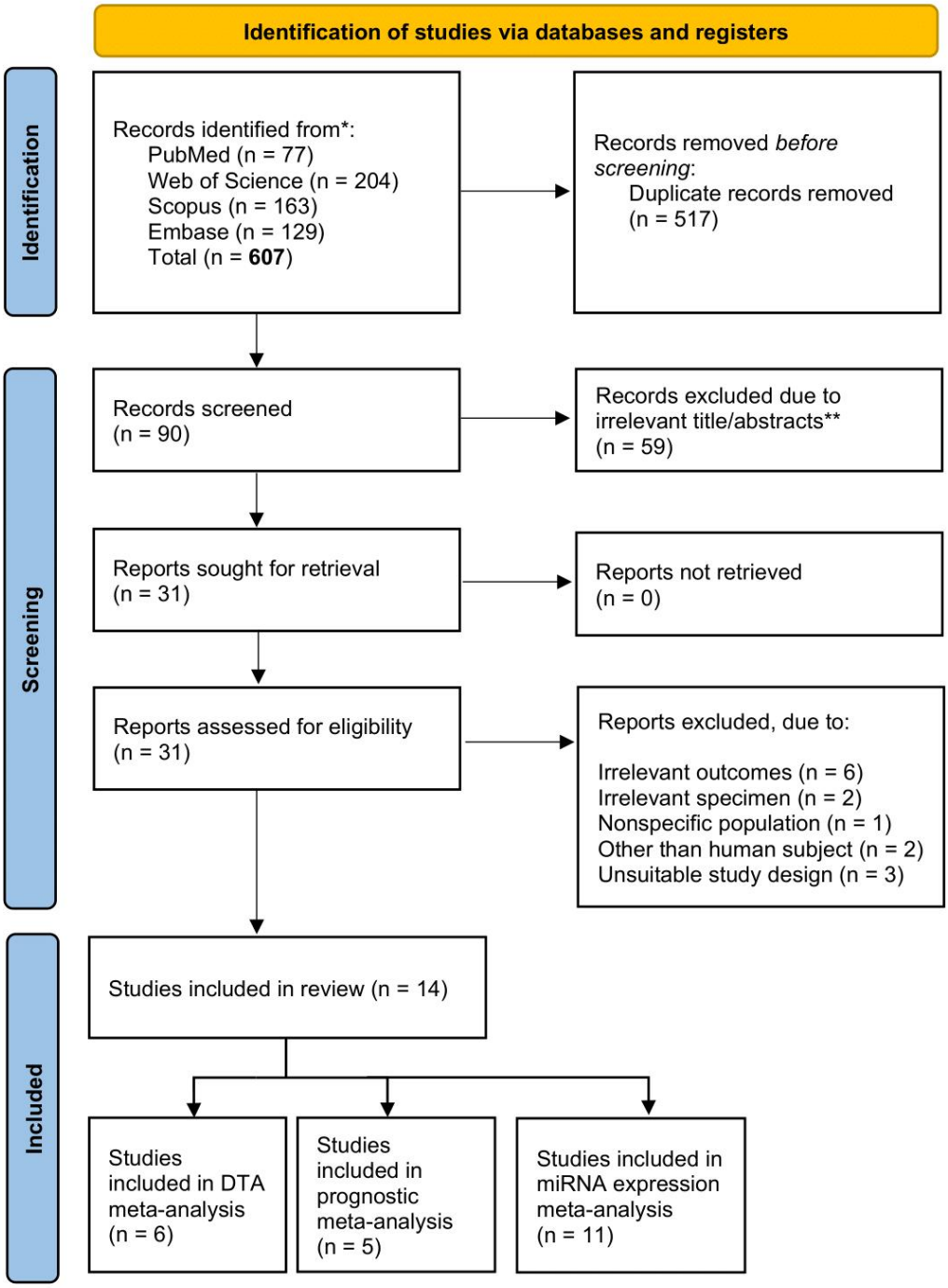

Figure 1. PRISMA flow diagram of included studies

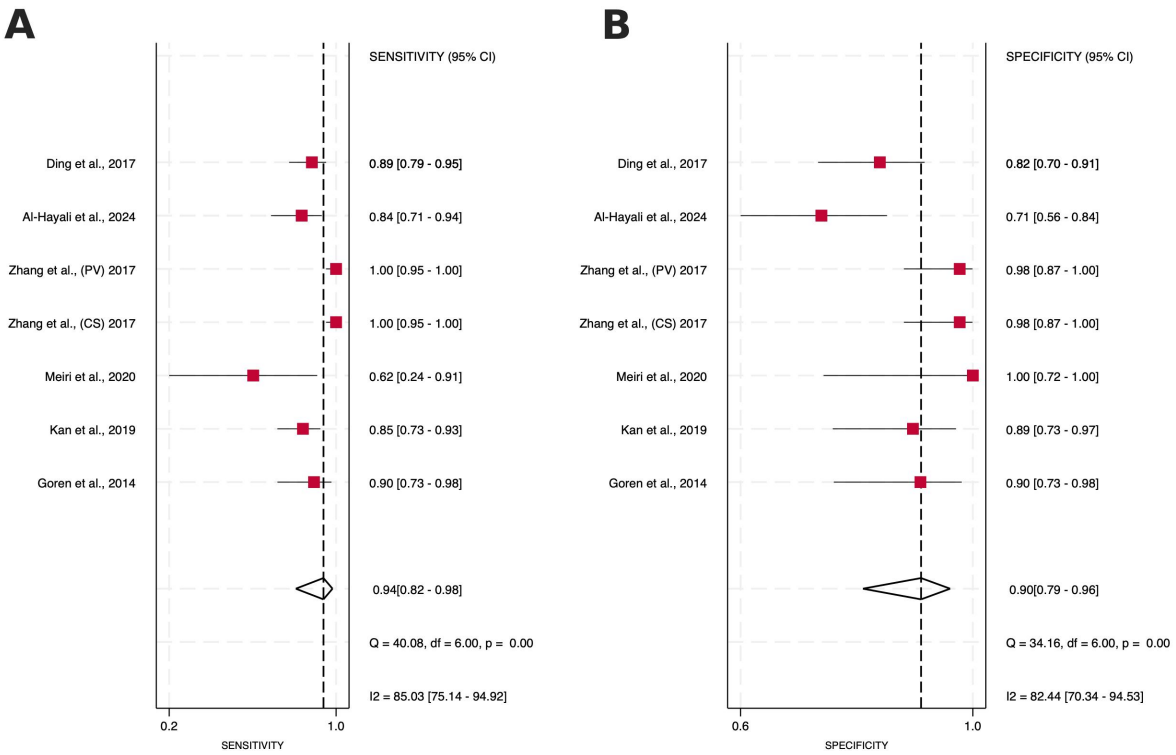

Figure 2. Forest plots summarizing the diagnostic accuracy of circulating miRNA-21 for heart failure (HF): (A) sensitivity and (B) specificity. Point estimates from six studies (seven independent datasets; Zhang et al., 2017 reported CS and PV cohorts separately) are represented as red squares with 95% confidence intervals (horizontal bars). The pooled summary estimate is illustrated by the diamond (width = 95% CI), while the dashed vertical line indicates the pooled value. Measures of between-study heterogeneity, including Cochran's Q and I^2, are provided below each panel.

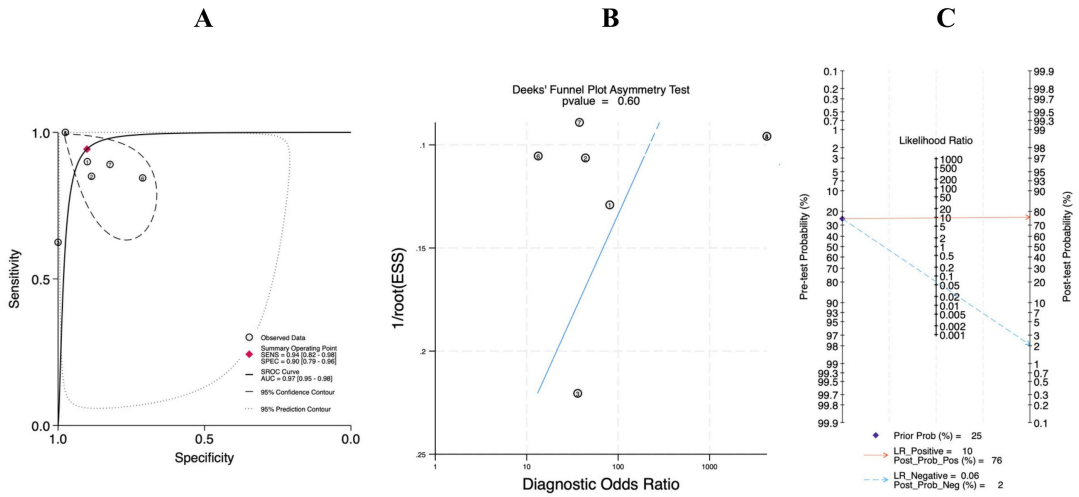

Figure 3. Diagnostic accuracy and clinical utility of circulating miRNA-21 for heart failure (HF). (A) SROC plot showing individual study estimates (circles), the bivariate summary point (diamond), fitted SROC curve, and 95% confidence/prediction regions. (B) Deeks' funnel plot for publication bias (p = 0.60). (C) Fagan's nomogram translating likelihood ratios to post-test probability (pre-test 25%: post-test 76% for LR+ = 10; 2% for LR− = 0.06).

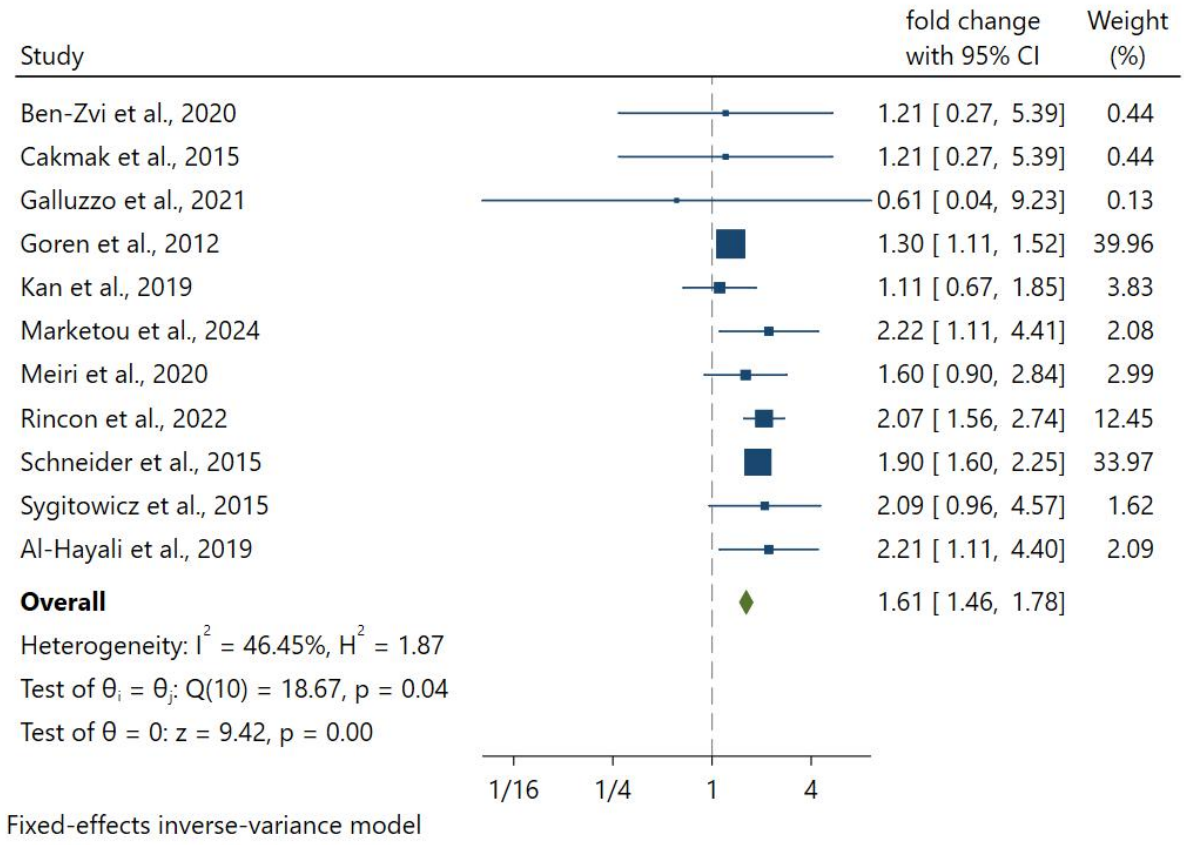

Figure 4. Forest plot depicting miRNA-21 expression levels in relation to HF events. Individual squares represent study-specific fold changes in circulating miR-21 expression among HF patients compared to non-HF controls. Horizontal lines indicate 95% confidence intervals, with the size of each square proportional to the inverse-variance weight of the respective study. The diamond shape represents the pooled effect estimate derived from a fixed-effects inverse-variance model, illustrating significantly elevated circulating miR-21 levels in HF patients (overall fold change 1.61; 95% CI 1.46–1.78; $p < 0.001$). The analysis reveals low-to-moderate heterogeneity between studies, with an I^2 value of approximately 46%. Abbreviations: HF: Heart failure; miRNA-21: MicroRNA-21; CI: Confidence interval.

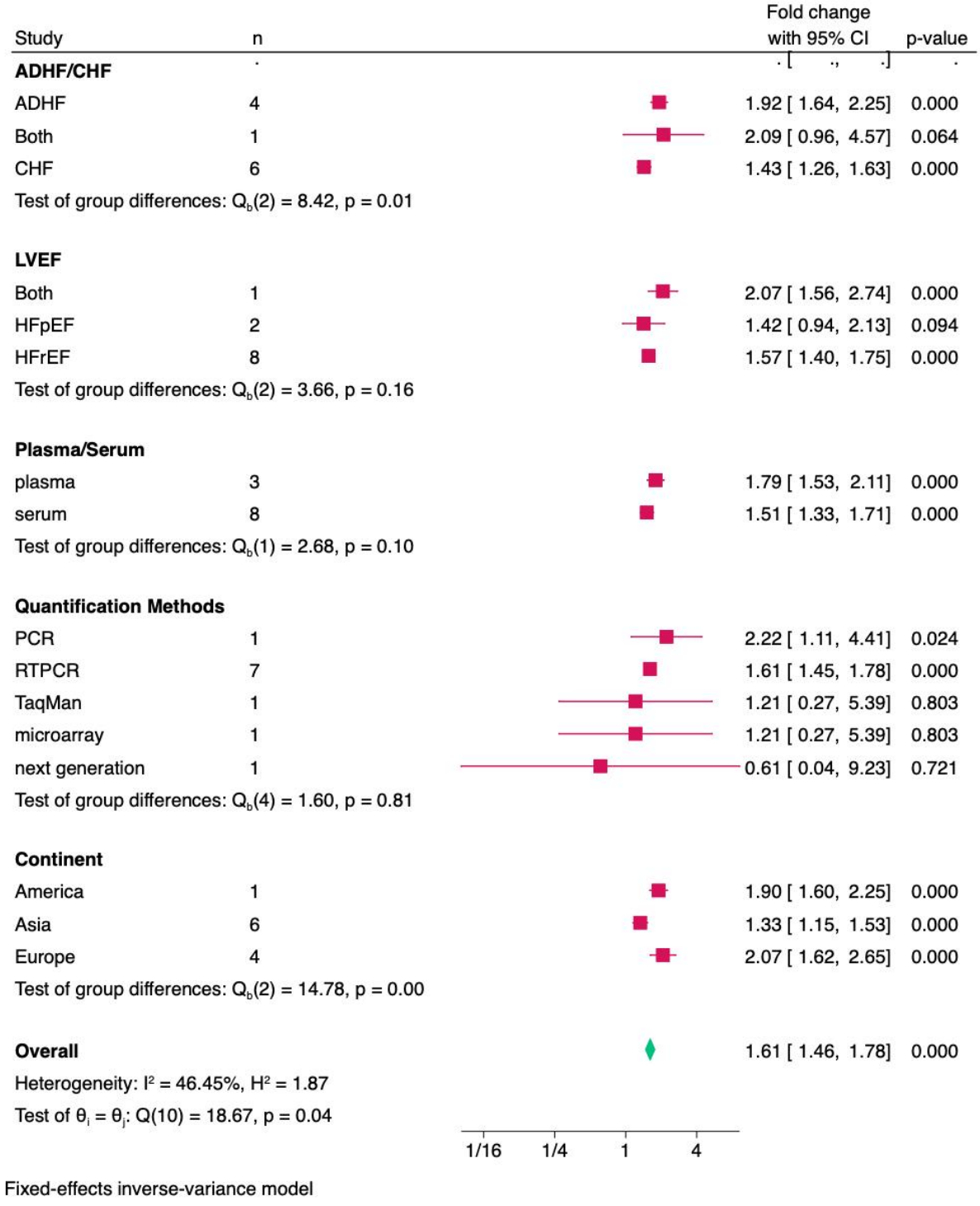

Figure 5. Subgroup analysis of circulating miRNA-21 expression levels in HF.

Forest plot shows fold changes (squares) with 95% confidence intervals across subgroups defined by onset (ADHF, CHF, both), LVEF phenotype (HFrEF, HFpEF, both), sample type (plasma vs. serum), quantification method, and continent; the diamond indicates the overall pooled fold change from a fixed-effects inverse-variance model. Abbreviations: HF: Heart failure; ADHF: Acute decompensated heart

failure; CHF: Chronic heart failure; HFrEF: Heart failure with reduced ejection fraction; HFpEF: Heart failure with preserved ejection fraction; LVEF: Left ventricular ejection fraction.

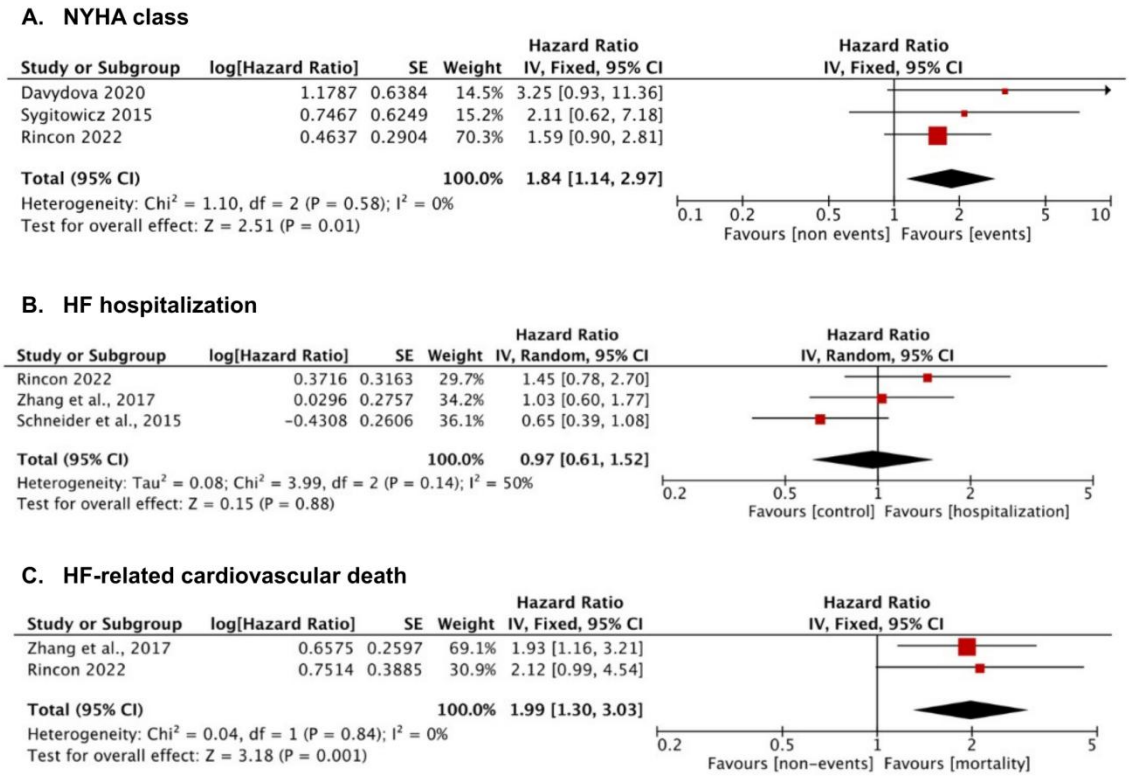

Figure 6. (A) Forest plot illustrating the relationship between miRNA-21 and NYHA classification. (B) Forest plot evaluating the association between miRNA-21 and heart failure-related hospitalizations. (C) Forest plot examining the relationship between miRNA-21 and heart failure-related cardiovascular mortality.

SUPPLEMENTAL DATA

Supplemental data are available at the following link:

https://www.bjbms.org/ojs/index.php/bjbms/article/view/13164/4091