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Novel and emerging therapies in acute and chronic heart failure

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Published in
Frontiers in Cardiovascular Medicine

ISSN 1664-8714
ISBN 978-2-83252-111-3
DOI 10.3389/978-2-83252-111-3

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Novel and emerging therapies in acute and chronic heart failure

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Citation

Triposkiadis, F., Boudoulas, K. D., Xanthopoulos, A., Karagiannis, G., Giamouzis, G., Skoularigis, J., eds. (2023). *Novel and emerging therapies in acute and chronic heart failure*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83252-111-3

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Editorial
PUBLISHED 23 March 2023
DOI 10.3389/fcvm.2023.1179352

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 04 March 2023
ACCEPTED 10 March 2023
PUBLISHED 23 March 2023

CITATION
Giamouzis G, Xanthopoulos A, Boudoulas KD, Karagiannis G, Skoularigis J and Triposkiadis F (2023) Editorial: Novel and emerging therapies in heart failure.
Front. Cardiovasc. Med. 10:1179352.
doi: 10.3389/fcvm.2023.1179352

Editorial: Novel and emerging therapies in heart failure

Grigorios Giamouzis[1,2]*, Andrew Xanthopoulos[1], Konstantinos Dean Boudoulas[3], Georgios Karagiannis[4], John Skoularigis[1,2] and Filippos Triposkiadis[1,2]

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KEYWORDS

heart failure, acute, chronic, novel therapies, emerging therapies, comorbidities, HFrEF – heart failure with reduced ejection fraction, HFpEF – heart failure with preserved ejection fraction

Editorial on the Research Topic
Novel and emerging therapies in heart failure

Heart failure (HF) has been named "the growing epidemic" (1, 2). Over the last decade, the annual number of HF hospitalizations has almost doubled with approximately 50% of patients being rehospitalized within 6 months of discharge (3). The complex array of physiologic, psychological, social, and health care delivery issues makes it a challenging chronic disease to manage. Understanding the epidemiology and pathophysiology of the syndrome, identifying the predictors and their strength of association with outcomes, and using the available diagnostic modalities cost-effectively are essential in order to implement novel therapeutic approaches to curb this epidemic.

This research topic was initiated by Triposkiadis et al. and published in 2022 in Frontiers in Cardiovascular Medicine. Our aim was to invite articles highlighting current research in novel or emerging therapeutic approaches -pharmacological and invasive- in both the acute and chronic setting of the disease, as well as potential interaction and/or barriers with concomitant cardiovascular or non-cardiovascular diseases. In this special issue, we present 7 original research articles, 3 systematic review articles and 1 opinion article that address such issues and investigate why, despite the emergence of novel therapeutic approaches that promise life prolongation and hospital length reduction, this patient population often has a poor prognosis and often requires rehospitalization. Below is a summary of these articles.

An increased number of HF patients suffer from chronic angina, which seriously impairs their quality of life (4). Cardiac shock wave therapy (CSWT) has recently become appealing due to the improvement in angina symptoms. In a systematic review and meta-analysis by Quan Qiu et al. the efficacy of CSWT on coronary artery disease (CAD) patients was evaluated. A total of 8 randomized controlled trials (RCTs) and 2 prospective cohort studies involving 643 patients ($n = 336$ CSWT and $n = 307$ control) were included in the study. CSWT was shown to moderately improve myocardial perfusion and cardiac function among patients with CAD, a finding that may provide the clinicians with a meaningful and valuable option for revascularisation, for instance in selected HF patients with ischemic cardiomyopathy.

Puerarin (7,40-dihydroxy-8-C-glucosylisoflavone) is the major bioactive ingredient of the root of Radix Puerariae and has been widely applied for the adjunctive management of CAD; its main drug delivery method is intravenous injection (5). Zunjiang Li et al. conducted a systematic review and meta-analysis that aimed to assess the adjunctive efficacy and safety of Puerarin injection (PI) in acute HF patients. Eight studies were included with a total of 614 patients with acute HF that demonstrated that adjunctive treatment with PI was superior to, and as safe as, conventional medicine alone. Specifically, PI increased the total effective rate and improved left ventricular ejection fraction (LVEF) as compared with conventional therapy without raising safety concerns, as there was no significant difference in adverse events between the two groups.

The pharmacological management of heart failure with preserved ejection fraction (HFpEF) continues to be an area of uncertainty due to multiple studies failing to show a clear benefit associated with therapies that have otherwise proven useful in the management of other HF subtypes. Therefore, both the 2021 ESC and the 2022 AHA/ACC/HFSA updated HF guidelines state that "in the absence of recommendations regarding disease-modifying therapies, treatment should be aimed at reducing symptoms of congestion with diuretics" (6, 7). Based on the results of 10 current RCTs comprising 10,334 patients, Danning Yang et al. performed a meta-analysis to illustrate the therapeutic impact of sodium-glucose cotransporter-2 (SGLT2) inhibitors in HFpEF patients. Treating HFpEF patients with SGLT-2 inhibitors was associated with a 22% reduction in the composite outcome of cardiovascular death or hospitalization for HF and had a similar improvement in health-related quality of life; interestingly, no statistical difference was observed in 6MWT distance. In an opinion article, Luis Tolento Cortes and Lisa Hong highlight the flaws of all recent RCTs regarding the use of ACEIs/ARBs/ARNIs, beta-blockers, aldosterone antagonists and SGLT2 inhibitors in patients with HFpEF. In a subsequent original research article, María Valero-Muñoz et al. identified the cardiac-specific features of protein and phosphoprotein changes in a murine model of HFpEF using mass spectrometry. Proteomics analysis of the left ventricular (LV) tissue showed that almost 900 proteins were expressed differentially between HFpEF and sham mice including changes in sarcomeric proteins, mitochondrial-related proteins, and NAD-dependent protein deacetylase sirtuin-3 (SIRT3). In summary, this study demonstrates marked changes in proteins related to mitochondrial metabolism and the cardiac contractile apparatus in HFpEF; the authors propose that SIRT3 may play a role in perpetuating these changes and, therefore, may be a target for drug development in HFpEF.

Yishu Wang et al. examined the effects of early phase 1 cardiac rehabilitation (CR) on cardiac function in patients with coronary heart disease (CHD) and acute HF using impedance cardiography (ICG). A total of 98 patients were recruited and randomized into two groups. The control group received standard pharmacotherapy and the CR group received standard pharmacotherapy combined phase 1 CR. NT-proBNP and hemodynamic parameters measured by ICG were estimated at baseline and at the end of the treatment period. Phase 1 CR resulted in a more pronounced reduction in NT-proBNP levels. Similarly, most hemodynamic parameters improved in the CR group, but not in the control group. These findings suggest that phase 1 CR in combination with the standard pharmacotherapy could improve hemodynamic characteristics by elevating cardiac output, ameliorating preload, improving systolic and diastolic function, and relieving afterload. Therefore, suitable stabilized patients with CHD and acute HF should undergo phase 1 CR.

In a phase 1, open-label, single-arm, first-in-human study, Lien-Cheng Hsiao et al. aimed to assess the safety and efficacy of combined intracoronary (IC) and intravenous (IV) transplantation of umbilical cord-derived mesenchymal stem cells (UMSC01) for heart repair in patient with a ST-elevation myocardial infarction (STEMI) with impaired LVEF (30%–49%) following successful reperfusion by percutaneous coronary intervention. In the 6 subjects who completed the study, there were no treatment-related serious adverse events or major adverse cardiovascular events during infusion or follow-up. NT-proBNP levels and wall motion scores decreased, whereas the LVEF increased significantly at the 12-month follow-up compared to the baseline values. This pilot study showed that combined IC and IV transplantation in this patient population appears to be safe, feasible, and potentially beneficial in improving heart function. Phase 2 studies will be needed to further investigate the effectiveness of dual-route transplantation of UMSC01 in STEMI patients.

In another interesting original research study by Bin He et al. the pathogenesis, immune-related pathways and important biomarkers involved in the progression of cardiomyopathy due to various etiologies were investigated. The authors reported that the hub genes (CD14, CCL2, and SERPINA3) can be used as markers to distinguish patients with cardiomyopathy. Furthermore, the authors demonstrated that the innate immune response, either dysregulation/imbalance of innate immune cells or activation of adaptive immune responses, were involved in the progression of HF.

Red blood cell distribution width (RDW) is a simple hematological parameter that reflects the heterogeneity of the red blood cell size (anisocytosis); higher levels of RDW have been associated with poor outcome in patients with HF (8, 9). Recent studies suggest that the favorable pivotal mechanism of SGLT-2 inhibitor in patients with HF is the stimulation of erythropoiesis *via* an early increase in erythropoietin (EPO) production, which leads to a rise in hematocrit (10). However, EPO has been also implicated in the pathophysiology of RDW increase in this patient population. In a very interesting mechanistic study by Nikolaos Katsiadas et al. the effects of SGLT-2 inhibitor administration on RDW in patients with HF and diabetes mellitus (DM) were examined. RDW increased with time in patients who received dapagliflozin and this increase was mainly attributed to the induction of hemopoiesis from dapagliflozin. Interestingly, increased baseline, but not post-SGLT2 inhibitor therapy, RDW values were independently associated with poor outcomes in patients with HF and DM.

Left bundle branch pacing (LBBP) is emerging as an effective alternative to achieve cardiac resynchronization therapy (CRT)

and improve heart function (11). In a retrospective study by Ying Gu et al. the feasibility and efficacy of LBBP in HF patients with a LVEF <50% and left bundle branch block (LBBB) was investigated; the study suggested that LBBP using a low and stable pacing threshold was feasible with a high successful implantation rate and was effective in correcting LBBB and improving LV structure and function.

Finally, Zhang Fang et al. aimed to evaluate the proper energy intake patterns and daily calorie intake in adult patients with HF in the United States of America. Among almost 1,000 participants, moderate malnutrition was more frequently related with mortality. Low-carbohydrate pattern (LCP) and median-carbohydrate pattern (MCP) diets had a lower risk of death as compared to a high-carbohydrate pattern (HCP) diet. There was no association between different amounts of calorie intake and all-cause mortality. Therefore, the relationship between energy intake and all-cause mortality may be influenced by energy intake patterns in HF patients.

Research in HF is an exciting and continuous endeavour. The hope is that this special edition on *Novel and Emerging Therapies in Heart Failure* will provide a meaningful contribution to the literature, will shed light to the understanding of HF, and will open new treatment pathways for the benefit of our patients.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Systematic Review
PUBLISHED 25 July 2022
DOI 10.3389/fcvm.2022.934598

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 02 May 2022
ACCEPTED 01 July 2022
PUBLISHED 25 July 2022

CITATION
Li Z, Fan Y, Huang C, Liu Q, Huang M, Chen B, Peng Z, Zhu W and Ding B (2022) Efficacy and safety of Puerarin injection on acute heart failure: A systematic review and meta-analysis.
Front. Cardiovasc. Med. 9:934598.
doi: 10.3389/fcvm.2022.934598

Efficacy and safety of Puerarin injection on acute heart failure: A systematic review and meta-analysis

Zunjiang Li[1,2], Ye Fan[1,2,3], Chunxia Huang[1,2], Quanle Liu[1,2,3], Manhua Huang[1,2], Baijian Chen[1,2,3], Zhe Peng[1,2], Wei Zhu[1,2,3]* and Banghan Ding[1,2,3]*

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Objective: This study aimed to assess the adjunctive efficacy and safety of Puerarin injection (PI) on acute heart failure (AHF) based on a systematic review and meta-analysis.

Methods: Nine databases were searched from March 1990 to March 2022 to identify randomized controlled trials (RCTs) related to the adjunctive treatment of PI for AHF. The Cochrane collaboration tool was used to assess the risk of bias in the included studies. Meta-analysis and subgroup and sensitivity analyses were conducted by RevMan 5.3 software. The evidence's certainty was evaluated by grading recommendations assessment, development, and evaluation (GRADE) methods.

Results: A total of 8 studies were included with a total of 614 patients with AHF. The meta-analysis demonstrated that adjunctive treatment with PI on AHF was superior to conventional medicine alone. It increased the total effective rate (RR = 1.38; 95% CI, 1.22–1.55; $p < 0.001$) and improved left ventricular ejection fraction [SMD = 0.85; 95% CI (0.62, 1.09); $p < 0.001$]. Regarding safety, a total of 11.9% (23/194) adverse reactions were observed in the PI group and 9.8% (19/194) adverse reactions in the control group, and there were no significant differences in the incident rate of adverse events between both groups [RR = 1.16; 95% CI (0.66–2.05); $p = 0.061$]. The outcomes' evidentiary quality was assessed as "moderate."

Conclusion: PI had an adjunctive effect on AHF combined with conventional medicine, and it seemed to be safe and more effective than the conventional medical treatment alone for improving the total clinical effective rate and left ventricular ejection fraction. But further well-designed RCTs are required to confirm the efficacy and safety

of XBP in treating AHF due to the poor methodological quality of the included RCTs.

Systematic Review Registration: [https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=327636], identifier [CRD42022327636].

KEYWORDS

acute heart failure, Puerarin injection, meta-analysis, systematic review, traditional Chinese medicine

Introduction

Acute heart failure (AHF) is a clinical complex syndrome characterized by rapid deterioration and reduction in ventricular function necessitating hospitalization (1, 2). It has a prevalence of more than 23 million worldwide, associated with significant mortality, morbidity, and healthcare expenditures (3, 4). Significant drug advances have been developed and recommended in the treatment of patients with AHF in the past decades, including diuretic drugs, positive inotropes, vasodilators, neurohormonal antagonists, mechanical circulatory support, respiratory management, etc., [2021; (3, 5)], while none of the treatments tested to date have been definitively proven to improve AHF survival (6). Regarding patients with acutely decompensated HF or HF with preserved ejection fraction, approximately 50% of HF patients with preserved ejection fraction die within 5 years (5), and up to one in six patients with acute decompensation HF die during admission or within 30 days after discharge (4). Thus a new and an alternative drugs management of AHF is still challenging and of imperative need.

Puerarin (*7,4′-dihydroxy-8-C-glucosylisoflavone*) is the major bioactive ingredient of the root of *Radix Puerariae*, which was isolated in the late 1950s (7). Puerarin injection (PI) has been widely applied for the adjunctive management of coronary heart disease treatment and its main drug delivery method is intravenous injection (8). Clinical and experimental research proved that PI combined with conventional treatment could further improve the curative unstable angina pectoris (8, 9). PI could dilate coronary artery, increase coronary blood flow, decrease heart rate, inhibit platelet aggregation, and improve microcirculation (8, 10, 11). Literatures continuously reported clinical adjunctive efficacy and safety of PI, as well as their experimental effect and mechanism in animal models on AHF, but they still lacked relevant reviews summarizing the efficacy and safety of PI in the treatment of AHF in terms of the quality of methodology and evidence.

In the present study, we aimed to clarify the efficacy and safety of PI as an adjunctive treatment for acute heart failure (AHF) based on the available evidence in clinical practice. We mainly focused on clarifying whether PI had an adjunctive effect by combined use with conventional treatment and evaluating the safety of PI regarding its combined use.

Data and methods

The effectiveness and safety of PI were critically assessed by a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (12).

Database for search

A total of 5 English databases (the MEDLINE *via* PubMed, the Cochrane Library, EMBASE, the Web of Science and Ovid database) and 4 Chinese databases [China Science and Technology Journal Database (VIP), Chinese Biomedical Literature Database (CBM), Wan-fang Database, China National Knowledge Infrastructure (CNKI)] were searched for identifying studies from March 1990 to March 2022.

Criteria for studies included

Type of participants (P)

Patients diagnosed with AHF in consistence with the AHF diagnostic criteria recognized at the time of publication of the study, regardless of age, gender, and course of the disease.

Type of interventions (I and C)

Control group: Conventional western medicine treatment, including diet and life regulation, diuretics, cardiotonic, oxygen inhalation, ECG monitoring, low-salt diet, restricted liquid intake etc. The treatment group was treated with PI in addition to the control group.

Type of outcome measures

Primary outcomes (O): ①Total clinical effective rate; ②left ventricular ejection fraction (LVEF); secondary outcomes: ①left

ventricular end-diastolic dimension (LVEDD); ②isovolumic relaxation time (IVRT); ③peak A velocity of the mitral inflow; ④peak E velocity of the mitral inflow; ⑤stroke volume SV; safety outcome: adverse events.

Types of studies (S)

Randomized controlled trials (RCTs) of PI in the treatment of AHF, without limit on method and language.

Exclusion criteria

①Repeated publications; ②case report; ③pure theoretical research; ④The data in the literature were wrong or incomplete.

Searching strategy

The MeSH terms of PICOS were combined to search in [Title/Abstract] by developing our search strategies sequentially. A combination of P+I, P+I+C, P+I+C+O, and P+I+C+O+S was used to search for studies. If the number of searched studies were small, we would search as P+I. The artificially screened studies according to the included and excluded criteria and the searching strategy are detailed in **Supplementary File 1**.

Data collection and analysis

Selection of studies and Kappa-coefficient analysis

After two review authors search out the articles, another two authors retrieved full text after screening the titles and abstracts, which meet with criteria of PICOS. Any discrepancies were handled by a discussion among all the authors. Then Kappa-coefficient analysis was performed regarding the level of agreement among the reviewers in article selection.

Data extraction and management

For data extraction, two reviewers independently identified the details for each study and presented them in a standardized form. The author's name, published year, sample size, initial characteristics of patients, treatment detail, criteria for AHF diagnosis, outcomes and adverse reactions, etc., were extracted by two authors independently.

Evaluation of risk of bias

The quality evaluation was assessed by the risk of bias assessment tool recommended by Cochrane Handbook 5.1. Seven aspects were assessed by two review authors, including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other sources of bias. The quality evaluation was judged as "high," "low," or "unclear" risk of bias. Any discrepancies were handled by consensus.

Data synthesis and analysis

The effect size was pooled using the Review Manager Software tool (RevMan, v.5.3; The Cochrane Collaboration). A fixed-effect model was chosen for the pool that had low heterogeneity, and a random-effects model was used where there was high heterogeneity. Mean deviation (MD) or Std mean difference (SMD) and 95% confidence intervals (CI) were utilized for continuous data, and relative risk (RR) with 95% CI were calculated for dichotomous data. Subgroup analysis and sensitivity analysis were also used to investigate potential sources of heterogeneity.

Sensitivity analysis

Sensitivity analysis was used to explore the significant heterogeneity that existed in studies, aiming to assess whether the conclusions were robust to the decision-making process. This study conducted a sensitivity analysis to observe whether the new effect-size results and heterogeneity changed significantly after removing single studies.

Evidence confidence

The grading recommendations assessment, development, and evaluation (GRADE) technique (13) were used to assess the evidence's certainty following the instructions of the website[1]. RCT evidence was initially classified as high quality, but it would be downgraded due to the risk of bias, inaccuracy, inconsistency, informality, and publication bias. The level of evidence was classified into four categories: "high," "moderate," "low," and "very low."

Results

Results of randomized controlled trials selection

A total of 75 related articles were initially detected. After 25 duplicate studies were eliminated, 50 RCTs were included for further screening. Then 39 studies were excluded without matching the inclusion requirements, and 3 non-RCT studies were eliminated after reviewing the article in detail. Finally, 8 studies (14–21) with a total of 614 patients with AHF were incorporated for systematic review and meta-analysis. Kappa-coefficient analysis suggested that the level of agreement among

1 https://www.gradepro.org/

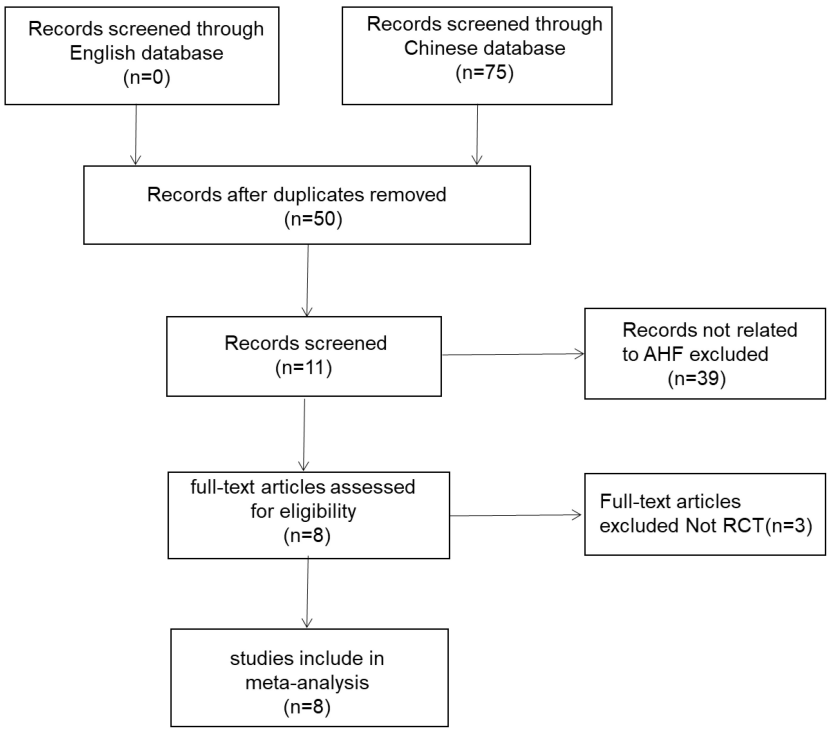

FIGURE 1
A PRISMA flow diagram of the literature screening and selection process.

the two reviewers in article selection had a high degree of consistency (Kappa = 0.805, **Supplementary Table 1**). **Figure 1** depicted the literature screening process and results.

Characteristics of the included randomized controlled trials

All 8 included RCTs were conducted in China between 2012 and 2019, the sample size ranged from 58 to 100, and the treatment duration varied from 7 days to 14 days, except in one study (21) which had no report on duration. All research interventions were Puerarin injection (PI) in combination with conventional western treatment, and the drug delivery methods of PI were intravenous injection in all the studies. In terms of the usage and dose of PI, 3 studies diluted 500 mg of Puerarin with 500 ml 5% glucose (14, 16, 17), 2 studies diluted 500 mg Puerarin with 250 ml 5% glucose (18, 21), and 3 studies diluted 200–400 mg with Puerarin with 500 ml of 5% glucose (15, 19, 20). Only 3 studies (14, 15, 17) reported that the AHF diagnostic criteria was inconsistent with acute heart failure diagnosis and treatment guide (2010 version) published by the Cardiovascular Disease Branch of Chinese Medical Association (22). None of the studies reported follow-up results. The basic characteristics of included RCTs are detailed in **Table 1**.

Risk of bias assessment

One trial (16) was rated as low risk for using random number tables to generate sequences, while the other studies (14, 15, 17–21) provided no details about the method of random sequences generation. All the included studies published complete data, and no selective outcomes were reported, so the risk of bias was considered "low." Beyond that, no studies mentioned the information about concealing of allocation, blinding of researchers, participants, and outcome evaluators, resulting in the risk of bias regarding performance, and detection was considered "unclear." The risk of other bias was considered "low," since no other obvious bias was observed in all RCTs. **Table 2** shows the results of the risk of bias of the included RCTs.

Meta-analysis results

Primary outcome measures of total effective rate

Six studies (14–17, 20, 21) involving 444 patients reported the total effective rate. The fixed-effects model was used for meta-analysis as there existed little heterogeneity between the studies (p = 0.83, I^2 = 0%). As shown in **Figure 2**, the results of the meta-analysis suggested that PI combined with

TABLE 1 Characteristics of included RCTs investigating the adjunctive effect of Puerarin injection (PI) on acute heart failure.

Included study (author/year/ language)	Sample size (E/C)	Average age (E/C)	Duration	Interventions		Usage and dose	AHF diagnostic criteria	Adverse events	Outcome
				Experiment group	Control group				
Ma (15)	40/40	60.35 ± 6.55/ 60.40 ± 6.53	20 days	PI plus CWT+M	CWT+M	200–400 mg diluted with 5% glucose 500 ml	a	Nausea, hypotension, vomiting, headache	①⑥
Zheng et al. (16)	42/42	60.6 ± 10.2/ 54.3 ± 13.5	14 days	PI plus CWT+M	CWT+M	500 mg diluted with 5% glucose 500 ml	NR	Nausea, hypotension, vomiting, headache	①②③⑤⑥
Zhang (20)	34/34	56.3 ± 5.8/ 56.8 ± 5.3	14 days	PI plus CWT+M	CWT+M	200–400 mg diluted with 5% glucose 500 ml	NR	Nausea, hypotension, vomiting, headache	①②③⑥
Xu (14)	33/33	61.37 ± 5.62/ 63.35 ± 4.13	7 days	PI plus CWT+L	CWT+L	500 mg diluted with 5% glucose 500 ml	a	No adverse events	①②③⑤⑥
Li (17)	29/29	60.21 ± 3.05/ 60.13 ± 3.11	14 days	PI plus CWT+M	CWT+M	500 mg diluted with 5% glucose 500 ml	a	NR	①②
Wu (19)	34/34	67.28 ± 3.10/ 66.03 ± 3.87	7 days	PI plus CWT+ rhBNP	CWT+ rhBNP	200–400 mg diluted with 5% glucose 500 ml	NR	NR	②
Wang (18)	50/50	58.05 ± 1.25/ 57.15 ± 1.46	14 days	PI plus CWT+M	CWT+M	500 mg diluted with 5% glucose 250 ml	NR	NR	②
Xiong (21)	45/45	58.96 ± 8.15/ 58.87 ± 8.21	NR	PI plus CWT+M	CWT+M	500 mg diluted with 5% glucose 250 ml	NR	Slow heart rate, hypotension, headache	①②③④⑥

E/C, Experimental group/ Control group; PI, Puerarin injection; CWT, conventional western treatment; M, Metoprolol; rhBNP, Recombined human; NR, Not report; ①: Total Effective Rate; ②LVEF: Left ventricular ejection fraction; ③LVEDD: Left ventricular end-diastolic dimension; ④SV: Stroke volume; ⑤NT-proBNP: N-terminal pro-B-type natriuretic peptide; ⑥Adverse events. a. Acute heart failure diagnosis and treatment guide (2010 version) published by Cardiovascular Disease Branch of Chinese Medical Association.

TABLE 2 The results of risk of bias of included RCTs.

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other source of bias
Ma (15)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Zheng et al. (16)	Low	Unclear	Unclear	Unclear	Low	Low	Low
Zhang (20)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Xu (14)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Li (17)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Wu (19)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Wang (18)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Xiong (21)	Unclear	Unclear	Unclear	Unclear	Low	Low	Low

conventional medical treatment increased the total effective rate by comparing with conventional medicine alone (RR = 1.38; 95% CI, 1.22–1.55; $p < 0.001$), indicating that PI had a favorable adjunctive effect on the total effective rate of AHF. Subgroup analyses according to PI doses showed that 200–400 mg/day (RR = 1.30; 95% CI, 1.09–1.55; $p = 0.003$) and 500 mg/day (RR = 1.42; 95% CI, 1.22–1.67; $p < 0.001$) of PI combined with conventional medicines treatments both increased the total effective rate compared with conventional medicine alone.

Primary outcome measures of left ventricular ejection fraction

Seven studies involving 534 patients reported the results of LVEF. The random-effects model was used for meta-analysis as there existed high heterogeneity between studies ($p < 0.001$, $I^2 = 96\%$). The results of the meta-analysis indicated that combining PI with a conventional medical treatment significantly improved LVEF (RR = 1.07; 95% CI, 0.87–1.27; $p < 0.001$, **Supplementary Figure 1**). Sensitivity analyses were

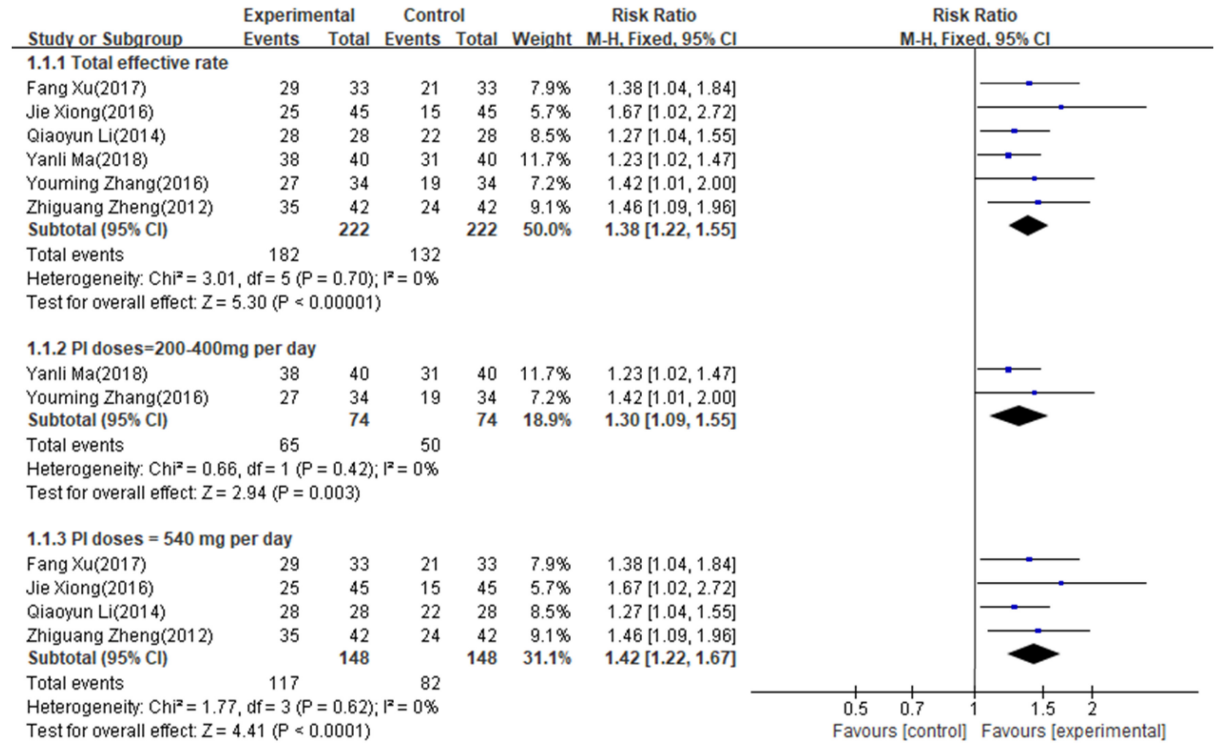

FIGURE 2
Forest plot of total effective rate (the total effective rate = effective rate + significant effective rate; invalid rate: patient's heart function, physical signs, and clinical AHF symptoms have not been improved or even worsen; effective rate: patient's heart function improved with 1 level, physical signs, and clinical AHF symptoms are relieved; significant effect rate: patient's heart function improved with 2 level or more, the heart rate decreased to normal level, physical signs, and clinical AHF symptoms have disappeared).

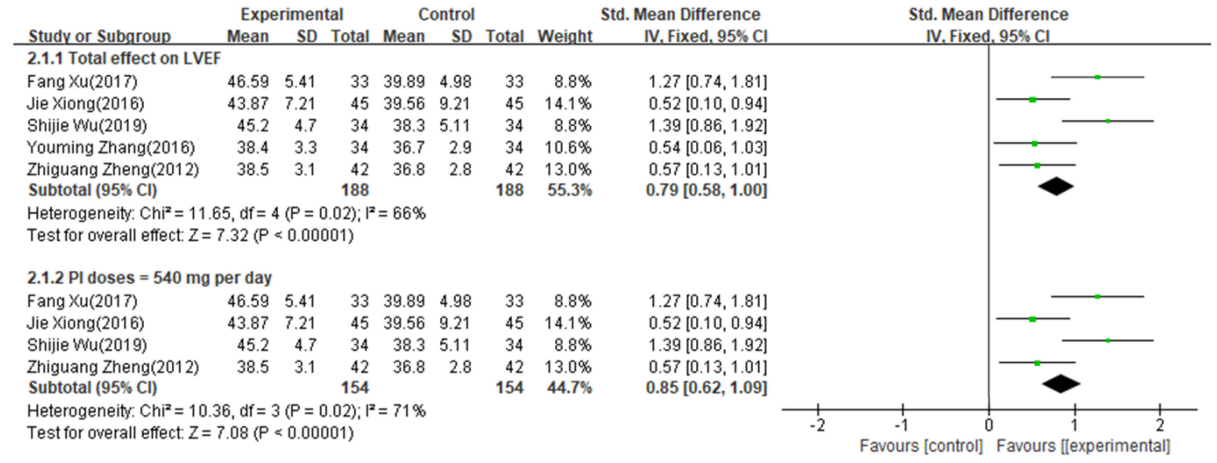

FIGURE 3
Forest plot of LVEF.

performed by excluding studies one by one. After removing the studies reported by "(17)" (17) and "(18)" (18), heterogeneity between studies was significantly reduced to 66%. As shown in **Table 1**, the sample size of the study "(17)" (17) and "(18)" (18) were the largest and smallest compared with other studies respectively, which might contribute to high heterogeneity. The results showed that the LVEF of patients with AHF was still significantly improved by the combined use of PI with conventional medical treatment (SMD = 0.79; 95% CI, 0.58–1.00; $p < 0.001$, **Figure 3**), and it indicated that combined

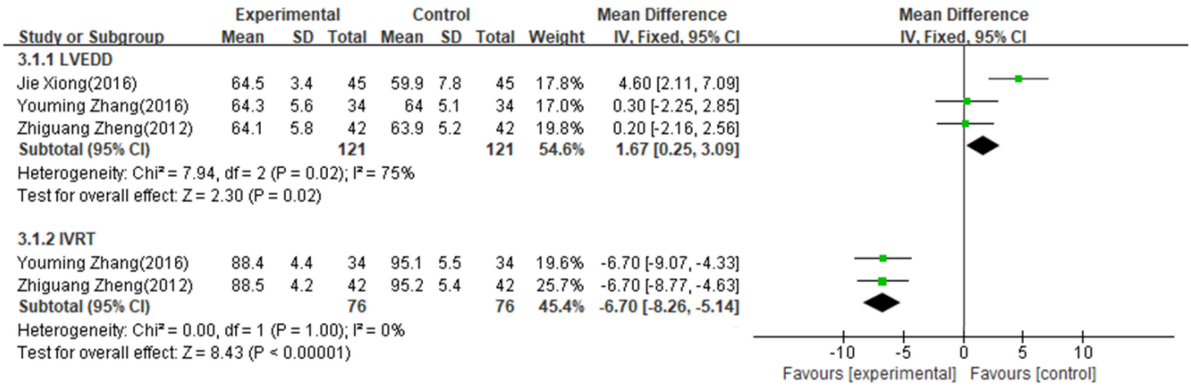

FIGURE 4
Forest plot of other heart function indicators.

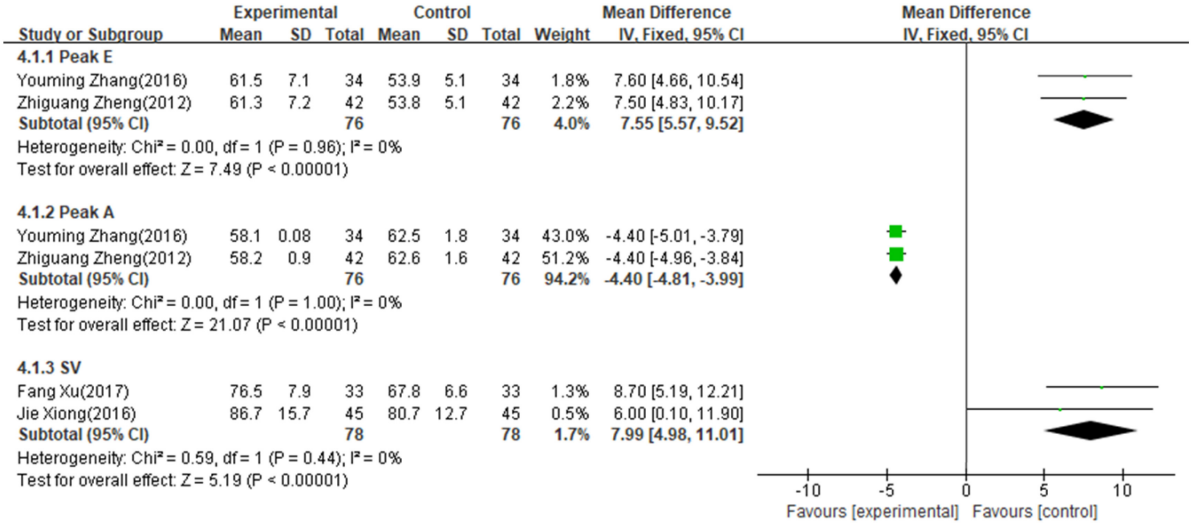

FIGURE 5
Forest plot of left ventricular diastolic function.

use of PI was beneficial for LVEF in patients with AHF. Subgroup analyses showed that 500 mg/day of PI combined with conventional medicines treatments also improved the LVEF compared with conventional medicine alone (SMD = 0.85; 95% CI, 0.62–1.09; $p < 0.001$, **Figure 3**). As "(15)" (15) did not report the LVEF valve, there were no sufficient studies ($\geq$ 2) for subgroup analyses on the dose of 200–400 mg/day.

Secondary outcome measures of other heart function indicators

Three studies (16, 20, 21) involving 242 patients reported the value of LVEDD and two studies (16, 20) involving 152 patients reported the value of IVRT. The fixed-effects model was used for meta-analysis on LVEDD ($p = 0.02$, $I^2 = 75\%$) and IVRT ($p = 1.00$, $I^2 = 0\%$) as there existed low to median heterogeneity between studies. As shown in **Figure 4**, the results of meta-analysis indicated that combining PI with conventional medicine treatment improved the heart function, including increased LVEDD (MD = 1.67; 95% CI, 0.25–3.09; $p < 0.001$, **Figure 4**) and decreased IVRT (MD = −6.70; 95% CI, −8.26 to −5.14; $p < 0.001$, **Figure 4**) when compared with conventional medicine alone.

Secondary outcome measures of left ventricular diastolic function

Two studies (16, 20) involving 152 patients reported the value of peak E, two studies (16, 20) involving 152 patients reported the value of peak A, and two studies (14, 21) involving 156 patients reported the value of SV. The fixed-effects model was used for meta-analysis on peak E ($p = 0.96$, $I^2 = 0\%$), Peak A ($p = 1.00$, $I^2 = 0\%$) and IVRT ($p = 0.44$, $I^2 = 0\%$) as there existed no heterogeneity between studies. As shown in **Figure 5**,

TABLE 3 The incidence rate of adverse effect.

Adverse effect	Studies	Total number of adverse effects	
		Experiment group	Control group
Nausea	(15, 16)	4	5
Hypotension	(15, 16, 21)	6	3
Vomiting	(15, 16)	4	4
Headache	(15, 16, 21)	3	1
Slow heart rate	(21)	2	2
No detailed classification	(20)	4	4
No adverse effect	(14)	0	0
Total events		23/194	19/194
Incident rate		11.9%	9.8%

the results of meta-analysis indicated that combining PI with conventional medicine treatment improved the left ventricular diastolic function, including increased peak E (MD = 7.55; 95% CI, 5.57–9.52; $p < 0.001$, **Figure 5**), decreased Peak A (MD = −4.40; 95% CI, −4.81 to −3.99; $p < 0.001$, **Figure 5**), and increased SV (MD = 7.99; 95% CI, 4.98–11.01; $p < 0.001$, **Figure 5**) when compared with conventional medicine alone.

Safety of adverse events comparison

Five studies (14–16, 20, 21) involving 388 patients reported adverse events. As detailed in **Table 3**, one study (14) reported no adverse reactions in both groups and one study (20) reported the total number of adverse effects without classification. Three studies (15, 16, 21) reported a detailed number of each kind of adverse reactions in both groups. In all, it reported a total of 11.9% (23/194) adverse reactions in the PI group and 9.8% (19/194) adverse reactions in the control group. All of the adverse reactions were modest, and no significant difference in the incident rate of adverse events was observed in both groups (RR = 1.16; 95% CI, 0.66–2.05; $p = 0.061$, **Figure 6**), indicating that adjunctive use of PI was safe as a conventional medical treatment.

Results of publication bias assess

We assessed publication bias on the results of total effective rate, LVEF, and adverse effect, as other results had less than three studies included. We detected that there is no publication bias on the results of total effective rate, LVEF, and adverse effect (**Figure 7**). But because of lacking access to the information on the clinical trial registry or study protocol, it could not rule out the potential of selectively reporting existing results. The published bias result of other results are provided in **Supplementary File 2**.

The quality of the evidence

The certainty of evidence on meta outcomes was assessed by the grading recommendations assessment, development, and evaluation (GRADE) methods, and it showed that evidentiary quality of meta results varied from "very low" to "moderate." The rationale for the downgrade was mainly due to small sample sizes and unclear risk of bias in the selected studies, as shown in **Table 4**.

Discussion

Traditional Chinese medicine (TCM) is proved to have an adjunctive effect in treating diseases when combined with the use of western medicine in improving the symptoms and quality of life of patients (23). Due to the side effects of western medicine therapy, TCM integration with routine western or conventional medical interventions plays a significant adjunctive role in enhancing the therapeutical effect and reducing the occurrence of adverse effects (24), thus the unique advantages of TCM have received increasing attention, but it still lacks systematic overviews to summarize the effectiveness of TCM based on the existing clinical evidence. Our systematic review and meta-analysis contained eight RCTs and revealed that PI combination therapy had an adjunctive effect in the treatment of patients with AHF, it could better increase the total effective rate, improved the heart function, and be safe for adjunctive use in treating AHF.

The adjunctive effect of Puerarin injection in treating acute heart failure

Our study also suggested that PI treatment in conjunction with conventional medical treatment was superior in increasing the total effective rate, improving heart function of the valve of LVEF, LVEDD, and IVRT, and improving the left ventricular diastolic function of the valve of Peak A, Peak E and stroke volume, in terms of comparing with conventional medical treatment alone. In addition, subgroup analysis was performed on the primary outcomes of total effective rate and LVEF. Interestingly, the results showed that adjunctive use of PI could both improve the LVEF value and increase the total effective rate regardless of the dose (200–400 mg/day or 500 mg/day). As previously reported, PI showed a satisfied clinical efficacy in the treatment of cardiovascular diseases that PI was more effective than using conventional western medical alone in the treatment of unstable angina pectoris (9). It is also more effective and relatively safe in the clinic for treating acute ischemic stroke and diabetic peripheral neuropathy (25, 26).

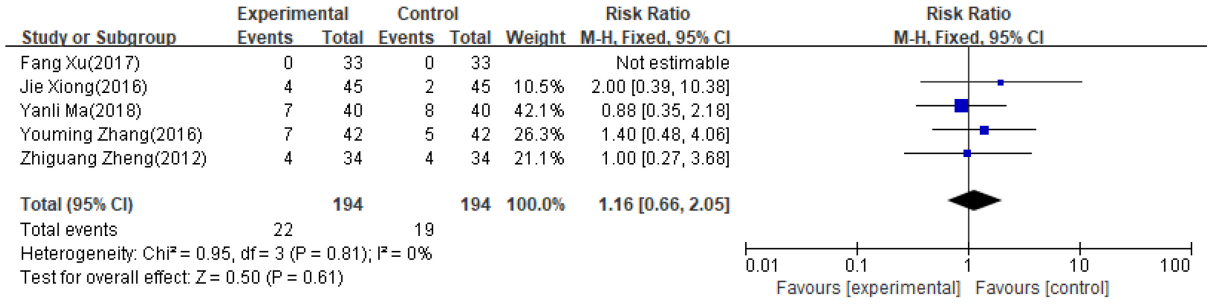

FIGURE 6
Forest plot of adverse effect.

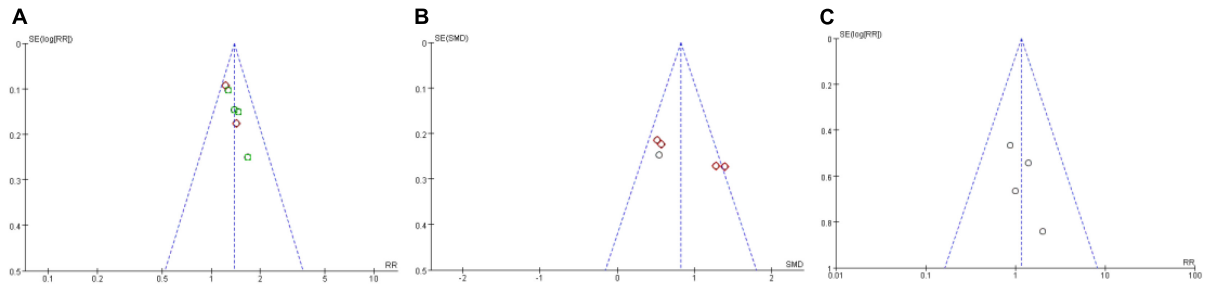

FIGURE 7
Funnel plot of publication bias assess. **(A)** Publication bias assess on total effective rate; **(B)** publication bias assess on LVEF; **(C)** Publication bias assess on adverse effect.

When we further explored the association between PI and favorable results in patients with AHF, it was proposed that PI had the effect of alleviating impaired heart function and inhibiting the levels of myocardial injury and inflammatory markers (27), as it was found that inflammation and heart function impaired in AHF resulted in neutral effects or worsening of clinical outcomes (28, 29). In addition, patients with AHF presented with similar congestion symptoms, which could lead to HF decompensation, which occurred owing to both fluid accumulation and redistribution, and further progress in the deterioration of AHF, thus decongestive therapy and diuretic drugs were recommended for AHF (1). PI was found that it could expand the coronary artery to promote coronary blood flow (10) and improve microcirculation to alleviate congestion symptoms (11), which might be the mechanism that PI could alleviate the AHF symptoms. Furthermore, clinical trials pointed out that a higher heart rate was a strong predictor of 1-year mortality of AHF, and reductions in coronary blood flow and myocardial oxygen consumption may be beneficial for AHF treatment (30, 31). Song et al reported that PI had the effect of decreasing heart rate and reducing myocardial oxygen consumption (32), which may also be the potential mechanism that PI had favorable results in patients with AHF.

The safe of Puerarin injection in conjunction with conventional medicine in treating acute heart failure

Regarding clinical safety, a total of 9.8% (19/194) adverse reactions occurred in the control groups while 11.9% (23/194) in the PI group, including nausea, hypotension, vomiting, headache, and slow heart rate. As 5 (62.5%) studies (14–16, 20, 21) reported the adverse effects and moderate evidence for safety assessment, we preliminary put forward the argument that combination therapy of PI was safe in treating AHF. But since the record for risk of bias assessment of included RCTs was "unclear," it implied that there is still a need for further eligible and critical clinical trials to validate the safety of PI.

The assessment of bias risk and evidence's confidence on the meta-results

The findings of meta-results were consistent with previously published research (33). To assess the credible clinical evidence of our results, evaluation of bias risk and evidence's confidence were performed. It showed that all the included studies lack

TABLE 4 The summary findings by the grading recommendations assessment, development, and evaluation (GRADE) methods.

Certainty assessment							Summary of findings			Comments
Participants (studies) follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Events		Anticipated absolute effects or relative effect (95% CI)	
							Control	Experiment		
Total Effective Rate 444 (6 RCTs)	Serious[a]	Not serious	Not serious	Not serious	None	⊕⊕⊕○ Moderate	132/222 (59.5%)	182/222 (82.0%)	RR 1.38 (1.22–1.55)	Risk of bias (-1[a])
LVEF 376 (5 RCTs)	Serious[a]	Not serious	Not serious	Not serious	None	⊕⊕⊕○ Moderate	188	188	SMD 0.79 higher (0.58–1.00)	Risk of bias (-1[a])
LVDD 242 (3 RCTs)	Serious[a]	Serious[b]	Serious[c]	Not serious	None	⊕⊕○○ Low	121	121	MD 1.67 higher (0.25–3.09)	Risk of bias (-1[a]) Inconsistency (-1[b]) Indirectness (-1[c])
IVRT 152 (2 RCTs)	Serious[a]	Serious[b]	Not serious	Not serious[e]	Yes[d]	⊕○○○ Very low	76	76	MD 6.7 lower (8.28 lower to 5.14 lower)	Risk of bias (-1[a]) Inconsistency (-1[b]) Imprecision (-1[e]) Publication bias (-1[d])
Peak A 152 (2 RCTs)	Serious[a]	Serious[b]	Not serious	Not serious[e]	Yes[d]	⊕○○○ Very low	76	76	MD 4.4 lower (4.81 lower to 3.99 lower)	Risk of bias (-1[a]) Inconsistency (-1[b]) Imprecision (-1[e]) Publication bias (-1[d])
SV 156 (2 RCTs)	Serious[a]	Serious[b]	Not serious	Not serious[e]	Yes[d]	⊕○○○ Very low	78	78	MD 7.99 higher (4.98 to 11.01)	Risk of bias (-1[a]) Inconsistency (-1[b]) Imprecision (-1[e]) Publication bias (-1[d])
Adverse Events 388 (5 RCTs)	Serious[a]	Not serious	Not serious	Not serious	None	⊕⊕⊕○ Moderate	19/194 (9.8%)	23/194 (11.9%)	RR 1.16 (0.66 to 2.05)	Risk of bias (-1[a])

CI, confidence interval; MD, mean difference; RR, risk ratio; SMD, Standardized mean difference. (a.) There exists unclear risk of bias as showed in Table 2; (b.) The sample size was too small; c. The direction of the effect is different as $50 < I2 \leq 75\%$; d. all plausible residual confounding would reduce the demonstrated effect; e. the number of studies is small. ⊕ The evidence's confidence upgrade 1 level; ○ the evidence's confidence downgrade 1 level.

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details in selection bias, blinding performance, and blinding outcome assessment (**Table 2**), which may result in the overstated effect of outcomes and reported bias in selected results. In addition, GRADE evaluation indicated that the confidence of the evidence was graded, which varied from very low to moderate quality for evidences (**Table 4**), and risk of bias, inconsistency, imprecision, and publication bias were mainly responsible for the downgrading of evidence because of the quality of included RCTs, thus larger RCTs with improved methodological quality in future are expected to further update the results of this systematic review.

Implications of prospective research and limitations of the present study

The adjunctive efficacy and safety of PI regarding curative effect among patients with AHF were for the first time systematically reviewed and evaluated in this study. At present, AHF treatment still lacks specific and effective medicine, leading to a relatively high recurrence rate, hospitalization rate, and mortality rate. We found that integrated use with PI could improve heart function, increased total effective rate, and was safe as the conventional western medication, which could be chosen by physicians when patients with AHF faced with unexpected treatment effects. The methodology of the present study was designed to a high standard according to the methodological quality of systematic reviews-2 (AMSTAR 2) by identifying relevant literature comprehensively, developing evaluation plans, and strict implementation, which could improve the accuracy and clinical applicability of the results of this study (34).

Although the results were encouraging, restrictions were unavoidably present in this study. Due to the small number and low to moderate quality of included studies, strictly designed trials according to the Consolidated Standards of Reporting Trials (CONSORT) statement also need to be further performed to verify the efficacy of PI as an adjunctive therapy for AHF. Duration included 7 days and 14 days, and the dose of PI included 500 mg/day and 200–400 mg/day, but we only did perform subgroup analysis of dose on the total effective rate due to small number of studies. Besides, the control group involves different conventional medical treatments, which potentially led to heterogeneity between the studies. Although there was no restriction on language when screening literature, the final included studies were all performed in China, which may lead to potential selection bias in the research. In addition, a few have data available for each outcome, for instance, the number of studies included in the meta-analysis of LVEDD, IVRT, Peak A, Peak E, and SV was 2-3/8 (25–37.5%), which limited the credibility of the above results. Thus, much more caution should be taken about the results until further trials in different populations and high-quality designed studies were performed to strengthen and update the results of the present meta-results.

Conclusion

In conclusion, PI plus CMT may be more beneficial than CMT alone for increasing the total effective rate, improve the heart function and left ventricular diastolic function. Also, it may be safe to combine PI with CMT in treating AHF. Regarding the very low to moderate evidence on the quality of meta-results, we should proceed with caution. Multi-center randomized controlled and double-blind trials are required with large sample sizes, rigorous design, and long follow-up period to confirm the efficacy and safety of PI in the future.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Author contributions

ZL and YF provided conceptualization, methodology, investigation, and writing—original draft. CH helped provide methodology, investigation, and formal analysis. QL and BC helped provide investigation, validation, data collection, and visualization. MH and ZP helped provide data collection and validation. BD and WZ provided conceptualization, funding acquisition, supervision, writing—review and editing, and project administration. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Department of Science and Technology of Guangdong Province (No. 2021A1515012224); clinical effect evaluation of kuanxiong aerosol on patients with "chest tightness and pain" in emergency-An open, randomized, controlled study [HT 2020-0974 (KY)]; clinical study of Astragalus injection on diuretic resistance in senile patients with acute heart failure (No. 20211162); and pre-hospital care-clinical study on the treatment of acute ST-segment elevation myocardial infarction by the combination of medicine and balance needle (No. AX046DS).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.934598/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 25 July 2022
doi: 10.3389/fcvm.2022.932193

Cardiac Shock Wave Therapy in Coronary Artery Disease: A Systematic Review and Meta-Analysis

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Objective: Coronary artery disease (CAD) has been one of the leading causes of morbidity and mortality worldwide. Cardiac shock wave therapy (CSWT) is a novel and non-invasive therapy for CAD. Therefore, we conducted a systematic review and meta-analysis to evaluate the efficacy of CSWT on CAD.

Methods and results: We performed a comprehensive search of electronic databases such as PubMed, Embase, the Cochrane Library, and Wanfang Data in October 2021. The results were reported as weighted mean difference (WMD) with a 95% confidence interval (CI). Statistical heterogeneity scores were assessed with the standard Cochran's *Q* test and the I^2 statistic. A total of 8 randomized trials and 2 prospective cohort studies, together involving 643 patients (n = 336 CSWT and n = 307 control), were included in our study. Eight studies with 371 patients showed significantly improved rest left ventricular ejection fraction (LVEF) with CSWT as compared to that of the control group (WMD 3.88, 95% CI 1.53–6.23, p = 0.001, I^2 = 51.2%). Seven studies with 312 patients reported left ventricular internal diameter in diastole (LVIDd) were markedly decreased in the CSWT group compared to the control group (WMD −1.81, 95% CI −3.23 to −0.39, p = 0.012, I^2 = 20.3%). The summed stress score significantly favored the CSWT group (WMD −3.76, 95% CI −6.15 to −1.37, p = 0.002, I^2 = 56.8%), but there was no significant difference for the summed rest score. Our data were acquired from studies without a perceived high risk of bias, so plausible bias is unlikely to seriously affect the main findings of the current study.

Conclusion: Based on data from our present meta-analysis, CSWT was shown to moderately improve myocardial perfusion and cardiac function among patients with CAD, which would provide the clinicians with a meaningful and valuable option.

Systematic Review Registration: The meta-analysis was registered on the Open Science Framework (OSF) (https://osf.io/r2xf9).

Keywords: cardiac shock wave therapy, coronary artery disease, meta-analysis, randomized controlled trials, efficacy

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Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: *29 April 2022*
Accepted: *21 June 2022*
Published: *25 July 2022*

Citation:
Qiu Q, Chen S, Qiu Y and Mao W (2022) Cardiac Shock Wave Therapy in Coronary Artery Disease: A Systematic Review and Meta-Analysis. Front. Cardiovasc. Med. 9:932193. doi: 10.3389/fcvm.2022.932193

INTRODUCTION

Coronary artery disease (CAD) is one of the most common and severe cardiovascular diseases and causes heavy economic and health burdens globally. CAD has affected nearly 20.1 million people ≥20 years of age in the United States (1). Although optimal medical therapy and revascularization have emerged as effective approaches for CAD treatment, an increased number of patients suffer from chronic angina, which seriously impairs the quality of life (2). Therefore, cardiac shock wave therapy (CSWT) has recently become appealing due to the improvement in angina symptoms.

Emerging evidence have suggested that CSWT, an application of low-intensity shock waves, showed beneficial effects on improvement in angina symptoms and exertional capacity in patients with CAD (3–5). *In vitro* and animal studies indicated that CSWT could exert anti-inflammatory effect, reduce oxidative stress, enhance angiogenesis, inhibit myocardial apoptosis and necroptosis, and regulate autophagy (6–10). However, in clinical studies, investigations into the efficacy of CSWT on cardiac functions and myocardial perfusion have yielded inconsistent and conflicting results. Some studies demonstrated that CSWT could enhance left ventricular (LV) systolic function and alleviate myocardial ischemia (11–13), whereas others found that there were no significant associations between CSWT and cardiac function or myocardial perfusion (14, 15). To address this issue, Burneikaitë et al. (16) and Yang et al. (17) performed meta-analyses in 2017 and 2020, respectively. However, they missed several important studies reported in Chinese, and additional studies have since been published. Moreover, the included studies in their meta-analyses were mainly single-arm studies. Therefore, we performed a meta-analysis to investigate the effect of CSWT on heart functions and myocardial perfusion in CAD based on random placebo-controlled trials.

METHODS

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (**Table 1**) (18). All the abstracts were screened by two independent methodologically trained reviewers (QQ and SJC). Discrepancies were resolved by discussion between the two researchers, if necessary, by a third reviewer (YGQ). The full-texts screening was evaluated in a similar manner to abstract screening. We used EndNote X8 (Clarivate, Pennsylvania, PA, United States) as literature management software for potentially eligible studies in the selection process.

Literature Search

A comprehensive systematic search strategy (**Supplementary Material 1**) was developed to retrieve relevant articles. Our objective was to identify all the randomized controlled trials (RCTs) and prospective cohort studies comparing the effect of CSWT on cardiac functions and myocardial perfusion with a placebo. PubMed, the Cochrane Library, Embase, and Wanfang Data were searched from January 1999 to November 2021 for English and Chinese language publications. Medical subject headings (MeSH terms) and keywords included cardiovascular disease, CAD, and angina pectoris combined with extracorporeal shockwave therapy.

Study Selection

We included studies in this meta-analysis fulfilling the following criteria: (1) randomized trials or prospective cohort studies; (2) studies involving patients with CAD confirmed by coronary angiography or CT angiography; (3) studies on the use of CSWT as the intervention; and (4) studies presenting all outcomes of interest.

Studies were excluded from the analysis if they were (1) basic science studies, case reports, letters, conference proceedings, reviews, or duplicated publications; (2) publications that did not report any outcomes of interest; (3) studies that lacked the placebo groups; and (4) trials investigating the efficacy of CSWT combination with stem cell therapy.

Study Quality Assessment and Risk of Bias

Two reviewers (QQ and YGQ) independently performed the quality evaluation. Assessment of the risk of bias in each included randomized trial was performed in accordance with the revised Cochrane risk-of-bias tool (RoB 2) (19). For cohort studies, we used the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool to assess the risk of bias (20). The risk of bias was evaluated in domains, including confounding, selection of participants into the study, classification of interventions, deviation from intended intervention, missing outcome data, measurement of the outcome, and selection of the reported result.

Data Extraction and Outcome Measures

Completed data from each study were extracted independently by two of the authors (QQ and SJC) using a standardized data extraction sheet. We extracted relevant information, including the first author, year of publication, trial design, trial duration, treatment regimen, patients' information, and characteristics of an outcome. Our primary outcome was global LV function and myocardial perfusion.

Statistical Analysis

We presented continuous data with normal distribution as mean value ± SD and non-normal data as median with interquartile range (IQR) (Q1, Q3). We analyzed results from randomized trials or prospective cohort studies that had placebo controls. We summarized all the continuous outcome data using weighted mean differences (WMDs) and their 95% confidence intervals (CIs). Heterogeneity was assessed using Cochran's Q test and expressed by I^2 statistic. If $I^2 \geq 50\%$ or the p-value < 0.05 for the Q-statistic, it indicated significant heterogeneity. The random-effects models were used in the presence of heterogeneity, and if there was no heterogeneity among studies, the fixed-effects models were performed. Publication bias was assessed by drawing a funnel plot and tested with Egger's test. Statistical analyses

TABLE 1 | PRISMA table.

Section and topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	p1
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	**Table 1**
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	p2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	p2
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	p3, 4
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p2–3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	p2 and **Supplementary Material 1**
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p2
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	**Table 3**
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	**Table 2**
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	p3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	p3
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis [e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)].	p3–4
	13b	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	p3–4
	13c	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	p5–6
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	–
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	–
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p4 and **Figure 1**
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	p4

(Continued)

TABLE 1 | (Continued)

Section and topic	Item #	Checklist item	Location where item is reported
Study characteristics	17	Cite each included study and present its characteristics.	**Table 3**
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	**Supplementary Material 2**
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	**Table 3**
Results of syntheses	20a	For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies.	p5
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	p5
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p5, 7, 8
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	p6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	–
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	p5
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	p6
	23b	Discuss any limitations of the evidence included in the review.	p8
	23c	Discuss any limitations of the review processes used.	p8
	23d	Discuss implications of the results for practice, policy, and future research.	p8
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	p1
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	–
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	–
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p8
Competing interests	26	Declare any competing interests of review authors.	p8
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	–

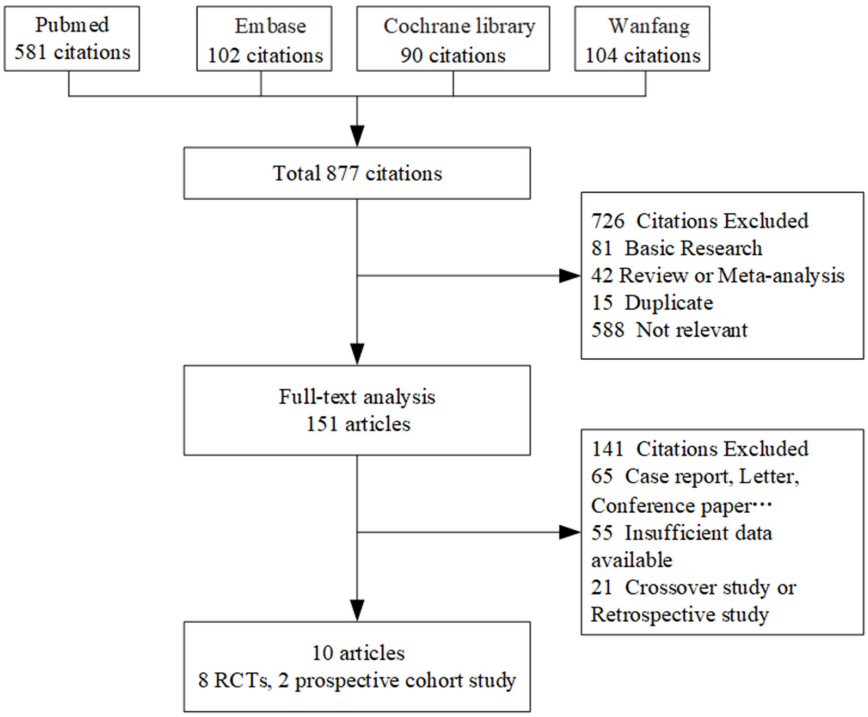

FIGURE 1 | Flow chart of the included studies. RCTs indicates randomized controlled trials.

were performed using STATA 15 (StataCorp LP, College Station, TX, United States).

RESULTS

Study Characteristics and Patient Population

By searching 4 databases, 877 eligible publications involving CSWT were identified and 726 records were initially excluded after screening the title and the abstract. Then, 151 records were included for a more thorough review using the inclusion and exclusion criteria described in the methods. Finally, 10 records (11, 21–29) were selected for review following the PRISMA statement. Among them, eight were RCTs and two were prospective cohort studies. The number of publications due to reasons for exclusion at each stage of the eligibility assessment is given in **Figure 1**. Several prospective placebo-controlled cohort studies and RCTs, which evaluated the effect of CSWT on relieving symptoms in patients with angina pectoris or CAD, were excluded from our study for lack of inadequate data on echocardiography or myocardial perfusion after full-text screening (30–32). In Yang's study (33), their data could not combine with others; therefore, we excluded it.

In total, 643 participants were included in this study with 336 cases treated with CSWT. Two publications were performed in Europe (Lithuania and Italy) and eight publications were performed in Asia (China and Japan) and published between 2010 and 2021. Investigators used CSWT as an alternative treatment option for stable angina, refractory angina, severe CAD, end-stage CAD, ischemic heart failure, and acute myocardial infarction (AMI). The reported mean or median age for studies were ranging from 56.6 to 71.4 years and 30.8% were women. The most commonly used CSWT operation protocols were the high-frequency treatment regimen completing nine CSWTs in 3 weeks and a low-frequency treatment regimen in which CSWT was performed three times weekly during the first week of each month within 3 months. **Table 2** describes the detailed CSWT protocols of included studies. The follow-up time of the studies ranged from 1 to 12 months. The common characteristics and the CSWT operation protocols of included studies are given in **Tables 2**, **3**, respectively.

Risk of Bias

Based on the methodological quality assessment (**Supplementary Material 2**), six studies were considered as having a moderate risk of bias and four studies were assessed as having a low risk of bias.

Meta-Analysis of Cardiac Shock Wave Therapy Effect on Left Ventricular Function

Eight studies with 371 patients reported changes of rest left ventricular ejection fraction (LVEF) by echocardiography. Meta-analysis showed significant improvement of rest LVEF due to CSWT (WMD 3.88, 95% CI 1.53–6.23, $p = 0.001$, $I^2 = 51.2\%$), as seen in **Figure 2A**. Seven studies with 312 patients reported left ventricular internal diameter in diastole (LVIDd) data, and the

result (**Figure 2B**) showed decreased LVIDd when comparing the CSWT group to the control group (WMD −1.81, 95% CI −3.23 to −0.39, $p = 0.012$, $I^2 = 20.3\%$).

Meta-Analysis of Cardiac Shock Wave Therapy Effect on Myocardial Perfusion

The meta-analysis of myocardial perfusion was based on the comparison between CSWT and placebo (control) on the parameters of the summed stress score (SSS) and the summed rest score (SRS) detected by single-photon emission CT. Four studies with 231 patients were included in the analysis of the effect of CSWT on the SSS, and the result (**Figure 3A**) of our meta-analysis showed significant improvement of the SSS in the CSWT group compared with placebo (WMD −3.76, 95% CI −6.15 to −1.37, $p = 0.002$, $I^2 = 56.8\%$). Only Liu and Jia reported the SRS data (**Figure 3B**), and there was no significant difference between the two groups (WMD −0.36, 95% CI −1.31 to 0.60, $p = 0.462$, $I^2 = 0.0\%$).

Publication Bias

Formal investigation using funnel plot and Egger's test revealed no publication bias in the meta-analyses for the effect of CSWT on LVEF and LVIDd (**Figures 4A–D**). The Egger's test results of LVEF and LVIDd showed *p*-values of 0.48 and 0.36, respectively (**Figures 4A,B**). The asymmetry of a funnel plot may result from different baseline characteristics of participants and from differences in the medical treatment of patients with CAD in all the studies (**Figures 4C,D**). We did not test publication bias for the meta-analyses of CSWT effect on the SSS and the SRS because too few studies were available to make a valid statistical test.

Sensitivity Analysis

Sensitivity analyses removing one study at a time revealed that the size and the direction of the pooled estimates of the effect of CSWT on LVEF and LVIDd were consistent for all the results (**Figures 5A,B**). Because there was a significant difference between the two groups on the SSS at baseline in one study, we excluded it and still found a significant difference comparing the CSWT group with placebo without significant heterogeneity (WMD −4.17, 95% CI −5.46 to −2.89, $p < 0.001$, $I^2 = 39.6\%$) (**Figure 6**).

DISCUSSION

The inconsistent conclusions about the effect of CSWT on cardiac function call for more rigorous studies to demonstrate the efficacy of CSWT for patients with CAD. By incorporating eight RCTs and two prospective cohort studies, our meta-analysis provided a relatively higher quality of evidence to increase the assurance of administering CSWT to patients with CAD. It is important to

TABLE 2 | The CSWT operation protocol of the included studies.

	Therapy regimen	Frequency and energy	Location	Device
Èelutkienë et al. (21)	9 sessions with 3 sessions per week; the first, fifth, and the ninth study weeks; 3-month period; 12 spots/session	100 impulses/spot	Whole LV	Cardiospec Medispec, Germantown, MD, United States
Weijing et al. (22)	Thrice weekly (first, third, and fifth days); the first, fifth, and the ninth study weeks; 3-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland
Jia et al. (11)	Thrice weekly (first, third, and fifth days); the first, fifth, and the ninth study weeks; 3-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic sessions	Modulith SLC; Storz Medical, Switzerland
Mengxian et al. (26)	Thrice weekly (first, third, and fifth days); the first, fifth, and the ninth study weeks; 3-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic sessions	Modulith SLC; Storz Medical, Switzerland
Kagaya et al. (23)	Second, fourth, and sixth days since AMI; 3 sessions in the ischemic border zone around the infarcted myocardium; 9 spots/session/day	200 impulses/spot; 0.09 mJ/mm^2	Ischemic border zone around the infarcted area	Modulith SLC; Storz Medical, Switzerland
Alunni et al. (24)	The first, fifth, and the ninth study weeks; 3-month period; 10 spots/session	100 impulses/spot; 0.09 mJ/mm^2	3 target sessions in the ischemic zone	Cardiospec Medispec, Germantown, MD, United States
Wang et al. (25)	Thrice weekly (first, third, and fifth days); first, fifth, and ninth study weeks; 3-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland
Wang et al. (25)	Thrice weekly (first, third, and fifth days); 1-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland
Zhang et al. (28)	Thrice weekly (first, third, and fifth days); 1-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland
Lan et al. (27)	Thrice weekly (first, third, and fifth days); 1-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland
Peng et al. (29)	Thrice weekly (first, third, and fifth days); first, fifth, and ninth study weeks; 3-month period; 9 spots/session	200 impulses/spot; 0.09 mJ/mm^2	Target ischemic session	Modulith SLC; Storz Medical, Switzerland

TABLE 3 | Characteristics of the included studies.

Study	Year	Trial type	Study population	Region	Age (mean)	M/F	Follow-up (m)	LVEF baseline (%)	Myocardial perfusion	CSWT/Con	Randomized methods	Control group
Èelutkienë et al. (21)	2019	RCT	Stable angina	Lithuania	67.2 ± 7.8/69.4 ± 7.8	45/14	6	46.5 ± 10.6/48.5 ± 9.0	8.5 (5.3; 12.8)/10.0 (4.0; 15.0)	30/29	Random number table	Sham procedure
Weijing et al. (22)	2021	RCT	Refractory angina	China	68.1 ± 6.7/68.9 ± 6.6	61/26	6	–	16.27 ± 7.64/16.45 ± 5.05	46/41	NR	Medical therapy
Jia et al. (11)	2021	RCT	Severe CAD	China	69.20 ± 11.33/71.40 ± 9.71	21/9	3	62.5 (60, 65)/62.5 (60, 65)	17.63 ± 7.86/11.23 ± 5.69	15/15	Random number table	Sham procedure
Mengxian et al. (26)	2012	RCT	Severe CAD	China	63.71 ± 8.60/66.45 ± 8.51	18/7	6	51.36 ± 4.27/50.18 ± 4.55	–	14/11	NR	Sham Procedure
Kagaya et al. (23)	2017	Cohort study	MI	Japan	65.0 ± 7.3/67.3 ± 12.8	27/5	12	58.7 ± 8.2/54.4 ± 12.3	–	17/25	NR	NR
Alunni et al. (24)	2015	Prospective cohort study	Refractory angina	Italy	70 ± 9.5/71 ± 5.3	63/9	6	56.4 ± 10.3/57.3 ± 9.6	–	43/29	NR	NR
Wang et al. (25)	2010	RCT	End-stage CAD	China	63 ± 10/69 ± 7	30/5	3	53.1 ± 12.8/54.3 ± 13.9	–	16/10	NR	NR
Wang et al. (25)	2010	RCT	End-stage CAD	China	63 ± 10/69 ± 7	30/5	1	56.1 ± 13.2/54.3 ± 13.9	–	9/10	NR	NR
Zhang et al. (28)	2021	RCT	RA	China	65.83 ± 6.3/64.4 ± 6.7	53/17	6	50.32 + 12.69/50.21 ± 10.01	320.10 ± 3.45/30.28 ± 2.34	38/32	NR	Medical therapy
Lan et al. (27)	2016	RCT	Ischemic HF	China	67 ± 6/66 ± 7	39/14	3	37.41 ± 5.87/38.31 ± 4.56	21.46 ± 9.51/23.58 ± 7.52	28/25	NR	Sham procedure
Peng et al. (29)	2018	RCT	Ischemic HF	China	62.5 ± 6.8/61.3 ± 7.2	100/80	3	44.40 ± 6.32/44.12 ± 12.52	22.91 ± 4.32/22.05 ± 4.07	90/90	NR	Sham procedure

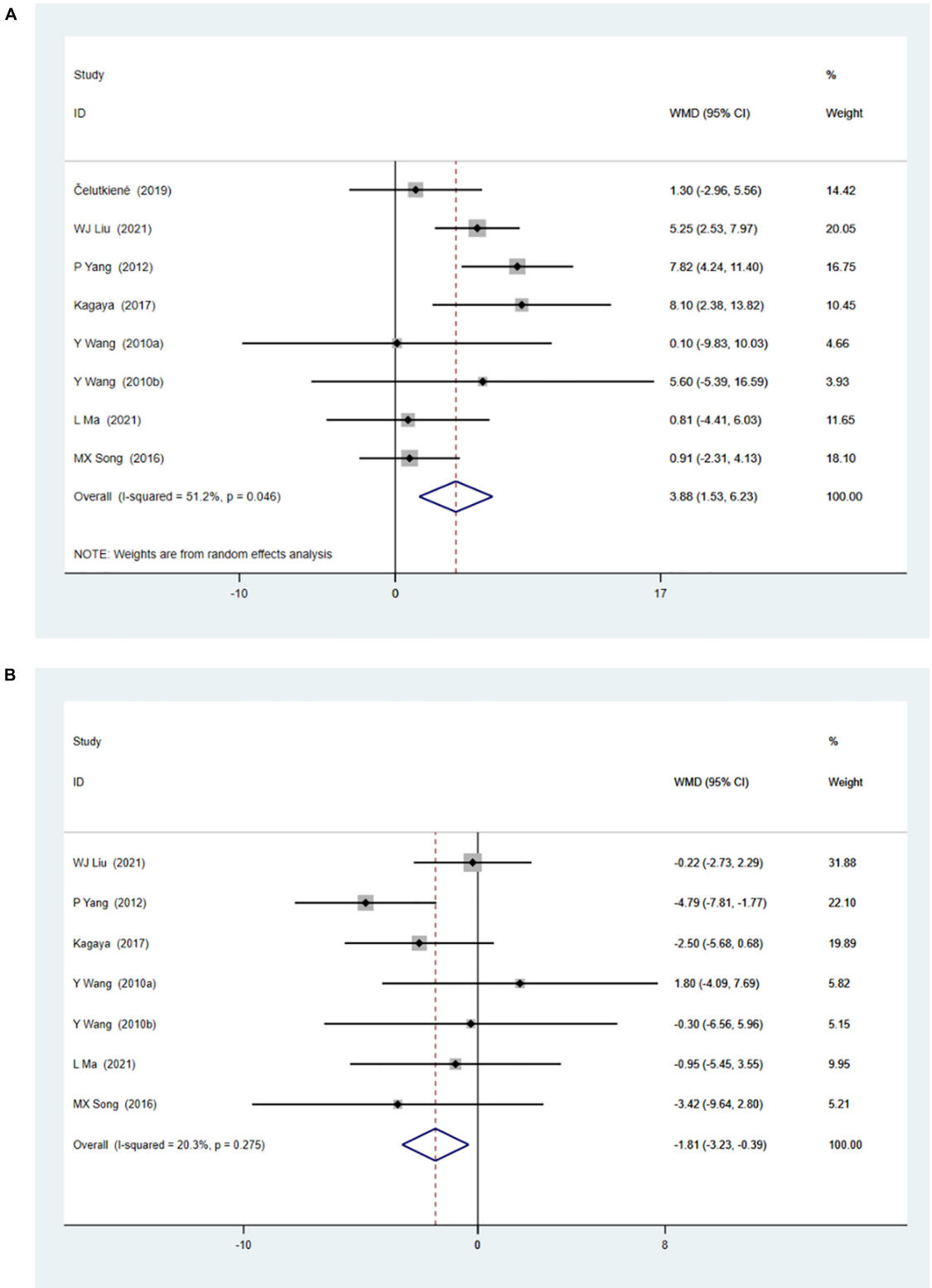

FIGURE 2 | (A) Forrest map of overall impact of cardiac shock wave therapy on LVEF (WMD 3.88, 95% CI 1.53–6.23, $p = 0.001$). The I^2 value revealed considerable heterogeneity across studies ($I^2 = 51.2\%$; $p = 0.046$). **(B)** Forrest map of overall impact of cardiac shock wave therapy on LVIDd (WMD –1.81, 95% CI –3.23 to –0.39, $p = 0.012$). The I^2 value revealed considerable heterogeneity across studies ($I^2 = 20.3\%$; $p = 0.275$).

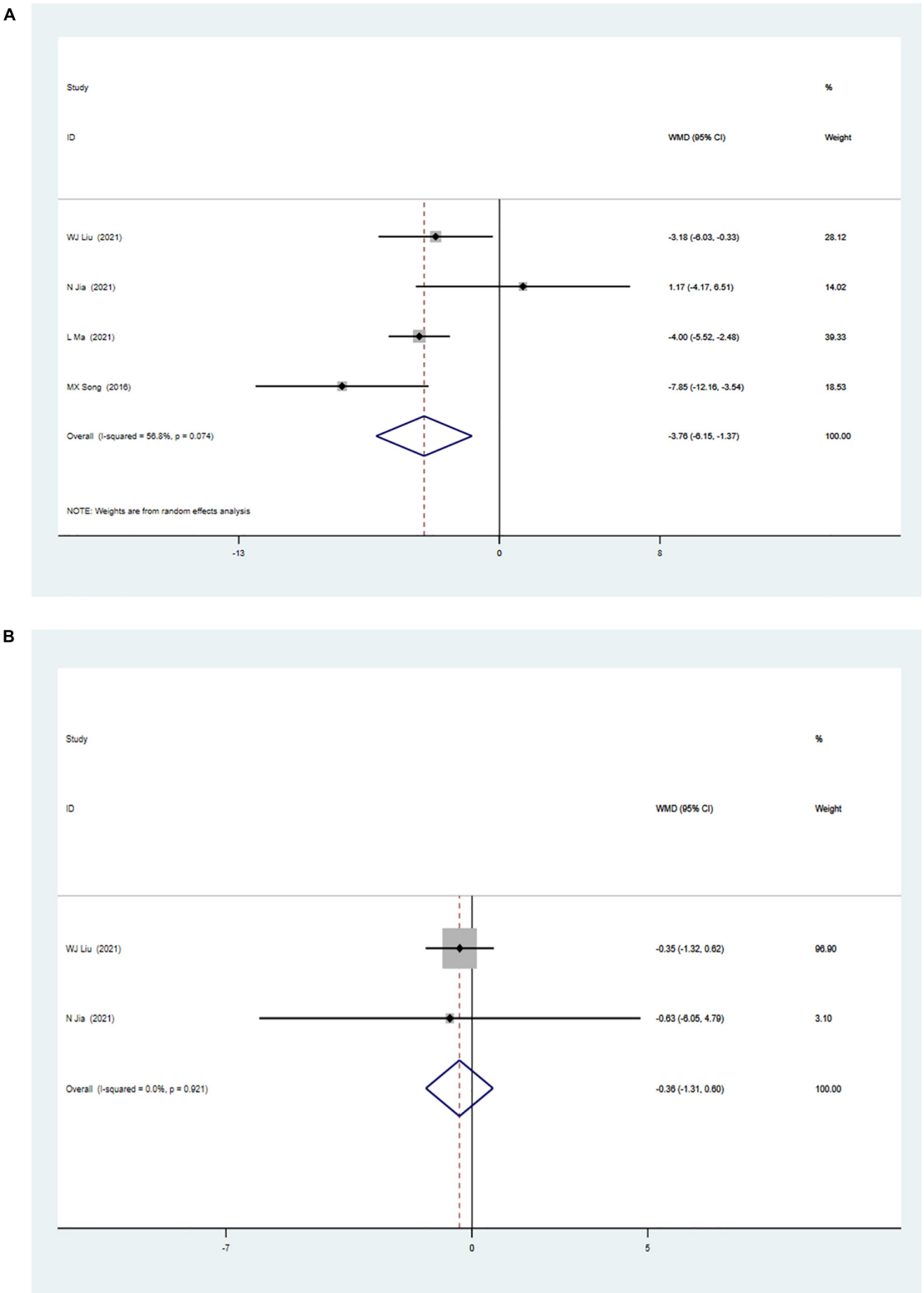

FIGURE 3 | (A) Forrest map of overall impact of cardiac shock wave therapy on SSS (WMD –3.76, 95% CI –6.15 to –1.37, $p = 0.002$). The I^2 value revealed considerable heterogeneity across studies ($I^2 = 56.8\%$; $p = 0.074$). **(B)** Forrest map of overall impact of cardiac shock wave therapy on SRS (WMD –0.36, 95% CI –1.31 to 0.60, $p = 0.462$). The I^2 value revealed considerable heterogeneity across studies ($I^2 = 0.0\%$; $p = 0.021$).

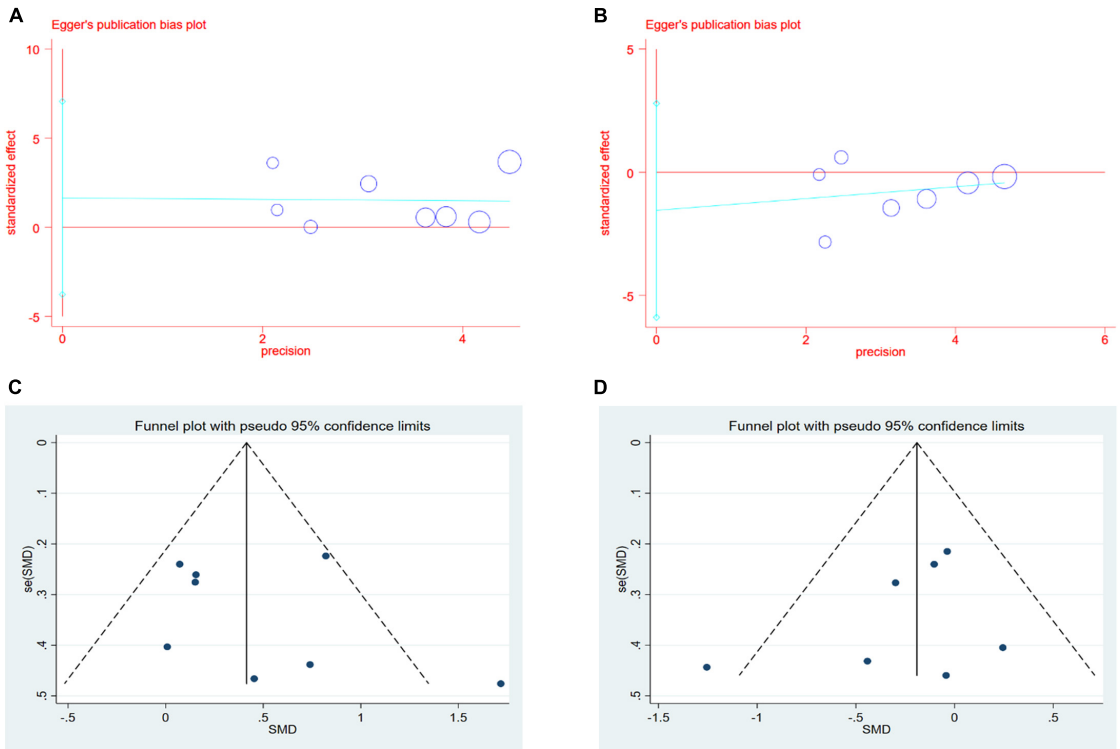

FIGURE 4 | (A,B) Results of Egger's test for LVEF and LVIDd. **(C,D)** Results of Funnel plot for LVEF and LVIDd.

note that CSWT could moderately improve myocardial function and prevent ventricular remodeling (supported by remarkably improved LVEF and decreased LVIDd). Besides, the analysis also revealed that CSWT may improve myocardial perfusion (supported by the decrease of the SSS).

The CSWT technology has proven to be safe in more than 60 medical centers worldwide. Accumulating evidence has demonstrated that CSWT does not cause an increase in the levels of cardiac biomarkers (i.e., troponin I, troponin T, creatine kinase-MB, B-type natriuretic peptide) and has no adverse effects on blood pressure, heart rate, and oxygen saturation in patients with CAD. Serious complications, including malignant arrhythmia and embolism, have not been observed after CSWT (11). In Kagaya's study (23), which enrolled 17 patients with AMI undergoing CSWT, 16 patients completed CSWT and survived 12 months after AMI without any adverse effects with only one patient dying of cardiac rupture independent of CSWT. In Ceccon's study (34), 15 patients with refractory angina receiving CSWT presented mild chest discomfort during treatment and with a rare appearance of major adverse effects related to CSWT. In Jia's study (11), 15 patients diagnosed with severe CAD were treated with CSWT and no severe adverse effects occurred. Since shock wave carries low-intensity energy, there are several contradictions to CSWT, including acute pericarditis, acute myocarditis, infectious endocarditis, deep venous thromboembolism, intracardiac thrombus, severe aortic valve stenosis, thoracic aortic aneurysm, thoracic aortic dissecting aneurysm, cardiac transplantation, pulmonary embolism, and mechanical heart valve replacement (11, 21).

Angina is the most common symptom of coronary heart disease, and it has been indicated that the mortality rate of refractory angina is 3–4% per year (35). Angina is one of the main indications of CSWT. CSWT generator produces pulse waves that could propagate through myocardial tissues and vascular endothelial cell membranes (6, 36). Many clinical trials have shown that CSWT could alleviate angina symptoms as assessed by the improved Seattle Angina Questionnaire score and the Canadian Cardiovascular Society grading of the angina pectoris, a decreased nitroglycerin dosage, and improved exercise tolerance assessed by increased 6-min walk distance (3, 11, 30).

Porcine, mice, or rat AMI or ischemic heart failure models were used in previous studies to demonstrate the improvement of LVEF or wall thickening fraction (6, 9, 37). At present, some studies indicated that CSWT could effectively improve myocardial function and perfusion in patients with stable angina and severe CAD at rest and during stress (11, 14, 21, 38, 39). Yang et al. (17) showed that CSWT significantly improved LVEF compared to baseline in their meta-analysis based on six single-arm studies and one cohort study, while the improvement may be due to optimal medical therapy and lifestyle modifications. Our study based on RCTs minimized various confounders and selection bias and had a higher level of statistical reliability. A recent meta-analysis of CSWT for stable CAD also reported that CSWT could improve LVEF (16). However, it should be

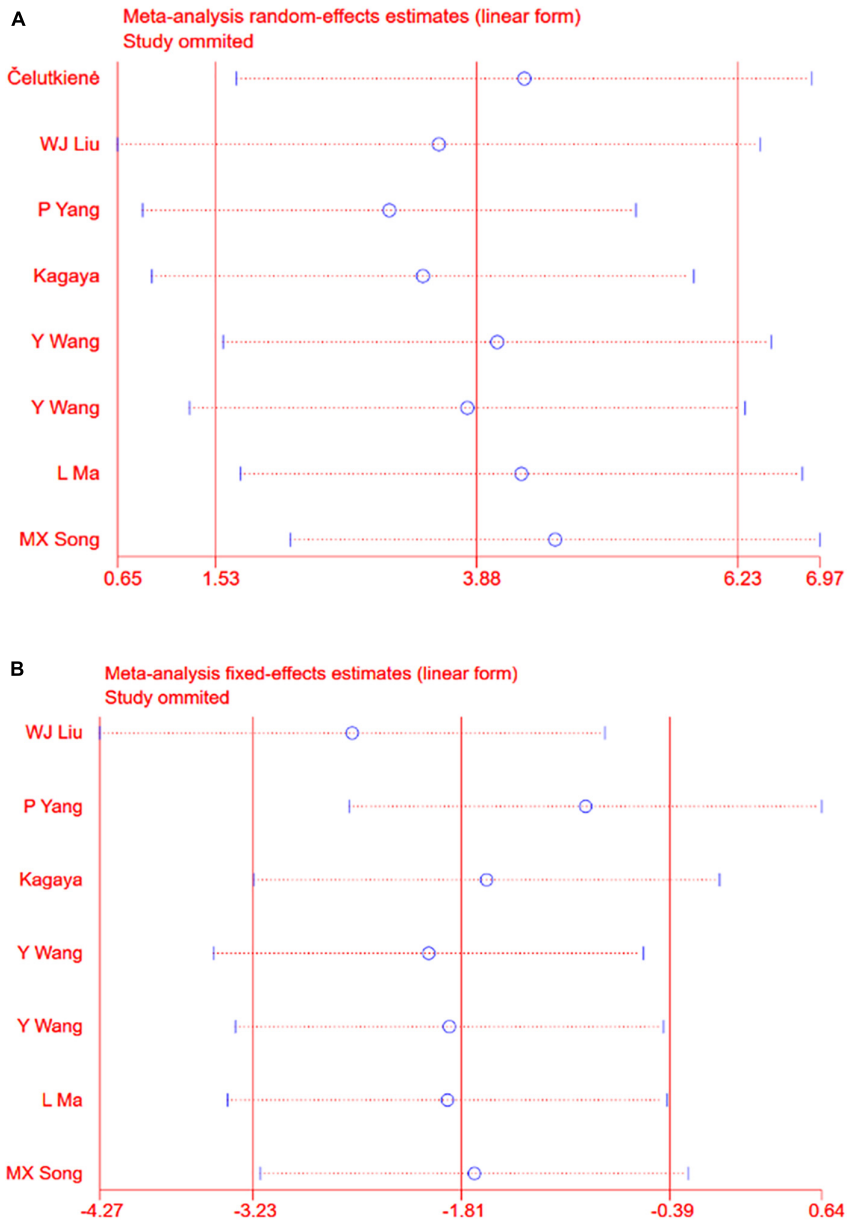

FIGURE 5 | (A) Sensitivity analysis for the LVEF. **(B)** Sensitivity analysis for the LVIDd.

noted that this study included single-arm, non-randomized, and randomized trials and they did not perform statistical analysis. Because RCTs are considered the gold standard evidence for determining the efficacy of interventions and our meta-analysis is based mainly on RCTs, our data have good internal validity.

There are two brands of CSWT machines used in the included studies. Both of the machines worked in a similar way to deliver shockwaves to the target ischemic zones in an R-wave-triggered manner. There were few head-to-head trials to compare the efficiency of the two different brands of machines. However, it seems that there is not much difference between the two kinds of machines. In this meta-analysis, Alunni's and Celutkiene's study (21, 24) chose the Cardiospec Medispec CSWT machine but the other studies used the Modulith SLC machine. Moreover, the included clinical trials did not share a uniform treatment protocol. Most of the studies included in the meta-analysis received two different courses of CSWT treatment, including a 1-month course (25–27) and a 3-month course (11, 21, 22, 24, 25). A total of 100 or 200 impulses were delivered to the target area with an energy flux density of 0.09 mJ/mm^2. Other parameters, such as location and frequency, differed subtly among these studies. We have acknowledged this as a limitation of the current meta-analysis, and future studies are needed to be performed according to the standard protocols.

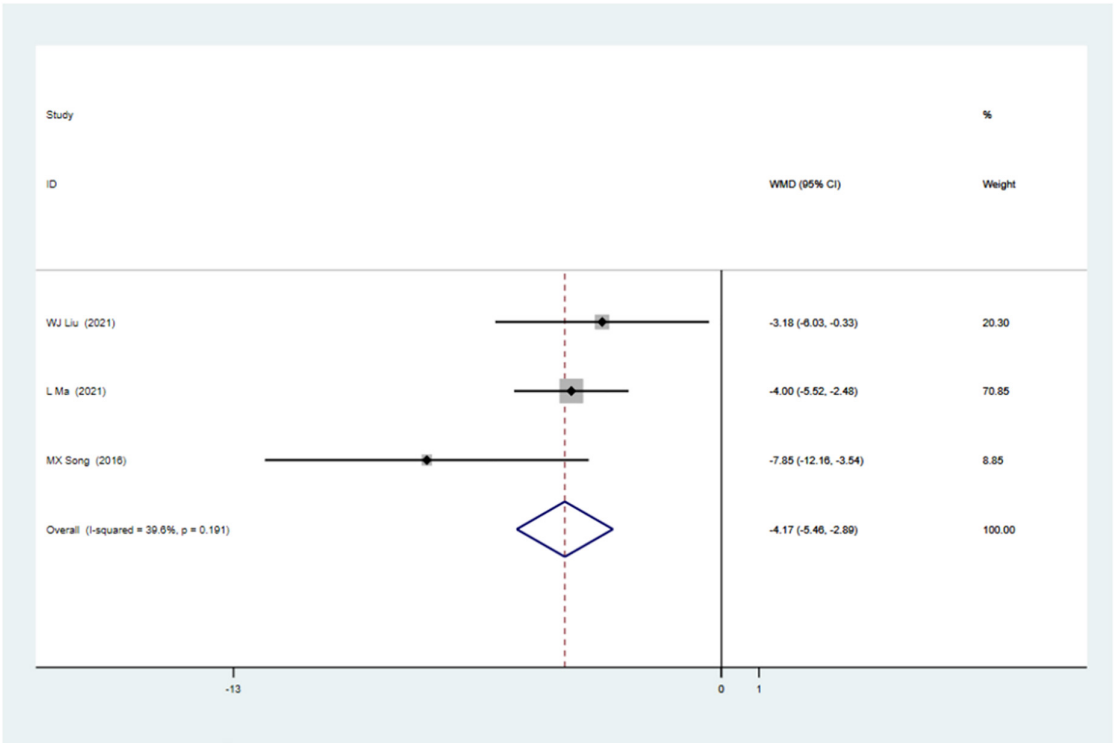

FIGURE 6 | Forrest map of updated impact of cardiac shock wave therapy on SSS (WMD –4.17, 95% CI –5.46 to –2.89, $p < 0.001$). The I^2 value revealed considerable heterogeneity across studies ($I^2 = 39.6\%$; $p = 0.191$).

Limitation

There are some limitations to this study. First, this meta-analysis is limited owing to a lack of large-scale RCTs and the long-term effect of CSWT on CAD. For example, Zhang et al. (28) conducted the largest RCT consisting of 180 participants and performed echocardiography evaluation before and 3 months after CSWT treatment. Time effect should be considered when evaluating the clinical outcomes of CSWT. Second, the sonographers who performed echocardiography affected the accuracy and precision of LVEF and LVIDd measurements, and this interpersonal variability had been shown to exist even when echocardiographic image acquisition was performed according to echocardiography guidelines. Moreover, it seemed possible that, due to sparse RCTs, this meta-analysis included all the known studies, and over half of the studies were conducted in Asia, which might influence the representativeness of the population.

CONCLUSION

Taken together, the present meta-analysis of these studies showed that CSWT appeared to be effective in improving myocardial perfusion and cardiac function in patients with CAD. CSWT is a promising therapeutic modality for the treatment of CAD. More high-quality RCTs with large samples and long-term follow-up are urgently needed to further confirm our results.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WM designed the study. QQ and SC screened and evaluated the studies and performed a comprehensive characterization of the studies. QQ performed the statistical analyses. YQ checked the included studies. YQ and SC checked the statistical analyses. QQ and WM wrote the manuscript. All authors have contributed to the article and approved the submitted version of the manuscript.

FUNDING

This study was supported by the Zhejiang Chinese Medical University Award 2021JKJNTZ024B.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.932193/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Opinion
PUBLISHED 27 July 2022
DOI 10.3389/fcvm.2022.943572

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 13 May 2022
ACCEPTED 08 July 2022
PUBLISHED 27 July 2022

CITATION
Tolento Cortes L and Hong L (2022) Heart failure with preserved ejection fraction—Out with the old and out with the new?
Front. Cardiovasc. Med. 9:943572.
doi: 10.3389/fcvm.2022.943572

Heart failure with preserved ejection fraction—Out with the old and out with the new?

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KEYWORDS

heart failure, preserved ejection fraction, HFpEF, pharmacology, heart failure management

Introduction

Reported 5-year mortality rates among patients diagnosed with heart failure are upwards of 50% (1, 2). Recent studies have demonstrated similar mortality rates between patients with reduced ejection fraction (HFrEF; EF ≤40%), mildly reduced ejection fraction (HFmrEF; EF 41%-49%) and preserved ejection fraction (HFpEF; EF ≥50%) (2, 3). While most guideline recommendations regarding pharmacotherapy pertain to patients with HFrEF, the above observation suggests a need for mortality-reducing therapy in all subtypes of heart failure (4, 5).

In the 2021 ESC update, advances in the pharmacological management of HFpEF are discussed and conclude that there are currently no convincing studies supporting morbidity/mortality benefits with HFpEF treatment as all studies have failed to achieve their primary endpoints (4). As previously accepted, the management of patients with HFpEF revolves around acute symptom management with agents such as diuretics in addition to the management of chronic comorbidities that may contribute to the progression of heart failure. However, medication management of HFpEF has recently been reassessed with some newer studies assessing the utility of various therapies in this population. Notably, these trials were conducted in patients with LVEF as low as 40% complicating the generalizability of results to patients with HFpEF as many of the study subjects would otherwise be categorized under HFmrEF. A brief synopsis of the results from select trials are summarized below and in Table 1.

Role of ACEI/ARB/ARNI therapy

The role of ACEI/ARB therapy in HFpEF stems from the PEP-CHF (perindopril), I-PRESERVE (irbesartan) and CHARM-Preserved (candesartan) studies. The PEP-CHF trial did not show any statistically significant differences in mortality/HF-related hospitalizations between those who received perindopril vs. placebo and was underpowered for the primary outcome. Notably, the study showed a benefit in the reduction of HF hospitalizations favoring perindopril at 1 year, but this benefit was not sustained as the difference was negligible when compared to placebo thereafter (6). The CHARM-Preserved trial reported that patients who received candesartan vs. placebo

TABLE 1 Summary of select clinical trials.

Trial	Interventions	Population	Primary outcomes	Results
PEP-CHF (6)	Perindopril vs. placebo	Adults ≥70 years old with HF, on diuretics with an ECG suggestive of diastolic dysfunction but LV wall motion index of 1.4 and LVEF ≥40%	Mortality or unplanned HF-related hospitalization in 1 year	23.6 vs. 25.1% HR 0.92 (95% CI 0.70–1.21; $p = 0.545$)
CHARM-preserved (7)	Candesartan vs. placebo	Adults ≥18 years old, NYHA class II–IV, history of HF hospitalization and LVEF >40%	Cardiovascular death or HF-related admission	22 vs. 24.3% Adjusted HR 0.86 (95% CI 0.74–1.00; $p = 0.051$)
I-PRESERVE (8)	Irbesartan vs. placebo	Adults ≥60 years old with HF symptoms, LVEF ≥45% and HF hospitalization in 6 months or evidence of HF or substrate of diastolic heart failure	Mortality and cardiovascular related hospitalizations	35.8% vs. 37% HR 0.95 (95% CI 0.86–1.05; $p = 0.35$)
PARAMOUNT-HF (9)	Sacubitril/valsartan vs. valsartan	Adults ≥40 years old, LVEF 45%, heart failure signs/symptoms, NT-proBNP >400 pg/ml, on diuretic therapy, SBP >140 mmHg or 160 mmHg if on ≥3 BP medications, eGFR ≥30 ml/min/1.73 m^2 and K <5.2 mmol/L	Change in NT-proBNP at 12 weeks	Change from baseline 22.7 vs. 3.2% Ratio of change 0.77 (95% CI 0.64–0.92; $p = 0.005$)
PARAGON-HF (10)	Sacubitril/valsartan vs. valsartan	Adults ≥50 years old, signs/symptoms of HF, NYHA class II–IV, EF ≥45% in last 6 months, elevated natriuretic peptides, structural heart disease and on diuretics.	Hospitalizations for HF and death from cardiovascular causes	37.1 vs. 42.2% RR 0.87 (95% CI 0.75–1.01; $p = 0.06$)
SENIORS (11)	Nebivolol vs. placebo	Adults ≥ 70 years old, LVEF≥40%	All-cause mortality or cardiovascular related hospitalization	31.1 vs. 25.3% HR 0.86 (95% CI 0.75–0.99; $p = 0.04$)
Aldo-DHF (12)	Spironolactone vs. placebo	Adults ≥ 50 years old, LVEF ≥50%, NYHA II–III, peak VO_2 ≤25 ml/min/kg, diastolic dysfunction on ECG or atrial fibrillation	Changes in diastolic function (Mean estimate of filling pressure improvement) and maximal exercise capacity (Mean Peak VO_2)	Diastolic function 12.1 vs. 13.6 Difference −1.5 (95% CI −2.0 to −0.9; $p < 0.001$) Exercise capacity 16.8 vs. 16.9 Difference 0.01 (95% CI −0.6 to 0.8; $p = 0.81$)
TOPCAT (13)	Spironolactone vs. placebo	Adults ≥50 years old, LVEF 45%, 1 HF sign/symptom, HF hospitalization within 1 year or BNP ≥100 pg/ml or NT-proBNP ≥360 pg/ml	Cardiovascular death, cardiac arrest or HF-related hospitalization	18.6 vs. 20.4% HR 0.89 (95% CI 0.77–1.04; $p = 0.14$)
EMPEROR PRESERVED (14)	Empagliflozin vs. placebo	Adults ≥18 years old, NYHA class II-IV, LVEF >40%, HF hospitalization in last 12 months or structural heart disease within 6 months, NT-proBNP ≥300 pg/ml without atrial fibrillation and on stable dose of diuretics	Cardiovascular death or HF-related hospitalization	13.8% vs. 17.1% HR 0.79 (95% CI 0.69–0.90; $p < 0.001$)

had a reduction in HF-related admissions after covariate adjustment ($p = 0.072$ before adjustment vs. $p = 0.047$ after adjustment) (7). Though, for the composite primary outcome including CV-related death and HF-related admissions, the observed difference was not significant despite covariate adjustment. Furthermore, the I-PRESERVE trial failed to find a difference in mortality or cardiovascular admissions in patients who received irbesartan vs. placebo (8). Reduction in HF hospitalization was seen in only one of these three trials, which had the most patients with HFmrEF and improved outcomes associated with use of ACEI/ARB therapy in HFpEF are likely derived from their benefit in the management of common comorbidities such as hypertension.

The role of ARNI in HFpEF was assessed in the PARAMOUNT-HF and PARAGON-HF trials. These trials compared sacubitril/valsartan vs. valsartan. While the PARAMOUNT-HF trial demonstrated a reduction in NT-proBNP (a marker for LV wall stress), the clinical relevance of this surrogate outcome is not clear and the PARAGON-HF trial demonstrated no difference in cardiovascular deaths or HF hospitalizations (9, 10). A subgroup analysis in the PARAGON-HF trial suggested a reduction in hospitalizations in patients with a LVEF ≤57% and sacubitril/valsartan carries an FDA-approved indication for HFpEF based on these results. The subgroup analysis included patients who would be categorized under HFmrEF but only a limited number of those who would fall within the parameters for HFpEF. The inclusion of patients with HFmrEF in these results precludes the ability to conclude the same benefit with sacubitril/valsartan exclusively among patients with HFpEF. Despite these data, there is insufficient evidence to support a strong recommendation for ARNI therapy in patients with HFpEF at this time. However, for patients with other chronic diseases where an ARB is indicated, ARNI therapy may be reasonable to consider, provided the patient can afford it.

Role of beta-blocker therapy

The role of beta-blocker therapy has not been extensively studied in patients with HFpEF. The SENIORS trial reported a reduction in all-cause mortality or cardiovascular-related hospitalizations associated with the use of nebivolol vs. placebo. However, the generalizability of this study to patients with HFpEF is limited as only ∼15% of participants had a LVEF >50% (11). Coupled with a high discontinuation rate secondary to drug intolerance in the SENIORS trial, it may be best to reserve beta-blocker therapy for patients with alternative indications where there is proven clinical benefit.

Role of aldosterone antagonists therapy

The role of spironolactone in HFpEF was assessed in the Aldo-DHF trial, which demonstrated an improvement in diastolic function (reported as an estimate of filling pressure), but not maximal exercise capacity at 12 months compared with placebo and the clinical relevance remained in question (12). The TOPCAT trial found no difference in the composite primary outcome of cardiovascular death, cardiac arrest and HF hospitalizations, but did find a reduction in the incidence of HF-related hospitalizations (13). Notably, this study included patients with EF ≥45%, meaning that the benefit of spironolactone was not exclusive to those with EF ≥50%. While the evidence to support use of an aldosterone antagonist in HFpEF is weak, given most patients in TOPCAT had HFpEF, the plausible reduction in HF hospitalization in this population, and the low medication cost, initiation of spironolactone in patients with HFpEF may be reasonable.

Role of SGLT2I therapy

The EMPEROR-PRESERVED trial demonstrated fewer events in the composite outcome of cardiovascular death and HF hospitalization with empagliflozin vs. placebo, regardless of diabetes. However, this effect was driven by the reduced incidence of HF-related hospitalizations with zero difference in all-cause mortality (14). This is yet another trial that did not exclusively study patients with EF ≥50%. Although a reduction in hospitalizations would otherwise support the initiation of SGLT2I therapy in patients with HFpEF, several patients included in the EMPEROR- PRESERVED trial had HFmrEF with subgroup analysis illustrating attenuation of this benefit as EF increased. Furthermore, the benefit among those with HFpEF may be offset by the high-cost of SGLT2I agents. In accordance with this discussion, the recently published 2022 AHA/ACC/HFSA guideline suggests that SGTL2-I can be beneficial in patients with HFpEF (2b; moderate strength recommendation and quality of evidence) (5).

Miscellaneous therapies

Additional trials have evaluated whether medications from other therapeutic classes play a role in the management of HFpEF. Digoxin was evaluated in the DIG-PEF trial and whilst a potential reduction in the composite outcome of mortality and hospitalizations was observed at 2 years, the benefit was not sustained at the conclusion of the study (37 months) (15). Cyclic guanosine monophosphate pathway stimulators were studied in

various trials (INDIE-HFpEF, VITALITY- HF-pEF, CAPACITY-HFpEF, and NEAT-HFpEF), but failed to show an increase in either exercise tolerance or quality of life (16–19). Lastly, the phosphodiesterase-5 inhibitor, sildenafil was studied in the RELAX trial, but also failed to show any benefit in exercise tolerance (20).

Conclusion

The pharmacological management of HFpEF continues to be an area of uncertainty due to multiple studies failing to show a clear benefit associated with therapies that have otherwise proven useful in the management of other HF subtypes. Currently, there are no approved medications that have demonstrated improved survival in patients with HFpEF. The initiation of therapies such as ACEI/ARB or beta-blockers is primarily based on their efficacy in the management of other comorbid conditions. The 2021 ESC guideline update recommends that "in the absence of recommendations regarding disease-modifying therapies, treatment should be aimed at reducing symptoms of congestion with diuretics." Likewise, the strong recommendation in the recently published 2022 AHA/ACC/HFSA guideline for use of diuretics, as needed in this patient population are concordant with those in the 2021 ESC guideline.

One of the most significant limitations in the current literature is the lack of data pertaining to those exclusively with EF $\geq$50% and future studies should aim to recruit only patients who meet this definition of HFpEF. In order to best evaluate the clinical implications of these therapies, studies should attempt to measure outcomes such as mortality, hospitalization, exercise tolerance and quality of life with adequate duration of follow up to ensure that any benefits seen early on are sustained. As we continue to search for therapies that may provide mortality/morbidity benefits to those with HFpEF, it is important for us as clinicians to understand the results and limitations of these pivotal clinical trials as they continue to emerge in order to make informed decisions in the interest of balancing risks and benefits to our patients. Until then, I guess we will stick with diuretics as our mainstay of therapy.

Author contributions

Information was gathered by both LT and LH. Both authors have read and agreed to the content of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 09 August 2022
DOI 10.3389/fcvm.2022.961920

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 05 June 2022
ACCEPTED 13 July 2022
PUBLISHED 09 August 2022

CITATION
Hsiao L-C, Lin Y-N, Shyu W-C, Ho M, Lu C-R, Chang S-S, Wang Y-C, Chen J-Y, Lu S-Y, Wu M-Y, Li K-Y, Lin Y-K, Tseng W-YI, Su M-Y, Hsu C-T, Tsai C-K, Chiu L-T, Chen C-L, Lin C-L, Hu K-C, Cho D-Y, Tsai C-H, Chang K-C and Jeng L-B (2022) First-in-human pilot trial of combined intracoronary and intravenous mesenchymal stem cell therapy in acute myocardial infarction.
Front. Cardiovasc. Med. 9:961920.
doi: 10.3389/fcvm.2022.961920

First-in-human pilot trial of combined intracoronary and intravenous mesenchymal stem cell therapy in acute myocardial infarction

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Background: Acute ST-elevation myocardial infarction (STEMI) elicits a robust cardiomyocyte death and inflammatory responses despite timely revascularization.

Objectives: This phase 1, open-label, single-arm, first-in-human study aimed to assess the safety and efficacy of combined intracoronary (IC) and intravenous (IV) transplantation of umbilical cord-derived mesenchymal stem cells (UMSC01) for heart repair in STEMI patients with impaired left ventricular ejection fraction (LVEF 30-49%) following successful reperfusion by percutaneous coronary intervention.

Methods: Consenting patients received the first dose of UMSC01 through IC injection 4-5 days after STEMI followed by the second dose of UMSC01 via IV infusion 2 days later. The primary endpoint was occurrence of any treatment-related adverse events and the secondary endpoint was changes of serum biomarkers and heart function by cardiac magnetic resonance imaging during a 12-month follow-up period.

Results: Eight patients gave informed consents, of whom six completed the study. None of the subjects experienced treatment-related serious adverse events or major adverse cardiovascular events during IC or IV infusion of UMSC01 and during the follow-up period. The NT-proBNP level decreased (1362 ± 1801 vs. 109 ± 115 pg/mL, p = 0.0313), the LVEF increased (52.67 ± 12.75% vs. 62.47 ± 17.35%, p = 0.0246), and the wall motion score decreased (26.33 ± 5.57 vs. 22.33 ± 5.85, p = 0.0180) at the 12-month follow-up compared to the baseline values. The serial changes of LVEF were 0.67 ± 3.98, 8.09 ± 6.18, 9.04 ± 10.91, and 9.80 ± 7.56 at 1, 3, 6, and 12 months, respectively as compared to the baseline.

Conclusion: This pilot study shows that combined IC and IV transplantation of UMSC01 in STEMI patients with impaired LVEF appears to be safe, feasible, and potentially beneficial in improving heart function. Further phase 2 studies are required to explore the effectiveness of dual-route transplantation of UMSC01 in STEMI patients.

KEYWORDS

intracoronary, intravenous, umbilical mesenchymal stem cell, acute myocardial infarction, human pilot trial

Introduction

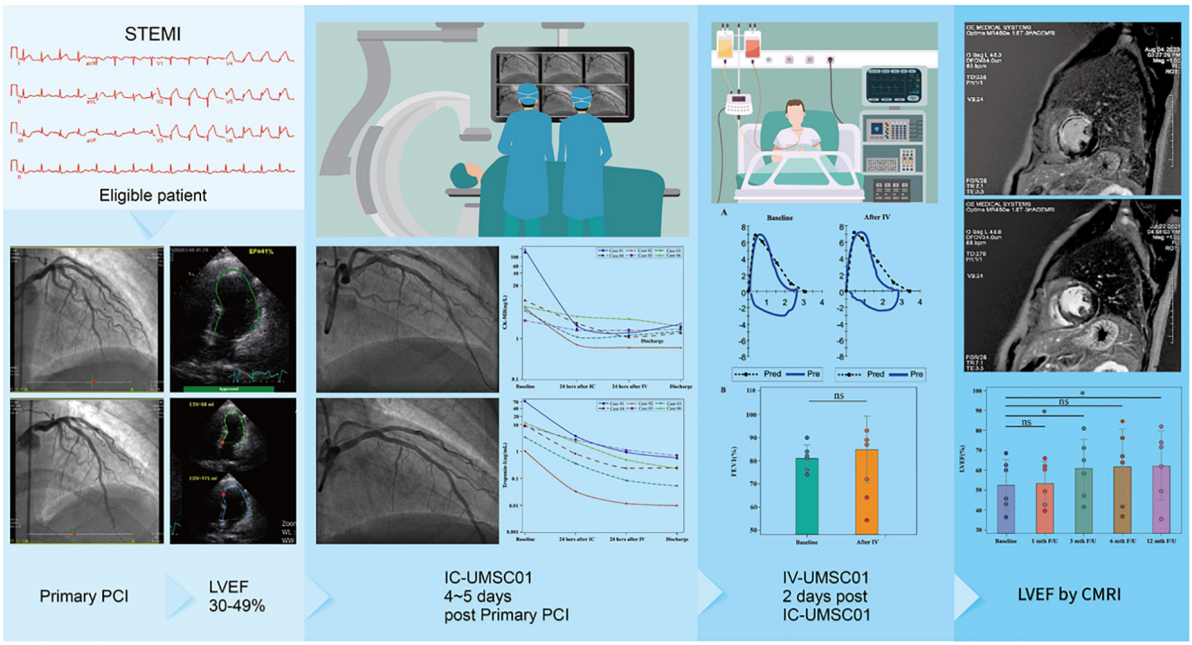
Acute myocardial infarction (AMI) elicits a robust cardiomyocyte death despite timely revascularization and optimal treatment (1). Acute injury initiates substantial inflammatory response, which causes cardiomyocyte damage and triggers cardiac fibrosis (2). Limitation of the initial injury and appropriate regulation of the immune response are essential to improve healing and reduce unfavorable left ventricular remodeling (3, 4). Stem cell-based therapies have been proposed as a promising strategy to reduce cardiomyocyte death and modulate immune reactions, leading to reduced myocardial scarring and improved cardiac function regardless of ischemic or non-ischemic cardiomyopathy (5–8).

Abbreviations: ACC, American College of Cardiology; AE, adverse event; AHA, American Heart Association; AMI, acute myocardial infarction; BMNC, bone marrow mononuclear cell; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CK-MB, creatine kinase-myocardial band; CMRI, cardiac magnetic resonance imaging; CXCR4, C-X-C Motif Chemokine Receptor 4; ECG, electrocardiography; FEV1, forced expiratory volume in 1 s; HLA, human leukocyte antigen; hUCS, human umbilical cord serum; IABP, intra-aortic balloon pump; IC, intracoronary; ICU, intensive care unit; Ig, immunoglobulin; IGF1R, insulin-like growth factor 1 receptor; IV, intravenous; LVEF, left ventricular ejection fraction; MACE, major adverse cardiovascular events; MOLLI, modified look-locker inversion recovery; MSC, mesenchymal stem cell; NT pro-BNP, amino-terminal pro-brain natriuretic peptide; PCI, percutaneous coronary intervention; PDGF-BB, platelet-derived growth factor-BB; RLVWMS, regional left ventricular wall motion score; SAE, serious adverse event; STEMI, ST-elevation myocardial infarction; SUSAR, suspected and unexpected serious adverse reaction; TIMI, thrombolysis in myocardial infarction; UC-MSC, umbilical cord-derived mesenchymal stem cell.

Bone marrow mononuclear cells (BMNCs) and circulating progenitor/stem cells delivered via the intracoronary (IC) or intravenous (IV) route constitute the most commonly used cell types, with doses ranging between 10^6 and 10^9 in published clinical studies (9–12). Recently, mesenchymal stem cells (MSCs) have drawn much attention because of their anti-inflammatory and immunomodulatory effects (13–16), and their effectiveness has been documented in many clinical trials (17, 18).

Previous studies in animals and humans have suggested that the dose of transplanted cells plays an important role in determining eventual myocardial function indices (19). Delivering sufficient cells by repeated transplantation might be necessary to overcome low retention and survival rates in the infarcted myocardium (7, 19). It has also been shown that three repeated doses of cells are superior to one dose of equivalent number of cells in relation to LVEF improvement, possibly due to greater antifibrotic and anti-inflammatory actions (20). There are various strategies regarding repeated transplantation, which involves the different combinations including deliver method, time interval and cell dose. Yao et al. demonstrated that repeated IC infusion of BMNCs 3 months after the first transfer is feasible and beneficial in patients with a large AMI (19). However, there has been no study in evaluating the feasibility and safety by combing IC and IV administration to achieve a sufficiently higher dose of umbilical cord-derived MSCs in patients with AMI.

To enhance the effect of cell-based therapy in post-AMI cardiac repair, we investigated umbilical-MSCs as a therapeutic



GRAPHICAL ABSTRACT
Eligible STEMI patients with a LVEF of 30-49% received the first dose of UMSC01 through IC injection 4-5 days after STEMI and the second dose of UMSC01 via IV infusion 2 days after IC. The culprit arteries were patent with comparable FEV1% and decreases in cardiac enzymes after IC + IV infusion. The LVEF increased at 12-month follow-up by cardiac MRI.

candidate. Although neither IC nor IV transplantation are ideal, they can complement each other (21). We hypothesized that a combined IC and subsequent IV umbilical-MSCs transplantation enables a synergistic anti-inflammatory and direct cell repair effect, which is beneficial in patients with AMI. Here, we first assessed the safety and explored the preliminary efficacy of umbilical cord-derived MSCs (UMSC01) by combining IC and IV stem cell administration.

Materials and methods

Study design and patients

This was a phase one, open-label, single-arm, single-center study in patients with acute ST-elevation MI (STEMI). Eligible patients who presented with first-ever STEMI were consecutively recruited. All of the subjects were hospitalized from the day of primary percutaneous coronary intervention (PPCI), until the third day after IV infusion of UMSC01. IC infusion of UMSC01 was performed 4–5 days via the index culprit artery after successful revascularization. The day of IC infusion of UMSC01 was designed as Day 0 and IV infusion of UMSC01 was carried out on Day 2. After discharge, all subjects were followed up at 1, 3, 6, and 12 months for endpoint evaluation, as shown in **Figure 1A**.

Male or female, aged between 20 and 76 years who presented with typical ischemic chest pain within 12 h of symptom onset and were diagnosed with first-time acute STEMI were considered eligible for the study. The infarct-related artery should be successfully revascularized by PPCI with a thrombolysis in myocardial infarction (TIMI) flow $\geq$ 2 suitable for cell infusion. After revascularization, STEMI patients should fulfill an echocardiography-determined left ventricular ejection fraction (LVEF) of $\geq$30% and <50% for enrollment. Patients with profound cardiogenic shock requiring mechanical support or those who had previous or incident significant valvular heart diseases were excluded. The full list of the inclusion and exclusion criteria can be found in online **Supplementary material**.

This study complied with the ethical principles of Declaration of Helsinki and was approved by the Research Ethics Committee of China Medical University Hospital (CMUH107-REC1-088). The trial was monitored by an independent data and safety monitoring board who met as planned to assess adverse events.

Preparation of UMSC01

On the day before administration, the cryopreserved UMSC01 cells were thawed in a 37°C water bath. The cell

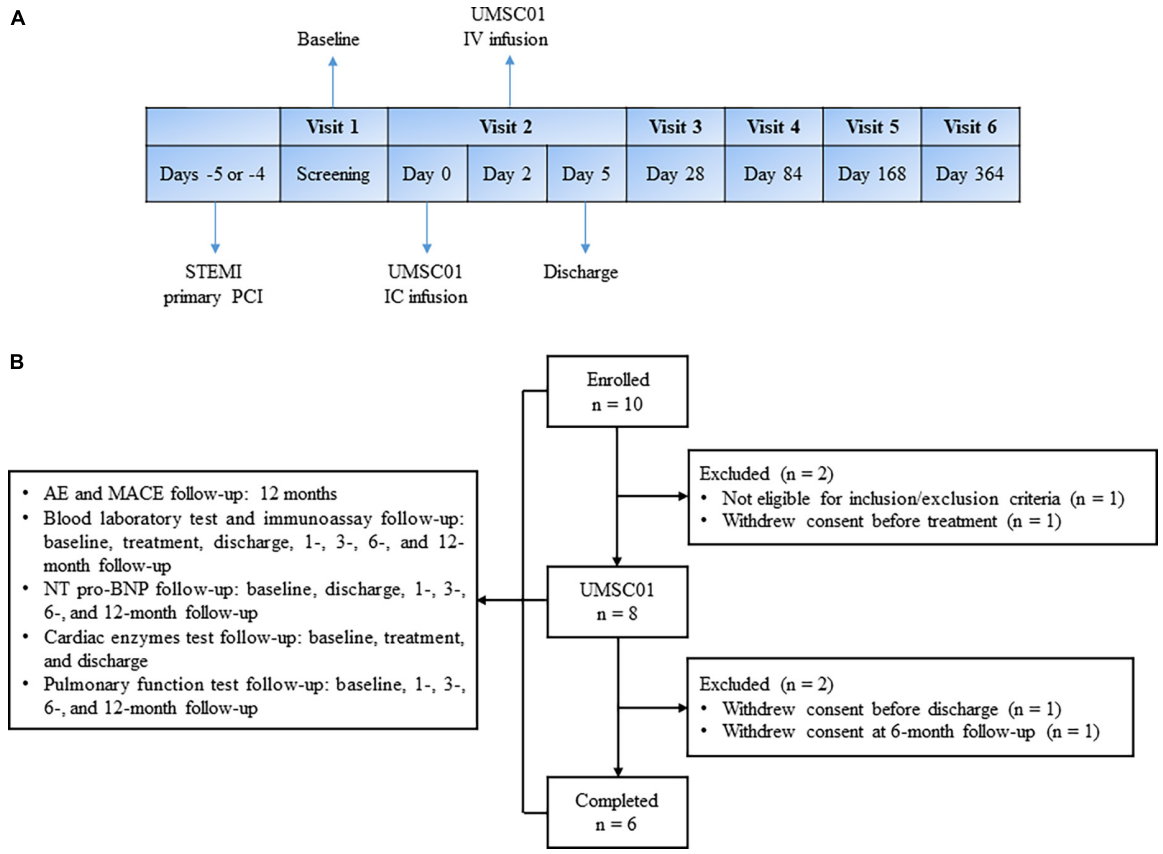


FIGURE 1
Study design and subject disposition. **(A)** Eligible patients who presented with first-ever ST elevation myocardial infarction (STEMI) were consecutively recruited. All of the subjects were hospitalized from the day of primary percutaneous coronary intervention (PCI), until the third day after IV infusion of UMSC01. IC infusion of UMSC01 was performed 4–5 days via the index culprit artery after successful revascularization. The day of IC infusion of UMSC01 was designed as Day 0 and IV infusion of UMSC01 was carried out on Day 2. After discharge, all subjects were followed up at 1, 3, 6, and 12 months for endpoint evaluation. **(B)** Between August 20, 2019, and August 2, 2020, we screened 10 patients with STEMI, of whom eight eligible patients provided written informed consent to participate in the clinical trial (NCT04056819). Two subjects withdrew consent during the follow-up period. Six subjects were followed up for the primary and secondary endpoints at 12 months. AE, adverse event; MACE, major adverse cardiovascular events; NT-pro-BNP, amino-terminal pro-brain natriuretic peptide.

suspension was washed three times to minimize residual reagents during the manufacturing process. The cell pellet was then resuspended in normal saline at a final cell density of $\sim 1 \times 10^7$ cells/mL for administration. The cell identity test showed that $\geq$95% of cells expressed CD73, CD90, and CD105, while the expression level of CD11b, CD19, CD45, CD34, and human leukocyte antigen-DR isotype (HLA-DR) was $\leq$2%. The final volume of UMSC01 for infusion (drug product) was resuspended in 0.9% saline containing a cell dose of either 1×10^7 for IC or 9×10^7 for IV infusion in AT-Closed Vial (Aseptic Technology, Gembloux, Belgium), while fulfilling the following product release criteria: cell viability by trypan blue (>70%), purity test by endotoxin examination (<0.25 EU/mL), and sterility test by gram staining and direct inoculation. The stepwise preparation of UMSC01 cells was described in the **Supplementary Methods**.

Intracoronary administration of UMSC01

We used the stop-flow technique for IC injection of UMSC01 with positioning of an over-the-wire balloon catheter within the segment of the previously deployed stent in the infarct-related artery (22). Briefly, 1×10^7 of UMSC01 in packed AT-Closed Vial® was mixed in 14 mL normal saline with 10,000 U/L heparin and directly injected using the over-the-wire balloon catheter in three treatment cycles. Each treatment cycle included 1.5 min of balloon inflation for cell injection, followed by 1.5 min balloon deflation for coronary reperfusion. Three cycles of cell injection were completed within 15 min. After completion of cell infusion, coronary angiography was repeated to confirm the patency of coronary artery flow. Then, patients were transferred to the intensive care unit

for overnight observation. Blood pressures, heart rate, oxygenation, and ECGs were continuously monitored throughout the procedure.

Intravenous administration of UMSC01

For IV infusion of UMSC01, 9×10^7 of UMSC01 mixed in 141 mL normal saline with 10,000 U/L heparin was administered via an antecubital vein at a flow rate of 2.5 mL/minute within 60 min. Blood pressures, heart rate, oxygenation, and ECGs were closely monitored throughout the procedure. Pulmonary function test with a ratio of the first second of forced expiration to the forced vital capacity (FEV1%) was measured before and 24 h after IV infusion. After completing the IV infusion of UMSC01, patients were transferred to the intensive care unit for overnight observation.

Study endpoints

The primary endpoints for the study were emergence of any suspected or unexpected serious adverse reaction and occurrence of major adverse cardiovascular events (MACE) including death, recurrent AMI, stroke, and target-vessel revascularization. The secondary endpoints were changes of the serum level of amino-terminal pro-brain natriuretic peptide (NT-proBNP) from baseline to discharge and at 1-, 3-, 6-, and 12-month follow-up and left ventricular function evaluated by cardiac magnetic resonance imaging (CMRI) from baseline to 1-, 3-, 6-, and 12-month follow-up.

Statistical analyses

All analyses were performed on patients who completed the entire study from the time of screening to the 12-month follow-up. Continuous variables are presented as mean and standard deviation, and categorical variables are presented as frequency and percentage. To compare the differences between baseline and follow-ups, paired *t*-tests were performed for efficacy endpoints to have more statistical power when the normality assumptions were not violated. Wilcoxon signed-rank tests were used for safety endpoints or non-normally distributed data. The Shapiro–Wilk method was adopted for normality tests because of small sample sizes. A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, United States).

Results

Subject disposition

Between August 20, 2019, and August 2, 2020, we screened 10 patients with ST-elevation AMI, of whom eight eligible patients provided written informed consent to participate in the clinical trial (NCT04056819). Two subjects withdrew consent during the follow-up period. Six subjects were followed up for the primary and secondary endpoints at 12 months. The subject disposition is shown in **Figure 1B**.

Patient characteristics

Baseline characteristics of the study subjects are shown in **Table 1**. All the enrolled subjects were males with a mean age of 61 ± 10 years. All the infarct-related arteries (left anterior descending artery in four patients and right coronary artery in two patients) underwent successful revascularization with TIMI 3 flow. The mean LVEF by echocardiography after PPCI was 43.0 ± 2.7 (range, 39.9-46.3%), which was associated with a baseline New York Heart Association functional class of II in all study subjects. None of the subjects had significant valvular heart disease, new-onset atrial fibrillation, or mechanical ventilation support. Following successful PPCI revascularization, all patients received guideline-directed medical treatment including dual-antiplatelet, β-blockers, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, and statins.

Characterization of UMSC01

The clinical grade of UMSC01 was certified and identified by surface markers, which showed uniformly high expression (≥95%) of CD73, CD90, and CD105 but low expression (≤2%) of CD11b, CD34, CD45, CD19, and HLA-DR (**Figure 2A**). The capacity of the potency assay for *in vitro* differentiation into mesodermal lineages of adipocytes, chondrocytes, and osteocytes was confirmed by Oil-Red-O, Alcian-Blue and Alizarin-Red staining, respectively (**Figure 2B**). The final drug substance and drug product of UMSC01 were released after passing all the criteria including cell viability, microbiological tests, endotoxin level, and Gram staining.

Primary endpoints for safety

There were no significant changes in the blood pressures, heart rates, or oxygenation status before and after IC injection or IV infusion of UMSC01 (**Supplementary Figure S1**). The culprit arteries remained patent at the end of IC infusion

TABLE 1 Baseline characteristics of the study subjects.

.	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Mean ± SD/n (%)
Age (year)	53	48	70	71	56	70	61 ± 10
Gender							
Male	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
Height (cm)	166	180	164	165	178	165	170 ± 7
Weight (kg)	83	78	58	61	96	62	73 ± 15
BMI (kg/m^2)	30.1	24.1	21.6	22.4	30.3	22.8	25.2 ± 3.9
Smoking	**Y**	**Y**	EX[a]	**Y**	**Y**	**Y**	5 (83.33)
Vital signs							
Systolic blood pressure (mmHg)	141	130	132	127	115	90	123 ± 18
Diastolic blood pressure (mmHg)	89	75	77	74	79	61	76 ± 9
Heart rate (bpm)	91	75	58	49	81	99	76 ± 19
Oximetry (%)	–	95	100	100	100	100	99 ± 2
Medical history							
Type 2 diabetes mellitus	N	N	N	N	**Y**	N	1 (16.67)
Hypertension	N	**Y**	**Y**	**Y**	**Y**	N	4 (66.67)
Dyslipidemia	**Y**	**Y**	**Y**	N	**Y**	N	4 (66.67)
Old myocardial infarction	N	N	N	N	N	N	0 (0.00)
Prior heart failure	N	N	N	N	N	N	0 (0.00)
Coronary artery disease	N	N	**Y**	N	N	N	1 (16.67)
ESRD	N	N	N	N	N	N	0 (0.00)
Stroke	N	N	N	N	N	N	0 (0.00)
COPD	N	N	**Y**	N	N	N	1 (16.67)
Liver cirrhosis	N	N	N	N	N	N	0 (0.00)
Cancer	N	N	N	N	N	N	0 (0.00)
Infarct-related artery							
LM	N	N	N	N	N	N	0 (0.00)
LAD	**Y**	**Y**	N	N	**Y**	**Y**	4 (66.67)
LCX	N	N	N	N	N	N	0 (0.00)
RCA	N	N	**Y**	**Y**	N	N	2 (33.33)
Culprit vessel TIMI flow after PCI							
0	N	N	N	N	N	N	0 (0.00)
1	N	N	N	N	N	N	0 (0.00)
2	N	N	N	N	N	N	0 (0.00)
3	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
Killip classification							
I	**Y**	N	**Y**	**Y**	**Y**	**Y**	5 (83.33)
II	N	N	N	N	N	N	0 (0.00)
III	N	N	N	N	N	N	0 (0.00)
IV	N	**Y**	N	N	N	N	1 (16.67)
NYHA functional classification							
I	N	N	N	N	N	N	0 (0.00)
II	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
III	N	N	N	N	N	N	0 (0.00)
IV	N	N	N	N	N	N	0 (0.00)
LVEF (%) by echocardiography after PCI	40.7	42.5	46.0	46.3	39.9	42.6	43.0 ± 2.7
Significant valvular heart disease	N	N	N	N	N	N	0 (0.00)
New onset atrial fibrillation	N	N	N	N	N	N	0 (0.00)

(Continued)

TABLE 1 (Continued)

.	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Mean ± SD/n (%)
Laboratory test at emergency room							
AST (IU/L)	116	13	16	13	52	15	38 ± 41
Creatinine (mg/dL)	0.95	1.59	1.05	1.14	0.91	1.19	1.14 ± 0.25
eGFR (mL/min/1.73 m^2)	83	47	70	63	86	60	68 ± 15
Sodium (mmol/L)	138	140	139	141	140	143	140 ± 2
Potassium (mmol/L)	3.3	3.9	3.8	4.1	4.0	4.2	3.9 ± 0.3
RBC (10^6/μL)	6.04	–	–	5.38	5.58	4.88	5.47 ± 0.48
WBC (10^3/μL)	13.7	18.2	8.2	15.0	10.5	5.3	11.8 ± 4.7
Hemoglobin (g/dL)	17.6	16.2	14.5	15.4	16.6	16.4	16.1 ± 1.1
Platelet count (10^3/μL)	219	288	213	233	249	251	242 ± 27
Troponin-I (ng/mL)	0.3769	0.2800	<0.0100	0.0371	<0.0100	0.2808	0.2437 ± 0.1451
Medications							
Aspirin	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
Clopidogrel	N	**Y**	N	N	N	N	1 (16.67)
Ticagrelor	**Y**	N	**Y**	**Y**	**Y**	**Y**	5 (83.33)
Glycoprotein IIb/IIIa inhibitors	N	N	N	**Y**	**Y**	N	2 (33.33)
Heparin	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
Beta blockers	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
ACEI/ARB	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
MRA	N	N	N	N	N	N	0 (0.00)
Statins	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	6 (100.00)
Vasopressor	N	N	N	N	N	N	0 (0.00)
Mechanical ventilation	N	N	N	N	N	N	0 (0.00)
IABP	N	**Y**	N	N	N	N	1 (16.67)

[a]Ex-smoker.

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin ii receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; IABP, intra-aortic balloon pump; LAD, left anterior descending artery; LCX, left circumflex artery; LM, left main; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York heart association; PCI, percutaneous coronary intervention; RBC, red blood cell; RCA, right coronary artery; SD, standard deviation; TIMI, thrombolysis in myocardial infarction; WBC, white blood cell.

in all subjects, and a steady decrease was seen in troponin-I (18.05 ± 27.46 vs. 1.66 ± 1.49 ng/L, p = 0.0313) and creatine kinase-MB (CK-MB; 30.7 ± 61.3 vs. 1.9 ± 1.0 ng/L, p = 0.0313) levels at 24 h compared to the baseline values (graphical abstract). The FEV1% was comparable (81.17 ± 5.74 vs. 84.83 ± 14.52%, p = 0.6875) at the end of IV infusion of UMSC01 (Graphical Abstract). There were significant differences in the levels of blood urea nitrogen (14 ± 3 vs. 19 ± 2 mg/dL, p = 0.0313), serum potassium (3.6 ± 0.2 vs. 4.2 ± 0.2 meq/L, p = 0.0313), white blood cell count (9.9 ± 2.9 vs. 6.5 ± 2.1 10^3/μL, p = 0.0313), platelet count (204 ± 32 vs. 227 ± 38 10^3/μL, p = 0.0313), and the plasma IgG (1050 ± 292 vs. 1225 ± 342 mg/dL, p = 0.0313) between baseline and 12-month follow-up, however these values were within normal ranges and were not associated with any clinically relevant events (**Table 2**). The carcinoembryonic antigen level (4.27 ± 4.19 vs. 2.71 ± 1.22 ng/mL, p = 0.2188) and immunology parameters including CD3 (55.3 ± 12.3 vs. 58.9 ± 6.1%, p = 0.4375), CD4/CD8 (2.8 ± 1.2 vs. 2.1 ± 0.8, p = 0.0938), anti-HLA antibodies (0.6 ± 0.4 vs. 0.6 ± 0.7%, p = 0.7500), and panel reactive antibody assay (0.4 ± 0.4 vs. 0.4 ± 0.5%, p = 0.6250) were not significantly different between baseline and at the 12-month follow-up. During the study period, none of the subjects experienced serious adverse events or major adverse cardiovascular events (**Table 3**). One patient had a unilateral inguinal hernia at 5-month of follow-up and was subsequently hospitalized for operation, which was adjudicated as a non-treatment related serious adverse event. There were three non-treatment related adverse events consisting of a non-ischemic chest pain, a mild superficial skin rash, and a localized eczema, respectively in three subjects.

Secondary endpoints for efficacy

The mean serum level of NT-proBNP decreased significantly at 12-month follow-up when compared to the value at baseline (1362 ± 1801 vs. 109 ± 115 pg/mL, p = 0.0313) (**Figure 3**). The stroke volume increased from 64.34 ± 11.44 ml/per beat to 84.11 ± 5.26 ml/per beat (p = 0.0033) and LVEF from

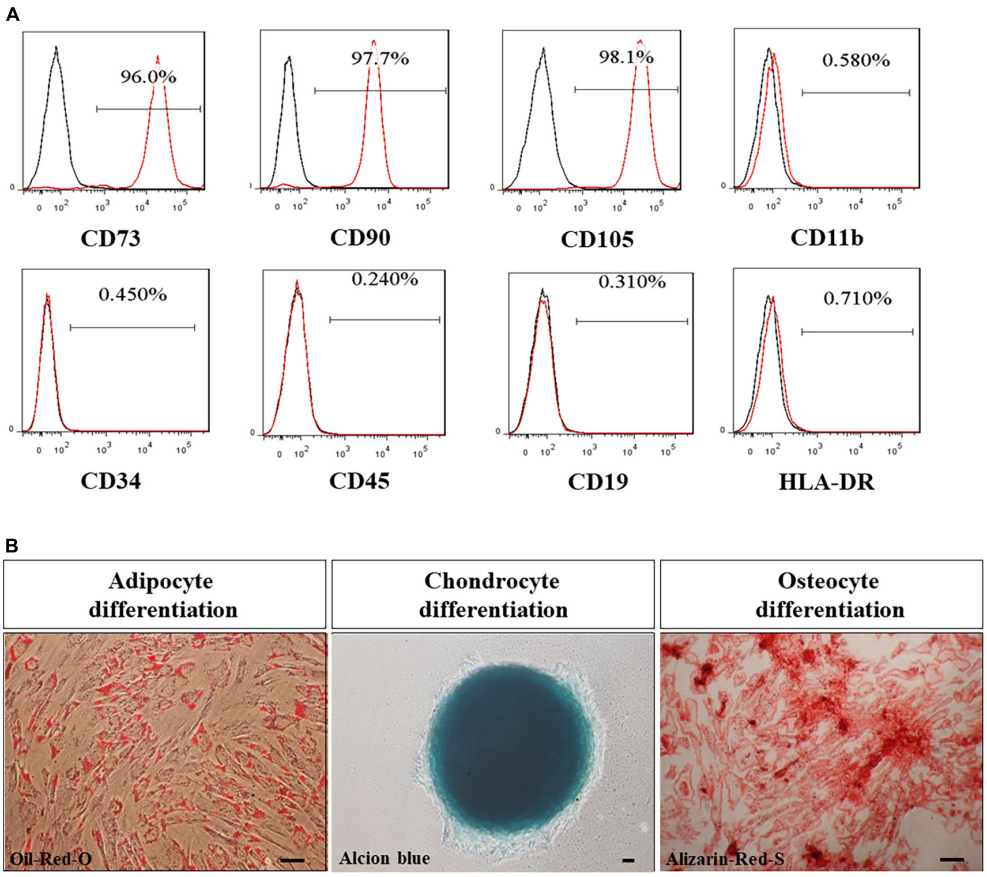


FIGURE 2
Characterization of human umbilical cord-derived mesenchymal stem cells (UMSC01). **(A)** The clinical grade of UMSC01 was certified and identified by surface markers, which showed uniformly high expression (≥95%) of CD73, CD90, and CD105 but low expression (≤2%) of CD11b, CD34, CD45, CD19, and HLA-DR. **(B)** The potency assay for the capacity of *in vitro* differentiation into mesodermal lineages of adipocytes, chondrocytes, and osteocytes was confirmed by Oil-Red-O, Alcian-Blue and Alizarin-Red staining, respectively. Bar = 50 μm. CD, cluster of differentiation; HLA, human leukocyte antigen.

52.67 ± 12.75% to 62.47 ± 17.35% (p = 0.0246), while the wall motion score decreased from 26.33 ± 5.57 to 22.33 ± 5.85 (p = 0.0180) at the 12-month follow-up compared to the baseline levels by CMRI (**Graphical Abstract** and **Figure 4**). The New York Heart Association functional classification improved from class II at baseline to class I at 12-month follow-up in all study subjects.

Discussion

We report a phase I clinical trial of combined IC and IV delivery of UMSC01 that reached the specified endpoints: STEMI patients safely received cell transplantation in this dual-route administration until the 12-month follow-up. To the best of our knowledge, this is the first-in-human study to demonstrate the safety and feasibility of a combined delivery method in AMI patients with STEMI and heart failure. Under the current study design, we observed an improvement in LVEF and functional status, which encourages a randomized double-blind phase II study.

Strengths of UMSC01

To explore the appropriate human MSCs for clinical applications, pluripotent-like markers for culturing MSCs that retain potent survival and self-renewal abilities should be thoroughly investigated. In our previous preclinical report (23), an insulin-like growth factor 1 receptor (IGF1R) expressing sub-population in human MSCs, including umbilical cord-MSCs, were cultured in platelet-derived growth factor-BB (PDGF-BB)-containing human umbilical cord serum (hUCS) and displayed longer survival and stronger proliferation potential. Through intercellular receptor transactivation between CXCR4 and IGF1R signaling pathways, implantation of IGF1R$^+$ MSCs showed significant improvement in

neurological function in a stroke model. In this clinical study, we translated the experimental setting by replacing the hUCS with commercially available platelet rich plasma containing high levels of PDGF-BB for culturing UMSC01 to treat the STEMI patients. The preliminary result found that UMSC01 administration might provide not only a safe but also a functional improvement strategy for STEMI patients in this pilot study. A larger-scale placebo-controlled trial is mandatory for demonstrating the definite clinical efficacy in the future.

In patients with AMI, a recent meta-analysis study demonstrated that transplantation of MSCs significantly improves left ventricular ejection fraction (LVEF) (24). Although Both BMNCs and MSCs contribute to an improvement of cardiac function in the clinical setting of AMI, it is important to know which type of stem cell could outperform the other (25). So far, there is no clinical trial with head-to-head comparison in evaluating the clinical efficacy between BMNCs and MSCs in patients with AMI. Interestingly, Hosseinpour et al. 25 reported that transplantation of MSCs might result in better LVEF improvement than BMNCs in a meta-analysis study. Among the different types of MSCs, umbilical cord-derived MSCs can be easily obtained and cultured (26, 27). Umbilical cord-derived MSCs have shown immunomodulatory and tissue-repair effects with low immunogenicity, which makes them ideal candidates for allogeneic adoptive transfer therapy (28, 29).

Mode of delivery

Stem cell-based cardiac therapy involves the transplantation of cells via various delivery methods. Currently, there are three main routes of cell implantation—intramyocardial (transepicardial or transendocardial), IC, and IV (7). Each delivery technique aims to transfer an adequate number of cells to the infarct site of the heart and to maximize the retention rate of cells, thus improving engraftment and facilitating robust therapeutic outcomes (30). Nevertheless, irrespective of the route of cell delivery, low retention remains a major hurdle limiting the beneficial effects of cell transplantation (31). To date, the vast majority of animal and clinical studies on stem cell therapy in cardiovascular disease have chosen a single delivery method. It is possible that combined IC and IV stem cell transplantation provides more benefits than IC or IV delivery alone (21). The combination of IC and IV for cell delivery, like IC alone, increases the number of cells homing to the injured area of the myocardium, while subsequent IV stem cell treatment allows for a higher cell dose and also exerts systemic anti-inflammatory effects to achieve better therapeutic outcome. Importantly, a study conducted by Liu et al. used a combined approach of cell delivery (IC injection with IV infusion) in a porcine model of chronic myocardial ischemia (32). Their results showed that umbilical cord-MSCs improved the left ventricular function, perfusion, and remodeling. In addition, there was a significant reduction in fibrosis and apoptosis (32). In our study design, we were able to deliver a total of 10^8 UMSC01 uneventfully. There was no any treatment-related adverse events, including coronary occlusion, immune reaction, or tumor formation. As a pilot phase one study, it is not possible to draw a solid conclusion regarding the efficacy of UMSC01 transplantation. Nevertheless, another randomized clinical trial is ongoing, which compares single with double IC infusion of umbilical cord-derived Wharton's jelly MSCs in patients with AMI (33). The result may provide better understanding of the effect of repeated transplantation of this cell type on myocardial function (LVEF).

Timing of delivery

Following STEMI, the key events include an acute inflammatory phase in the first 4 days, resolution and repair phase within 2 weeks, and a remodeling phase after 2 weeks (34, 35). Blunting the infarct-triggered inflammation and promotion of late healing are important for the reduction of abnormal cardiac remodeling (36–38). It has been extensively studied in previous studies regarding time of delivery in stem cell therapy after AMI (39–43). For BMNCs, it was suggested to deliver stem cells 3–7 days after AMI for improvement of myocardial function (24). Consistently, a meta-analysis also shows a higher efficacy if MSCs transplantation was performed within the first week following AMI (24). In our study, IC treatment on the 4–5th day post-MI ensured a stable post-AMI condition and was able to modulate the acute immune response and shape acute inflammation. The IV cell administration on the 6–7th day post-MI improved the myocardial repair process and modified scar replacement. A longer stem cell treatment period that covered the golden first 2 weeks after AMI potentially augmented the regeneration effect.

Cell therapy for acute myocardial infarction in post-BAMI era

In most stem cell-treated AMI trials, transplanted cells were BMNCs and the benefits in cardiac regeneration were mixed and uncertain (10). To date, the autologous bone marrow cell therapy in AMI trial (BAMI) is the largest study of stem-based cell therapy for AMI in the world. Initially, BAMI was designed to establish whether BMNCs reduced mortality in patients with STEMI by IC infusion. Unfortunately, the low number of cases was

TABLE 2 Serial laboratory tests during the study period.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Mean ± SD	*P*-value[a]
AST (IU/L)								
Baseline	109	26	36	67	41	53	55 ± 30	–
After IC injection	58	16	20	18	24	20	26 ± 16	**0.0313**
After IV infusion	56	17	26	19	13	17	25 ± 16	**0.0313**
1-Month follow-up	52	19	23	18	19	21	25 ± 13	**0.0313**
3-Month follow-up	66	16	27	20	17	19	28 ± 19	**0.0313**
6-Month follow-up	30	23	21	28	15	29	24 ± 6	**0.0313**
12-Month follow-up	39	32	25	17	16	15	24 ± 10	**0.0625**
ALT (IU/L)								
Baseline	122	14	15	29	54	25	43 ± 41	–
After IC injection	91	20	17	25	48	24	38 ± 28	**0.4688**
After IV infusion	95	20	23	23	31	22	36 ± 29	**0.4688**
1-Month follow-up	82	14	25	16	41	27	34 ± 25	**0.2500**
3-Month follow-up	81	14	33	18	26	25	33 ± 25	**0.3750**
6-Month follow-up	40	33	19	35	19	36	30 ± 9	**1.0000**
12-Month follow-up	55	27	18	12	14	16	24 ± 16	**0.2188**
BUN (mg/dL)								
Baseline	14	10	12	13	19	14	14 ± 3	–
After IC injection	12	11	12	16	16	10	13 ± 3	**0.5000**
After IV infusion	–	11	13	17	11	10	12 ± 3	**0.9375**
1-Month follow-up	26	10	13	13	16	19	16 ± 6	**0.3750**
3-Month follow-up	16	15	14	19	22	16	17 ± 3	**0.0313**
6-Month follow-up	18	12	12	20	22	16	17 ± 4	**0.0625**
12-Month follow-up	20	19	16	21	20	18	19 ± 2	**0.0313**
Creatinine (mg/dL)								
Baseline	0.77	0.79	0.77	1.08	0.85	1.06	0.89 ± 0.15	–
After IC injection	0.72	0.93	0.82	0.98	0.83	1.09	0.90 ± 0.13	**0.9063**
After IV infusion	0.81	0.98	0.85	0.89	0.87	1.19	0.93 ± 0.14	**0.3438**
1-Month follow-up	1.01	1.09	1.04	1.25	0.95	1.25	1.10 ± 0.13	**0.0313**
3-Month follow-up	0.89	1.06	1.05	1.49	0.86	1.02	1.06 ± 0.23	**0.0938**
6-Month follow-up	0.89	0.93	0.95	1.31	0.84	0.97	0.98 ± 0.17	**0.1563**
12-Month follow-up	0.82	1.13	0.86	1.36	0.77	1.05	1.00 ± 0.23	**0.2188**
eGFR (mL/min/1.73 m^2)								
Baseline	106	105	100	67	93	69	90 ± 18	–
After IC injection	114	87	93	75	96	67	89 ± 17	**0.9375**
After IV infusion	100	82	89	84	91	60	84 ± 13	**0.3125**
1-Month follow-up	77	72	71	57	82	57	69 ± 10	**0.0313**
3-Month follow-up	89	75	70	46	92	72	74 ± 16	**0.0938**
6-Month follow-up	89	87	78	54	95	77	80 ± 14	**0.1563**
12-Month follow-up	98	69	88	52	105	70	80 ± 20	**0.2500**
Sodium (mmol/L)								
Baseline	138	141	139	141	141	136	139 ± 2	–
After IC injection	137	139	139	141	139	139	139 ± 1	**0.8750**
After IV infusion	135	140	139	140	141	139	139 ± 2	**0.7500**
1-Month follow-up	139	143	140	142	142	139	141 ± 2	**0.0313**
3-Month follow-up	138	141	141	144	142	139	141 ± 2	**0.1250**
6-Month follow-up	139	141	142	141	142	140	141 ± 1	**0.1250**
12-Month follow-up	141	142	139	143	139	140	141 ± 2	**0.2500**

(Continued)

TABLE 2 (Continued)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Mean ± SD	*P*-value[a]
Potassium (mmol/L)								
Baseline	3.3	3.9	3.7	3.7	3.3	3.7	3.6 ± 0.2	–
After IC injection	3.5	3.8	3.8	3.4	3.5	4	3.7 ± 0.2	**0.5938**
After IV infusion	3.8	3.8	3.9	3.4	3.5	4.2	3.8 ± 0.3	**0.2813**
1-Month follow-up	3.6	4.4	3.5	3.7	3.8	3.9	3.8 ± 0.3	**0.1875**
3-Month follow-up	4.1	4.1	4.2	4	4.5	4.2	4.2 ± 0.2	**0.0313**
6-Month follow-up	3.6	4.1	3.9	3.8	3.9	4	3.9 ± 0.2	**0.0313**
12-Month follow-up	4.2	4.4	4.1	4.1	4.2	3.9	4.2 ± 0.2	**0.0313**
RBC (10^6/uL)								
Baseline	5.14	4.67	6.87	4.79	4.89	4.57	5.16 ± 0.86	–
After IC injection	5.17	4.53	6.24	4.23	4.59	4.08	4.81 ± 0.80	**0.0625**
After IV infusion	5.48	4.57	6.10	4.37	4.47	4.14	4.86 ± 0.76	**0.0938**
1-Month follow-up	5.16	4.77	6.55	5.20	4.64	4.21	5.09 ± 0.80	**0.8438**
3-Month follow-up	5.04	4.57	6.44	4.88	4.87	3.84	4.94 ± 0.85	**0.0938**
6-Month follow-up	5.21	5.04	6.61	4.70	4.92	3.88	5.06 ± 0.89	**0.6875**
12-Month follow-up	5.07	4.79	6.58	4.40	4.79	4.07	4.95 ± 0.87	**0.1563**
WBC (10^3/μL)								
Baseline	11.0	15.2	8.1	8.1	9.4	7.7	9.9 ± 2.9	–
After IC injection	7.2	11.1	6.4	6.8	9.9	6.8	8.0 ± 2.0	**0.0625**
After IV infusion	8.1	11.4	5.8	6.9	11.0	8.1	8.6 ± 2.2	**0.2188**
1-Month follow-up	7.4	7.2	5.1	5.9	7.8	5.1	6.4 ± 1.2	**0.0313**
3-Month follow-up	6.7	9.1	5.9	7.2	8.3	4.8	7.0 ± 1.6	**0.0313**
6-Month follow-up	6.7	8.0	5.5	5.8	6.7	6.9	6.6 ± 0.9	**0.0313**
12-Month follow-up	6.0	10.7	6.0	5.2	6.0	4.9	6.5 ± 2.1	**0.0313**
Hemoglobin (g/dL)								
Baseline	15.6	13.8	15.1	14.1	15.3	15.4	14.9 ± 0.7	–
After IC injection	15.9	13.7	14.2	12.9	14.3	13.5	14.1 ± 1.0	**0.0938**
After IV infusion	16.7	13.4	13.9	13.4	14.0	13.8	14.2 ± 1.3	**0.1563**
1-Month follow-up	15.7	14.9	15.2	15.2	14.3	13.8	14.9 ± 0.7	**0.8750**
3-Month follow-up	15.3	13.7	14.6	13.7	14.9	12.5	14.1 ± 1.0	**0.0313**
6-Month follow-up	16.0	14.6	15.2	13.7	14.7	12.7	14.5 ± 1.2	**0.7500**
12-Month follow-up	15.7	15.0	15.3	13.1	14.8	13.0	14.5 ± 1.2	**0.6875**
Platelet count (10^3/μL)								
Baseline	173	262	192	180	209	205	204 ± 32	–
After IC injection	204	467	211	161	214	203	243 ± 111	**0.2500**
After IV infusion	232	517	231	174	233	229	269 ± 123	**0.0625**
1-Month follow-up	172	293	193	189	224	247	220 ± 45	**0.0938**
3-Month follow-up	175	313	204	324	251	253	253 ± 59	**0.0313**
6-Month follow-up	182	352	238	230	216	263	247 ± 58	**0.0313**
12-Month follow-up	195	301	224	202	212	227	227 ± 38	**0.0313**
Carcinoembryonic antigen (ng/mL)								
Baseline	3.16	3.69	1.84	3.37	1.00	12.57	4.27 ± 4.19	–
After IC injection	2.86	2.63	2.15	2.46	0.98	10.71	3.63 ± 3.53	**0.1563**
After IV infusion	3.67	2.64	1.84	3.28	0.99	9.84	3.71 ± 3.16	**0.3125**
1-Month follow-up	2.26	3.22	1.80	2.32	0.82	7.18	2.93 ± 2.22	**0.0313**
3-Month follow-up	2.44	3.18	1.85	4.02	1.34	5.44	3.05 ± 1.51	**0.5625**
6-Month follow-up	2.77	2.69	2.41	2.45	0.79	4.17	2.55 ± 1.08	**0.1563**
12-Month follow-up	3.20	3.31	2.10	2.23	0.95	4.48	2.71 ± 1.22	**0.2188**

(Continued)

TABLE 2 (Continued)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Mean ± SD	*P*-value[a]
CK (IU/L)								
Baseline	2163	289	331	610	186	335	652 ± 753	–
24 h after IC injection	231	47	80	129	123	118	121 ± 62	**0.0313**
24 h after IV infusion	128	42	94	70	83	96	86 ± 29	**0.0313**
Discharge	150	38	107	79	62	69	84 ± 39	**0.0313**
CD3 (%)								
Baseline	52.6	76.8	49.8	41.1	50.6	60.6	55.3 ± 12.3	–
After IC injection	60.5	59.7	–	42.3	–	43.7	51.6 ± 9.9	**0.6250**
After IV infusion	58.9	–	39.5	36.8	64.7	68.1	53.6 ± 14.5	**0.6250**
1-Month follow-up	58.6	55.7	60.7	41.0	60.3	74.1	58.4 ± 10.6	**0.5625**
3-Month follow-up	60.8	67.1	48.6	34.8	54.8	63.1	54.9 ± 11.8	**1.0000**
6-Month follow-up	69.3	63.7	54.4	52.1	54.6	64.0	59.7 ± 6.9	**0.3125**
12-Month follow-up	57.5	64.8	56.5	48.9	60.6	65.3	58.9 ± 6.1	**0.4375**
CD4/CD8								
Baseline	2.1	1.8	4.0	3.4	4.1	1.2	2.8 ± 1.2	–
After IC injection	1.8	2.0	–	3.4	–	1.9	2.3 ± 0.8	**0.6250**
After IV infusion	1.6	–	5.1	2.5	3.7	1.7	2.9 ± 1.5	**1.0000**
1-Month follow-up	1.6	1.3	4.4	2.3	4.4	1.1	2.5 ± 1.5	**0.3125**
3-Month follow-up	1.5	1.6	4.1	2.0	4.0	0.8	2.3 ± 1.4	**0.0625**
6-Month follow-up	1.7	1.5	3.0	2.6	2.6	0.6	2.0 ± 0.9	**0.0313**
12-Month follow-up	2.0	1.4	3.4	2.4	2.0	1.2	2.1 ± 0.8	**0.0938**
IgG (mg/dL)								
Baseline	759	1100	1430	1020	689	1300	1050 ± 292	–
After IC injection	931	1050	1490	806	677	922	979 ± 280	**0.5625**
After IV infusion	967	1210	1440	930	649	1380	1096 ± 302	**0.4375**
1-Month follow-up	970	1120	1530	1180	932	1760	1249 ± 329	**0.0313**
3-Month follow-up	892	1250	1650	1270	943	1570	1263 ± 311	**0.0313**
6-Month follow-up	917	1210	1790	1060	914	1510	1234 ± 352	**0.0313**
12-Month follow-up	823	1180	1740	1160	947	1500	1225 ± 342	**0.0313**
IgM (mg/dL)								
Baseline	208.0	140.0	72.5	97.4	30.1	44.9	98.8 ± 66.2	–
After IC injection	226.0	169.0	71.0	94.1	19.1	25.2	100.7 ± 82.0	**1.0000**
After IV infusion	272.0	174.0	70.3	105.0	20.6	36.2	113.0 ± 95.2	**0.6875**
1-Month follow-up	235.0	160.0	74.2	129.0	24.6	47.0	111.6 ± 78.8	**0.1563**
3-Month follow-up	220.0	146.0	75.4	161.0	28.9	41.8	112.2 ± 75.4	**0.2188**
6-Month follow-up	208.0	133.0	82.2	110.0	18.9	37.8	98.3 ± 68.7	**1.0000**
12-Month follow-up	181.0	116.0	80.5	119.0	21.7	37.9	92.7 ± 58.7	**0.4375**
Anti-HLA antibodies (%)								
Baseline	0.7	0.2	1.0	0.4	0.4	1.1	0.6 ± 0.4	–
After IC injection	1.9	0.7	0.5	0.1	0.3	0.5	0.7 ± 0.6	**0.9063**
After IV infusion	1.7	0.4	0.7	0.3	3.9	0.4	1.2 ± 1.4	**0.6875**
1-Month follow-up	2.4	0.9	2.4	1.7	1.6	0.1	1.5 ± 0.9	**0.0938**
3-Month follow-up	0.7	0.1	0.8	0.7	0.1	0.6	0.5 ± 0.3	**0.3750**
6-Month follow-up	0.8	1.9	0.7	1.0	0.5	0.9	1.0 ± 0.5	**0.5625**
12-Month follow-up	0.3	0.2	1.9	0.5	0.1	0.8	0.6 ± 0.7	**0.7500**

(Continued)

TABLE 2 (Continued)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Mean ± SD	*P*-value[a]
Panel reactive antibody assay (%)								
Baseline	0.40	0.20	0.30	0.20	0.30	1.20	0.4 ± 0.4	–
After IC injection	1.20	0.10	0.40	0.20	0.40	0.70	0.5 ± 0.4	**0.8125**
After IV infusion	1.00	0.10	0.40	0.10	0.50	0.10	0.4 ± 0.4	**1.0000**
1-Month follow-up	0.60	0.30	1.50	0.90	0.50	0.10	0.7 ± 0.5	**0.3125**
3-Month follow-up	0.20	0.10	0.20	0.20	0.10	0.50	0.2 ± 0.1	**0.0625**
6-Month follow-up	0.30	0.70	0.50	1.20	0.40	0.80	0.7 ± 0.3	**0.3438**
12-Month follow-up	0.10	0.10	1.30	0.20	0.10	0.30	0.4 ± 0.5	**0.6250**

[a]Wilcoxon signed-rank test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CD, cluster of differentiation; CK, creatine kinase; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; Ig, immunoglobulin; IC, intracoronary; IV, intravenous; RBC, red blood cell; WBC, white blood cell; SD, standard deviation.

TABLE 3 Adjudication of treatment related adverse events among study subjects.

	Case 01	Case 02	Case 03	Case 04	Case 05	Case 06	*n* (%)
SUSAR[a]	N	N	N	N	N	N	0 (0.00)
SAE							
Death	N	N	N	N	N	N	0 (0.00)
Life-threatening[b]	N	N	N	N	N	N	0 (0.00)
Hospitalization[c]	N	N	N	N	N	**Y**	1 (16.67)
Disability/Incapacity	N	N	N	N	N	N	0 (0.00)
Congenital anomaly/Birth defect	N	N	N	N	N	N	0 (0.00)
AE[d]							
Chest pain	N	N	N	N	N	**Y**	1 (16.67)
Severity	–	–	–	–	–	*Mild*	
Relationship with study IP	–	–	–	–	–	*Unrelated*	
Eczema	N	**Y**	N	N	N	N	1 (16.67)
Severity	–	*Mild*	–	–	–	–	
Relationship with study IP	–	*Unrelated*	–	–	–	–	
Inguinal hernia	N	N	N	N	N	**Y**	1 (16.67)
Severity	–		–	–	–	*Moderate*	
Relationship with study IP	–		–	–	–	*Unrelated*	
Rash	N	N	N	N	**Y**	N	1 (16.67)
Severity	–	–	–	–	*Mild*	–	
Relationship with study IP	–	–	–	–	*Unrelated*	–	
MACE	N	N	N	N	N	N	0 (0.00)

[a]The nature and severity of which is not consistent with the applicable drug information.
[b]The subject was at risk of death at the time of event.
[c]The subject required hospitalization or prolonged existing hospitalization.
[d]Any untoward medical occurrence in a patient or clinical investigation subject administered a harmaceutical drug and which did not necessarily have to have a causal relationship with this treatment.
AE, adverse event; MACE, major adverse cardiovascular events; IP, investigational product; SAE, serious adverse event; SUSAR, suspected and unexpected serious adverse reaction; TEAE, treatment emergent adverse event.

unable to show a significant difference in improving survival between patients and the case–control group. In contrast to BMNCs, MSCs show consistent findings of improvement in cardiac function. MSCs, especially UMSC01, have multiple differentiation capabilities, immune exemption, easy access, large-scale expansion, and ethical advantages. The work by Gao et al. (29) demonstrated that IC delivery of UMSC01 could significantly improve myocardial viability and cardiac function in patients with STEMI. Furthermore, in our study, we showed that UMSC01 delivery using this novel administration method is safe and feasible in human beings. Although we did not include a control group in this trial, the enrolled patients showed an improvement in cardiac function, regional wall motion, heart failure symptoms, and biomarkers, indicating that UMSC01 transplantation might be beneficial in post-AMI cardiac repair.

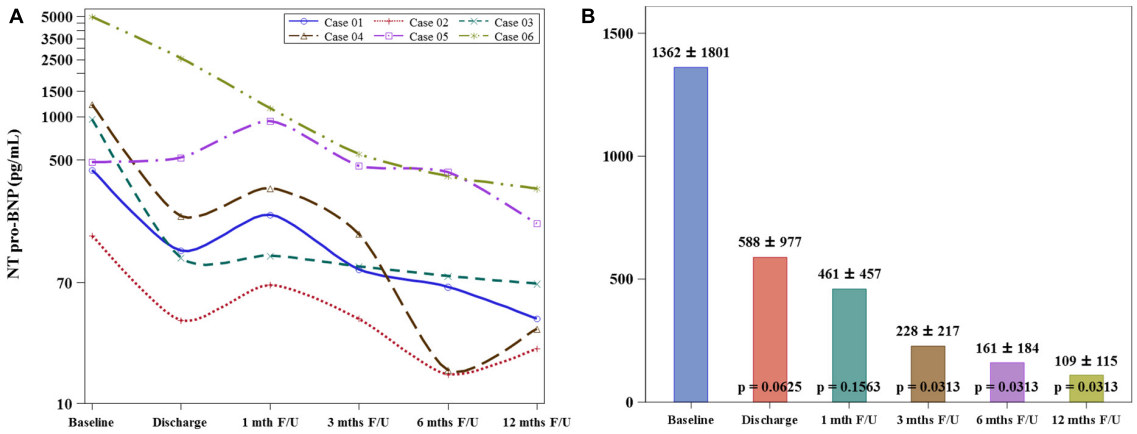


FIGURE 3
Comparison of NT pro-BNP levels between baseline and 12-month follow-up. **(A)** The serum level of NT pro-BNP of individual study patients shows a consistent declination pattern from baseline to 12-month follow-up. **(B)** The mean serum level of NT-proBNP decreased significantly at 12-month follow-up when compared to the value at baseline.

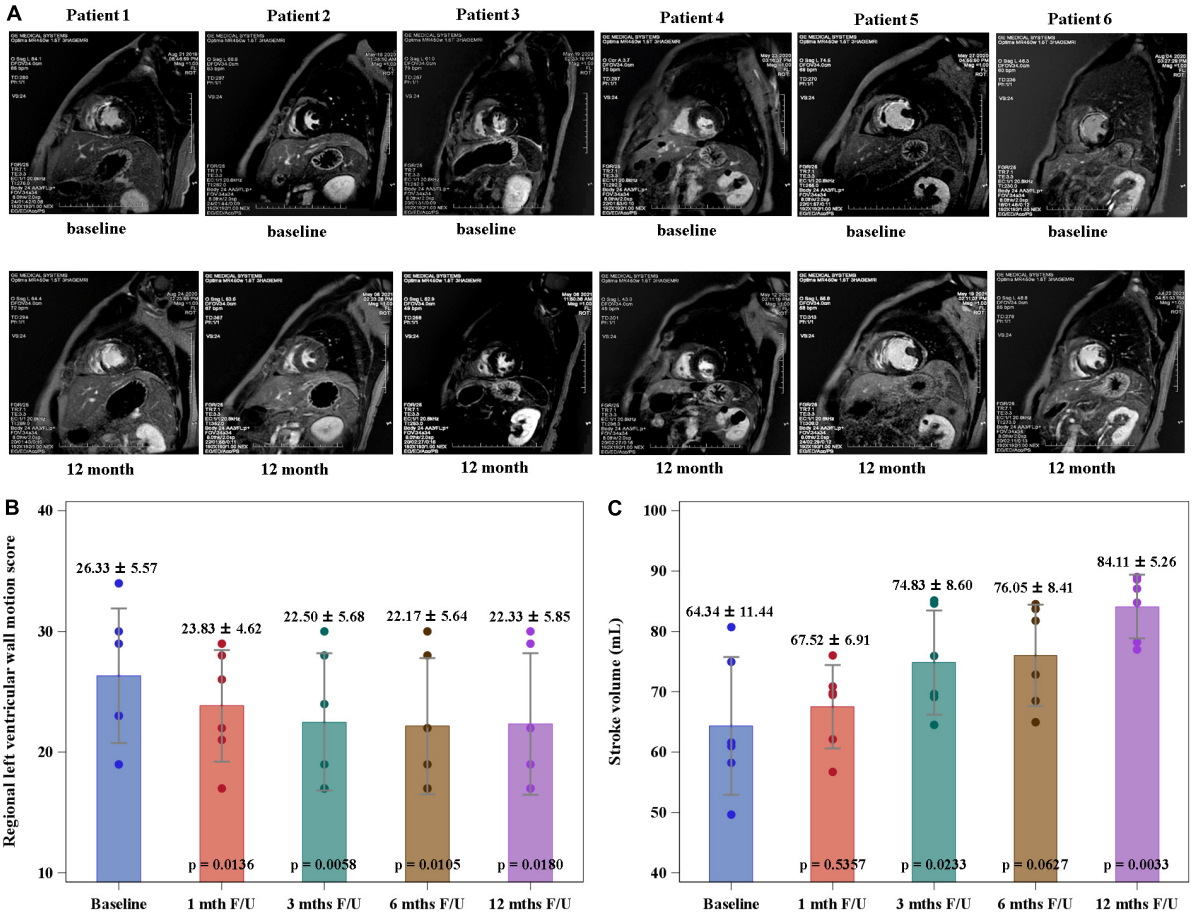


FIGURE 4
Regional left ventricular wall motion score (RLVWMS) and stroke volume evaluated by cardiac magnetic resonance imaging (CMRI). **(A)** The representative CMRIs of individual study patient at baseline and 12-month follow-up. The RLVWMS decreased **(B)** and the stroke volume increased **(C)** at 12-month follow-up compared to the baseline levels.

Over the last two decades, almost 100 blinded and unblinded AMI clinical trials worldwide have shown an excellent safety profile of cell-based therapy but have reported mixed and uncertain results on its potential benefits (9, 10, 21, 44). Of note, the number of enrolled patients in many of the clinical trials were not sufficient to test the benefits of stem cell transplantation when combined with the current guideline-directed AMI therapy (44, 45). The BAMI trial failed to demonstrate that BMNC therapy improves survival in patients with AMI due to low enrollment and low mortality rates. However, the BAMI researchers observed the clinical benefit of reduced heart failure associated hospitalization in patients receiving BMNC therapy (45). In a recent meta-analysis study, Attar et al. further confirmed that BMNC therapy improved clinical outcomes in terms of reinfarction and hospitalization for heart failure (46). Thus, it is crucial to identify a better cell type, optimal dose, and route of transplantation to strengthen the therapeutic effect and achieve a statistical significance in the post-BAMI era (10).

Study limitations

This study had some limitations. First, although this pilot trial was designed to determine whether the novel approach of combined IC and IV delivery of UMSC01 in STEMI patients is safe, the sample size of six analyzable subjects was relatively small. Second, placebo-treated patients were not included in this phase I study. The efficacy of UMSC01 transplantation remains unclear. However, stem cell-treated patients exhibited improvement in heart function after a 12-month follow-up period. Third, this study focused mainly on the safety and feasibility of the combined IC and IV methods and thus did not provide comprehensive multimodality imaging in the assessment of efficacy. Lastly, in this pilot study, although we observed no significant changes of several immunology parameters including CD3, CD4/CD8, anti-HLA antibodies, and panel reactive antibody assay between baseline and at the 12-month follow-up, it is indeed necessary to measure more immunology/inflammatory markers such as IL-6 and IL-10 in the future phase II study.

Conclusion

In this pilot study, our approach of IC injection combined with IV infusion of UMSC01 in STEMI patients with impaired LVEF appears to be safe, feasible, and potentially beneficial in improving cardiac systolic function and heart failure symptoms up to 12 months after treatment. As this is the first trial of dual-route transplantation of stem cells in humans, larger randomized and placebo-controlled phase II studies are required to demonstrate the efficacy of this novel approach.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethics Committee of China Medical University Hospital (CMUH107-REC1-088). The trial was monitored by an independent data and safety monitoring board who met as planned to assess adverse events. The patients/participants provided their written informed consent to participate in this study.

Author contributions

L-CH, Y-NL, and W-CS: conception and design, provision of study material or patients, data analysis and interpretation, manuscript writing, and final approval of manuscript. MH, C-RL, S-SC, Y-CW, J-YC, S-YL, K-YL, and Y-KL: provision of study material or patients, collection of data, data analysis and interpretation, and final approval of manuscript. M-YW, W-YT, M-YS, C-TH, C-KT, L-TC, C-LC, C-LL, and K-CH: data analysis and interpretation, final approval of manuscript. D-YC and C-HT: financial support and administrative support. K-CC and L-BJ: financial support, conception and design, provision of study material or patients, data analysis and interpretation, manuscript writing, and final approval of manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Ever Supreme Bio Technology Co., Ltd. and Ministry of Economic Affairs ROC A + program. This study was supported in part by Taiwan Ministry of Health and Welfare Clinical Trial Center (MOHW110-TDU-B-212-124004) and China Medical University Hospital (DMR-111-105). The funders had no role in the study design, data collection and

analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

C-HT was the founder of Ever Supreme Bio Technology. W-CS, D-YC, K-CC, and L-BJ were stockholders of the Ever Supreme Bio Technology. W-CS was employed by Ever Supreme Bio Technology and China Medical University Hospital. C-TH, C-KT, L-TC, and C-LC were employed by Ever Supreme Bio Technology.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.961920/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 23 August 2022
DOI 10.3389/fcvm.2022.990016

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 09 July 2022
ACCEPTED 02 August 2022
PUBLISHED 23 August 2022

CITATION
Gu Y, Li Y, Zhu Y, Lin X, Tian T, Zhang Q, Gong J, Wang L and Li J (2022) Cardiac resynchronization therapy in heart failure patients by using left bundle branch pacing. *Front. Cardiovasc. Med.* 9:990016. doi: 10.3389/fcvm.2022.990016

Cardiac resynchronization therapy in heart failure patients by using left bundle branch pacing

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Background: Left bundle branch pacing (LBBP) is emerging as an effective alternative to achieve cardiac resynchronization therapy (CRT) and improve heart function. The purpose of our study was to investigate the feasibility and efficacy of LBBP in heart failure patients with left ventricular ejection fraction (LVEF) <50% and left bundle branch block (LBBB).

Methods: All patients with complete LBBB and LVEF <50% were retrospectively included in the study from April 2018 to April 2021 and underwent CRT *via* LBBP implantation. ECG, pacing parameters, the New York Heart Association (NYHA) functional class, echocardiographic measurements, and complications were recorded and analyzed at implant and during follow-up of 1, 6, and 12 months.

Results: Left bundle branch pacing was successful in all 34 patients (mean age 65.6 ± 11.2 years, 67.6% men). A significant decrease in QRS duration (QRSd) was observed after the LBBP operation for 1 month (153.2 ± 1.7 vs. 111.9 ± 2.6 ms, $p < 0.01$). LBB capture threshold and R-wave amplitude remained stable at 12-month follow-up when compared with implantation values (0.62 ± 0.13 V @ 0.4 ms vs. 0.73 ± 0.21 V @ 0.4 ms, 12.02 ± 5.68 mV vs. 8.58 ± 4.09 mV, respectively). LVEF increased significantly (35.28 ± 1.70% vs. 51.09 ± 1.71%, $p < 0.01$) accompanied with reduced left ventricular end-diastolic dimension (LVEDd; 65.3 ± 1.99 vs. 53.58 ± 2.07 mm, $p < 0.01$) and left atrial dimension (LAD; 49.03 ± 1.32 vs. 40.67 ± 1.58 mm, $p < 0.01$). Normalized LVEF (LVEF ≥ 50%) was found in 70.5% of patients at 12 months. The NYHA classification, brain natriuretic peptide (BNP), and 6-minute walk test (6MWT) were significantly improved at follow-up of 12 months (all $p < 0.01$ vs. baseline). No deaths or heart failure hospitalizations were observed during the follow-up period.

Conclusion: The current work suggested that LBBP was feasible with a high success implantation rate and effective to correct LBBB and improved left ventricular structure and function with a low and stable pacing threshold.

KEYWORDS

left bundle branch pacing, cardiac resynchronization therapy, left bundle branch block, heart failure, pacing threshold

Introduction

Cardiac resynchronization therapy (CRT) by biventricular pacing (BVP) was widely used to provide clinical benefits in heart failure patients with decreased left ventricular ejection fraction (LVEF) and left bundle branch block (LBBB) (1–3). Though ventricular dyssynchrony and heart failure symptoms could be improved, approximately one-third of the patients had no response to traditional CRT. Compared with BVP, His Bundle Pacing (HBP) might correct LBBB and achieve better ventricular resynchronization and heart functional improvements (4, 5). However, HBP was found to require higher LBBB correction capture thresholds, lower R wave amplitudes, and smaller implant success rates, which limited the widespread application of the HBP technique (6, 7).

As an innovative technique, left bundle branch pacing (LBBP) has emerged to be an alternative method by pacing the left bundle branch bypassing the block region, resulting in physiological pacing and achieving electrical synchrony of the left ventricle. The first case of successful cardiac resynchronization by LBBP was conducted by Huang et al. (8). Increasing evidence showed that LBBP could develop relatively narrow QRS duration (QRSd), fast peak left ventricular activation time (LVAT), and LBBB correction with a low and stable pacing output (9, 10). While the clinical benefits and adverse effects had been described in several case reports and works, the clinical outcome of our center had not been reported.

The aim of the present study was to identify the clinical efficacy and safety of LBBP in heart failure with LBBB.

Methods

Study population

This was a retrospective, non-randomized, and single-center study performed between April 2018 and April 2021. Patients who met the following criteria were included (1) ECG with complete LBBB according to Strauss criteria (11); (2) LVEF $<$50% with heart failure symptoms; and (3) life expectancy $>$1 year. Patients with an age $\leq$18 years and pregnancy were excluded. The study was performed in accordance with the principles established in the Declaration of Helsinki and approved by the Ethics Committee of Jinling Hospital, Nanjing University School of Medicine. All subjects provided written informed consent.

Implant procedure

The conduction of LBBP was performed according to the previous reports (12, 13). First, the His bundle was marked using the Select Secure Lead (model 3830, Medtronic, Minneapolis, MN) through the C315 His delivery sheath (Medtronic, Minneapolis, MN). Subsequently, the lead was directed toward the ventricular side 1–2 cm along the line from His site to the right ventricular (RV) apex at right anterior oblique (RAO) 30° and then deeply screwed into the inter-ventricular septum. Once a right bundle branch block (RBBB) morphology was achieved with paced QRSd in lead V1, further lead advancement was stopped. Sti-LVAT was recorded as the interval from the pacing stimulus to the peak of the R wave in leads V4–V6 at high and low outputs (14). Finally, the depth of the lead in the septum was determined by contrast injection through the sheath at the left anterior oblique (LAO) 30°.

Data collection and follow-up

All enrolled patients were followed in our center at 1-, 6-, and 12 months post-operation. Baseline demographics and medical history were documented at enrollment. Bipolar R-wave amplitude, unipolar LBB capture threshold, and unipolar pacing impedance were collected at implant and follow-up visits. Electrocardiographic and echocardiographic parameters were recorded, such as QRSd, LVEF, left ventricular end-diastolic dimension (LVEDd), left atrial dimension (LAD), degree of mitral regurgitation (MR), and tricuspid regurgitation (TR, mild as first degree, moderate as second degree, and severe as third degree). The measurement of QRSd was achieved from the onset of the intrinsicoid R wave noted in lead V1 or V2 (15). Brain natriuretic peptide (BNP) levels, the New York Heart Association (NYHA) functional class, and the 6-min walk test (6MWT) were measured and compared at baseline and follow-up. Procedure-related complications, data regarding the significant increase of pacing threshold, lead dislodgement and perforation, infections, embolism, stroke, heart failure rehospitalizations, and death were collected during operation and post-operation visits.

Statistical analysis

Statistical analysis was performed by SPSS version 22.0 software. Shapiro-Wilk test was used to evaluate the normality of the quantitative data. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range). The difference between 2 groups was analyzed by paired Student's t-test, whereas the difference between multiple groups was analyzed by one-way ANOVA or Kruskal–Wallis H test. Categorical variables were presented as percentages. A p-value < 0.05 was considered to indicate a statistical significance.

TABLE 1 Baseline characteristics.

	LBBP ($n = 34$)
Age (years)	65.6 ± 11.2
Male, *n* (%)	23 (67.6)
Hypertension, *n* (%)	21 (61.8)
Diabetes, *n* (%)	9 (26.5)
AF, *n* (%)	6 (17.6)
AV block, *n* (%)	2 (5.9)
ICM, *n* (%)	7 (20.6)
Intrinsic QRS duration (ms)	153.2 ± 6.7
Hb (g/L)	132.6 ± 20.5
Cr (umol/L)	83.2 (61.2, 113.9)
BNP (pmol/L)	385.8 (170.1, 896.6)
Baseline LVEF (%)	35.3 ± 9.9
NYHA functional class	
II, *n* (%)	4 (11.8)
III, *n* (%)	18 (52.9)
IV, *n* (%)	12 (35.3)
Medicine history	
Beta-blockers, *n* (%)	27 (79.4%)
ACE inhibitors/ARB, *n* (%)	21 (61.8%)
Diuretics, *n* (%)	31 (91.2%)

Results

Baseline characteristics of heart failure patients

A total of 34 patients was included in the study, who had symptomatic heart failure with decreased LVEF (35.3 ± 9.9%) and LBBB with wide QRSd (153.2 ± 1.7 ms). The average age was 65.6 ± 11.2 years old, and 23 of these patients (67.6%) were men. Six patients had paroxysmal atrial fibrillation (AF), two patients had a first-degree atrioventricular block (AVB), and 7 patients had ischemic cardiomyopathy (ICM). The baseline characteristics of the subjects are shown in Table 1.

ECG and pacing parameters in patients with LBBP implantation

All 34 patients successfully underwent CRT using LBBP (Figures 1A,B). An ECG showed that QRSd significantly decreased upon pacing the left bundle branch (Figures 1C,D). As shown in Table 2 and Supplementary Figure S1A, QRSd narrows dramatically from 153.2 ± 1.7 ms at baseline to 111.9 ± 2.6 ms during 1-month of follow-up and then stays stably narrow at 6 months (107.8 ± 2.4 ms) and 12 months (104.7 ± 3.4 ms, all $p < 0.01$). The sti-LVAT remained the same at both low and high outputs when LBB was captured (Figures 1E,F). The average sti-LVAT was 80.4 ± 3.1 ms after LBBP perforation. The mean unipolar LBB capture threshold was 0.73 ± 0.21 V @ 0.4 ms (@ = at) upon the time of implantation and decreased and remained stable at 1 month (0.61 ± 0.32 V @ 0.4 ms), 6 months (0.56 ± 0.12 V @ 0.4 ms), and 12 months (0.62 ± 0.13 V @ 0.4 ms). The R-wave amplitudes were 8.58 ± 4.09, 8.93 ± 3.62, 11.40 ± 3.50, and 12.02 ± 5.68 mV at implantation, 1, 6, and 12 months. Unipolar pacing impedance was decreased rapidly over the first-month post-implantation and thereafter remained steady during follow-up (Table 2).

NYHA functional class and echocardiographic parameters

As shown in Figure 2A, compared with baseline BNP, patients with LBBP show no significant change in BNP at 1-month follow-up, whereas they have a significantly lower BNP at 6- and 12-month follow-up. Consistent with BNP, clinical heart function concerning NYHA and 6MWT was demonstrated to be improved during a follow-up period of 6 and 12 months (Figures 2B,C). LVEF was improved from a mean value 35.28 ± 1.70% at baseline to 50.26 ± 1.51% on follow-up of 6 months ($p < 0.01$) and increased to 51.09 ± 1.71% at 12 months ($p < 0.01$) after LBBP implantation (Figure 2D). An LVEF improvement >5% from baseline was defined as an LBBP response, and LVEF ≥ 50% was considered as a super-response. As shown in Figure 2E, 55.8 and 70.5% of patients have normalized LVEF (LVEF ≥ 50%) at 6 months and 12 months (Figure 2E). As shown in Supplementary Figure S1B, the mean change of LVEF in the general population is 2.44 ± 1.96% at 1-month follow-up, 11.47 ± 5.03% at 6-month follow-up, and 11.89 ± 5.05% at 12-month follow-up (both $p < 0.01$ with 1-month follow-up). For super-responders, the average change of LVEF was 2.44 ± 1.89% at 1-month follow-up, 11.44 ± 3.37% at 6-month follow-up, and 13.89 ± 2.18% at 12-month follow-up (both $p < 0.01$ with 1-month follow-up). The means of LVEDd and LAD were significantly lower at 6 months (65.3 ± 1.99 vs. 55.57 ± 1.81 mm and 49.03 ± 1.32 vs. 41.14 ± 2.98 mm, both $p < 0.05$) and 12 months (65.3 ± 1.99 vs. 53.58 ± 2.07 mm and 49.03 ± 1.32 vs. 40.67 ± 1.58 mm, both $p < 0.01$) of follow-up, whereas there was no non-significant reduction in LVEDd and LAD after 1-month LBBP implantation (Figures 2F,G). In addition, MR and TR were shown to be ameliorated at 6 and 12 months follow-up (Figure 2H; Supplementary Figure S1C).

Complications of LBBP

There were no major acute adverse effects during device implantation. No lead displacements and LV perforations were documented. An increase in LBBP capture threshold >1.0 V @

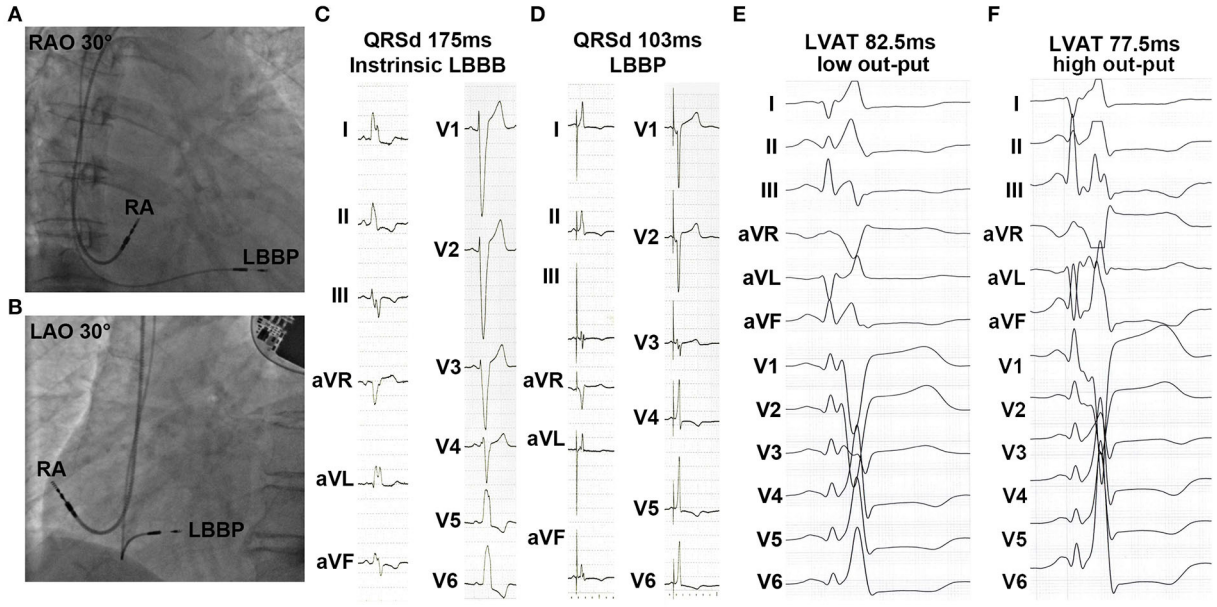

FIGURE 1
Characteristics of left bundle branch pacing (LBBP) during implantation. **(A,B)** Right anterior oblique (RAO) and left anterior oblique (LAO) fluoroscopic images showed the sites of LBBP pacing lead. **(C,D)** Baseline QRS duration (QRSd) and paced QRSd upon pacing left bundle branch. **(E,F)** Sti-left ventricular activation time (LVAT) at low and high outputs.

TABLE 2 Electrophysiological and pacing parameters.

	At implant	At 1 months	At 6 months	At 12 months
QRSd (ms)	153.2 ± 1.7	111.9 ± 2.6	107.8 ± 2.4	104.7 ± 3.4
Pacing threshold (V @ 0.4 ms)	0.73 ± 0.21	0.61 ± 0.32	0.56 ± 0.12	0.62 ± 0.13
R-wave amplitude (mV)	8.58 ± 4.09	8.93 ± 3.62	11.40 ± 3.50	12.02 ± 5.68
Impedance (Ω)	612.90 ± 156.74	458.00 ± 110.59	400.00 ± 46.34	475.25 ± 77.15

0.4 ms was not observed in any of the patients. None of the patients presented infection, LV thrombosis, and stroke during the follow-up period. During the follow-up of 12 months, no deaths or heart failure hospitalizations were observed.

Discussion

In this retrospective, single-center, and observational study, we explored the feasibility, effectivity, and safety of a novel pacing technique in heart failure patients with cardiac resynchronization indications by using LBBP. The major findings of the current work are as follows: (1) the success rate of LBBP was high in patients with heart failure and LBBB. (2) The QRSd was significantly reduced after LBBP implantation and kept narrow during the follow-up period. (3) Obvious improvements in clinical heart function and echocardiographic response were found in CRT implantation *via* LBBP. (4) LBBP showed low and stable pacing thresholds with long-term follow-up.

A previous study showed that CRT *via* BVP was a traditional strategy to ameliorate prognosis and decrease mortality of chronic patients with heart failure (16). However, the anatomy of the coronary sinus differs from an individual, which contributed to difficulties in placing LV lead into the optimal vein branches. Owing to the anatomical features of the left bundle branch that has fasciculus widely under the endocardium of the left side of the interventricular septum, pacing the left bundle branch is easy by screwing the pacing lead helix through the interventricular septum to the left ventricular subendocardium. LBBP operation was successfully achieved in all 34 heart failure cases, which revealed that LBBP had a high implant success rate and was feasible at implant.

Cardiac resynchronization therapy is recommended for symptomatic patients with heart failure in sinus rhythm with a QRSd ≥150 ms and LBBB QRS morphology and with LVEF ≤35% despite optimal medical treatment in order to

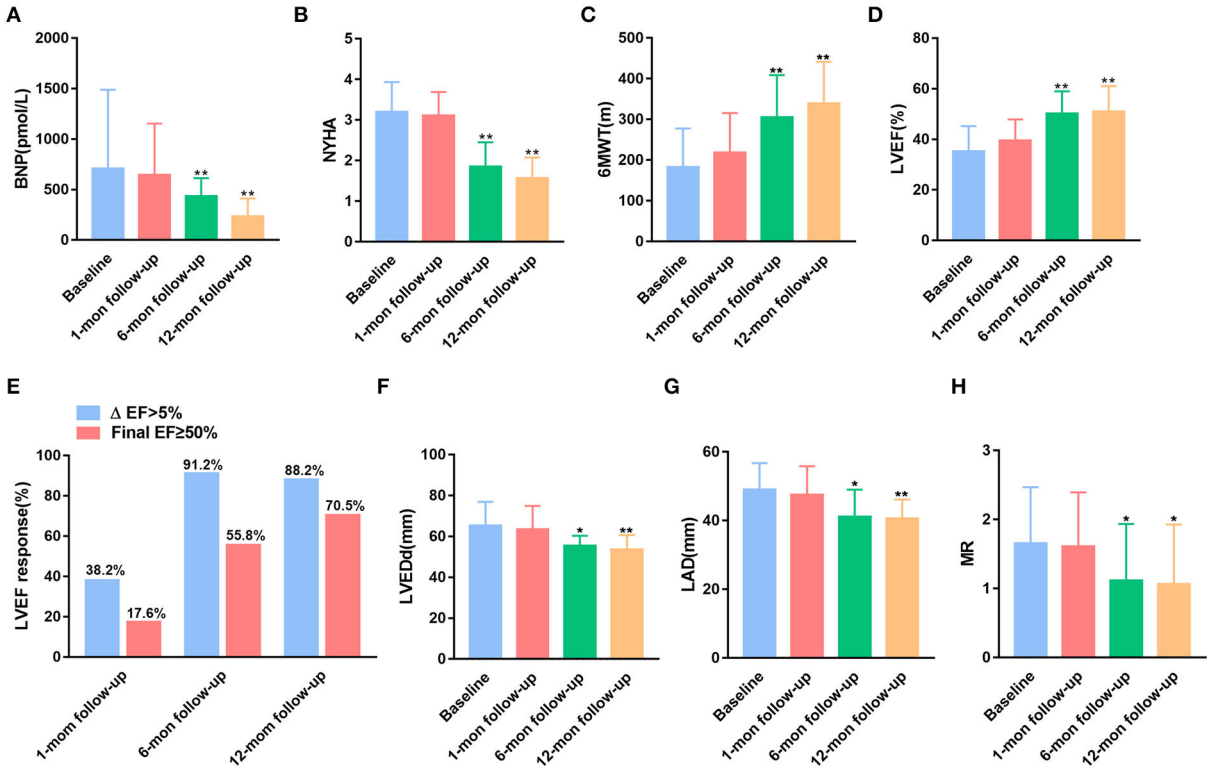

FIGURE 2
Comparisons of the New York Heart Association (NYHA) functional class and echocardiographic parameters at baseline and during the follow-up period. **(A)** Brain natriuretic peptide (BNP). **(B)** The NYHA classification. **(C)** 6-Minute walk test (6MWT). **(D)** Left ventricular ejection fraction (LVEF). **(E)** LVEF response rate. **(F)** Left ventricular end-diastolic dimension (LVEDd). **(G)** Left atrial dimension (LAD). **(H)** Mitral regurgitation (MR). *$p < 0.05$, and **$p < 0.01$ with baseline.

improve symptoms and reduce morbidity and mortality (3). Compared with BVP and HBP, LBBP was demonstrated to be easier to operate and improve LVEF with a low and stable threshold. Our data showed that LBBP pacing thresholds were 0.73 ± 0.21 V @ 0.4 ms at implant and then slightly decreased and remained stable and low (under 1 V @ 0.4 ms) during follow-up. Meanwhile, the mean QRSd had shortened by ~42 ms with LBBP operation for 1 month and was kept narrow in 12 months of follow-up. It has been reported that a narrower QRSd could lead to better mechanical synchronization of the ventricle (17). Pacing distal to the site of LBBB could correct LBBB and restore normal physiological left ventricular activation, which resulted in QRSd reduction (18). Thus, our study suggested that effective electrical and mechanical resynchronization was obtained from CRT through LBBP.

After 1 month of operation, nearly 38.2% of patients had a 5% increase of LVEF from baseline, and only 17.6% of patients had normalized LVEF. With the extension of follow-up, a significant increase of LVEF was observed in these heart failure patients with LBBB requiring CRT by LBBP. Super-response to LBBP implantation was achieved in 55.8 and 70.5% of patients after 6- and 12-month follow-up, respectively. The results were similar to the project conducted by Huang et al. (19), in which 75% of the non-ischemic population had normalized LVEF (≥50%) at 1 year by using LBBP. Apart from the high echocardiographic response, improved clinical manifestations were also achieved during long-term follow-up that included modified NYHA functional class, decreased BNP, and increased 6MWT. These results suggested that cardiac systolic function was improved during long-range follow-up visits of LBBP implantation.

Though LBBP was demonstrated to deliver effective cardiac resynchronization by correcting LBBB, pacing the left bundle branch did not allow normal physiological activation of both ventricles due to delayed LV lateral wall activation. However, we found that QRSd was decreased and maintained normal after LBBP implantation, and ventricular function did not deteriorate during follow-up, the results of our study might lie in heart failure patients with typical LBBB meeting Strauss criteria and the majority had dilated cardiomyopathy and were included in the present work. In addition, no adverse operative-related complications occurred in the process of implantation and follow-up, which revealed that LBBP was a safe approach to physiological pacing.

In conclusion, we identified that LBBP is a rational method of physiological pacing in heart failure patients with LBBB, as it leads to improvements in ventricular structure and function. Low and stable capture thresholds are associated with LBBP. However, the present work was conducted in a single-center with a small cohort. Large-scale, long-term, and randomized controlled clinical trials remain to be done to further estimate the clinical advantages and safety of LBBP in comparison with BVP and HBP in CRT candidates.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Jinling Hospital Affiliated to Nanjing University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL and LW conceived and designed the experiment. YG, YL, and YZ analyze the data. XL and TT performed the statistical analysis. JL and YG wrote the manuscript. JL, QZ, and JG revised the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors thank all patients who participated in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.990016/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Comparisons of QRS duration (QRSd) and TR at implantation and during follow-up. **(A)** The intrinsic and paced QRSd during left bundle branch pacing (LBBP). **(B)** The change of left ventricular ejection fraction (LVEF) in the general population and super-responders. **(C)** Mitral regurgitation (MR) at baseline and follow-up. $^{*}p < 0.05$, and $^{**}p < 0.01$ with baseline.

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 24 August 2022
DOI 10.3389/fcvm.2022.958895

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 01 June 2022
ACCEPTED 01 August 2022
PUBLISHED 24 August 2022

CITATION
Wang Y, Xiao Y, Tang J, Liu Y, Li H, Peng Z, Xu D and Shen L (2022) Effects of early phase 1 cardiac rehabilitation on cardiac function evaluated by impedance cardiography in patients with coronary heart disease and acute heart failure.
Front. Cardiovasc. Med. 9:958895.
doi: 10.3389/fcvm.2022.958895

Effects of early phase 1 cardiac rehabilitation on cardiac function evaluated by impedance cardiography in patients with coronary heart disease and acute heart failure

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Purpose: The purpose of the study was to access the impact of phase 1 cardiac rehabilitation (CR) on cardiac function and hemodynamic changes in patients with coronary heart disease (CHD) and acute heart failure (AHF).

Materials and methods: A total of 98 patients with CHD and AHF were recruited and randomized into two groups. Control group received standard pharmacotherapy and CR group received standard pharmacotherapy combined phase 1 CR. NT-proBNP and hemodynamic parameters measured by impedance cardiography (ICG) were estimated at baseline and at the end of treatment period.

Results: Phase 1 CR combined routine medical treatment could lower NT-proBNP levels. The percentage of high-risk patients was significantly decreased in CR group, although the post-treatment NT-proBNP level between control group and CR group showed no significant differences. Similarly, most hemodynamic parameters improved in the CR group, but not in the control group, suggesting that phase 1 CR in combination with the standard pharmacotherapy improved hemodynamic characteristics by elevating cardiac output, ameliorating preload, improving systolic and diastolic function, and relieving afterload, although the post-treatment hemodynamic parameters showed no statistically significant differences between the control group and the CR group.

Conclusion: Phase 1 CR combined routine medication can improve cardiac function and hemodynamic characteristics in patients with CHD and AHF. Thus, recommendation of phase 1 CR to stable patients is necessary.

KEYWORDS

phase 1 cardiac rehabilitation, impedance cardiography, acute heart failure, cardiac function, hemodynamics

Introduction

Coronary heart disease (CHD) is one of the most common causes of heart failure (HF). Despite the improvement in long-term prognosis of patients with HF due to the development of pharmacotherapy, intervention, implantable cardioverter defibrillator, and cardiac resynchronization therapy, the mortality and re-admission rate of patients with HF, however, remain high. Thus, improving prognosis and outcomes of patients with HF is of high priority.

Cardiac rehabilitation (CR), an accessible and economical therapy, has attracted progressive attention in the recent years. European Society Of Cardiology (ESC) and American Heart Association/American College of Cardiology (AHA/ACC) have recognized CR as a class I recommendation for patients with HF (1). CR includes three phases: inpatient CR (phase 1 CR), early stage of outpatient CR (phase 2 CR), and long-term community-based CR (phase 3 CR). Phase 1 CR (or inpatient CR) provides hospitalized patients with cardiac rehabilitation and preventive measures including exercise training, patient education, and behavior interventions. Several guidelines and expert consensus recommend phase 1 CR to hemodynamical stable patients with acute heart failure (AHF) and patients with HF recurrence (2–5).

It has been demonstrated that phase 1 CR contributed to alleviate symptoms (6), improve functional capacity and activity of daily living (7–9), shorten hospital stay length (10, 11), and reduce re-admission rate (11, 12) and all-cause mortality (11, 13). Specifically, patients receiving phase 1 CR had a 26% increase in 6-min walk test (6MWT) compared with controls (14), suggesting an enhancement in cardiopulmonary function and exercise capacity. Consistently, early movement within 48 h improved oxygen uptake efficiency slope in patients with acute myocardial infarction (AMI) (15). Besides, early movement training also ameliorated the inflammatory level in patients with AMI (16).

However, the prevalence of phase 1 CR remains low. A cross-sectional investigation including 454 hospitals revealed that only 24% hospitals provide phase 1 CR program (17). Meanwhile, the awareness of phase 1 CR in patients remains relatively insufficient (18). Therefore, there is a need to promote phase 1 CR.

The continuous monitoring and evaluation of patients with HF in phase 1 CR is critical, but an accurate and efficient method lacks. Impedance cardiography (ICG), a non-invasive approach of constant monitoring instantaneous changes in thoracic electrical impedance based on the Ohm's law, can provide reliable hemodynamic values and has been used to estimate cardiac function in patients with HF (19). Previous studies have confirmed the accuracy of ICG by comparing it to echocardiography (20). Meanwhile, as the change of hemodynamics status happens prior to occurrence of symptoms, the feature that ICG can capture small hemodynamic changes and thus can identify asymptomatic abnormalities makes ICG a more sensitive method than echocardiography. Most importantly, the intensity and duration of phase 1 CR can be adjusted promptly based on patients' condition reflected by ICG-measured hemodynamic changes. Furthermore, the prospective evaluation and identification of cardiac decompensation by ICG test (PREDICT) study finds that the combination of parameters measured by ICG can predict the short-term mortality and re-admission rate of patients with HF (21). Thus, ICG can be applicated as a useful approach in evaluating the effectiveness of phase 1 CR.

However, the effects of phase 1 CR on hemodynamic changes in patients with AHF remain unknown. In this study, we attempt to explore the impact of phase 1 CR on cardiac function and hemodynamics in patients with CHD and AHF through the pre- and post-treatment hemodynamic changes detected by ICG.

Materials and methods

Study sample

This study was approved by the Clinical Research Ethics Committee, the Second Xiangya Hospital of Central South University, China. All participants provided informed consent.

This study was a randomized controlled trial. A total of 106 patients with CHD and AHF who were admitted for treatment in cardiac care units from 2019 to 2020 were recruited and randomly assigned to one of two treatment groups, the control group or the CR group.

Randomization and allocation sequence was based on a block size fixed to 2 and generated through a computerized random number generator by a staff not involved in the trial. The patients in control group were treated with ordinary standard medical treatment. The patients in CR group received medical treatment plus 1-week phase 1 CR program during hospitalization.

The inclusion criteria were as follows: (1) age $>$ 18 years; (2) the percutaneous transluminal coronary intervention revealed $>$ 75% narrowing of the proximal anterior descending artery or three main coronary arteries; (3) the echocardiography showed enlarged heart with the diagnosis meets the criteria of left ventricular end diastolic diameter (LVEDd) $>$ 5 cm; (4) the left ventricular ejection faction (LVEF) between 30 and 50%; (5) apparent clinical signs and symptoms of AHF appeared; and (6) laboratory test showed elevated NT-proBNP level.

The exclusion criteria were as follows: (1) any life-threatening comorbidities, (2) patients with unstable hemodynamics; (3) acute phase of pulmonary diseases, including asthma attacks, pulmonary embolism, pneumothorax, and impaired cognition, (4) severe infections, such as infectious

endocarditis and septicemia; (5) uncontrolled arrhythmia; (6) severe valvular disease; (7) trauma or surgical history in the past 6 months; (8) aortic dissection; (9) cancer; (10) cognitive limitation; and (11) refuse to provide consent. Patients with the main diagnosis other than CHD and AHF were also excluded.

Phase 1 cardiac rehabilitation program

Phase 1 CR was performed under the instruction and observation of experienced physicians and adjusted according to the patients' conditions. The specific procedure was followed by the fourth edition of guidelines for CR and secondary prevention program (22).

Phase 1 CR began when patients fitted those following conditions: (1) no chest pain in the past 8 h; (2) no evident symptoms or signs of decompensated heart failures; (3) no new onset arrhythmia nor changes on electrocardiograph (ECG); and (4) no elevation of NT-proBNP.

The phase 1 CR program lasted for 7 days. On each day, the duration is 30 min, including 10-min warm-up activity, 10-min aerobic exercise, and 10-min Meridians patting or flexibility training according to the patients' conditions.

The evaluation of the phase 1 CR program is the combination of targeted heart rate, which is to raise heart rate to 20 ± 5 beat per minute above the rest heart rate, and Borg scale, a rating of perceived exertion scale.

The criteria of termination of phase 1 CR were as follows: (1) chest pain, palpitations, dyspnea, sweating, and other obvious discomfort symptoms; (2) ECG showed frequent ventricular tachycardia, atrial tachycardia, atrial fibrillation, and other malignant arrhythmias; (3) systolic blood pressure did not increase but decreased by 10 mmHg or more, or systolic blood pressure elevated by 180 mmHg; and (4) the patient requested to stop.

Data collection

The echocardiography was performed by experienced physician within 24 h of admission.

The hemodynamic parameters were detected by experienced staff using ICG (CSM3000, the Cheer Sails Medical). ICG was performed after 5 min of rest in the supine position. The electrodes were placed following the instructions. The hemodynamic variables included cardiac output (CO), cardiac index (CI), stroke volume (SV), stroke index (SI), thoracic fluid content (TFC), pre-ejection period (PEP), left ventricular ejection time (LVET), systolic time ratio (STR), systemic vascular resistance (SVR), stroke systemic vascular resistance (SSVR), and stroke systemic vascular resistance index (SSVRI). The venous blood was obtained for detection of NT-proBNP followed by ICG measurements.

Statistics

The data were analyzed by SPSS version 20.0. Measurement data followed normal distribution were expressed as mean ± standard deviation, whereas non-normal distributional data were expressed as median (interquartile range). Enumeration data were expressed as proportion and evaluated by Chi-square test. Paired *t*-test was used to compare the changes in normal distributional parameters, and Kruskal–Wallis test was used to compare the changes in non-normal distributional parameters. A repeated measures ANOVA was used to compare the pre- and post-treatment changes in control and CR group. Spearman's correlation analysis was used to evaluate the correlation between the parameters determined by ICG and blood NT-proBNP. A value of $p < 0.05$ was considered statistically significant.

Results

Basic characteristics

The study included 106 patients (average age 66.96 ± 2.76 years old, 72.4% male). The control group contained 53 patients, 52 of whom finished the treatment. The CR group contained 53 patients, 46 of whom were analyzed (**Figure 1**). The fundamental data are shown in **Table 1**. There were no differences in height, weight, body mass index (BMI), blood pressure and rest heart rate, companion diseases, and medical history between control and CR group before treatment. The echocardiography showed comparable in cardiac structure and cardiac function between two groups (**Table 2**).

T-proBNP levels in pre- and post-phase 1 cardiac rehabilitation

As shown in **Table 3**, there were no differences in NT-proBNP level between control and CR group before treatment.

In control group, the NT-proBNP level decreased from 1913.62 (926.33; 4,378.22) pg/ml to 1,439.61 (283.7275; 2,594.87) pg/ml after the treatment ($p < 0.05$). In CR group, the NT-proBNP level decreased from 2,643.00 (1,527.59; 4,360.00) pg/ml to 1,889.00 (704.85; 3,315.00) pg/ml after the treatment ($p < 0.05$).

After treatment, the NT-proBNP level in CR group was non-significantly lower than that of control group.

The level of NT-proBNP was found to correlated with the short-term prognosis of patients with AHF and that patients with NT-proBNP higher than 5,180 pg/ml had higher risk of sudden death (23). Thus, the subgroup analyses defined patients with NT-proBNP > 5,180 pg/ml as high NT-proBNP group, whereas NT-proBNP ≤ 5,180 pg/ml as low NT-proBNP

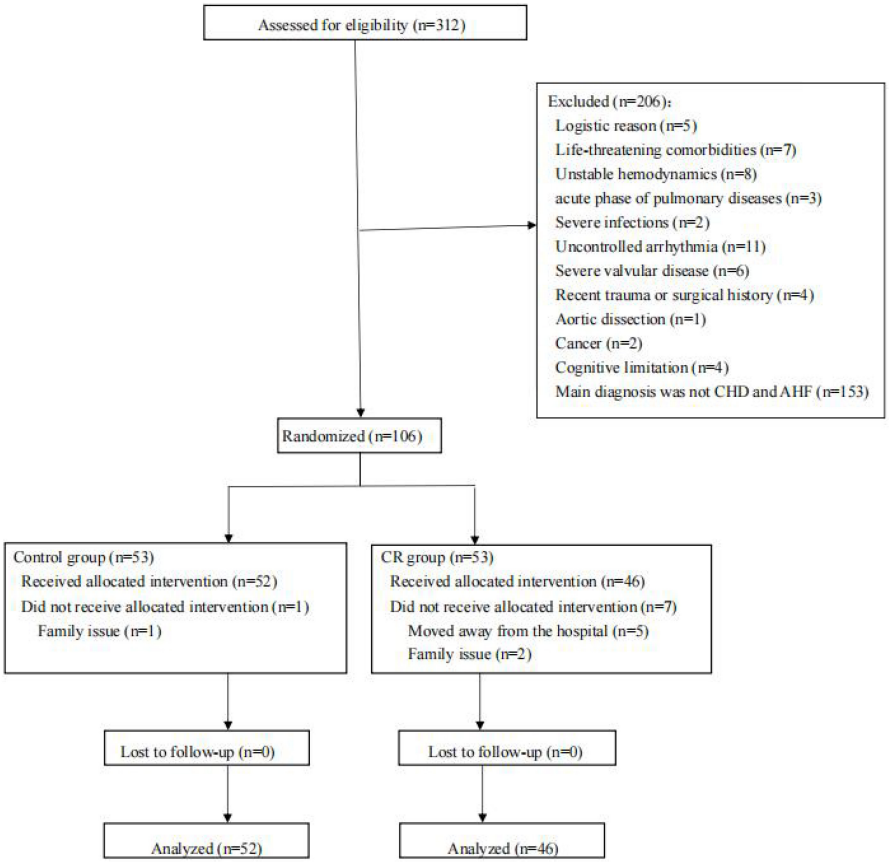

FIGURE 1
Flow diagram of patients.

group. Before the treatment, 22.4% of patients (control group 19.2%, CR group 26.1%) were divided into high NT-proBNP group, while after the treatment, 8.2% of patients (control group 13.5%, CR group 2.2%) have NT-proBNP higher than 5,180 pg/ml. In control group, the patients with high NT-proBNP group decreased by 5.7%, whereas in CR group, that percentage decreased by 23.4%. In addition, the number and the percentages of patients with NT-proBNP higher than 5,180 pg/ml in the CR group significantly decreased ($p < 0.05$), which suggested that phase 1 CR could further lower plasma NT-proBNP level and improve short-term prognosis of patients with CHD and AHF based on the routine medicine.

Impedance cardiography parameters comparison pre- and post-phase 1 cardiac rehabilitation

Cardiac output parameters

Table 4 showed the following cardiac output parameters: cardiac output (CO), cardiac output index (CI), stroke volume (SV), and stroke volume index (SI) of control group and CR group. As it showed, there were no differences between two groups before treatment. After the treatment, no significant changes were found between two groups.

In control group, the cardiac output parameters showed no changes before and after the treatment, whereas in CR group, CO, CI, and SV increased after the treatment ($p < 0.05$). It indicated that routine treatment had limitations in improving cardiac output in short term, while medical treatment combined phase 1 CR could improve cardiac output in patients with CHD and AHF.

Afterload parameter

As Table 5 showed, there were no differences in TFC between two groups before and after the treatment. After the treatment, the TFC of control group decreased from 0.034 (0.029; 0.036)/Ω to 0.030 (0.025; 0.035)/Ω ($p > 0.05$), at the same time, the TFC of CR group decreased from 0.035 (0.029;0.041)/Ω to 0.031 (0.029;0.035)/Ω ($p > 0.05$). However, the statistics showed no significant changes in TFC before and after treatment in both groups.

TABLE 1 Comparison of baseline clinical characteristics of patients.

Parameters (unit)	All patients (n = 98)	Control group (n = 52)	CR group (n = 46)	P-value
Age (years)	66.96 ± 2.76	65.26 ± 2.80	68.38 ± 2.73	0.990
Gender [male, n (%)]	71 (72.4%)	39 (75.0%)	32 (69.6%)	0.548
Height (cm)	164.22 ± 1.27	162.35 ± 1.43	165.77 ± 1.14	0.166
Weight (kg)	67.92 ± 2.64	65.30 ± 2.84	70.12 ± 2.48	0.703
BMI (kg/m^2)	24.20 ± 0.82	23.79 ± 0.89	24.55 ± 0.76	0.747
Rest HR (beat/minute)	68.29 ± 2.00	66.83 ± 2.38	69.50 ± 1.69	0.892
SBP (mmHg)	122.01 ± 3.11	120.78 ± 3.30	123.04 ± 2.96	0.340
DBP (mmHg)	69.97 ± 1.82	65.04 ± 2.01	74.08 ± 1.67	0.778
MAP (mmHg)	84.59 ± 1.76	81.04 ± 1.83	87.54 ± 1.70	0.710
Accompany disease, n (%)				
Hypertension	69 (70.4%)	36 (69.2%)	33 (71.7%)	0.827
Diabetes	72 (73.5%)	41 (78.9%)	31 (67.4%)	0.253
Medication, n (%)				
Furosemide	77 (78.6%)	42 (80.8%)	35 (76.1%)	0.573
Loop diuretics	80 (81.6%)	44 (84.6%)	36 (78.3%)	0.445
Aspirin	98 (100.0%)	52 (100.0%)	46 (100.0%)	1.000
Clopidogrel	98 (100.0%)	52 (100.0%)	46 (100.0%)	1.000
Statins	98 (100.0%)	52 (100.0%)	46 (100.0%)	1.000
Beta blocker	82 (83.7%)	44 (84.6%)	38 (82.6%)	0.789
ACEI/ARB/ARNI	88 (89.8%)	47 (90.4%)	41 (89.1%)	0.838
Digoxin	41 (41.8%)	21 (40.4%)	20 (43.5%)	0.757

Data are expressed as mean ± standard, or percentages. n, number; CR, cardiac rehabilitation; BMI, body mass index; rest HR, heart rate at rest; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor neprilysin inhibitors.

To further determine whether phase 1 CR has influence on TFC level, we divided patients into two subgroups: patients with TFC $\geq$ 0.035/Ω were defined as high-preload group, whereas patients with TFC $<$ 0.035/Ω were defined as low-preload group. Before the treatment, 50.0% of patients (50.0% in control group, 50.0% in CR group) were in high-preload subgroup. After the treatment, 29.6% of patients (30.8% in control group, 28.3% in CR group) had TFC higher than 0.035/Ω. The percentage of patients with high preload was decreased in both control group and CR group.

To further identify the effects of phase 1 CR under same preload situation, we compared TFC changes of high- or low-preload settings in control group and CR group. The results are shown in Table 6. In control group, TFC changes were not statistically significant in either high- or low-preload settings. In CR group, though no significant change was found in low-preload subgroup, the TFC level decreased from 0.0395 (0.0365; 0.0427)/Ω to 0.0315 (0.0290; 0.0350)/Ω ($p < 0.05$) in high-preload setting. Taken together, these findings indicated that phase 1 CR plus routine treatment decrease TFC in high-preload patients.

Contraction parameters

As Table 7 showed, there were no differences in PEP, LVET, and STR between two groups before and after the treatment.

Medical treatment decreased the PEP in control group from 101.00 (90.50; 117.00) to 97.00 (86.50; 113.50) ms ($p > 0.05$), whereas phase 1 CR plus medication shorter PEP from 114.00 (109.00; 133.00) to 102.00 (87.00;115.00) ms ($p < 0.05$).

Pre- and post-treatment LVET showed no differences in both control and CR groups.

The STR in control group was shorter from 0.40 (0.30; 0.40) to 0.30 (0.30; 0.40) ($p > 0.05$), whereas in CR group, the STR was changed from 0.50 (0.35; 0.50) to 0.40 (0.30; 0.50) ($p < 0.05$). The results suggested that routine treatment plus phase 1 CR

TABLE 2 Comparison of baseline parameters of echocardiography between control group and CR group.

Parameters (unit)	control group (n = 52)	CR group (n = 46)	P-value
LAD (mm)	34.09 ± 1.15	32.31 ± 0.84	0.142
LVED (mm)	59.87 ± 0.89	61.00 ± 1.27	0.580
IVST (mm)	9.91 ± 0.32	9.81 ± 0.14	0.062
LVPWT (mm)	9.57 ± 0.25	9.42 ± 0.16	0.254
LVEF (%)	35.73 ± 16	36.13 ± 10	0.745

Data are expressed as mean ± standard. LAD, left atrial diameter; LVED, left ventricular end-diastolic diameter; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction.

TABLE 3 Comparison of NT-proBNP of patients with CHD and AHF before and after the treatment.

Parameter (unit)	Control group (n = 52)			CR group (n = 46)			P^{*}	$P^{\#}$
	Pre-treatment	Post-treatment	P	Pre-treatment	Post-treatment	P		
NT-proBNP (pg/ml)	1913.62 (926.33; 4378.22)	1439.61 (283.7275; 2594.87)	0.004	2643.00 (1527.59; 4360.00)	1889.00 (704.85; 3315.00)	0.000	0.536	0.188

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; NT-proBNP, N-terminal pro brain natriuretic peptide.
p: values of comparison between changes of pre-treatment and post-treatment observed in CR group or control group.
$^{*}p$: values of comparison between baseline parameter in CR group versus those in control group.
$^{\#}p$: values of comparison between parameter after treatment observed in CR group versus those in control group.
$p < 0.05$ was considered statistically significant.

TABLE 4 Comparisons of parameters of cardiac output measured by impedance cardiography of patients with CHD and AHF before and after the treatment.

Parameters (unit)	Control group (n = 52)				CR group (n = 46)				P^{*}	$P^{\#}$
	Pre-treatment	Post-treatment	Changes	P	Pre-treatment	Post-treatment	Changes	P		
CO (L/min)	4.05 (3.72;4.87)	4.70 (3.70;5.10)	0.40 (−0.18;0.80)	0.204	3.90 (3.55;4.80)	4.70 (4.05;5.05)	0.20 (0.05;0.95)	0.005	0.775	0.801
CI (L/min/m$^{2)}$	2.65 (2.07;2.80)	2.75 (2.22;3.17)	0.20 (−0.75;0.58)	0.184	2.30 (2.05;2.65)	2.70 (2.35;2.85)	0.20 (0.00;0.55)	0.008	0303	0.688
SV (mL)	57.50 (47.00;69.50)	61.10 (50.25;79.00)	5.00 (−10.25;16.25)	0.414	54.00 (42.50;70.00)	68.00 (54.50;75.50)	5.00 (−2.00;18.50)	0.03	0.623	0.698
SI (mL/m^2)	36.50 (25.75;44.50)	36.00 (30.50;47.75)	2.50 (−5.75;10.00)	0.408	30.00 (25.75;36.50)	35.50 (29.50;43.00)	1.50 (−0.33,9.00)	0.057	0.214	0.473

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; CO, cardiac output; CI, cardiac index; SV, stroke volume; SI, stroke index.
p: values of comparison between changes of pre-treatment and post-treatment observed in CR group or control group.
$^{*}p$: values of comparison between baseline parameter in CR group versus those in control group.
$^{\#}p$: values of comparison between parameter after treatment observed in CR group versus those in control group.
$p < 0.05$ was considered statistically significant.

TABLE 5 Comparison of preload parameter measured by impedance cardiography of patients with CHD and AHF before and after the treatment.

Parameter (unit)	Control group (n = 52)				CR group (n = 46)				P^{*}	$P^{\#}$
	Pre-treatment	Post-treatment	Change	P	Pre-treatment	Post-treatment	Change	P		
TFC (1/Ω)	0.034 (0.029;0.036)	0.030 (0.025;0.035)	−0.002 (−0.005;0.001)	0.141	0.035 (0.029;0.041)	0.031 (0.029;0.035)	−0.001 (−0.008;0.004)	0.100	0.205	0.234

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; TFC, Thoracic Fluid Content.
p: values of comparison between changes of pre-treatment and post-treatment observed in CR group or control group.
$^{*}p$: values of comparison between baseline parameter in CR group versus those in control group.
$^{\#}p$: values of comparison between parameter after treatment observed in CR group versus those in control group.
$p < 0.05$ was considered statistically significant.

TABLE 6 Comparison of preload of patients with CHD and AHF before and after the treatment under same preload setting.

TFC (/Ω)	Control group (n = 52)		CR group (n = 46)	
	High-preload subgroup (TFC ≥ 0.035/Ω) (n = 26)	Low-preload subgroup (TFC < 0.035/Ω) (n = 26)	High-preload subgroup (TFC ≥ 0.035/Ω) (n = 20)	Low-preload subgroup (TFC < 0.035/Ω) (n = 26)
Pre-treatment	0.0360 (0.0350;0.0400)	0.0290 (0.0250;0.0310)	0.0395 (0.0365;0.0427)	0.0290 (0.0260;0.0310)
Post-treatment	0.0320 (0.0258;0.0408)	0.0280 (0.0245;0.0325)	0.0315 (0.0290;0.0350)	0.0305 (0.0290;0.0330)
P	0.161	0.720	0.010	0.155

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; TFC, Thoracic Fluid Content.
p-values of comparison between TFC changes of pre-treatment and post-treatment observed in high or low-preload settings in control group or CR group.
$p < 0.05$ was considered statistically significant.

shorter PEP and STR, thus improving constriction function of left ventricular.

Moreover, to identify the effects of phase 1 CR in improving left ventricular constriction, subgroup analysis was made. STR > 0.4 was defined as dysfunctional constriction. Before the treatment, 39.8% of patients (control group 21.2%, CR group 60.8%) were defined as dysfunctional constriction, while after the treatment, 16.3% of patients (control group 5.8%, CR

TABLE 7 Comparisons of cardiac function of contraction measured by impedance cardiography of patients with CHD and AHF before and after the treatment.

Parameters (unit)	Control group (n = 52)				CR group (n = 46)				P^*	$P^{\#}$
	Pre-treatment	Post-treatment	Changes	P	Pre-treatment	Post-treatment	Changes	P		
PEP (ms)	101.00 (90.50;117.00)	97.00 (86.50;113.50)	−4.00 (−20.00;11.50)	0.387	114.00 (109.00;133.00)	102.00 (87.00;115.00)	−12.00 (−23.00;-3.00)	0.001	0.057	0.473
LVET (ms)	270.00 (257.00;306.00)	280.00 (259.00;313.50)	7.00 (−8.50;40.25)	0.227	248.00 (242.00;289.00)	266.00 (242.00;296.00)	12.00 (−17.00;34,00)	0.140	0.139	0.247
STR (–)	0.40 (0.30;0.40)	0.30 (0.30;0.40)	0.00 (−0.10;0.00)	0.178	0.50 (0.35;0.50)	0.40 (0.30;0.50)	−0.10 (−0.10;0.00)	0.006	0.067	0.158

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; PEP, pre-ejection period; LVET, left ventricular ejection time; STR, systolic time ratio.
p: values of comparison between changes of pre-treatment and post-treatment observed in CR group or control group.
$^*p$: values of comparison between baseline parameter in CR group versus those in control group.
$^{\#}p$: values of comparison between parameter after treatment observed in CR group versus those in control group.
$p < 0.05$ was considered statistically significant.

TABLE 8 Comparison of afterload parameters measured by impedance cardiography of patients with CHD and AHF before and after the treatment.

Parameters (unit)	Control group (n = 52)				CR group (n = 46)				P^*	$P^{\#}$
	Pre-treatment	Post-treatment	Changes	P	Pre-treatment	Post-treatment	Changes	P		
SSVR (dynes/cm^5/beat)	244.05 (213.77;352.90)	236.30 (207.37;278.77)	−21.65 (−69.58;42.73)	0.313	300.70 (210.40;352.07)	251.45 (175.35;318.85)	−42.45 (−61.88; −6.60)	**0.016**	0.413	1.000
SSVRI (dynes/cm^5/m^2/beat)	155.00 (129.12;203.30)	141.35 (129.80;159.80)	−16.05 (−52.13;23.53)	0.156	174.40 (150.00;203.52)	141.60 (114.60;194.32)	−27.40 (−37.20; −4.65)	**0.010**	0.349	0.737
SVR (dynes/cm^5)	1290.80 (1138.60;1506.60)	1199.10 (994.87;1451.45)	−140.05 (−291.23;63.58)	0.126	1319.70 (1179.20;1510.80)	1151.80 (987.60;1285.00)	−71.50 (−334.55; 58.30)	**0.028**	0.873	0.584

Data are expressed as median (interquartile range). CR, cardiac rehabilitation; SSRV, stroke systemic vascular resistance; SSRVI, stroke systemic vascular resistance index; SVR, systemic vascular resistance.
p: values of comparison between changes of pre-treatment and post-treatment observed in CR group or control group.
$^*p$: values of comparison between baseline parameter in CR group versus those in control group.
$^{\#}p$: values of comparison between parameter after treatment observed in CR group versus those in control group.
$p < 0.05$ was considered statistically significant.

TABLE 9 Correlation between change of hemodynamic parameters measured by impedance cardiology and change of NT-proBNP in patients with CHD and AHF.

	All group R	P	Control group R	P	CR group R	P
ΔCO	−0.3	0.895	0.101	0.709	−0.344	0.117
ΔCI	0.087	0.605	0.117	0.667	0.035	0.878
ΔSV	−0.019	0.912	0.069	0.799	−0.212	0.344
ΔSI	0.022	0.904	0.074	0.786	−0.093	0.742
ΔTFC	−0.148	0.376	−0.404	0.121	0.074	0.744
ΔSSVR	−0.074	0.693	−0.114	0.674	0.076	0.789
ΔSSVRI	−0.078	0.765	−0.122	0.654	0.112	0.690
ΔSVR	−0.127	0.446	−0.223	0.407	0.125	0.579
ΔPEP	0.053	0.777	0.073	0.789	−0.129	0.646
ΔLVET	0.040	0.831	0.022	0.936	0.207	0.460
ΔSTR	0.092	0.622	0.036	0.896	0.260	0.349

Δ = post-treatment–pre-treatment.
$p < 0.05$ was considered statistically significant.

group 28.3%) had dysfunctional constriction. The standardized percentage of patients with constriction problem decreased 15.1 and 32.0%, respectively, in control group and CR group, which implied that phase 1 CR can improve constriction function of left ventricular.

Pressure load parameters

As Table 8 showed, there were no differences in SVR, SSVR, and SSVRI between two groups before and after the treatment.

In control group, the SVR, SSVR, and SSVRI showed no differences before and after the treatment. In CR group, the

SSVR decreased from 300.70 (210.40; 352.07) dynes/cm^5/beat to 251.45 (175.35; 318.85) dynes/cm^5/beat ($p < 0.05$), SSVRI decreased from 174.40 (150.00;203.52) dynes/cm^5/m^2/beat to 141.60 (114.60;194.32) dynes/cm^5/m^2/beat ($p < 0.05$), and SVR decreased from 1,319.70 (1,179.20; 1,510.80) dynes/cm^5 to 1,151.80 (987.60; 1,285.00) dynes/cm^5 ($p < 0.05$). The results above suggested that phase 1 CR plus routine treatment could decrease system resistance to lower pressure load.

The correlation between changes in impedance cardiography parameters and NT-proBNP

Spearman's correlation analysis was used to identify the correlation between the changes in ICG parameters and NT-proBNP (**Table 9**); however, no correlation was observed between these two items.

Discussion

Previous studies have demonstrated that hemodynamic parameters measured by ICG can reflect the hemodynamic characteristics of patients with HF. In this study, we found that both standard pharmacotherapy and phase 1 CR combined routine medical treatment could lower NT-proBNP levels in patients with CHD and AHF. However, the number and the percentages of high-risk patients in CR group significantly decreased after the treatment. Meanwhile, most hemodynamic parameters improved after the treatment in CR group, but not in control group, suggesting that phase 1 CR plus standard pharmacotherapy improved hemodynamic characteristics by elevating SV, CO, and CI, decreasing TFC in high-preload patients, shortening PEP and STR, and lowering SVR, SSVR, and SSVRI, although the post-treatment hemodynamic parameters showed no statistically significant differences between control group and CR group. Most importantly, the decrease of standardized percentage of patients with dysfunctional problem in CR group was higher than that in control group, indicating an improvement in systolic and diastolic function of left ventricular.

NT-proBNP and cardiac rehabilitation

Large amounts of studies have demonstrated that the level of NT-proBNP is a predictor of outcome of patients with HF (24, 25). Normally, the higher level of NT-proBNP, the higher of New York Heart Association (NYHA) functional classes, the worse of prognosis of patients with HF (26). Meanwhile, in this study, both routine treatment and routine treatment combined phase 1 CR could decrease the level of NT-proBNP effectively in patients with CDH and AHF. Subgroup analysis demonstrated that the number and the percentages of patients with higher level of NT-proBNP in the CR group significantly decreased ($p < 0.05$), suggesting that phase 1 CR plus medication can further lower the levels of NT-proBNP in patients with CHD and AHF, which implies that phase 1 CR may improve the outcome in patients with HF. Previous studies have found that phase 1 CR could improve the symptoms of patients with HF. A perspective study showed that phase 1 CR improved the percentage of patients in NYHA class I and class II from 19.6 and 35.2% in the admission to 24.8 and 54.1% in the dismission, respectively, and the percentage of patients with NYHA class III decreased from 44.2 to 19.6% (6). Similarly, Taya et al. found that the high-intense intermittent training decreased the serum level of BNP in patients with HF from 432 (812) pg/ml to 254 (400) pg/ml ($p < 0.001$) (27). Those results imply that early movement can improve the symptoms of patients with HF. Moreover, Motoki et al. demonstrated that phase 1 CR improved the daily activity function in patients with acute decompensated HF (7).

Impedance cardiography and cardiac rehabilitation

Cardiac function

Several reports have demonstrated the accuracy of SV and CO detected by ICG (28, 29). We found that patients in CR group had a significant improvement in SV, CO, and CI and a non-significant improvement in SI. However, no significant differences were observed after the treatment in all parameters above mentioned between control group and CR group. The improvement of post- and pre- treatment SV in the CR group, not in control group, implied that early movement may improve pump function of heart. Consistent with our finding, Chursina et al. reported that free-load bicycle exercise improved LVEF in patients with ischemic cardiomyopathy (30, 31).

Preload

Thoracic fluid content is used to reflect the preload or volume load in ICG. Previous studies have found that TFC is negatively correlated with pulmonary capillary wedge pressure (32). The patients with severer symptoms and higher NYHA levels had higher TFC level in AHF (33). Moreover, TFC in patients with HF had a significant positive correlation with re-admission rate and risk of death in 2 months (34). However, there is no research deciphering the effect of phase 1 CR on TFC. In this study, phase 1 CR plus routine medical treatment only showed a decrease tendency in TFC. Interestingly, in patients with TFC $\geq$ 0.035/Ω, phase 1 CR plus medical treatment significantly decreased TFC. However, such improvement was not discovered in patients with TFC < 0.035/Ω. Taken together, our findings suggested that phase 1 CR decreased preload of heart in patients with high preload. Consistently, Gielerak

et al. found that 8-week CR could decrease TFC, elevate the maximum oxygen uptake, and improve exercise tolerance in patients with HF (35). Moreover, the decrease in TFC had positive correlation with the improvement in 6-min walk test (36). All the results above suggest that CR can improve cardiac function by decreasing TFC level.

Contraction

Pre-ejection period refers to the time period of isovolumic contraction and LVET refers to time period of left ventricular isometric contraction. The decreased contraction would result in prolonging PEP and shortening LVET. STR is the ratio of PEP and LVET. Thus, STR can reflect the efficiency of ventricular contraction and left ventricular function.

In this study, the significant decrease of PEP and STR in CR group, not in control group, implied that phase 1 CR combined medical treatment improved left ventricular contraction in patients with CHD and AHF, although no significant differences have been found between control group and CR group. Emerging studies have demonstrated the correlation of STR and risk of death in patients with HF. Sadauskas et al. reported that STR $\geq$ 0.55 is an indicator of higher risk to death in 6 months in patients with recurrent HF (OR = 0.29) (37). Moreover, in PREDICT trial, a multicenter trial including 2,316 patients, the hemodynamic parameters including STR were identified as a predictor of short-term clinical events, such as HF recurrent decompensation (21). The findings above suggest that STR could be used to warn the risk of adverse cardiovascular events.

Furthermore, STR is related to short-term outcome of patients with HF (21). Thompson reported the negative correlation between STR and LVEF (r = –0.54; p < 0.001) (38). Vijayaraghavan et al. analyzed ICG parameters and quality of life in 64 patients with chronic HF and pointed out that shorter PEP was associated with the improvement in NYHA level (39), suggesting that PEP could be a reflection of symptoms in patients with HF.

In addition, STR can imply diastole function. In IMPEDDANS study, ICG was used to evaluate diastolic dysfunction in patients with arterial hypertension. Nazario Leao et al. found that PEP, LVET, and STR had good discriminative ability in discovering left ventricular diastole dysfunction. Amid them, the sensibility of STR was 99% and the specificity was 90%. The threshold of diastole dysfunction is PEP $\leq$ 104 ms, LVET $\geq$ 320 ms, and STR $\leq$ 0.31 (40). Although we did not focus on diastolic improvement, the results of our study implicated that phase 1 CR combined medical treatment could shorten PEP and STR, which suggests the improvement of phase 1 CR on diastole function in patients with CHD and AHF worthy further investigation.

Afterload

Systemic vascular resistance, SSVR, and SSVRI are the parameters that reflect pressure load in ICG. Cotter et al. found an elevation in SVRI in patients with AHF (41), which was due to the activation of neuroendocrine. The elevation of SVRI contributed to the persistence of blood pressure and perfusion of important organs under the circumstance of decreased contractility. Overload SVRI may cause elevated afterload, decreased CI, increased left ventricular end-diastolic pressure and pulmonary capillary wedge pressure, and eventually leading to pulmonary edema. While decreasing SVR properly can increase SV, which could improve pulmonary congestion, however, there is no research indicating the effects of phase 1 CR on cardiac afterload. In this research, the significant decrease of SVR, SSVR, and SSVRI in CR group, but not in control group, suggested that phase 1 CR combined medical treatment could reduce afterload, at least to some extent, in patients with CHD and AHF, although no significant differences of post-treatment SVR, SSVR, and SSVRI have been found between control group and CR group.

Correlation of BNP and impedance cardiography

Previous studies identified some correlations between BNP and ICG parameters. As Pomenta et al. reported, the TFC measured by ICG is an independent predictor of BNP in patients with AHF, despite the severe contraction dysfunction and NYHA levels (34). However, in this study, we did not determine the correlation between NT-proBNP and ICG parameters.

Long-term outcomes

Despite that 1-week period of phase 1 CR improved cardiac function and hemodynamic characteristics in patients with CHD and AHF, whether the short-term benefits remain in the long run still needs to be further studied. Of note, though emerging studies have suggested that long-term exercise can improve the outcomes in patients with HF, the participation of long-term exercise is relatively low. Whether patients completed phase 1 CR would choose to continue phase 2 and phase 3 CR depends on the willing of patients, the recommendation of physicians, and the convenience, that is, whether there are facilities near the their communities (5). Thus, facilities need to be built and physicians should take the responsibility to recommend patients to further exercise movements.

Limitations

This study has several limitations. Frist, the sample amount was relatively small due to the limitation of collection time and

one-center study. Second, this study did not compare the effects of phase 1 CR in population with HF with preserved EF, HF with might reversed EF, and HF with reserved EF. Therefore, more studies with larger population and multicenters were needed to confirm the effects of phase 1 CR on cardiac function and hemodynamics in patients with CHD and AHF.

Conclusion

In this study, we find that phase 1 CR plus routine medication can improve cardiac function and hemodynamic parameters in patients with CHD and AHF in short term. Thus, it is important to recommend phase 1 CR to patients once they are stable.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Clinical Research Ethics Committee, the Second Xiangya Hospital of Central South University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Funding

This work was supported by the National Nature Scientific Funding of China (no. 82072555), Chinese Cardiovascular Association- Access fund (2019-CCA-ACCESS-023), and the National Nature Scientific Funding of Hunan Province and Changsha (2021JJ30948 and 202203013201).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 25 August 2022
DOI 10.3389/fcvm.2022.966968

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 12 June 2022
ACCEPTED 01 August 2022
PUBLISHED 25 August 2022

CITATION
Valero-Muñoz M, Saw EL, Hekman RM, Blum BC, Hourani Z, Granzier H, Emili A and Sam F (2022) Proteomic and phosphoproteomic profiling in heart failure with preserved ejection fraction (HFpEF). *Front. Cardiovasc. Med.* 9:966968. doi: 10.3389/fcvm.2022.966968

Proteomic and phosphoproteomic profiling in heart failure with preserved ejection fraction (HFpEF)

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Although the prevalence of heart failure with preserved ejection fraction (HFpEF) is increasing, evidence-based therapies for HFpEF remain limited, likely due to an incomplete understanding of this disease. This study sought to identify the cardiac-specific features of protein and phosphoprotein changes in a murine model of HFpEF using mass spectrometry. HFpEF mice demonstrated moderate hypertension, left ventricle (LV) hypertrophy, lung congestion and diastolic dysfunction. Proteomics analysis of the LV tissue showed that 897 proteins were differentially expressed between HFpEF and Sham mice. We observed abundant changes in sarcomeric proteins, mitochondrial-related proteins, and NAD-dependent protein deacetylase sirtuin-3 (SIRT3). Upregulated pathways by GSEA analysis were related to immune modulation and muscle contraction, while downregulated pathways were predominantly related to mitochondrial metabolism. Western blot analysis validated SIRT3 downregulated cardiac expression in HFpEF vs. Sham (0.8 ± 0.0 vs. 1.0 ± 0.0; $P < 0.001$). Phosphoproteomics analysis showed that 72 phosphosites were differentially regulated between HFpEF and Sham LV. Aberrant phosphorylation patterns mostly occurred in sarcomere proteins and nuclear-localized proteins associated with contractile dysfunction and cardiac hypertrophy. Seven aberrant phosphosites were observed at the z-disk binding region of titin. Additional agarose gel analysis showed that while total titin cardiac expression remained unaltered, its stiffer N2B isoform was significantly increased in HFpEF vs. Sham (0.144 ± 0.01 vs. 0.127 ± 0.01; $P < 0.05$). In summary, this study demonstrates marked

10.3389/fcvm.2022.966968

changes in proteins related to mitochondrial metabolism and the cardiac contractile apparatus in HFpEF. We propose that SIRT3 may play a role in perpetuating these changes and may be a target for drug development in HFpEF.

KEYWORDS

HFpEF – heart failure with preserved ejection fraction, proteomics, phosphoproteomics, titin, mitochondria, metabolism, SIRT3

Introduction

Heart failure (HF) is a clinical syndrome caused by abnormalities in the heart that limit its ability to fill or eject blood (1). Heart failure with preserved ejection fraction (HFpEF) is symptomatic clinical HF where left ventricular (LV) ejection fraction (EF) is preserved (LVEF $\geq$ 50%), and presently accounts for about 50% all HF clinical presentations. However, unlike HF with reduced EF (HFrEF), where LVEF is $<$ 50%, there are limited evidence-based therapies for HFpEF (2–4). In addition to its escalating prevalence, HFpEF morbidity (5) and mortality (6) continues to increase. Central to HFpEF is the involvement of both cardiac and extra-cardiac abnormalities (7, 8). In contrast to HFrEF, HFpEF is highly associated with comorbidities and as such is a heterogenous multisystem disorder involving the heart, pulmonary, renal, adipose tissue, skeletal muscle, immune/inflammatory signaling and the vascular system (9, 10). Patients with HFpEF are generally older, more often female and have a predominance of comorbidities, such as hypertension, obesity, type 2 diabetes, atrial fibrillation, renal dysfunction, etc. (11, 12). However, the specific etiologies by which patients develop HFpEF are variable. Thus, a precision-based approach is needed to identify pathogenic mechanisms in HFpEF (10, 13).

Proteomic studies are powerful tools that allow for large-scale characterization of the entire protein phenotype in a biological system (14). Alterations in proteome patterns, such as global changes in protein expression and post-translational modifications (PTMs), are often indicative of marked changes in functional stages in health and disease (15). Thus, investigating the varying patterns of the proteome may provide insights into pathogenic pathways (16) and these protein signatures may facilitate rapid screening of the efficacy of novel treatments and aid in drug development (17, 18).

Previous proteomic studies have identified protein changes in dilated cardiomyopathy, atherosclerosis, and atrial fibrillation (19–23) and these types of studies likely provided a deeper mechanistic understanding of the molecular pathways in HF. For example, cardiac tissue from patients with HFrEF demonstrated protein modifications associated with cardiac metabolism, cardiac remodeling, and impaired cardiac contractility (24–27). Additionally, differentially regulated pathways by proteomic signatures were observed in HFrEF vs. HFpEF patients, which is consistent with the predominant view that the underlying pathophysiology in these two diseases are largely different, and thus the variable response to therapies. This difference is exemplified by Adamo et al., where blood samples from both HFrEF and HFpEF patients demonstrated increased growth factor signaling and increased angiogenesis markers, while proteomic signatures from only HFpEF patients showed increased humoral immunity and those from HFrEF patients showed increased extracellular matrix remodeling markers, consistent with active cardiac remodeling (28). These findings underscore the potential that high-performance proteomics, in combination with clinical assessment, may identify unique targets in specific groups of HF patients.

Although HFpEF is greatly impacted by the obesity and diabetes pandemic, hypertension remains the most prevalent and modifiable risk factor in HFpEF and is implicated in both its pathogenesis and prognosis (12, 29). Hypertensive HFpEF pathophysiology extends beyond the emphasis on LV hypertrophy development and diastolic dysfunction to impaired myocardial contractility, left atrial myopathy, cardiomyocyte remodeling, macro- and microvascular dysfunction, to systemic inflammation, fibrosis, and collagen deposition. However, despite this knowledge a paucity of therapies exists for HFpEF. Here, we applied a deep quantitative proteomics and phosphoproteomic profiling approach to identify molecular protein signatures that are altered in HFpEF in a well characterized murine model of hypertension-associated HFpEF, the *SAUNA* model (SAlty drinking water/Unilateral Nephrectomy/Aldosterone), which recapitulates the human HFpEF phenotype (30–37) (**Supplementary Figure 1**). Using an unbiased and comprehensive analysis, we report

Abbreviations: ACTA1, skeletal alpha-actin; EF, ejection fraction; GLUT1, glucose transporter 1; GLUT4, glucose transporter 4; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IVST, interventricular septum wall thickness; LV, left ventricle; LVEF, left ventricle ejection fraction; LVEDD, left ventricle end diastolic diameter; LVESD, left ventricle end systolic diameter; MFN1, mitofusin 1; MS/MS, tandem mass spectrometry; MYH7, beta-myosin heavy chain; MYH9, myosin heavy chain 9; OXCT1, succinyl-CoA:3-ketoacid coenzyme A transferase 1; PM1, tropomyosin alpha-1 chain; PTM, post-translational modification; RWT, relative wall thickness; SLC16A1, monocarboxylate transporter 1; SIRT3, sirtuin-3; TFAM, transcription factor A mitochondrial; TWT, total wall thickness.

a systematic, large-scale study of pathway, metabolic and organelle level changes that occur in the left ventricle of this HFpEF murine model.

Material and methods

All procedures related to the handling and surgery of the mice conformed to the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

SAUNA model of HFpEF

As previously described (30–33, 35–37), eight-week-old male C57BL/6J mice (Jackson Laboratories) were anesthetized with 80–100 mg/Kg ketamine and 5–10 mg/Kg xylazine intraperitoneally. Mice (20–25 g) then underwent uninephrectomy, received either a continuous infusion of saline (Sham) or *d*-aldosterone (0.30 μg/h, Sigma-Aldrich, St. Louis, MO, United States; HFpEF) for 4 weeks via osmotic minipumps (Alzet, Durect Corp., Cupertino, CA, United States) and were maintained on 1% sodium chloride drinking water.

Physiological measurements

Blood pressure and echocardiographic measurements were performed at the end of the 4 weeks. Systolic blood pressure was measured using a non-invasive tail-cuff blood pressure analyzer (BP-2000 Blood Pressure Analysis System; Visitech Systems Inc., Apex, NC, United States). Transthoracic echocardiography was performed using a Vevo770 High-Resolution *in vivo* Micro-Imaging System and a Real-Time Micro Visualization 707B Scanhead (VisualSonic Inc., Toronto, ON, Canada) as previously described (33). Briefly, interventricular septum wall thickness (IVST), left ventricle (LV) posterior wall thickness (LVPWT), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), and LV ejection fraction (LVEF) were measured. As a measure of systolic function and cardiac contractility fractional shortening (FS) was calculated as follows (LVEDD-LVESD/LVEDD) × 100. Total wall thickness (TWT) was derived from an average of the IVST and LVPWT. Relative wall thickness (RWT) was calculated as 2× LVPWT/LVEDD. LV mass was calculated using the formula described by Kiatchoosakun et al. (38). As diastolic function is sensitive to heart rate (HR) and loading conditions, HR was maintained at ∼350 bpm during these measurements (39). Pulse wave measurements were then recorded and analyzed blinded to group.

Histopathological analyses

Paraffin-embedded sections (5 μm) of the mid-LV were stained with hematoxylin and eosin (H&E, Sigma-Aldrich) to measure LV cardiac myocyte cross-sectional area. Microscopy images (BZ-9000 BioRevo microscope, Keyence Corp. of America, Itasca, IL, United States) were analyzed blinded to group identity using ImageJ measuring software (National Institutes of Health, Bethesda, MD, United States).

Tissue sample preparation for proteomics and phosphoproteomics

Left ventricle samples from 4 mice/group were processed as previously described (22, 40–42). Briefly, freshly thawed samples were homogenized on ice in with a mixer mill MM 400 (Retsch USA Verder Scientific Inc., Newtown, PA, United States) in 10 volumes of 8 M urea, 50 mM ammonium bicarbonate, 2 mM dithiothreitol, and protease and phosphatase inhibitor cocktails (Roche Applied Science, Indianapolis, IN, United States). Tissue homogenate was then sonicated with a probe sonicator (Branson Ultrasonics Corporation, North Billerica, MA, United States) and centrifuged. After centrifugation, supernatant was decanted and total protein in each sample was determined using a modified "microtiter plate" version of the Bradford assay (Sigma-Aldrich). For phosphoproteomics experiments, aliquots containing 300 μg of protein were alkylated with 5 mM iodoacetamide for additional 45 min at room temperature in the dark. Samples were then diluted eight-fold with 50 mM ammonium bicarbonate and digested overnight with sequencing-grade trypsin (#90057, Thermo Fisher Scientific Inc., Waltham, MA, United States). Digestion was stopped by acidification to a final concentration of 1% (v/v) formic acid and the peptide solutions were desalted using disposable C18 Sep-Pak syringes (Waters Corporation, Milford, MA, United States) and lyophilized to dryness following manufacturer's instructions.

Tandem mass tag (TMT) labeling

Peptide concentrations were determined by a colorimetric peptide assay kit (Thermo Fisher Scientific Inc., Waltham, MA, United States) and an aliquot of 100 μg was placed in 100 μl of 100 mM triethylammonium bicarbonate. Peptides were labeled with 0.4 mg of TMT label (TMT10plex^{TM} Isobaric Label Reagent Set, Thermo Fisher Scientific Inc., Waltham, MA, United States). All samples were labeled in the same TMT-batch, representing reporter tags 126C, 127N, 127C, 128C, 129N, 129C, 130N, and 131N. Labeled samples were pooled, and 95% was set aside for phosphopeptide enrichment. The remaining 5% of

labeled peptides and the phosphopeptide enriched samples were analyzed separately by mass spectrometry.

Phosphopeptide enrichment

Phosphopeptides were selectively enriched by binding to titanium dioxide (TiO_2) beads (Titansphere Phos-TiO Bulk 10 μm, GL Sciences, Tokyo, Japan) (43). Briefly, peptides were resuspended in 200 μl 80% acetontirile, 6% trifluoroacetic acid and incubated for 10 min with 10 μl of slurry containing TiO_2 beads. Unbound peptides and supernatant were decanted, and the beads were washed three times with a wash buffer containing 50% acetonitrile and 1% trifluoroacetic acid. After final decanting, the beads were incubated for 10 min with elution solution containing 25% ammonium hydroxide and 50% acetonitrile and the eluate was carefully removed and dried prior to mass spectrometry analysis.

Mass spectrometry analysis

Tryptic peptide mixtures and enriched phosphopeptides were analyzed by nano-scale high-performance liquid chromatography (Proxeon EASY-Nano system, Thermo Fisher Scientific Inc., Waltham, MA, United States) and online nano electrospray ionization tandem mass spectrometry (Q-Exactive HF-X mass spectrometer; Thermo Fisher Scientific Inc., Waltham, MA, United States). Briefly, samples were loaded in aqueous 0.1% (v/v) formic acid via a trap column (75 μm i.d. × 2 cm, Acclaim PepMap100 C18 3 μm, 100 Å, Thermo Fisher Scientific) and peptides were resolved over an Easy-Spray analytical column (50 cm × 75 μm ID, PepMap RSLC C18, Thermo Fisher Scientific) by an increasing mobile phase B. Mobile phase A consisted of 2% acetonitrile and 0.1% formic acid, and organic phase B contained 80% acetonitrile and 0.1% formic acid. Reverse phase separation was performed over 120 min at a flow rate of 300 nl/min. Eluted peptides were ionized directly into the mass spectrometer using a nanospray ion source. The mass spectrometer was operated in positive ion mode with a capillary temperature of 300 C, and with a potential of 2,100 V applied to the frit. Tandem mass spectrometry (MS/MS) was performed using high-energy collision-induced disassociation and 10 MS/MS data-dependent scans (45,000 resolution) were acquired in profile mode alongside each profile mode full-scan mass spectra (120,000 resolution) as reported previously (44). The automatic gain control (AGC) for MS scans was 1×10^6 ions with a maximum fill time of 60 ms. The AGC for MS/MS scans was 3×10^4, with 80 ms maximum injection time, 0.1 ms activation time, and 33% normalized collision energy. To avoid repeated selection of peptides for MS/MS a dynamic exclusion list was enabled to exclude all fragmented ions for 60 s.

Protein identification

Data files (RAW format) were searched using the standard workflow of MaxQuant (version 1.3.0.5)[1] under standard settings using the entire Swiss-Prot mouse database[2] downloaded January 24, 2019, allowing for two missed trypsin cleavage sites, carbamidomethylation of cysteine (fixed) and variable oxidation of methionine, protein N-terminal acetylation and phosphorylation of STY residues. Precursor ion tolerances were 20 ppm for first search and 4.5 ppm for a second search. The MS/MS peaks were de-isotoped and searched using a 20-ppm mass tolerance. A stringent false discovery rate threshold of 1% was used to filter candidate peptide, protein, and phosphosite identifications. The datasets generated for this study have been deposited and publicly available at the PRIDE Archive, proteomics data repository (European Bioinformatics Institute, European Molecular Biology Laboratory) with the data set identifier PXD033501.

Bioinformatics analysis

The searched intensity data were filtered, normalized, and clustered using *Omics Notebook* (45). Filtering was performed to remove any proteins or phosphopeptides not quantified in at least 70 percent of samples, with 2,905 and 281 proteins and phosphopeptides passing the filter, respectively. After filtering, both datasets showed low levels of sparsity and no missing value imputation was performed. The LIMMA R package was used for LOESS normalization and differential expression analysis (46). A combined ranked list for both sets was generated where duplicate gene entries were removed to keep the entry with the highest absolute rank value.

GSEA analysis

Gene Set Enrichment analysis (GSEA) software from the fgsea R package was used to compute gene set enrichment after ranking proteins by differential expression in HFpEF vs. Sham (45, 47, 48). Briefly, GSEA was used in rank mode along with gene sets downloaded from the Bader Lab (Mouse_GOBP_AllPathways_no_GO_iea_October_01_2018_symbol.gmt)[3] (49, 50). GSEA results were visualized using the Enrichment Map app (Version 3.1) in Cytoscape (Version 3.6.1) and highly related pathways were grouped into a theme and labeled by AutoAnnotate (version 1.2). For the merged gene set analyses, we applied an enrichment $P < 0.01$ and FDR ≤ 0.1 cutoffs and calculated overlap between gene set annotations

1 http://maxquant.org/
2 www.uniprot.org/taxonomy/10090
3 https://baderlab.org/GeneSets

using a combination of Jaccard and overlap coefficients with a cutoff of 0.375.

Titin isoform analysis

Additional studies were performed to investigate changes in titin isoforms in HFpEF. Briefly, LV protein lysates from Sham (N = 7) and HFpEF (N = 11) mice were extracted and electrophoresed in 1% agarose gels using a SE600X vertical gel system (Hoefer Inc., Holliston, MA, United States) as previously described (51). Gels were run at 15 mA constant current, stained with Neuhoff's Coomassie (52), and then scanned using Epson Perfection V750 PRO scanner (Epson America Inc., Los Alamitos, CA, United States) and analyzed using One-D scan EX analysis software (Scanalytics Inc., Rockville, MD, United States). The integrated optical density of titin and total myosin heavy chain (MHC) was determined as a function of the slope of the linear range between integrated optical density and loaded volume (53). The expression of compliant N2BA titin, stiffer N2B titin and total titin (TT) was normalized to the expression of total MHC. The expression of titin degraded product (T2) was normalized to the TT expression.

SIRT3 immunoblotting analysis

Protein lysates were extracted from LV tissue using in ice-cold RIPA buffer as previously described (54). Equal amounts of protein were then subjected to electrophoresis in SDS-polyacrylamide gel under reducing conditions and blotted to polyvinylidene difluoride (PVDF) membranes using the Bio-Rad Transblot Turbo Transfer System (Hercules, CA, United States). The membranes were blocked in 5% BSA, 0.1% Tween-20 in tris-buffered saline for 1 h at room temperature and then incubated overnight at 4°C with rabbit anti-SIRT3 antibody (Cell Signaling Technology, Inc., Danvers, MA, United States, #5490; 1:1.000). Membranes were then washed with tris-buffered saline and incubated with respective horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h in room temperature: anti-rabbit antibody (R&D system, HAF008; 1:5,000). Immune complexes were detected with the enhanced chemiluminescence ECL detection system (Bio-Rad, #1705060) in the ImageQuant LAS 4000 biomolecular imaging system (GE Healthcare, Pittsburgh, PA, United States). The intensity of bands for each protein was normalized to the loading control mouse anti-GAPDH (Abcam, Ab8245; 1:10.000).

Statistical analysis

Proteomics and phosphoproteomics differential analysis were based on a moderated t-test and performed using R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria) (45, 55). For histology analysis, titin isoform studies and SIRT3 expression, data are shown as mean ± SEM and statistical significance of differences was assessed using the Student's t-test (two sided). In those cases when data were not sampled as a normal distribution, non-parametric Mann–Whitney U test was used. $P \leq 0.05$ values were considered significant. These statistical tests were performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, United States).

Results

Mouse model of HFpEF

As previously described (30–33, 35–37), salty drinking water, unilateral nephrectomy, and chronic exposure to aldosterone (*SAUNA*) induced hypertension associated HFpEF in mice. Compared to Sham, HFpEF mice demonstrated a moderate increase in systolic blood pressure (137.8 ± 7.0 mmHg vs. 115.4 ± 6.0 mmHg; $P < 0.05$), lung congestion (4.5 ± 0.1 vs. 4.0 ± 0.1 $P < 0.01$), and LV hypertrophy, measured by the LV weight-to-total body weight ratio (3.7 ± 0.1 mg/g vs. 3.3 ± 0.1 mg/g; $P < 0.05$). Additionally, cardiomyocyte size was increased 1.2-fold in HFpEF mice vs. Sham; $P < 0.05$ (**Supplementary Figure 2**).

Echocardiography demonstrated preserved LVEF and increased LV mass (107.5 ± 4.9 mg vs. 78.2 ± 7.9 mg in Sham;

TABLE 1 Characteristics and echocardiographic parameters of HFpEF (*SAUNA*) mice 4 weeks after *d*-Aldosterone or saline (Sham) infusion.

	HFpEF	Sham
Systolic blood pressure (mmHg)	137.8 ± 7.0*	115.4 ± 6.0
Wet-to-dry lung ratio	4.5 ± 0.1**	4.0 ± 0.1
Heart weight-to-body weight (mg/g)	3.7 ± 0.1*	3.3 ± 0.1
Left ventricle structure and function		
LV mass (mg)	107.5 ± 4.9*	78.6 ± 7.9
Total wall thickness (mm)	1.0 ± 0.0***	0.8 ± 0.1
Posterior wall thickness (mm)	1.0 ± 0.1*	0.8 ± 0.1
Relative wall thickness	0.7 ± 0.1***	0.5 ± 0.0
LV end-systolic diameter (mm)	1.1 ± 0.2*	1.6 ± 0.1
LV end-diastolic diameter (mm)	3.0 ± 0.2	3.3 ± 0.1
LV ejection fraction (%)	91.1 ± 1.3	83.1 ± 3.0
LV fractional shortening	62.1 ± 2.3	52.0 ± 3.5
E/A	1.9 ± 0.2	1.7 ± 0.2
Early filling deceleration time (ms)	21.0 ± 3.0	17.6 ± 2.6
Isovolumetric relaxation time (ms)	24.3 ± 2.6*	14.4 ± 1.6

Data are expressed as mean ± SEM. A, peak late transmitral flow velocity; E, peak early transmitral flow velocity; LV, left ventricular (N = 5 mice/group), *$P < 0.05$ vs. Sham; **$P < 0.01$ vs. Sham; ***$P < 0.005$ vs. Sham. Statistical analysis by two-tailed Student's t-test.

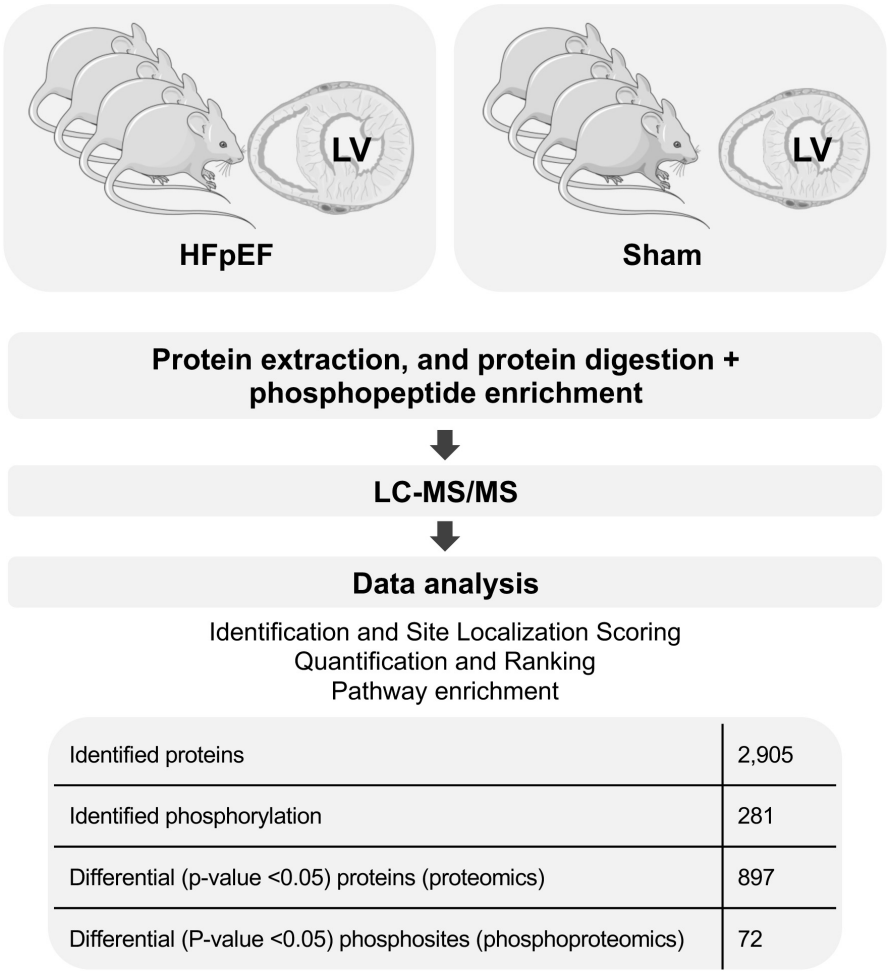
$P < 0.05$; **Table 1**). Wall thickness was significantly increased in HFpEF and there was evidence of concentric hypertrophy, as demonstrated by the increased relative wall thickness (0.7 ± 0.1 vs. 0.5 ± 0.0 in Sham; $P < 0.005$). As previously shown (33), LV end-systolic dimensions and end-diastolic dimensions were also decreased in HFpEF (**Table 1**). HFpEF mice had impaired diastolic function, characterized by an increase in isovolumetric relaxation time (24.3 ± 2.6 ms vs. 14.4 ± 1.6 ms in Sham; $P < 0.05$).

Comparison to human HFpEF: Recently, two clinical scores (HFA-PEFF and H2FPEF) were developed to standardize the clinical diagnosis of human HFpEF. However, a discrepancy exists between these scores (56). The H2FPEF score largely includes clinical parameters whereas the HFA-PEFF score includes predominantly echocardiographic measures and natriuretic peptides. The HFA-PEFF score can rule in human HFpEF with high specificity (93%) and positive predictive value (98%) when the score is high (5–6 points) (57). As such, the translational utility of the HFpEF *SAUNA* mouse model was demonstrated in the context of this HFpEF score with a HFA-PEFF score of ≥ 6 as described by Withaar et al. (58), where a score of ≥ 5 is a high probability of clinical HFpEF.

Proteome profile of the left ventricle in HFpEF

To achieve comprehensive evaluation of the cardiac signaling that is seen in HFpEF, a global quantitative proteome and phosphoproteome profile was performed in LV cardiac tissue obtained from HFpEF mice and their respective Shams ($N = 4$ mice/group; **Figure 1**).

Proteomics analysis found a total of 2,905 identified proteins that were then used for comparative analysis (**Supplementary Table 1**). Among them, 897 proteins were differentially expressed between HFpEF and Sham LV, with 19% of these



Identified proteins	2,905
Identified phosphorylation	281
Differential (p-value <0.05) proteins (proteomics)	897
Differential (P-value <0.05) phosphosites (phosphoproteomics)	72

FIGURE 1
General proteomics and phosphoproteomics workflow. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

being predominantly higher in HFpEF than in Sham ($P < 0.05$; **Figures 2A,B**).

Systematic evaluation of the datasets revealed abundant changes in sarcomeric proteins, namely skeletal alpha (α)-actin (ACTA1; $P = 0.000039$), beta (β)-myosin heavy chain (MYH7; $P = 0.006963$), myosin heavy chain 9 (MYH9; $P = 0.000408$), tropomyosin alpha (α)-1 chain (TPM1; $P = 0.048698$); the mitochondria-related proteins mitofusin 1 (MFN1; $P = 0.001059$), mitochondrial dynamin like GTPase (*aka* optic atrophy protein 1, OPA1; $P = 0.046441$) and transcription factor A mitochondrial (TFAM; $P = 0.005837$); and the NAD-dependent protein deacetylase sirtuin-3 (SIRT3; $P = 0.000914$), recently implicated in cardiac function and cardiac stress responsiveness in HFpEF (59, 60) (**Figure 2B** and **Supplementary Table 1**).

Impaired mitochondrial function and oxidative metabolism of energy substrates in HFpEF

There was an extensive *reduction* in the abundance of proteins involved in cardiac metabolism in the LV of HFpEF mice, including the oxidation of free fatty acid (FFA), pyruvate, and ketone bodies. Significant changes are summarized in **Figure 3**. These include:

(I) β-oxidation related enzymes, implicated in FFA metabolism to acetyl-CoA, such as acyl-CoA dehydrogenase (ACAD) family member 11 (ACAD11; $P = 0.00002$), long-chain specific ACAD (ACADL; $P = 0.00449$), short-chain specific ACAD (ACADS; $P = 0.00896$), short-branched chain specific ACAD (ACADSB; $P = 0.011317$), 3-ketoacyl-CoA thiolase (ACAA2, $P = 0.00609$), enoyl-CoA hydratase (ECHS1, $P = 0.02647$), hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex (HADH) beta (β)-subunit (HADHB, $P = 0.00729$) and HADH alpha (α)-subunit (HADHA, $P = 0.03235$).

(II) the pyruvate oxidation enzyme pyruvate dehydrogenase X component (PDHX, $P = 0.00961$), which is part of the pyruvate dehydrogenase complex that catalyzes pyruvate to acetyl-CoA; and

(III) the ketone metabolism enzyme succinyl-CoA:3-keto-acid coenzyme A transferase 1 (OXCT1, $P = 0.01237$), which catalyzes ketone bodies and produces acetyl-CoA for the tricarboxylic acid (TCA) cycle.

These cumulative results suggest that the energy substrates for mitochondrial oxidative metabolism may be inefficient in HFpEF. Interestingly, although there were no significant alterations in the protein signature of fatty acid and glucose transporters (CD36 and GLUT1 and 4, respectively) in HFpEF, there was an upregulation of the ketone bodies transporter monocarboxylate transporter 1 (SLC16A1, $P = 0.00376$) in the LV of HFpEF.

Additional analysis revealed that the mitochondrial proteins involved in the TCA cycle were also significantly decreased in the LV of HFpEF mice. These mitochondrial enzymes, namely citrate synthase (CS, $P = 0.008068$),

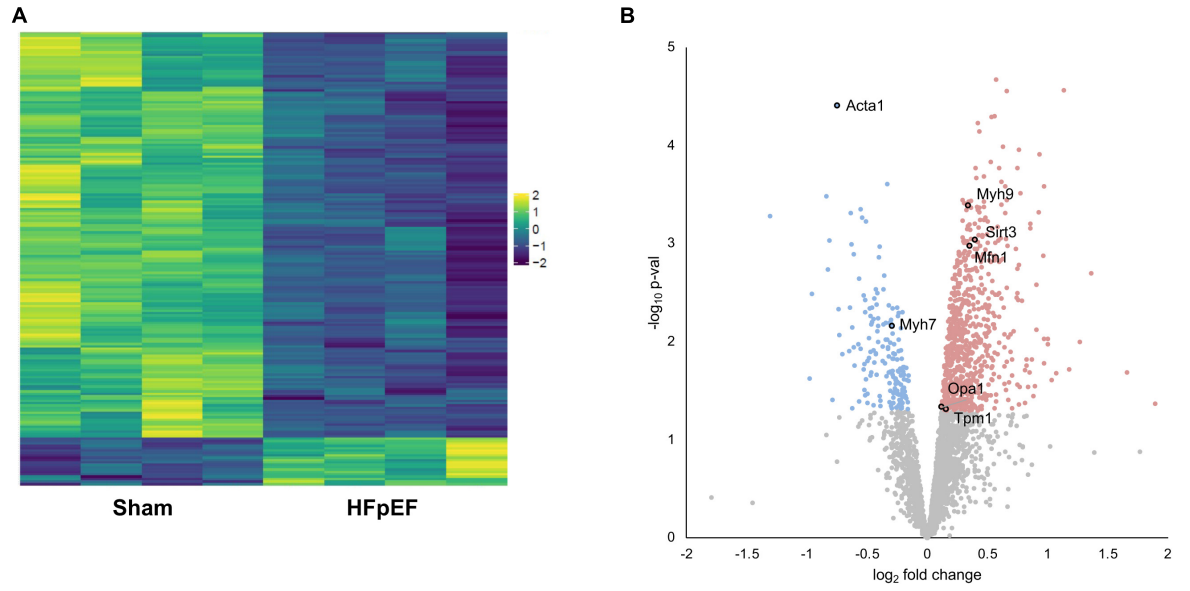


FIGURE 2
Proteomics analysis. **(A)** Heatmap of the differentially expressed proteins. **(B)** Volcano plot presenting log-transformed *p*-values (*t*-test) associated with individual significantly altered proteins plotted against log-transformed fold change in abundance between the left ventricles in Sham and HFpEF mice. Blue and red dots represent up-regulation and down-regulation in HFpEF ($N = 4$) vs. Sham ($N = 4$), respectively.

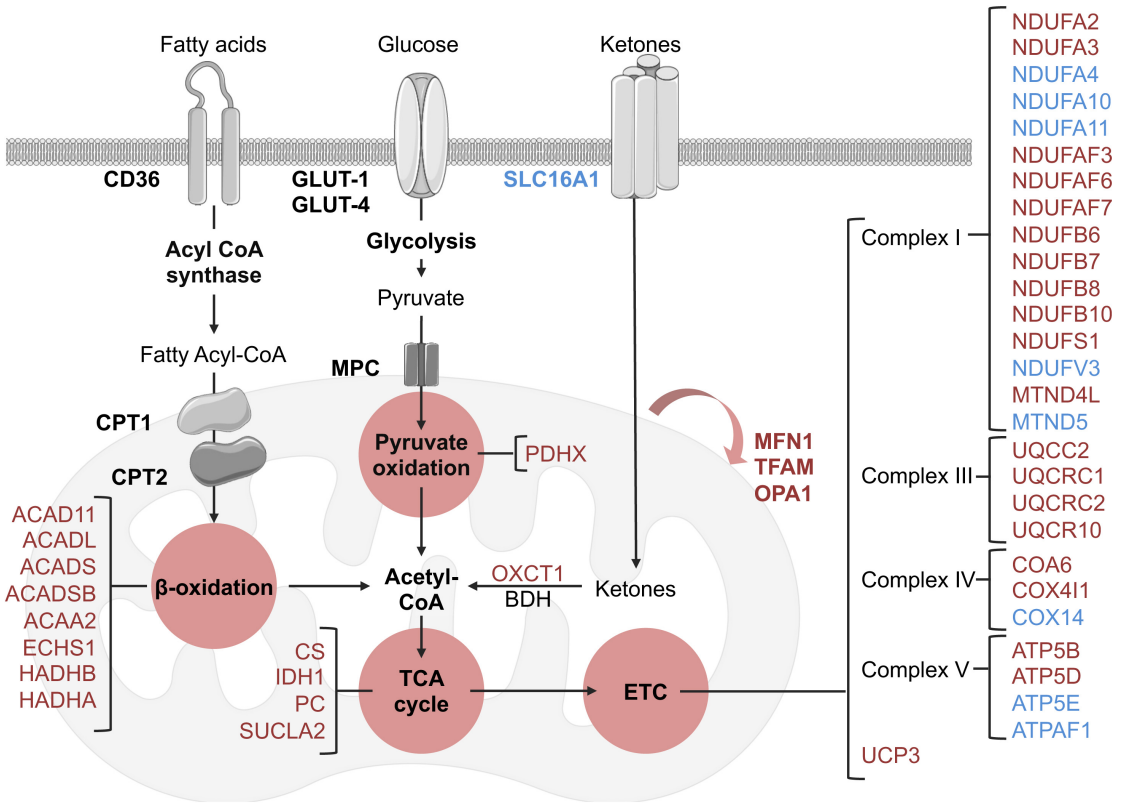


FIGURE 3
Schematic overview of identified metabolic and mitochondrial related targets in HFpEF mice in the proteome dataset. Blue and red represent significant ($P < 0.05$) up-regulation and down-regulation in HFpEF vs. Sham, respectively. Black targets are unchanged.

succinyl-CoA ligase beta subunit (SUCLA2, $P = 0.02523$), isocitrate dehydrogenase (IDH1, $P = 0.00105$) and pyruvate carboxylase (PC, $P = 0.00206$), are required to catalyze acetyl-CoA and produce essential intermediates for the biosynthesis process, and most importantly, high energy molecules such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) for the electron transport chain (ETC). Subsequent analysis then showed that 27 proteins involved in the ETC (namely the respiratory complex I, III, IV, and V) were also differentially expressed between HFpEF and Sham. Of these 27 proteins, 19 proteins were significantly reduced in the HFpEF, suggesting impaired ETC, which was consistent with an additional reduction of the uncoupling protein 3 (UCP3, $P = 0.02765$).

Lastly, additional proteins involved in mitochondrial biogenesis (transcription factor A, TFAM, $P = 0.00584$) and fusion (mitofusin-1, MFN1, $P = 0.001056$ and dynamin-like 120 kDa protein, OPA1, $P = 0.04644$) were similarly decreased in the LV tissue from HFpEF mice.

These findings (**Figure 3**) suggest that mitochondrial dysfunction may lead to inefficient metabolism of energy substrates, possibly contributing to an energy deficit and thus affecting cardiac function in HFpEF.

Pathway enrichment analysis

Pathway enrichment analyses of the proteomics and phosphoproteomics combined datasets were performed by means of GSEA, which detects biology-driven gene sets of canonical pathways from databases of molecular signatures (61). These analyses revealed that the most relevant and over-represented (enriched) biological annotations in the LV from HFpEF to be: (I) processes involving immune system modulation, (II) cardiac muscle cell development and differentiation, and (III) muscle contraction (**Table 2**). These processes included positive regulation of cytokine production (GO:0001819; $P = 0.0000$), striated muscle contraction (Wikipathway; $P = 0.0000$), positive regulation of adaptive immune response (GO:0002821; $P = 0.00578$), cardiac muscle cell development (GO:0055013; $P = 0.03158$) and cardiac muscle cell differentiation (GO: 0055007; $P = 0.03571$). In contrast, the downregulated pathways were related to a multitude of GO terms associated with cellular metabolism (**Table 3**). This is consistent with the earlier data from **Figure 3**, where pathways and processes involving acetyl-CoA metabolic process (GO:0006084, $P = 0.00000$), fatty acid metabolic process (GO:0006631, $P = 0.00000$),

10.3389/fcvm.2022.966968

TABLE 2 Biological annotations terms enriched in significantly up-regulated proteins of the proteome dataset.

Name	Group	*P*-value	Size	ES
Positive regulation of cytokine production	GO:0001819	0.00000	57	−0.34
Pallium development	GO:0021543	0.00000	32	−0.38
Platelet degranulation	Reactome pathway	0.00000	56	−0.33
Response to elevated platelet cytosolic ca2 +	Reactome pathway	0.00000	58	−0.33
Signaling by ROBO receptors	Reactome pathway	0.00000	98	−0.26
Striated muscle contraction	Wikipathway	0.00000	30	−0.42
Positive regulation of adaptive immune response	GO:0002821	0.00578	18	−0.47
Regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	GO:0002822	0.00585	18	−0.47
Positive regulation of wound healing	GO:0090303	0.00595	16	−0.51
Intrinsic pathway for apoptosis	Reactome pathway	0.00633	15	−0.54
Positive regulation of response to wounding	GO:1903036	0.00671	20	−0.44
Integrin pathway	Biocarta pathway	0.00690	23	−0.39
Nucleus organization	GO:0006997	0.00893	31	−0.36
Foxo pathway	PID pathway	0.01500	16	−0.48
G2 m checkpoints	Reactome pathway	0.01754	52	−0.28
Coagulation	Hallmark Pathway	0.01818	54	−0.29
Complement and coagulation cascades	Wikipathway	0.01829	21	−0.45
Cerebral cortex development	GO:0021987	0.02143	27	−0.39
Rho GTPases activate PKNs	Reactome pathway	0.02158	25	−0.40
Positive regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	GO:0002824	0.02222	17	−0.47
Regulation of adaptive immune response	GO:0002819	0.02367	19	−0.46
Spermatid development	GO:0007286	0.02717	16	−0.44
Spermatid differentiation	GO:0048515	0.02924	16	−0.44
Fc epsilon receptor signaling	Reactome pathway	0.02985	50	−0.27
Cardiac muscle cell development	GO:0055013	0.03158	38	−0.31
Regulation of production of molecular mediator of immune response	GO:0002700	0.03550	17	−0.42
Cardiac muscle cell differentiation	GO:0055007	0.03571	41	−0.30
Activation of MAPK activity	GO:0000187	0.03593	21	−0.38
Cardiac cell development	GO:0055006	0.03659	38	−0.31
Regulation of blood coagulation	GO:0030193	0.04380	25	−0.35
Regulation of expression of SLITS and ROBOS	Reactome pathway	0.04762	82	−0.25

ES, enrichment score. Results are sorted by the nominal *P*-value in an ascending order.

acyl-CoA biosynthesis process (GO:0071616, $P = 0.0000$), fatty acid oxidation (GO:0019395, $P = 0.00111$), coenzyme metabolic process (GO:0006732, $P = 0.01015$) were significantly reduced in HFpEF.

Phospho-proteome profile of the left ventricle in HFpEF

We next investigated the phosphoproteomics dataset. Phosphoproteomics analysis profiled 281 mouse reference protein sequences, of which 240 mapped to serine, 37 mapped to threonine and 3 mapped to tyrosine residues, consistent with the expected 90:9:1 cellular distribution ratio (22). The abundance of 72 phosphosites was differentially altered (elevated or reduced) between HFpEF and Sham ($P < 0.05$; **Figures 4A,B**).

Aberrant phosphorylation patterns occurred on proteins linked to disparate subcellular compartments, ranging from sarcomeric proteins (LIM domain-binding protein 3, LDB3; myozenin 2, MYOZ2; titin, TTN), to nuclear-localized proteins (BAG family molecular chaperone regulator 3, BAG3; high mobility group protein HMG-I/HMG-Y, HMGA1) with established links to cardiac contractile function, cardiac hypertrophy and/or cardiomyopathy (**Figure 4B** and **Supplementary Table 2**).

Left ventricular titin expression and phosphorylation in HFpEF

Despite global proteomics not showing a significant change in total titin in the LV between HFpEF and Sham mice,

TABLE 3 Biological annotations terms significantly enriched in down-regulated proteins of the proteome dataset.

Name	Group	*P*-value	Size	ES
Regulation of tp53 activity	Reactome pathway	0.00000	23	0.60
Purine nucleoside bisphosphate metabolic process	GO:0034032	0.00000	43	0.51
Acetyl-coA metabolic process	GO:0006084	0.00000	16	0.64
Negative regulation of lipid metabolic process	GO:0045833	0.00000	17	0.63
Ribonucleoside bisphosphate metabolic process	GO:0033875	0.00000	43	0.51
Monocarboxylic acid catabolic process	GO:0072329	0.00000	46	0.53
Nucleoside bisphosphate metabolic process	GO:0033865	0.00000	43	0.51
Fatty acid metabolic process	GO:0006631	0.00000	91	0.48
Carboxylic acid catabolic process	GO:0046395	0.00000	78	0.46
Monocarboxylic acid metabolic process	GO:0032787	0.00000	138	0.45
Organic acid catabolic process	GO:0016054	0.00000	78	0.46
Acyl-CoA biosynthetic process	GO:0071616	0.00000	15	0.67
Thioester biosynthetic process	GO:0035384	0.00000	15	0.67
Sulfur compound metabolic process	GO:0006790	0.00000	99	0.44
Carboxylic acid metabolic process	GO:0019752	0.00000	259	0.41
Oxoacid metabolic process	GO:0043436	0.00000	264	0.41
Organic acid metabolic process	GO:0006082	0.00000	268	0.41
Cellular monovalent inorganic cation homeostasis	GO:0030004	0.00000	17	0.66
Response to nitrogen compound	GO:1901698	0.00000	196	0.39
Small molecule metabolic process	GO:0044281	0.00100	463	0.34
Thioester metabolic process	GO:0035383	0.00109	39	0.53
Fatty acid oxidation	GO:0019395	0.00111	38	0.52
Positive regulation of ion transmembrane transporter activity	GO:0032414	0.00111	34	0.51
Cilium assembly	GO:0060271	0.00115	22	0.59
Protein trimerization	GO:0070206	0.00121	17	0.66
Sulfur compound biosynthetic process	GO:0044272	0.00229	26	0.58
Protein dephosphorylation	GO:0006470	0.00231	25	0.59
Protein localization	Reactome pathway	0.00310	84	0.42
Acyl-CoA metabolic process	GO:0006637	0.00327	39	0.53
Fatty acid catabolic process	GO:0009062	0.00328	39	0.51
Activation of GTPase activity	GO:0090630	0.00362	16	0.61
Response to oxygen-containing compound	GO:1901700	0.00400	256	0.35
Fatty acid beta-oxidation	GO:0006635	0.00439	31	0.54
Lipid oxidation	GO:0034440	0.00443	38	0.52
Monovalent inorganic cation homeostasis	GO:0055067	0.00473	20	0.62
Laminin interactions	Reactome pathway	0.00486	15	0.63
Small molecule catabolic process	GO:0044282	0.00509	110	0.40
Lipid modification	GO:0030258	0.00536	45	0.50
Dephosphorylation	GO:0016311	0.00553	34	0.52
Ion channel transport	Reactome pathway	0.00553	34	0.51
Metabolism of water-soluble vitamins and cofactors	Reactome pathway	0.00559	32	0.54
Response to organonitrogen compound	GO:0010243	0.00604	178	0.38
Cellular amino acid metabolic process	GO:0006520	0.00609	98	0.41
Positive regulation of transporter activity	GO:0032411	0.00661	37	0.51
Cilium organization	GO:0044782	0.00685	23	0.58
Nucleoside bisphosphate biosynthetic process	GO:0033866	0.00823	19	0.56
Neurotransmitter transport	GO:0006836	0.00894	27	0.54
Positive regulation of ion transmembrane transport	GO:0034767	0.00966	49	0.46

(Continued)

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TABLE 3 (Continued)

Name	Group	*P*-value	Size	ES
Cell projection assembly	GO:0030031	0.00968	49	0.46
Long-chain fatty acid metabolic process	GO:0001676	0.00980	17	0.60
Response to drug	GO:0042493	0.01006	137	0.38
Cell projection organization	GO:0030030	0.01006	155	0.37
Coenzyme metabolic process	GO:0006732	0.01015	126	0.38
Fatty acid metabolism	Reactome pathway	0.01053	72	0.42
Mitochondrial fatty acid beta-oxidation	Reactome pathway	0.01114	28	0.52
Cellular response to hormone stimulus	GO:0032870	0.01148	75	0.43
Pyrimidine-containing compound metabolic process	GO:0072527	0.01214	17	0.59
Cellular response to oxygen levels	GO:0071453	0.01350	26	0.52
Plasma membrane bounded cell projection assembly	GO:0120031	0.01609	47	0.46
Purine nucleoside bisphosphate biosynthetic process	GO:0034033	0.01667	19	0.56
Negative regulation of cellular response to TGFbeta stimulus	GO:1903845	0.01914	15	0.58
Ribonucleoside bisphosphate biosynthetic process	GO:0034030	0.01932	19	0.56
Transmission across chemical synapses	Reactome pathway	0.01967	45	0.45
Nephron development	GO:0072006	0.01975	15	0.59
Cellular response to endogenous stimulus	GO:0071495	0.02018	163	0.35
Positive regulation of transmembrane transport	GO:0034764	0.02030	62	0.42
Plasma membrane bounded cell projection organization	GO:0120036	0.02113	149	0.36
Cellular response to organic substance	GO:0071310	0.02200	323	0.33
Response to organic substance	GO:0010033	0.02200	448	0.32
Branched-chain amino acid catabolism	Reactome pathway	0.02241	21	0.54
Regulation of coenzyme metabolic process	GO:0051196	0.02281	18	0.56
Neuronal system	Reactome pathway	0.02318	59	0.42
Cellular response to inorganic substance	GO:0071241	0.02341	34	0.48
Negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	GO:0090101	0.02392	19	0.55
FCgamma receptor dependent phagocytosis	Reactome pathway	0.02540	25	0.51
Fatty acid biosynthetic process	GO:0006633	0.02549	23	0.52
Cellular response to nitrogen compound	GO:1901699	0.02554	108	0.38
Negative regulation of TGFbeta receptor signaling pathway	GO:0030512	0.02599	15	0.58
Positive regulation of cation transmembrane transport	GO:1904064	0.02612	46	0.45
Negative regulation of organelle organization	GO:0010639	0.02764	87	0.39
Regulation of muscle organ development	GO:0048634	0.02772	34	0.46
Dicarboxylic acid metabolic process	GO:0043648	0.02793	42	0.46
Positive regulation of striated muscle tissue development	GO:0045844	0.02818	21	0.52
Positive regulation of muscle tissue development	GO:1901863	0.02904	21	0.52
Cellular amino acid biosynthetic process	GO:0008652	0.03012	17	0.56
Positive regulation of muscle organ development	GO:0048636	0.03030	21	0.52
Signal release	GO:0023061	0.03111	26	0.49
Heterotrimeric G-protein signaling pathway-GI alpha and GS alpha mediated pathway	Panther pathway	0.03222	22	0.50
Response to endogenous stimulus	GO:0009719	0.03307	215	0.33
Protein complex oligomerization	GO:0051259	0.03313	163	0.36
Neurotransmitter secretion	GO:0007269	0.03410	16	0.57
Signal release from synapse	GO:0099643	0.03431	16	0.57
Regulation of transporter activity	GO:0032409	0.03434	71	0.40
Regulation of transmembrane transporter activity	GO:0022898	0.03441	68	0.40
Cellular response to lipid	GO:0071396	0.03470	67	0.40
Regulation of TGFbeta receptor signaling pathway	GO:0017015	0.03477	21	0.52

(Continued)

TABLE 3 (Continued)

Name	Group	*P*-value	Size	ES
Regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	GO:0090092	0.03528	35	0.46
NABA basement membranes	MSIGDB	0.03534	19	0.53
Coenzyme biosynthetic process	GO:0009108	0.03560	67	0.40
Cellular response to chemical stimulus	GO:0070887	0.03600	417	0.31
Response to organic cyclic compound	GO:0014070	0.03858	123	0.36
Transition metal ion transport	GO:0000041	0.03943	19	0.54
Regulation of striated muscle tissue development	GO:0016202	0.04013	34	0.46
Response to ammonium ion	GO:0060359	0.04152	23	0.49
Positive regulation of ion transport	GO:0043270	0.04280	73	0.39
Opioid signaling	Reactome pathway	0.04282	22	0.52
Cell-cell adhesion	GO:0098609	0.04366	66	0.40
Positive regulation of sodium ion transport	GO:0010765	0.04380	16	0.55
Regulation of muscle tissue development	GO:1901861	0.04402	34	0.46
Blood vessel morphogenesis	GO:0048514	0.04516	48	0.42
Regulation of NIK/NF-kappaB signaling	GO:1901222	0.04535	19	0.52
Regulation of response to drug	GO:2001023	0.04642	15	0.55
Alpha-amino acid metabolic process	GO:1901605	0.04674	55	0.41
Negative regulation of cell proliferation	GO:0008285	0.04689	84	0.37
Activation of cysteine-type endopeptidase activity involved in apoptotic process	GO:0006919	0.04711	20	0.50
Integrin signaling pathway	MSIGDB	0.04718	26	0.48
Cooperation of PDCL (PHLP1) and TRIC CCT in G-protein beta folding	Reactome pathway	0.04785	15	0.54
Response to peptide	GO:1901652	0.04876	84	0.37
Glutamine family amino acid metabolic process	GO:0009064	0.04901	20	0.50

ES, enrichment score. Results are sorted by the nominal *P*-value in an ascending order.

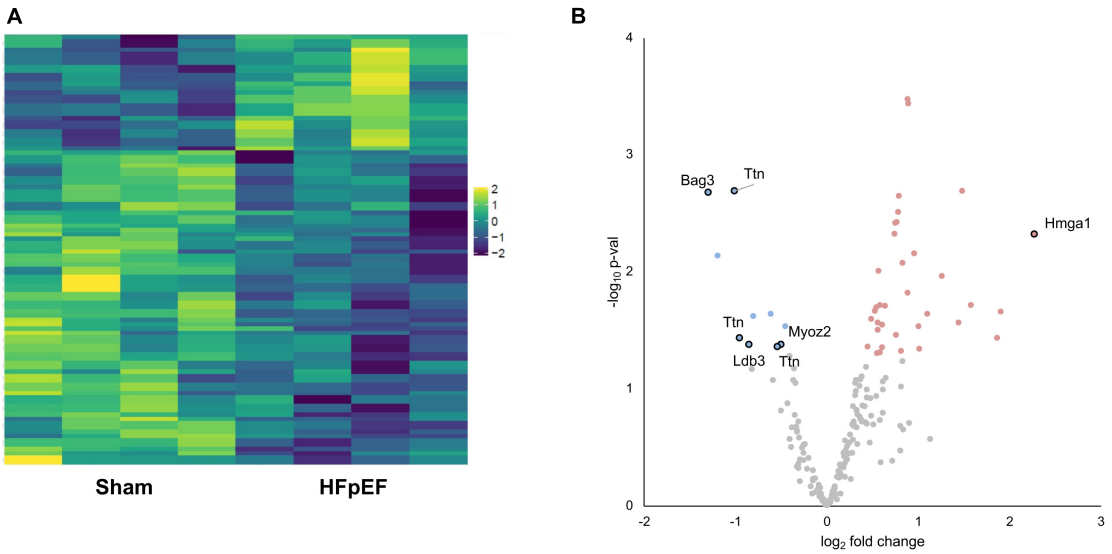

FIGURE 4
Phosphoproteomics analysis. **(A)** Heatmap of the differentially expressed phosphosites. **(B)** Volcano plot presenting log-transformed *p*-values (*t*-test) associated with individual significantly altered phosphosites plotted against log-transformed fold change in abundance between the left ventricles in Sham and HFpEF mice. Blue and red dots represent up-regulation and down-regulation in HFpEF ($N = 4$) vs. Sham ($N = 4$), respectively.

extensive phosphorylation changes across titin were observed in HFpEF vs. Sham. We identified 22 titin phosphosites (including 76% serines, 19% threonines, and 4% tyrosines) in the phospho-dataset, and among them, seven phosphosites were $P < 0.05$, all considered class 1 (localization probability 0.75–1.00) (**Table 4**). Interestingly, five of these were located at the z disk binding region (S262, S264, T266, S1411, and S1415) while the remainder were residues in the C-terminal region (S34464, T34467), suggesting changes in the mechano-sensing activity of titin. These regions are known to act as titin "hotspots," which respond to mechanical stress and regulate specific actions such as activating the hypertrophic gene program or interacting with the protein quality control machinery (62, 63).

Additionally, high-resolution gel electrophoresis was performed to further examine other potential switches in titin isoform expression which may affect titin stiffness, i.e., to quantitatively detect changes in the stiffer N2B or the compliant N2BA isoforms of titin (**Figure 5A**). As expected, there were no changes in total titin (TT) expression between HFpEF and Sham mice. However, as previously described (64), N2B expression was significantly increased in the LV from HFpEF compared to Sham mice (0.144 ± 0.010 vs. 0.127 ± 0.010; $P < 0.05$). Neither N2BA expression, N2BA/N2B ratio nor titin degradation were differentially altered between HFpEF and Sham mice (**Figure 5B**).

Left ventricular expression of SIRT3 in HFpEF mice

Accumulating evidence suggests that SIRT3 plays a critical role in the development of HF (65), particularly in HFpEF (60, 66, 67). As the global proteomics dataset showed a decreased in SIRT3 in the LV from HFpEF mice vs. Sham ($P = 0.000914$), we thus performed additional immunoblot analysis to validate these findings. Indeed, SIRT3 expression was significantly decreased in the LV from HFpEF mice vs. Sham (0.8 ± 0.0 vs. 1.0 ± 0.0; $P < 0.001$; **Figure 6A**).

Discussion

Heart failure with preserved ejection fraction is a complex disease involving several sub-phenotypes within a heterogeneous HFpEF syndrome (10, 13, 68). Of all the comorbidities in HFpEF, hypertension remains the most common, and is implicated in both the pathogenesis and the prognosis of the disease (12, 29). However, the exact biological mechanisms that underlie hypertension associated HFpEF remain largely unclear. In this study, we investigated the proteomic and phosphoproteomics profile underlying HFpEF in a clinically relevant murine model of hypertension associated HFpEF. The *SAUNA* model of HFpEF model fulfils the

TABLE 4 Significantly changed titin phosphosites.

Feature	Log FC (Sham/HFpEF)	*P* value	Position	Site probability (%)	Peptide sequence
Ttn_A2ASS6.55	−1.01327	0.00203	S262	0.99876	QLPHKTPPRIPPKPKSRSPTPPSIAAKAQLA
Ttn_A2ASS6.56	−1.01327	0.00203	S264	0.99836	PHKTPPRIPPKPKSRSPTPPSIAAKAQLARQ
Ttn_A2ASS6.60	−1.01327	0.00203	T266	0.99033	KTPPRIPPKPKSRSPTPPSIAAKAQLARQQS
Ttn_A2ASS6.36	−0.96294	0.03670	S34464	0.96352	VTSPPRVKSPEPRVKSPETVKSPKRVKSPEP
Ttn_A2ASS6.40	−0.96294	0.03670	T34467	0.81504	PPRVKSPEPRVKSPETVKSPKRVKSPEPVTS
Ttn_A2ASS6.29	−0.54438	0.04350	S1411	0.79632	PTPEAVSRIRSVSPRSLSRSPIRMSPAMSPA
Ttn_A2ASS6.30	−0.54438	0.04350	S1415	0.96095	AVSRIRSVSPRSLSRSPIRMSPAMSPARMSP
Ttn_A2ASS6.27	−0.41168	0.05240	S283	0.89160	PSIAAKAQLARQQSPSPIRHSPSPVRHVRAP
Ttn_A2ASS6.28	−0.41168	0.05240	S290	0.95098	QLARQQSPSPIRHSPSPVRHVRAPTPSPVRS
Ttn_A2ASS6.23	0.42746	0.06498	S34451	0.82907	TLTVQKARVIEKAVTSPPRVKSPEPRVKSPE
Ttn_A2ASS6.24	0.42746	0.06498	S34457	0.98108	ARVIEKAVTSPPRVKSPEPRVKSPETVKSPK
Ttn_A2ASS6.38	0.31502	0.10499	T33859	0.99883	LTQDDLEMVRPARRRTPSPDYDLYYYRRRRR
Ttn_A2ASS6.31	−0.31311	0.19004	S34107	0.99068	DAERRSPTPERTRPRSPSPVSSERSLSRFER
Ttn_A2ASS6.32	−0.33175	0.22516	S34109	0.76541	ERRSPTPERTRPRSPSPVSSERSLSRFERSA
Ttn_A2ASS6.25	1.12758	0.27026	S1406	1.00000	APTYMPTPEAVSRIRSVSPRSLSRSPIRMSP
Ttn_A2ASS6.26	1.12758	0.27026	S1408	1.00000	TYMPTPEAVSRIRSVSPRSLSRSPIRMSPAM
Ttn_A2ASS6.41	0.46224	0.28856	Y33864	0.93320	LEMVRPARRRTPSPDYDLYYYRRRRRSLGDM
Ttn_A2ASS6.33	−0.27602	0.31791	S34112	0.88295	SPTPERTRPRSPSPVSSERSLSRFERSARFD
Ttn_A2ASS6.22	0.19164	0.43120	S307	0.93434	VRHVRAPTPSPVRSVSPAGRISTSPIRSVKS
Ttn_A2ASS6.42	−0.06749	0.79212	S301	0.99417	RHSPSPVRHVRAPTPSPVRSVSPAGRISTSP
Ttn_A2ASS6.43	−0.06749	0.79212	S307	0.93434	VRHVRAPTPSPVRSVSPAGRISTSPIRSVKS
Ttn_A2ASS6.58	−0.06749	0.79212	T299	0.99713	PIRHSPSPVRHVRAPTPSPVRSVSPAGRIST

Results are sorted by the nominal *P*-value in an ascending order. Highlighted values indicate significantly regulated phosphorylation between Sham and HFpEF.

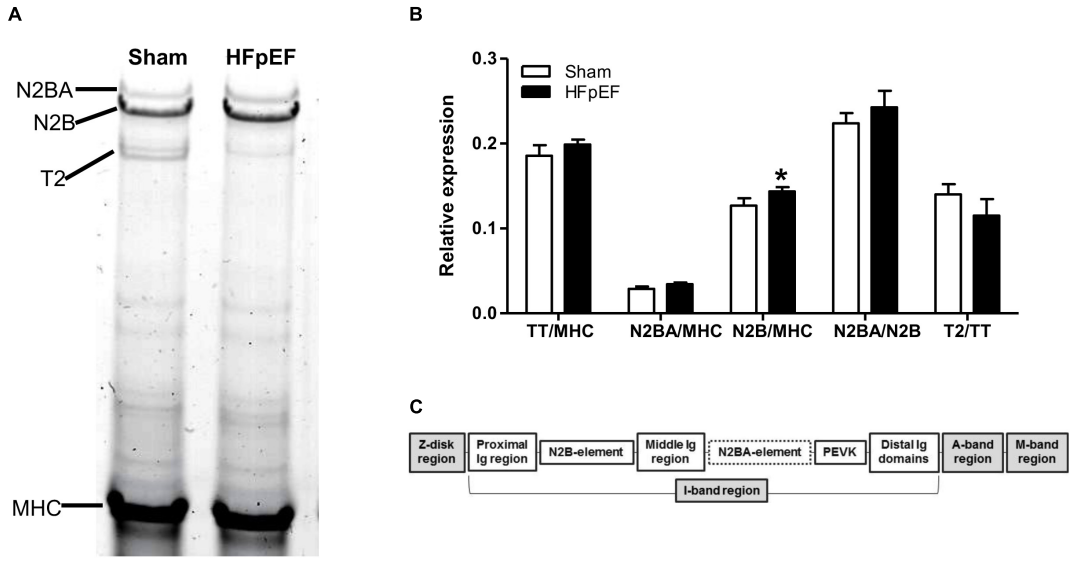

FIGURE 5
Titin isoform expression. **(A)** Representative image of 1% agarose gel for titin analysis. **(B)** Quantitative analysis of total titin (TT) and titin isoforms N2BA and N2B relative to total myosin heavy chain (MHC), the ratio of N2BA to N2B, and titin degradation product (T2) relative to TT, in the left ventricles from Sham (N = 7) and HFpEF mice (N = 11). Data are presented as mean ± SEM. Unpaired T-test was performed, $^*P < 0.05$ vs. Sham mice. **(C)** Overview of the titin molecule structure.

criteria for a "high probability of HFpEF" based on HFA-PEFF diagnostic algorithm for human HFpEF (58, 69).

In the present study, extensive proteomics and phosphoproteomics analysis permitted in-depth screening of the changes in protein expression, post-translational modifications (i.e., phosphorylation), and pathway alterations in HFpEF. These included but were not limited to: (I) changes in cardiac metabolism, where the predominant components were the mitochondrial metabolic processes and mitochondrial dysfunction; (II) alteration in cardiac contractile function-related proteins; (III) overexpression of pathways related to immune modulation; and (IV) a significant decrease in SIRT3 expression, that was validated by immunoblotting.

We found marked changes in signatures of protein expression related to mitochondrial function and oxidative metabolism of energy substrates in HFpEF. There was a significant decrease in targets related to mitochondrial substrate oxidation, suggesting that cardiac mitochondrial metabolic function is impaired in HFpEF. Interestingly, there was an upregulation of the ketone bodies transporter SLC16A1 in the LV of HFpEF, but this was not accompanied by comparable changes in ketone metabolism enzymes. Although not investigated in this study, these findings may contribute to the metabolic impairment seen in HFpEF by increasing the transport of ketone bodies into the mitochondria, but without a compensatory catabolic response. We hypothesize that this mismatch in mitochondrial substrate intake and utilization results in mitochondrial ketone bodies accumulation which may detrimentally affect cardiac function (70). Ketone bodies are thought to be a relevant energy source in both preclinical HFrEF models (71) and advance HFrEF patients (72). Additionally, it has been shown that HFpEF patients have significantly higher circulating ketone levels than HFrEF patients (73) suggesting that some of the beneficial effects of SGLT2 inhibitors in HFpEF may be due to enhanced ketone bodies availability and cardiac utilization (74–76), a process known as "thrifty substrate/fuel hypothesis" (77). We also observed decreased OXCT1 (aka SCOT, succinyl-CoA:3-ketoacid CoA transferase) expression in HFpEF hearts (**Figure 3**). OXCT1 allows cells to utilize energy stored in ketone bodies thus its decrease in HFpEF hearts supports a role for ketone body cardiac metabolism. Similarly, others have shown worse HF in pre-clinical models with cardio-specific deletion of OXCT1 (78).

Proteomic evaluation of PTMs is essential to understand the function of many proteins in physiological and pathophysiological settings. PTMs are regulators of protein structure and function and, in the heart the predominant PTM is phosphorylation, followed by acetylation (79), and it is also recognized that many proteins are regulated by phosphorylation independently of their expression (80).

Titin is a major cardiac protein regulated by phosphorylation and facilitates myocardial passive tension by conditioning cardiomyocyte-derived stiffness (81). Titin regulates cardiomyocyte stiffness both at the transcriptional and post-transcriptional level. At the transcriptional level, titin shifts from its compliant isoform N2BA toward its stiff isoform N2B, which contributes to the impaired diastolic function that is seen in HFpEF (33, 64, 82). In the present study,

translational and PTMs in titin are apparent in the LV of HFpEF hearts. The stiffer N2B isoform was significantly increased in HFpEF mice. However, it is notably that the N2B isoform is also the predominant isoform expressed in the LV of rodents (83). At the post-transcriptional level, despite comparable global proteomics expression between HFpEF mice and Sham, phosphoproteomics analysis showed that titin was one of the proteins with the greatest alterations in phosphorylation in HFpEF mice. Similar to a Dahl salt-sensitive rat study (84), in these *SAUNA* HFpEF mice most of the significantly hyper-phosphorylated titin residues were located at the Z-disk binding region of the titin protein (**Figure 5C**). Interestingly, it has been suggested that titin may be part of a Z-disk macromolecular machinery acting as a node for hypertrophic signaling (85). As such, our findings that the myofilament and myofilament-associated proteins viz. ACTA1, MYH7, MHY9, TPM1, and MYOZ2 were differentially expressed in both global and phosphoproteomics dataset, support the premise that alterations in sarcomeric and myofilament regulating proteins play a central role in HFpEF. Of these proteins, MYH7, TPM1 and MYOZ2 are known to be important in hypertrophic cardiomyopathy (86–88), and may play a similar role in HFpEF. However, although their function in muscle contraction is well known (89, 90), their role in cardiac hypertrophy and adverse cardiac remodeling remains elusive. It has been hypothesized that changes in cardiac architecture may be a compensatory response that eventually fails, resulting in a re-induction of fetal genes, fibrosis replacing necrotic and apoptotic cardiac cells, and a shift in metabolic substrates (91). However, additional studies are warranted to identify the precise role these myofilament-associated proteins play in HFpEF.

Because of its size, titin has more phosphorylation sites than other smaller proteins and hundreds of phosphorylation sites have been predicted based on proteomic analysis (83), Z-disk Similarly, multiple kinases are also involved in titin phosphorylation (92), representing more opportunities for the regulation of the cardiomyocyte structure and function. However, the effect that a specific phosphorylation pattern has on the function of titin is largely dependent on the specific structural domain which is modified within the protein (93). For example several studies have focused on the "spring-like" I-domain, including the N2bus and PEVK regions, likely due to the mechanically active nature of this specific domain, where phosphorylation may modulate passive and active tension of the sarcomere (85, 92–94). Conversely, the proline-directed kinases, including extracellular signal-regulated kinase-1/-2 (ERK1/2) and cyclin-dependent protein kinase-2 (Cdc2) were able to regulate the phosphorylation status of non-extensible Z-disk (95, 96) and C-terminal (M-band) (97) regions (98). Although additional studies using site-specific methods are needed (92), it has been suggested that changes in the phosphorylation status of these regions may have an important function, not only during developmental stages, but also regulating the binding of titin to other and M-band proteins, as well as the assembly and turnover of these binding partners (99, 100).

In addition to phosphorylation, HFpEF is also associated with hyperacetylation of mitochondrial proteins in the myocardium (101, 102). In the mitochondria, the acetylation state of key enzymes involved in mitochondrial metabolism, oxidative stress defense and mitochondrial dynamics is regulated by the mitochondrial, NAD-dependent protein deacetylase SIRT3 (103–106). SIRT3 interacts with at least 84 mitochondrial proteins involved in many aspects of mitochondrial biology, such as maintaining mitochondrial integrity and function (67, 106). In the present study, the global proteomics data set showed decreased expression of SIRT3 in the LV of HFpEF mice, which was also confirmed by immunoblotting. Others have shown that reduced SIRT3

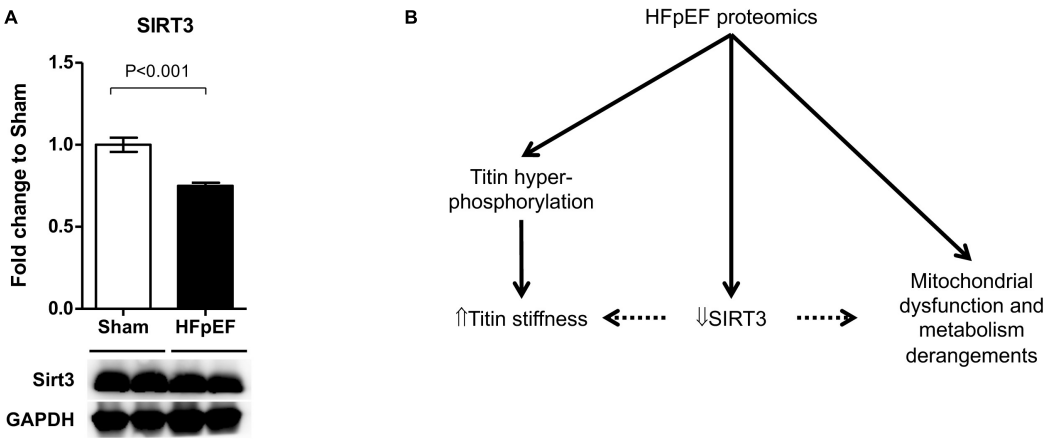

FIGURE 6
SIRT3 regulation in HFpEF. **(A)** SIRT3 protein expression in the left ventricles from HFpEF mice (N = 11) and Sham (N = 7). Data are presented as mean ± SEM. Statistical analysis by two-tailed Student's t-test. **(B)** Overview of proposed SIRT3 regulatory system in HFpEF.

expression is related to reduced NAD+ bioavailability in HFpEF, and that cardiomyocyte specific SIRT3 knockout mice developed worse diastolic dysfunction in HFpEF (60). Additional studies using whole-body knockout or transgenic mice similarly showed that SIRT3 is required to maintain cardiac contractile function under pro-hypertrophic or ischemic stress (107–110). A recent study showed that a deficit of cardiac NAD+ exists not only pre-clinical HFpEF models but also in patients with HFpEF, and that increasing NAD+ levels with nicotinamide improved diastolic dysfunction (111). The authors hypothesized the beneficial effects were mediated, partly by increasing deacetylation of proteins that regulate the mechano-elastic properties of cardiac myocytes such as titin and sarco/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a). Although not investigated in the present study, SIRT3 may also play a role in cardiomyocyte stiffness and impaired diastolic function in HFpEF, possibly by titin acetylation (59). SIRT 3 may be a future target since, compared to younger subjects, exercise increases SIRT3 protein expression in muscle, which is decreased in older sedentary individuals (112). Interestingly, of the 7 mammalian sirtuins described, SIRT3 is the only analog whose increased expression associates with longevity in humans (113–115). Since HFpEF is highly associated with aging, and exercise training is effective in improving the quality of life in HFpEF patients (116), future studies are warranted to explore the role of SIRT3 expression in muscle in patients with HFpEF.

In conclusion, untargeted proteomics have demonstrated a key role of protein PTMs in metabolism, cell preservation and sarcomere function in the heart (117). In the present study marked proteomics and phosphoproteomics changes occurred in the heart in HFpEF mice which were related to altered mitochondrial metabolism and sarcomere contractility. It is possible that SIRT3 plays a pivotal role in HFpEF, by regulating mitochondrial metabolism and titin stiffness but this requires further study (**Figure 6B**).

Data availability statement

The datasets presented in this study can be found online at the PRIDE Archive, proteomics data repository (European Bioinformatics Institute, European Molecular Biology Laboratory) with the data set identifier PXD033501. Raw unedited gels images are shown in **Supplementary Figure 3**.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

Author contributions

MV-M and FS contributed to the conception and design of the study. MV-M and ES performed the surgeries and physiological measurements. MV-M, ES, and ZH performed the molecular analysis. RH and BB performed the proteomic sample preparation and carried out the mass spectrometry and bioinformatics analysis. MV-M, ES, and FS wrote the first draft of the manuscript. RH wrote sections of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

Funding

This work was supported by the National Institute of Health RO1HL145985 to FS and R35HL144998 to HG.

Conflict of interest

FS was a full-time employee of Eli Lilly and Co, Indianapolis, IN and holds a joint, academic appointment at Boston University School of Medicine. All the work in this publication is from The Sam Lab, Whitaker Cardiovascular Institute at Boston University School of Medicine, Boston, MA. This work was funded by NIH RO1HL145985 awarded to FS. None of the work was funded nor supported by Eli Lilly and Co. FS has no other conflicts to disclose regarding the work in this manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.966968/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 06 September 2022
DOI 10.3389/fcvm.2022.973279

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 20 June 2022
ACCEPTED 04 August 2022
PUBLISHED 06 September 2022

CITATION
He B, Quan L-P, Cai C-Y, Yu D-Y, Yan W, Wei Q-J, Zhang Z, Huang X-N and Liu L (2022) Dysregulation and imbalance of innate and adaptive immunity are involved in the cardiomyopathy progression. *Front. Cardiovasc. Med.* 9:973279. doi: 10.3389/fcvm.2022.973279

Dysregulation and imbalance of innate and adaptive immunity are involved in the cardiomyopathy progression

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Background: Cardiomyopathy is known to be a heterogeneous disease with numerous etiologies. They all have varying degrees and types of myocardial pathological changes, resulting in impaired contractility, ventricle relaxation, and heart failure. The purpose of this study was to determine the pathogenesis, immune-related pathways and important biomarkers engaged in the progression of cardiomyopathy from various etiologies.

Methods: We downloaded the gene microarray data from the Gene Expression Omnibus (GEO). The hub genes between cardiomyopathy and non-cardiomyopathy control groups were identified using differential expression analysis, least absolute shrinkage and selection operator (LASSO) regression and weighted gene co-expression network analysis (WGCNA). To assess the diagnostic precision of hub genes, receiver-operating characteristic (ROC) curves as well as the area under the ROC curve (AUC) were utilized. Then, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis and Gene Ontology (GO) analysis were conducted on the obtained differential genes. Finally, single-sample GSEA (ssGSEA) and Gene Set Enrichment Analysis (GSEA) were utilized to analyze the infiltration level of 28 immune cells and their relationship with hub genes based on gene expression profile data and all differential gene files.

Results: A total of 82 differentially expressed genes (DEGs) were screened after the training datasets were merged and intersected. The WGCNA analysis clustered the expression profile data into four co-expression modules, The turquoise module exhibited the strongest relationship with clinical traits, and nine candidate key genes were obtained from the module. Then we intersected DEGs with nine candidate genes. LASSO regression analysis identified the last three hub genes as promising biomarkers to distinguish the cardiomyopathy group from the non-cardiomyopathy control group. ROC curve analysis in the validation dataset revealed the sensitivity and accuracy of three hub genes as marker genes. The majority of the functional enrichment analysis results were concentrated on immunological and inflammatory pathways. Immune infiltration analysis revealed a significant correlation between regulatory T cells,

type I helper T cells, macrophages, myeloid-derived suppressor cells, natural killer cells, activated dendritic cells and the abundance of immune infiltration in hub genes.

Conclusion: The hub genes (CD14, CCL2, and SERPINA3) can be used as markers to distinguish cardiomyopathy from non-cardiomyopathy individuals. Among them, SERPINA3 has the best diagnostic performance. T cell immunity (adaptive immune response) is closely linked to cardiomyopathy progression. Hub genes may protect the myocardium from injury through myeloid-derived suppressor cells, regulatory T cells, helper T cells, monocytes/macrophages, natural killer cells and activated dendritic cells. The innate immune response is crucial to this process. Dysregulation and imbalance of innate immune cells or activation of adaptive immune responses are involved in cardiomyopathy disease progression in patients.

KEYWORDS

cardiomyopathy, weight gene co-expression network analysis (WGCNA), biomarkers, immune cell infiltration, LASSO regression

Introduction

Cardiomyopathies are a diverse set of cardiac muscle illnesses characterized by electrical or mechanical abnormalities, typically exhibiting abnormal ventricular dilation or hypertrophy, thus contributing to the decline in systolic and diastolic function in heart failure (1). The etiology of cardiomyopathy is diverse, and its classification varies by country. For example, cardiomyopathy is classified according to etiology in the American Heart Association classification, while the European Society of Cardiology classification is based on a combination of morphology and hemodynamics. Currently, cardiomyopathy is mainly divided into primary and secondary cardiomyopathies. Primary cardiomyopathy can be classified as hereditary, such as arrhythmogenic right ventricular cardiomyopathy (ARVC), hypertrophic cardiomyopathy (HCM), acquired, such as inflammatory cardiomyopathy (ICM), and mixed, such as hereditary and acquired dilated cardiomyopathy (DCM). Secondary cardiomyopathy mainly includes alcoholic, ischemic, and perinatal cardiomyopathy (2, 3). Currently, it is not clear whether cardiomyopathy of different etiologies involves common mechanisms in molecular genetics changes or pathogenesis. Though, understanding these mechanisms is critical for managing and treating cardiomyopathy. However, to the best of our knowledge, we are innovatively combining cardiomyopathy with various etiologies to explore the key genetic changes or pathogenesis of cardiomyopathy progression compared to non-cardiomyopathy individuals by bioinformatics method.

The development of various phenotypes in cardiomyopathy depends on the complex interactions between individual genetic genotypes, multiple cellular signaling pathways, and environmental stressors. Although the pathogenesis of cardiomyopathy varies by etiology, inflammation and the immune system both play important roles in mediating irreversible damage to the myocardium (4, 5). When the heart is damaged or stressed, innate immune cells, such as neutrophils and monocytes, will migrate to the damage site and release mediators such as reactive oxygen species (ROS) and proteases to remove the factors that cause heart damage. However, after injury, Cardiomyocytes will further secrete pro-inflammatory cytokines that can trigger adaptive immunity and aggravate the inflammatory response (6). Current studies have shown that myocardial inflammation involves multiple inflammatory pathways, such as the TNF/NF-κβ pathway associated with cardiac infection and injury, pattern recognition receptors expressed by macrophages such as Toll-like receptors (TLRs), and oxidative and stress-activated caspase-1 inflammasome pathway and so on (7). In conclusion, inflammation plays a vital function in cardiomyopathy progression and pathogenesis. Therefore, the regulation of inflammation remains a promising target for treating cardiomyopathy of different etiologies. To find out the key immune-related pathway involved in them, our study further elucidates the inflammatory infiltration mechanism of cardiomyopathy.

In this study, we merged and intersected the data from cardiomyopathy groups with various etiologies so that the differentially expressed genes (DEGs) obtained by screening were more representative. Next, we intersected DEGs with the candidate key genes determined using weighted gene co-expression network analysis (WGCNA). Following this, the hub genes that distinguish the cardiomyopathy group from the non-cardiomyopathy control group were screened out using the least absolute shrinkage and selection operator (LASSO) regression on the basis of the intersection genes. Combining these methods increases the accuracy of the targeted

signature genes for screening. Most importantly, we validated the screened hub genes expression level and diagnostic ability in the cardiomyopathy and non-cardiomyopathy control group in a large independent sample dataset (validation group). In addition, we conducted KEGG pathway enrichment analysis, Gene Ontology (GO) on DEGs, and Gene Set Enrichment Analysis (GSEA) on all differential gene files to identify their inflammatory and immune-related signaling pathways. Finally, using single-sample GSEA (ssGSEA), we investigated the infiltration of 28 immune cells based on expression profile data and their connection with hub genes. The current study would help us understand the cardiomyopathy pathogenesis and identify novel predictive and treatment targets for cardiomyopathy.

Materials and methods

GEO data download

We downloaded the Microarray expression data from the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) (8). The dataset for this analysis is divided into validation and training datasets. The training dataset included the following: GSE42955 (9) (12 cases of DCM, 12 cases of ICM, and five cases of controls), where GPL6244 platform of Affymetrix Human Gene 1.0 ST Array served as the foundation, in addition to GSE29819 (10) (12 cases of ARVC, 12 cases of DCM, and 12 cases of controls), where GPL570 platform of Affymetrix Human Genome U133 Plus 2.0 Array served as the foundation. The 17 controls of the training dataset (GSE42955 and GSE29819) were derived from non-diseased donor hearts, which could not be transplanted for technical reasons. The large sample dataset was used as the validation dataset: GSE5406 (11) (86 DCM, 108 ICM, and 16 controls), where GPL96 platform of Affymetrix Human Genome U133A Array served as the foundation. The 16 controls of the validation dataset (GSE5406) were from non-diseased normal hearts that had normal left ventricular function. The training and validation datasets can be subdivided into cardiomyopathy (Treat) and non-cardiomyopathy control (Con) groups.

Data merging, intersecting, and screening of DEGs

The data from GSE42955 and GSE29819 datasets were merged and intersected using "sva" and "limma" R software packages (version 4.2.0), and the data were probe-annotated, batch-corrected, and normalized. Probe annotation files provided by researchers were employed to translate probes in each dataset into gene symbols. We determined the amount of gene expression in a given tissue by using the average numbers of probes that correspond to the same gene symbol. A systematic evaluation of ComBat's performance demonstrates that it outperforms other tools. Therefore, we used ComBat to eliminate batch effects between the two datasets (12). Both "pheatmap" package and "ggplot2" package were deployed to create DEGs heatmaps and volcano plots, respectively (13). The screening criteria of DEGs were customized to log fold change (FC) > 1 and $P < 0.05$ (14, 15).

Building gene co-expression networks and finding the most relevant modules for clinical traits

From the expression profiling data of the merged datasets, a weighted gene co-expression network was created using the WGCNA package of the R program (16). We used the "goodSampleGenes" function to check the data's integrity and the "pickSoftThreshold" function to verify the ideal soft threshold (β) to correlate to a scale-free network more closely. After obtaining the matrix data, we converted it into a topological overlap matrix, and then gene clustering was performed, and the clustering results were identified by dynamic shearing module. Next, a hierarchical clustering dendrogram was built after calculating the module eigengenes (MEs) and combining related modules in the clustering tree based on the MEs. The modules were subsequently merged with phenotypic data to generate heatmaps. Subsequently, the correlations between genes and modules (MM) and the importance of genes (GS) were calculated. Finally, correlation histograms and scatter plots were drawn according to the MM and GS values.

Hub gene screening, expression level verification, and diagnostic ability of the hub gene

According to the screening criteria (absolute value of MM > 0.80, absolute value of GS > 0.50), candidate key genes were picked from the modules with the greatest connectivity. Using R's "venn" package, we intersected the candidate key genes with the DEGs. Lastly, the final hub gene was screened by LASSO regression (17). The expression levels of the obtained hub gene in the training datasets were compared between the control group and the cardiomyopathy group using a boxplot, which was validated on an independent, large-sample dataset (GSE5406). The accuracy of the hub gene as a marker gene for cardiomyopathy and control groups was also assessed using the Receiver-operating characteristic (ROC) curve, and the diagnostic ability was further validated in the validation dataset.

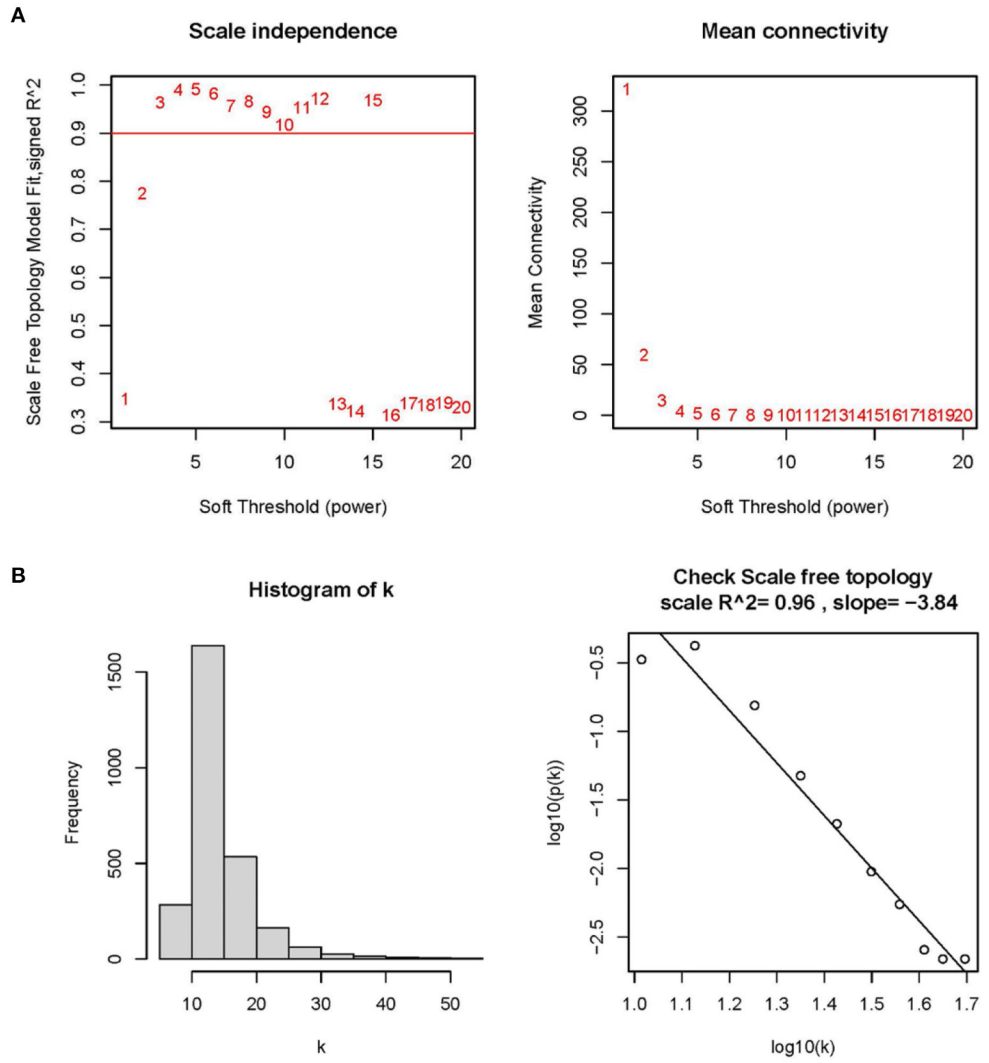

FIGURE 1
Determining optimal soft thresholds (β) in WGCNA. **(A)** Examination of the average connectivity under various β and scale-free fitting index. The red line implies that the corresponding soft threshold is 10 when the correlation coefficient is 0.9. **(B)** Connectivity distribution histogram and a scale-free network correlation coefficient of 0.96 checked at $\beta = 3$.

GO, KEGG, and GSEA functional enrichment analysis

KEGG enrichment analysis and GO were conducted on DEGs using the "clusterProfiler" and "enrichplot" packages of the R software (18). For all differential gene files, we used the immune-related gene sets that we obtained from the Molecular Signature Database (MsigDB) for GSEA enrichment (19). The top five significantly enriched immune gene sets were displayed. Considered statistically significant P values were adjusted at < 0.05 ($q < 0.05$).

ssGSEA enrichment analysis to assess immune cell infiltration on profile data expression and its association with hub genes

With the ssGSEA algorithm, we assessed the correlation of gene expression profiles with the 28 immune cells (20). The differential expression levels of 28 immune infiltrating cells in the cardiomyopathy and non-cardiomyopathy control groups were visualized using Violin plots and heatmaps. The degree of association between the 28 immune cells and hub gene was evaluated

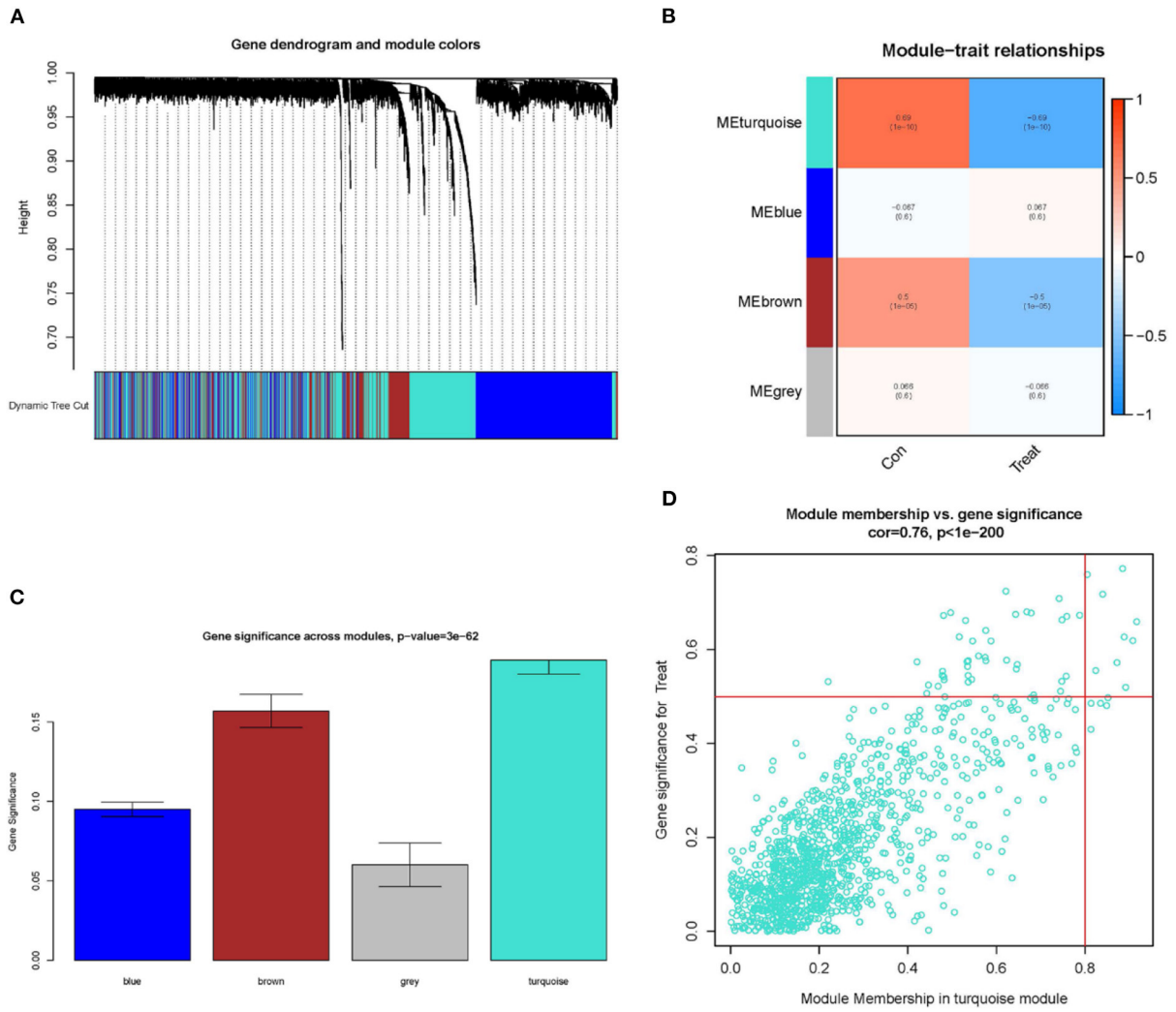

FIGURE 2
Constructing WGCNA modules and screening candidate key genes. **(A)** Gene clustering dendrogram: each branch represents a gene, and each color below represents a co-expression module. **(B)** Module-trait relationships heatmap, with turquoise modules significantly associated with controls. **(C)** Distributing gene significance in each module. **(D)** In the turquoise module, a scatter plot of gene module members vs. gene significance is shown, GS > 0.5 and MM > 0.8 are candidate key genes.

by Spearman correlation and visualized using R software's "ggplot2" package.

Results

WGCNA analysis for the construction of a co-expression network for the identification of important modules and genes

To improve the data quality, the samples are clustered, missing values are filled in, outliers are removed, and normalized the data. Therefore, when the optimal soft threshold $\beta = 3$ is selected after clustering, the constructed network is more like the scale-free network (Figures 1A,B). Subsequently, a topological overlap matrix was derived. Using dynamic hybrid shearing, we obtained gene modules, which were then clustered to create four gene modules (Figure 2A). A heatmap was used to show the correlation of the above modules with clinical traits in the control group and the cardiomyopathy group and genes' importance in each module. The strongest correlation (cor) among them was found between the turquoise module and the control group (cor = 0.69; P = 1e-10) and the highest gene importance within the turquoise module (Figures 2B,C). Lastly, according to the scatter plot, between GS and MM in the turquoise module have a strong correlation (cor = 0.76; P = 1e-200). Under the screening conditions of GS > 0.5 and MM

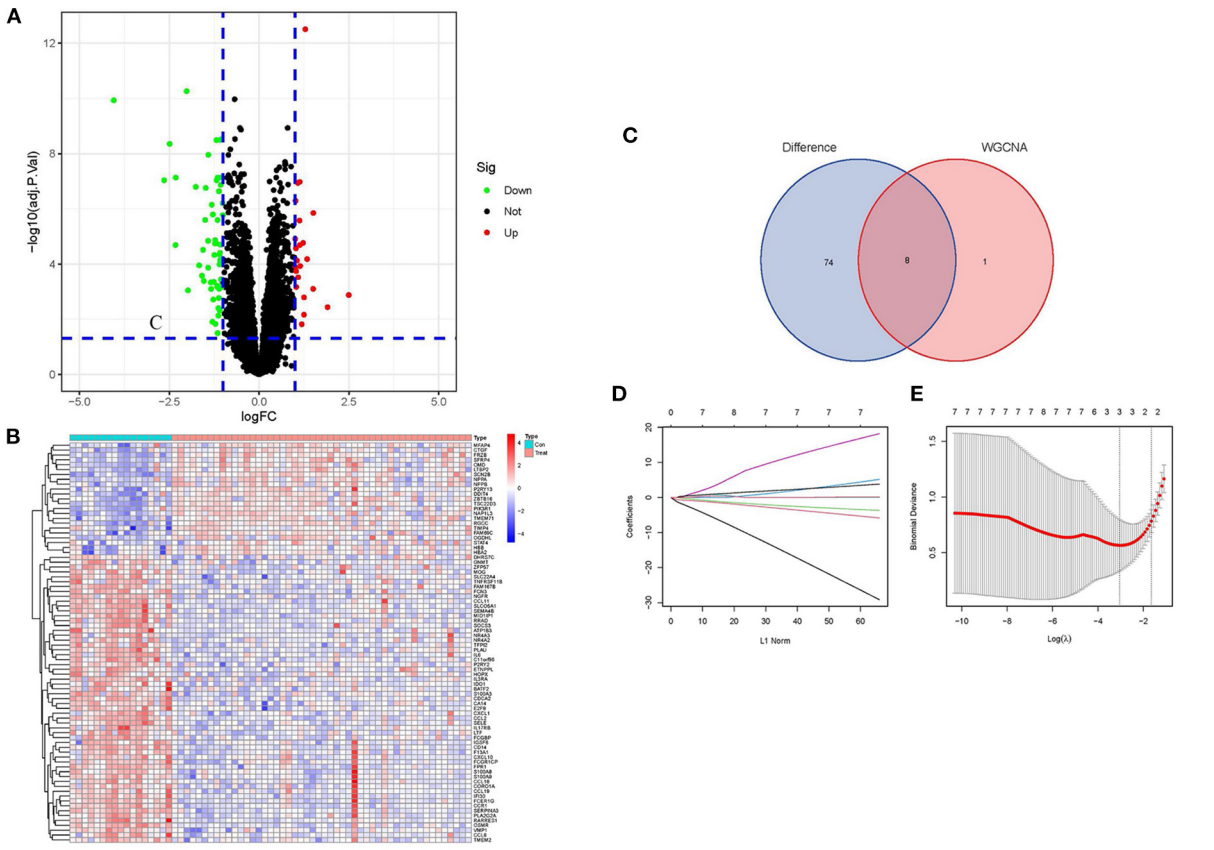

FIGURE 3
Screening of DEGs and identification of Hub genes. **(A)** Volcano plots of DEGs expression in control and cardiomyopathy groups. **(B)** Heatmap of DEGs expression in cardiomyopathy and control groups. **(C)** Venn diagram of the DEGs' intersection and candidate genes of the turquoise module. **(D)** Map of the regression coefficients of the eight genes in LASSO model. **(E)** Three hub genes screened by 10-fold cross-validation in the LASSO regression model.

> 0.8, 9 candidate key genes belonging to the turquoise module were obtained then used for subsequent analysis (Figure 2D, Supplementary File 4).

Screening of differential genes and identification of hub genes

Based on the DEGs' screening criteria (log fold change (FC) > 1 and adjusted-P < 0.05), a total of 82 DEGs were obtained (Supplementary File 3). DEGs expression in the samples was displayed in volcano plots and heatmaps (Figures 3A,B). By intersecting the DEGs with the nine candidate key genes from the turquoise module, we were able to get eight intersection genes (Figure 3C, Supplementary File 4). Finally, we performed LASSO regression analysis for the intersection genes, and the final three hub genes were identified as follows: *CD14*, *CCL2*, and *SERPINA3* (Figures 3D,E, Supplementary File 4).

Identification of the expression level of the hub gene and its diagnostic value

Using boxplots, the expression levels of the three hub genes were determined. In the training dataset, the cardiomyopathy group's expression levels of *CD14*, *CCL2*, and *SERPINA3* were significantly lower than the ones in the control group. P values were all <0.001 (P < 0.001) (Figure 4A). Then, in a separate large-sample validation dataset, we further validated the expression levels of these three hub genes (GSE5406), and the results were like those of the training group, *CD14* (P < 0.05), *CCL2* (P < 0.01), and *SERPINA3* (P < 0.001). The expression difference of SERPINA was most significant in the validation group (Figure 4B). Our next step was to assess the precision of the three hub genes as markers for discriminating between cardiomyopathy and control groups using ROC curves. In the training datasets, the area under the ROC curve (AUC) values of *CD14*, *CCL2*, and *SERPINA3* genes were all > 0.95, indicating that these genes have high diagnostic values as marker genes

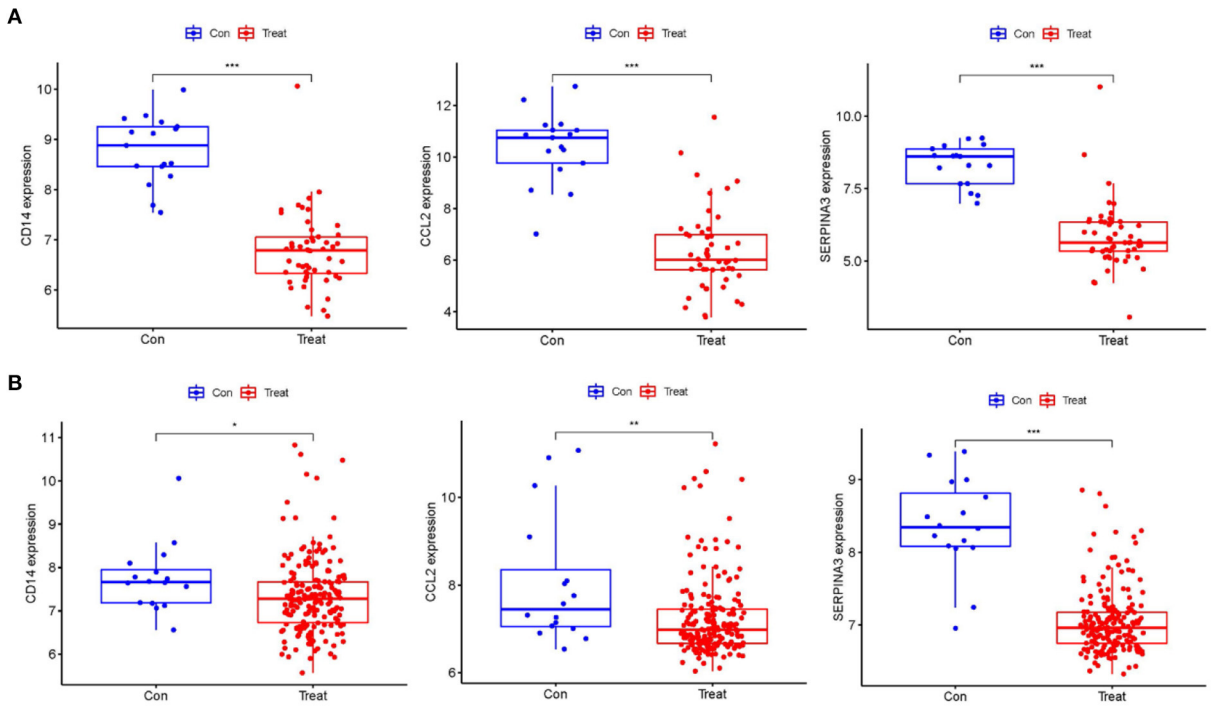

FIGURE 4
Identification of the expression level of the Hub gene. **(A)** The expression levels of hub genes, *CD14*, *CCL2* and *SERPINA3* in the training datasets were significantly lower in the cardiomyopathy group than in the control group. **(B)** The hub gene expression was verified in the large sample validation dataset (GSE5406), and the expressions of *CD14*, *CCL2* and *SERPINA3* in the cardiomyopathy group were significantly lower than those in the control group, of which *SERPINA3* had the most significant difference. "***", "**", "*" represent $P < (0.001, 0.01, 0.05)$.

(Figure 5A). In the validation dataset, the AUC values of the three genes *CD14*, *CCL2*, and *SERPINA3* were 0.673, 0.704, and 0.939, respectively. Data from the large sample validation dataset indicated that all three genes could be used as marker genes, with *SERPINA3* having the highest diagnostic sensitivity (Figure 5B).

Functional enrichment analysis of DEGs

KEGG enrichment analysis and GO were done to comprehend the related signaling pathways and important biological functions involved in DEGs (Supplementary File 5). Biological functions are mainly enriched in defense and inflammatory processes, such as leukocyte chemotaxis and migration. In terms of cellular composition, it is mainly enriched in the extracellular matrix. The molecular functions are primarily enriched in receptor activities, including G protein-coupled receptors, signaling receptor activators, etc. (Figures 6A–C). The KEGG signaling pathway enrichment analysis revealed that DEGs were mainly abundant in inflammation-related signaling pathways, such as chemokine, cytokine-cytokine receptor interaction and IL-17 signaling pathways (Figures 7A–C). These findings uncovered cellular mechanisms and abnormal signaling pathways implicated in the development of cardiomyopathy.

Immune signature gene set enrichment analysis

GSEA enrichment analysis was performed on all differential gene files using the immune signature gene set in MsigDB database to identify the underlying immune-related mechanisms during cardiomyopathy progression. A total of 1,281 gene sets were enriched ($q < 0.05$, Supplementary File 6). We showed the top five most significantly enriched gene sets, among which the gene sets CD4+ T cells, CD8+ T cells, and naive T cells have high enrichment scores in the cardiomyopathy group but low scores in the control group. In contrast, natural killer cells and regulatory T cells were highly enriched in the control group. These findings demonstrated the importance of adaptive immune cell in the progression of cardiomyopathy, while regulatory immune cells and innate immune cells may play a role in heart protection (Figures 8A,B).

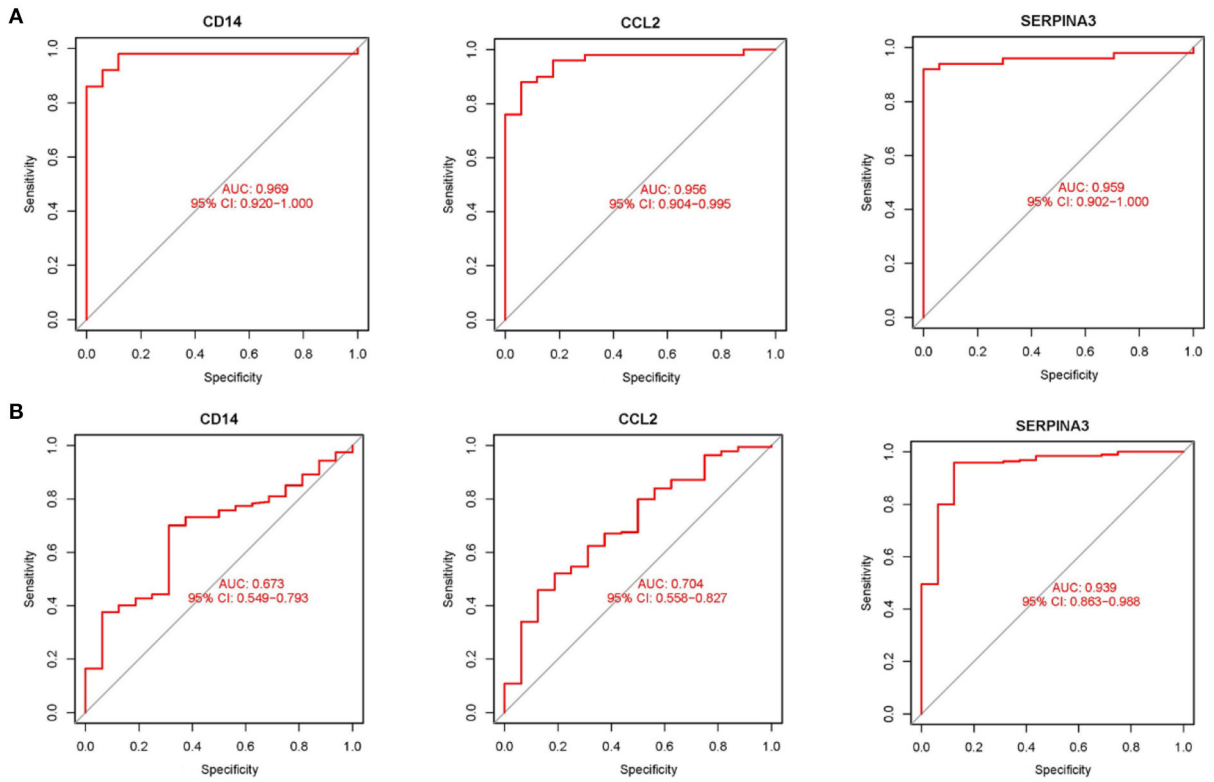

FIGURE 5
Validation hub genes are used as marker genes **(A)** Diagnostic ability of hub genes in the training datasets. The area under ROC curve (AUC) was used to evaluate the discriminating ability of hub gene in cardiomyopathy and control groups. **(B)** The validation results of hub gene in the large sample validation dataset (GSE5406) were similar to those of the training datasets.

ssGSEA analysis of immune cell infiltration and its correlation with hub genes

By using ssGSEA analysis, we first compared immune cell infiltration between cardiomyopathy and control groups. The distribution of the 28 immune cells in the expression profile samples is shown in Figure 9A and Supplementary File 7. The immune cell infiltration analysis results showed that regulatory T cells, myeloid-derived suppressor cells, type 1 helper T cells, and macrophages were lower in the cardiomyopathy group than in the control group (Figure 9B). Then we evaluated the association of 28 immune cells with hub genes, among which regulatory T cells were associated with *CD14* ($P < 0.01$), *CCL2* ($P < 0.001$) and *SERPINA3* ($P < 0.001$), activated dendritic cells, myeloid-derived suppressor cells with *CD14* ($P < 0.001$), *CCL2* ($P < 0.001$) and *SERPINA3* ($P < 0.01$), natural killer cells with *CD14* ($P < 0.001$), CCL2 ($P < 0.001$) and SERPINA3 ($P < 0.001$), macrophages were positively correlated with *CD14* ($P < 0.01$), *CCL2* ($P < 0.001$) and *SERPINA3* ($P < 0.01$) (Figure 9C). These findings may demonstrate the critical role of regulatory and innate immune cells in heart protection.

Discussion

Recently, WGCNA analysis has replaced DEGs-based screening approaches because of their deficiencies. For example, traditional methods are only used to study a small number of datasets, and the correlation between genes is "one size fits all," which is prone to overlook essential regulatory core molecules in the regulatory process of biological systems. By contrast, the core molecules associated with clinical traits can be identified based on the weighted gene co-expression network (21). LASSO provides a dimensionality reduction impact, as a regression analysis approach, compared to classical logistic and Cox regression (17). In this study, we analyzed data by WGCNA to determine candidate genes that are strongly related to the clinical traits of the cardiomyopathy group and the control group. Following, we intersected DEGs with the candidate key genes to identify divergent and highly correlated intersecting genes between the two groups. Eventually, LASSO regression analysis identified three hub genes: *CD14*, *CCL2*, and *SERPINA3*. The expression level of these three hub genes was significantly lower in the cardiomyopathy group when compared to the control group. The AUC represents its sensitivity and

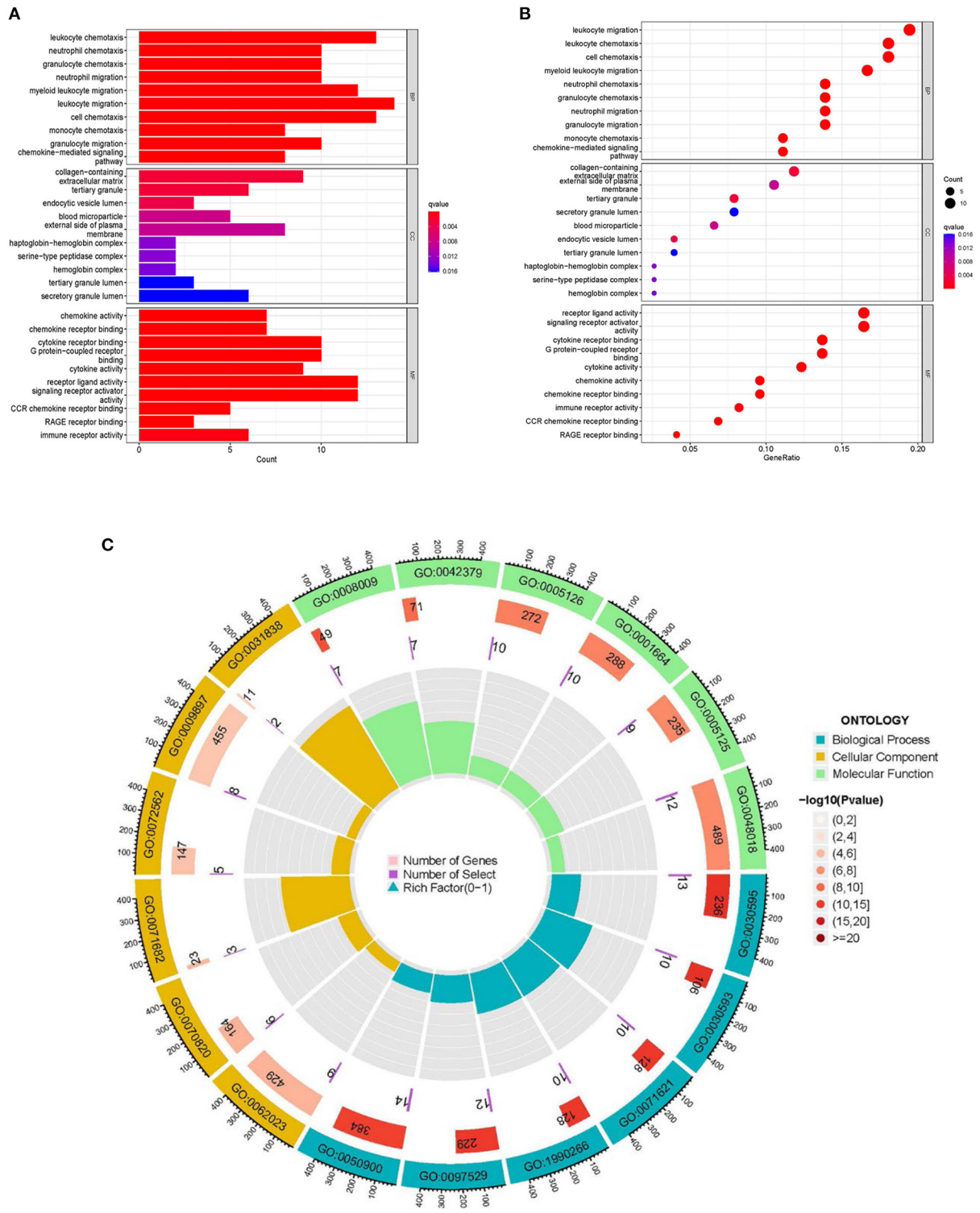

FIGURE 6
GO enrichment analysis of DEGs. **(A)** The histogram of GO enrichment analysis; the redder the color, the more significant the enrichment. **(B)** Bubble diagram representing GO enrichment analysis; the size of bubbles represents the number of enriched genes, and the redder the color of the bubbles, the more significant the enrichment is. **(C)** The first circle indicates that BP, CC, MF are represented by different colors and the top six enriched GO:ID are taken. The second circle represents the number of genes in different GO:ID genome backgrounds, where different colors represent the significant degree of DEGs enrichment. The third circle represents the number of genes enriched by DEGs. The fourth circle represents the proportion of genes.

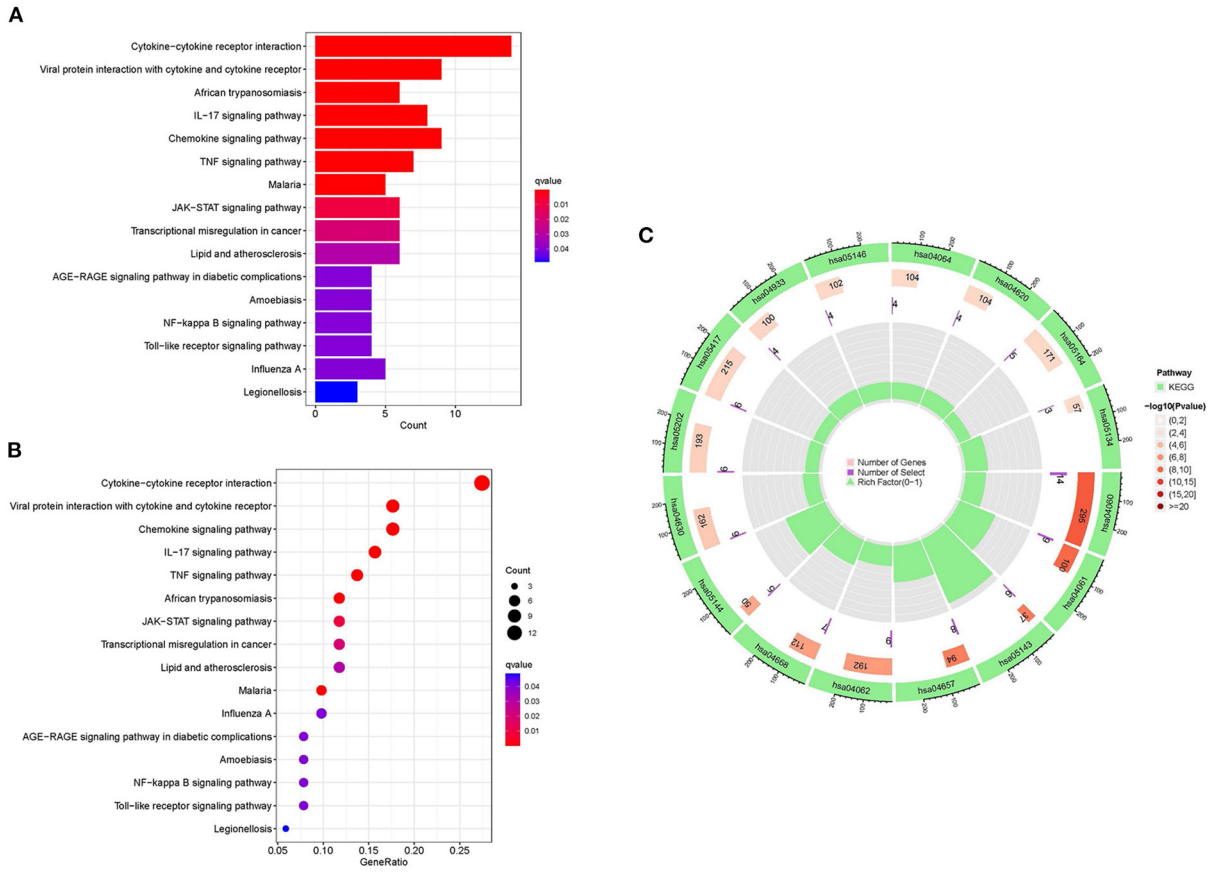

FIGURE 7
KEGG enrichment analysis of DEGs. **(A)** The histogram of KEGG enrichment analysis; the redder the color, the more significant the enrichment. **(B)** Bubble diagram representing KEGG enrichment analysis; the size of bubbles indicates the number of enriched genes, and the redder the color of bubbles, the more significant the enrichment is. **(C)** The first circle represents the enriched KEGG:ID. The second circle represents the number of genes in different KEGG:ID pathway backgrounds, where different colors represent the significant degree of DEGs enrichment. The third circle represents the number of genes for which DEGs are enriched in the pathway. The fourth circle represents the proportion of genes.

accuracy as a marker gene. Among them, *SERPINA3* had the highest diagnostic efficiency and was the most strikingly differentially expressed gene. In addition, it is worth noting that previous research has focused on cardiomyopathies of a single etiology, which can improve the understanding of the pathological mechanisms of specific diseases, but may lack the exploration of common pathological mechanisms involved in cardiomyopathies of different etiologies. However, our current study is innovatively investigation to combine cardiomyopathy with varied etiologies which examined common hub genes and their diagnostic ability as marker genes and how they contribute to the immune infiltration pattern of various cardiomyopathies. Especially, we have assessed the diagnostic ability and accuracy not only in the training dataset, but also in the large sample validation dataset as well. In this way, our results are very reliable and trustworthy.

The innate immune system functions as an "early warning system," allowing the host to differentiate between non-self and self accurately and quickly. It is activated by "pattern recognition receptors" found on many cells, including cardiomyocytes. Cardiomyocytes express various pattern recognition receptors like Toll-like and *CD14* receptors. The cardiac innate immune system relies on these pattern recognition receptors to respond to various forms of myocardial injury (22, 23). The *CD14* receptor encoded by the *CD14* gene is a leucine-rich receptor mainly expressed on the surface of natural cells, especially monocytes/macrophages. It can also act as a receptor in a soluble form (sCD14) on cells that do not express CD14 on their surface, such as dendritic cells (24). It plays an important protective role as a pattern recognition molecule to recognize a variety of inflammatory mediators (25). One of the CC chemokines, called monocyte chemoattractant protein-1 (*CCL2; MCP-1*), is important for the migration of monocytes, memory T cells, and natural killer cells (26, 27). Several studies have shown that monocytes/macrophages are involved in the pathogenesis and occurrence of cardiovascular disease by retaining and

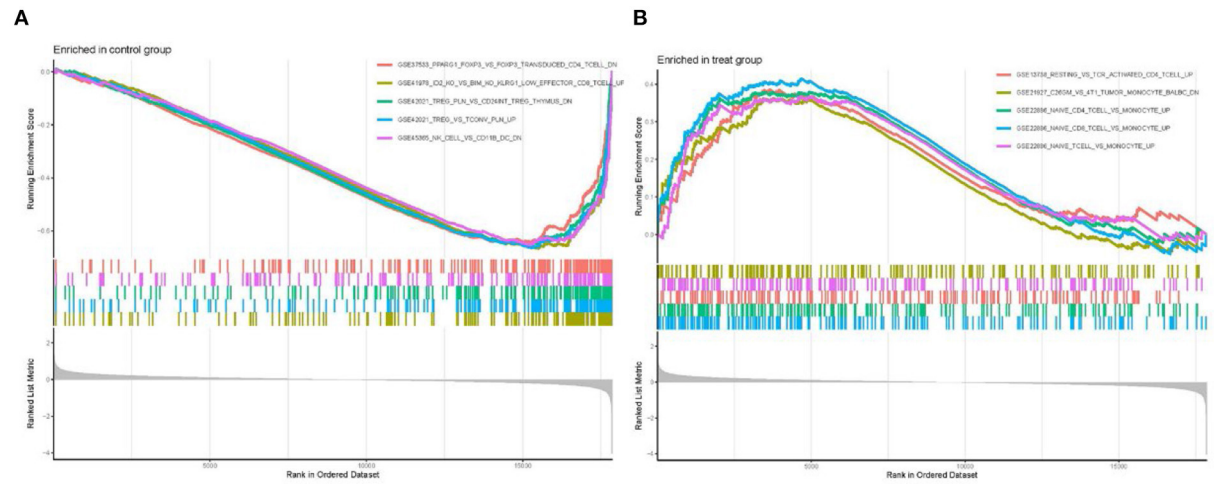

FIGURE 8
Enrichment of GSEA immune signature gene set. **(A)** Immune gene set scores in the control group. **(B)** Immune gene set scores in the cardiomyopathy group.

activating *CCL2*. As in ischemic cardiomyopathy, *CCL2* has a persistent chronic expression (28). In our study, the expression level was lower compared to that of the control group, and it may be used as a target molecule to distinguish chronic cardiomyopathy from normal myocardium, or its dysregulation promotes cardiomyopathy development. Unquestionably, this needs to be verified in large samples and further experiments.

As a member of the serine protease inhibitor superfamily, *SERPINA3*, also known as α1-antichymotrypsin, is implicated in oxidative stress, apoptotic cell death and inflammatory responses (29). The *SERPINA3* gene expression is regulated by cytokines such as IL1 and IL6. In addition, the *SERPINA3* gene expression as part of inflammatory responses can regulate immune cells via mast cell chymotrypsin, leukocyte elastase, and neutrophil cathepsin G (30). Inadequate regulation of *SERPINA3* can result in prolonged or excessive cathepsin G activity, eventually causing tissue injury (31). Studies have shown that *SERPINA3* is associated with systemic inflammation and oxidative stress. In stable heart failure, excess levels can have detrimental effects on cardiac function and increased mortality or cardiac accidents. *SERPINA3* may be as well utilized as a heart failure predictive biomarker with great potential (32, 33). Consistent with previous studies, our study showed that the number of samples expressing *SERPINA3* in the cardiomyopathy group was significantly greater compared to the control group. However, in comparison with the control group, the expression level was significantly lower, so this specifies that the dysregulation or imbalance of *SERPINA3* gene expression is involved in heart disease progression in those with cardiomyopathy.

Furthermore, the difference in *SERPINA3* expression levels between the two groups, and its ability to serve as a marker gene to distinguish the cardiomyopathy group from the control group, was significantly greater than that of *CD14* and *CCL2*. Thus, these merits further investigation into the diagnostic and therapeutic potential of SERPINA3 in cardiomyopathy. Most importantly, we found that both *CCL2* and *CD14*, which are closely related to monocytes/macrophages, and *SERPINA3*, regulated by cytokines IL1 and IL6, appear to be involved in the innate immune response. We conjecture that their dysregulation and imbalance may contribute to the cardiomyopathy progression. To better understand this finding, we further performed an enrichment analysis of the data to explore the immune-related pathways involved.

GO enrichment analysis of DEGs exhibited that the biological functions were primarily concentrated in the immune and inflammatory response. However, this is consistent with our previous presentation, which stated that after cardiac injury triggers inflammatory and immune responses, it promotes tissue healing and remodeling by activating compensatory mechanisms. Though, remodeling and inflammation become chronic over time, decreasing cardiac function and heart failure (34). Cardiac fibrosis occurs in the progression of different etiologies of cardiomyopathy, and enrichment analysis of cellular component shows that the extracellular matrix is closely related to fibrosis (35). The molecular function enrichment of signaling receptor activator activity and G protein-coupled receptors represent central physiological functions involved in cardiomyocyte growth, metabolism, and functional regulation. The KEGG signaling pathway showed that

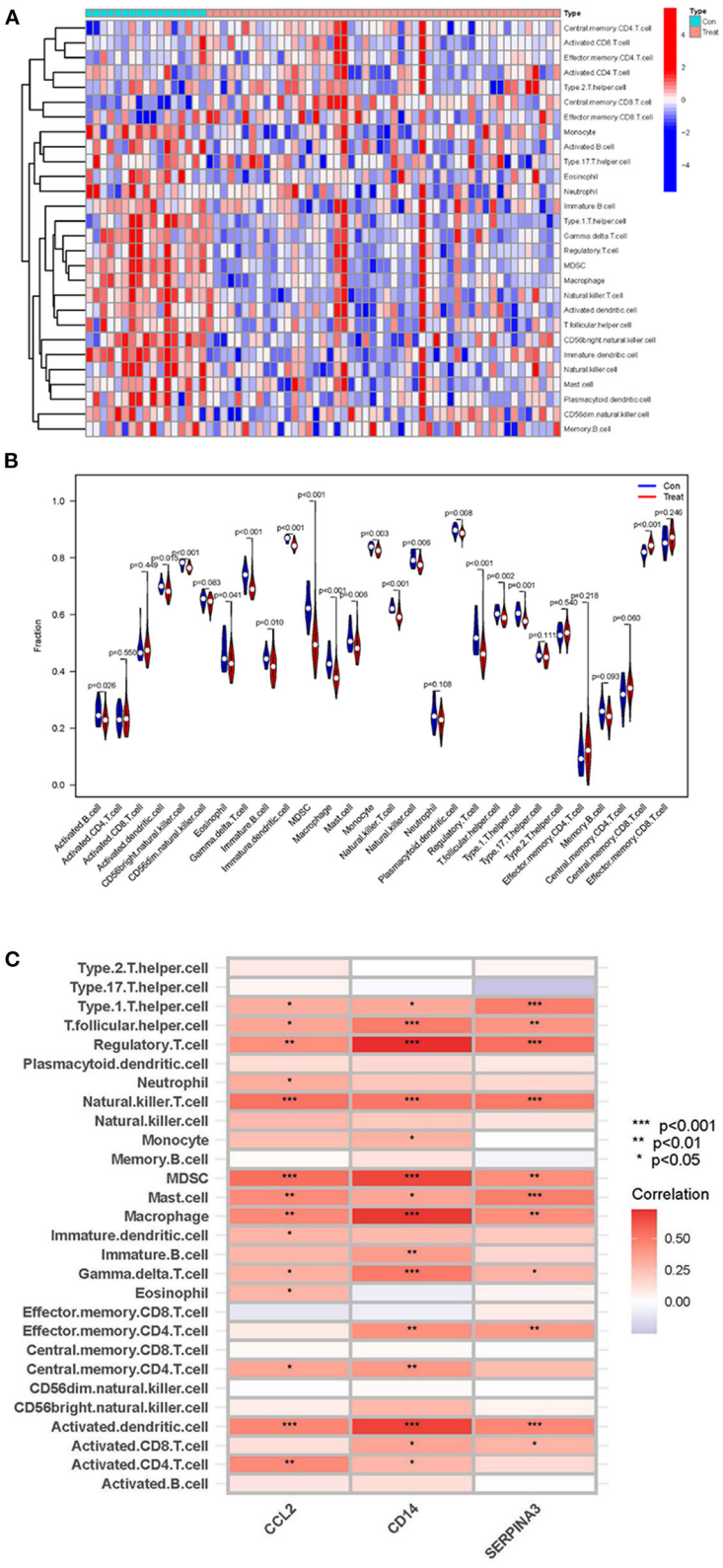

FIGURE 9
ssGSEA analysis of immune cell infiltration and its correlation with hub genes. **(A,B)** Heatmaps and violin plots showing the differences and distribution of 28 immune cells in cardiomyopathy and control groups. **(C)** The relation between immune cell infiltration and three hub genes; the redder the color, the more significant the difference. "***", "**", "*" represent $P < (0.001, 0.01, 0.05)$.

DEGs were predominantly enriched in chemokine, cytokine-cytokine receptor interaction and IL-17 signaling pathway. Cytokines participate in the coordination of the immune system during the host's defense, and their release after organism injury triggers innate and adaptive immunity (36). Cardiomyopathy may be triggered by cytokines, which are believed to have a role in the pathophysiology of several forms of cardiac dysfunction (37, 38). Th17 cells are a distinct subset of CD4+ T helper cells that primarily produce IL-17 cells, which link adaptive and innate immune responses. Activating IL-6, transforming growth factor-β and IL-1 in a pro-inflammatory cytokine milieu enables naive CD4+ T cells to differentiate and prime Th17 cells (36). Studies have found that the Th17/CD4+ T cells' imbalance may play a crucial part in the process of myocardial injury, and the higher the proportion of Th17 cells, the more obvious the decrease in cardiac function. However, the high expression of IL-17 can aggravate the induction of ventricular hypertrophy and myocardial fibrosis, leading to ventricular remodeling (39, 40). Finally, GSEA enrichment analysis of expression profile files showed that CD8+T cells, CD4+T cells and naive T cells had high enrichment scores in the cardiomyopathy group, while natural killer cells and regulatory T cells had high enrichment scores in the control group. CD4+ T cell subsets now include TH1 cells, TH2 cells, TH17 cells and regulatory T cells. Regulatory T cells, expressing CD25 and the transcription factor FoxP3, have immunomodulatory properties that help suppress inflammation and autoimmune diseases (41). As an adaptive immune response, TH1, TH2 cells, and the previously described Th17 cell subtype have been implicated in the progression of myocardial disease in numerous studies (4, 5). These findings highlighted the crucial significance of genes associated with adaptive immune cells in the incidence and development of cardiomyopathy, while regulatory and innate immune cells may be involved in the protection of the heart.

Finally, we analyzed the infiltration of 28 immune cells in the cardiomyopathy group and the non-cardiomyopathy control group using the ssGSEA algorithm. The findings indicated that in the cardiomyopathy group, myeloid-derived suppressor cells, regulatory T cells, type I helper T cells, and macrophages were lower compared to the control group. Further analysis of the most relevant immune infiltrating cells involved in the three hub genes showed that regulatory T cells, myeloid-derived suppressor cells, activated dendritic cells, macrophages and natural killer cells were most closely related to the hub genes. According to many scholars' studies, it demonstrated that that regulatory T cells, myeloid-derived progenitor cells, monocytes/activated macrophages, or dendritic cells can play a suppressive regulatory function in the myocardial disease development (42, 43). These results are consistent with our study. Based on the above analysis, we further proved that the control group was more involved in the innate immune response, while the adaptive immune response played an important role in the cardiomyopathy group. Our studies suggest that the heart may protect itself through regulatory T cells and innate immune cells; however, dysregulation and imbalance of innate immune cells and activation of adaptive immune responses are involved in cardiomyopathy disease progression in patients. These findings deepen our understanding of the immune system's role in cardiomyopathy progression. It can better guides scholars for clinical drug development and might help efforts to develop more targeted specific immunotherapy for cardiomyopathy.

Because of its limitations, this study can be validated further in prospective and larger-sample studies to eliminate invasive diagnostics and to give a guideline for early detection and focused medication development for cardiomyopathy of diverse etiologies. The reasons are as follows: Firstly, this study was retrospective and did not include all etiologies of cardiomyopathy, such as HCM, so a larger prospective study is needed to validate our conclusions. Secondly, the data set sample of the training group in this study is still small, so the accuracy of the assessment and prediction of the disease Hub gene can be improved by increasing the sample size. In addition, there are some other limitations that need to be highlighted. For example, further experimental validation using animal models of cardiomyopathy or tissue samples from human cardiomyopathy patients is needed. Moreover, the present study can only support the correlation analysis between cardiomyopathy and immune cells and between Hub genes and immune cells, but cannot reveal the cause-and-effect relationship. Finally, the subjects in this study may differ in terms of geography, race, living environment, genetic variation and susceptibility to cardiomyopathy. All these factors may have an impact on the study of cardiomyopathy.

In conclusion, with the intersection of multiple cardiomyopathy gene sets with different etiologies and WGCNA analysis and LASSO regression analysis, we screened out a key module (turquoise module) and three hub genes involved in cardiomyopathy progression (*CD14*, *CCL2*, and *SERPINA3*). After that, we combined bioinformatics analyses of GO, KEGG, GSEA, and ssGSEA. This study demonstrated differential expression of hub genes and their diagnostic ability as marker genes, but it also contributed to the common immune infiltration pattern of various cardiomyopathies and provided insights into the underlying immunomodulatory mechanisms.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

BH was engaged to write and conceptualize the original draft and were responsible for methodology. L-PQ, C-YC, D-YY, WY, Q-JW, ZZ, and X-NH were responsible for software. LL was responsible for reviewing and editing. The published version of the work has been reviewed and approved by all authors.

Funding

This study was sponsored by funds from the Chinese National Natural Science Foundation of China (81560076), Guangxi Natural Science Foundation Youth Program (2018JJB140358), Middle-aged and Young Teachers in Colleges and Universities in Guangxi Basic Ability Promotion Project (2021KY0534), and The First Batch of High-level Talent Scientific Research Projects of the Affiliated Hospital of Youjiang Medical University for Nationalities in 2019 (R20196316).

Acknowledgments

It was the GEO network that helped make this study possible. We are grateful for the freely available data from the GEO network.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.973279/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Systematic Review
PUBLISHED 08 September 2022
DOI 10.3389/fcvm.2022.942125

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 12 May 2022
ACCEPTED 15 August 2022
PUBLISHED 08 September 2022

CITATION
Yang D, Zhang Y, Yan J, Liu M and An F (2022) SGLT-2 inhibitors on prognosis and health-related quality of life in patients with heart failure and preserved ejection fraction: A systematic review and meta-analysis. *Front. Cardiovasc. Med.* 9:942125. doi: 10.3389/fcvm.2022.942125

SGLT-2 inhibitors on prognosis and health-related quality of life in patients with heart failure and preserved ejection fraction: A systematic review and meta-analysis

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Background: Heart failure with preserved ejection fraction (HFpEF) is becoming the main subtype of heart failure, but lacks proven effective therapies. Sodium-glucose cotransporter-2 (SGLT-2) inhibitor, a new kind of oral glucose-lowering agent, shows a great effect on improving cardiovascular outcomes. Based on the results of current RCTs, we perform this meta-analysis to illustrate the therapeutic impact of SGLT2i in HFpEF patients.

Methods: We systematically searched the online database and 10 RCTs were involved. The primary outcome was the prognosis outcome of HFpEF patients, including a composite outcome of cardiovascular (CV) death and hospitalization for heart failure (HHF), CV mortality, HHF, and all-cause mortality. Main secondary outcomes included improvement of KCCQ-TSS (Kansas City Cardiomyopathy Questionnaire and total symptom score) and 6-Minute Walk Test (6MWT). All pooled results were calculated by the random-effects model. Statistical heterogeneity was assessed using the chi-squared test and was quantified using the I-squared statistic.

Results: Ten RCTs comprising 10,334 patients were involved in. Incidence of composite outcome was reduced in SGLT-2 inhibitor group compared with placebo (HR: 0.78, 95% CI: 0.69–0.88, $p = 0.00$). Improvement of KCCQ-TSS was also more pronounced in the SGLT-2 inhibitor group (MD: 2.74, 95% CI: 1.30–4.18, $p = 0.00$). No statistical difference was observed in 6MWT.

Conclusion: Treating HFpEF patients with SGLT-2 inhibitors is associated with reducing the composite outcome of CV death and HHF and improving health-related quality of life. Further studies with more evidence are in need to confirm this conclusion.

KEYWORDS

sodium-glucose cotransporter-2 inhibitors, heart failure with preserved ejection fraction, prognosis, health-related quality of life, meta-analysis

Introduction

Heart failure with preserved ejection fraction (HFpEF), previously known as diastolic heart failure, is a subtype of heart failure characterized as left ventricular ejection fraction (LVEF) ≥50%. Until now, HFpEF has become the predominant form of heart failure worldwide, especially in aged population (1, 2). Despite recognizing the complexity of the specific clinical syndrome in the recent 20 years, the pathophysiology of HFpEF has not been illustrated explicitly. In consideration of the unclear mechanism, nor do people find a treatment that has a convincing clinical benefit in reducing mortality or morbidity of HFpEF. Compared with the recommended quadruple regimen of HFrEF, no Ia or Ib class recommendations were released in the 2021 ESC guideline (3).

Sodium-glucose cotransporter-2 (SGLT-2) inhibitor is a new class of oral glucose-lowering agents that can promote glucosuria and thus reduce serum glucose and lower blood pressure. A large amount of evidence has emerged that other than increasing glucosuria excretion in T2DM patients, SGLT-2 inhibitors can significantly improve cardiovascular outcomes, including decreasing the incidence of cardiovascular (CV) death and hospitalization for heart failure (HHF) (4–6). In the field of heart failure, meta-analysis of several large-scale RCTs has convinced that SGLT-2 inhibitors can reduce all-cause and cardiovascular death in HFrEF patients (7, 8).

As a result of the potential benefits, large-scale RCTs were also implemented to determine the effect SGLT-2 inhibitors might have on HFpEF. EMPEROR-Preserved trial, released in 2021, showed that empagliflozin reduced the combined cardiovascular risk in HFpEF patients, which made it the first large RCT to achieve a positive endpoint (9). Therefore, collecting existing RCTs, we performed this systematic review and meta-analysis to evaluate the prognosis of HFpEF population treated with SGLT-2 inhibitors. To understand health-related quality of life (HRQoL) and exercise capacity in the population, we also evaluated the improvement of the Kansas City Cardiomyopathy Questionnaire (KCCQ) score,6-Minute Walk Test (6MWT), and NT-proBNP levels.

Methods

This systematic review and meta-analysis followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (10) and the Cochrane Handbook for Systematic Reviews of Interventions (11). The entire process of our meta-analysis abides by PICOS criteria.

Data sources and search methods

Two independent investigators (DN.Y. and Y.Z.) independently searched the following online databases: PubMed, Embase, Cochrane Library, ClinicalTrials.gov, and SinoMed from the establishment of the databases till 5 May 2022. Search terms included MeSH terms "Heart Failure", "Heart Failure, Diastolic", "Sodium-Glucose Transporter 2 Inhibitors" and all relevant entry terms. A detailed searching strategy is shown in Supplementary materials. To ensure no relevant publications were overlooked, we also manually searched for qualifying publications in the reference lists of eligible articles. Only randomized controlled trials could be involved. No date limit was put in this meta-analysis.

Endpoints and study selection

The primary outcome of this meta-analysis was the prognosis outcome of HFpEF patients, including a composite outcome of CV death and HHF, CV mortality, HHF, and all-cause mortality. The main secondary outcome was the improvement of KCCQ-TSS (Kansas City Cardiomyopathy Questionnaire, Total Symptom Score) and 6MWT. Other secondary outcomes included improvement of KCCQ-CSS (Clinical Summary Score), KCCQ-OSS (Overall Summary Score), KCCQ-PL (Physical Limitation), and NT-proBNP level.

The studies included in our meta-analysis must meet all of the following criteria: (a) randomized, double-blind, placebo-controlled trials; (b) involve the HFpEF population, defined as patients with symptoms and signs of HF, with evidence of structural and/or functional cardiac abnormalities and/or raised natriuretic peptides (NPs), and with an LVEF > 40%; (c) compare SGLT-2 inhibitors with another placebo, or add SGLT-2 inhibitors in a standard diabetic therapy; (d) report any of the following outcomes: a composite outcome of CV death and HHF, all-cause mortality, KCCQ score of subscales, 6MWT, and NT-proBNP level. Publications were excluded from the meta-analysis if they were (a) conference reports, reviews, case reports, or summaries; (b) studies published in a language other than English or Chinese; and (c) head-to-head studies compared SGLT-2 inhibitors with other glucose-lowering agents.

Data collection and quality assessment

Two reviewers (DN.Y. and Y.Zh.) independently extracted data of interest using an electronic data collection form designed preciously. The extracted data mainly include the following: (a) Baseline characteristics of included studies: trial name and trial number, study design, subgroup population, details of treatments, publication time, and follow-up duration; (b) Baseline characteristics of patients: gender, age, NYHA class,

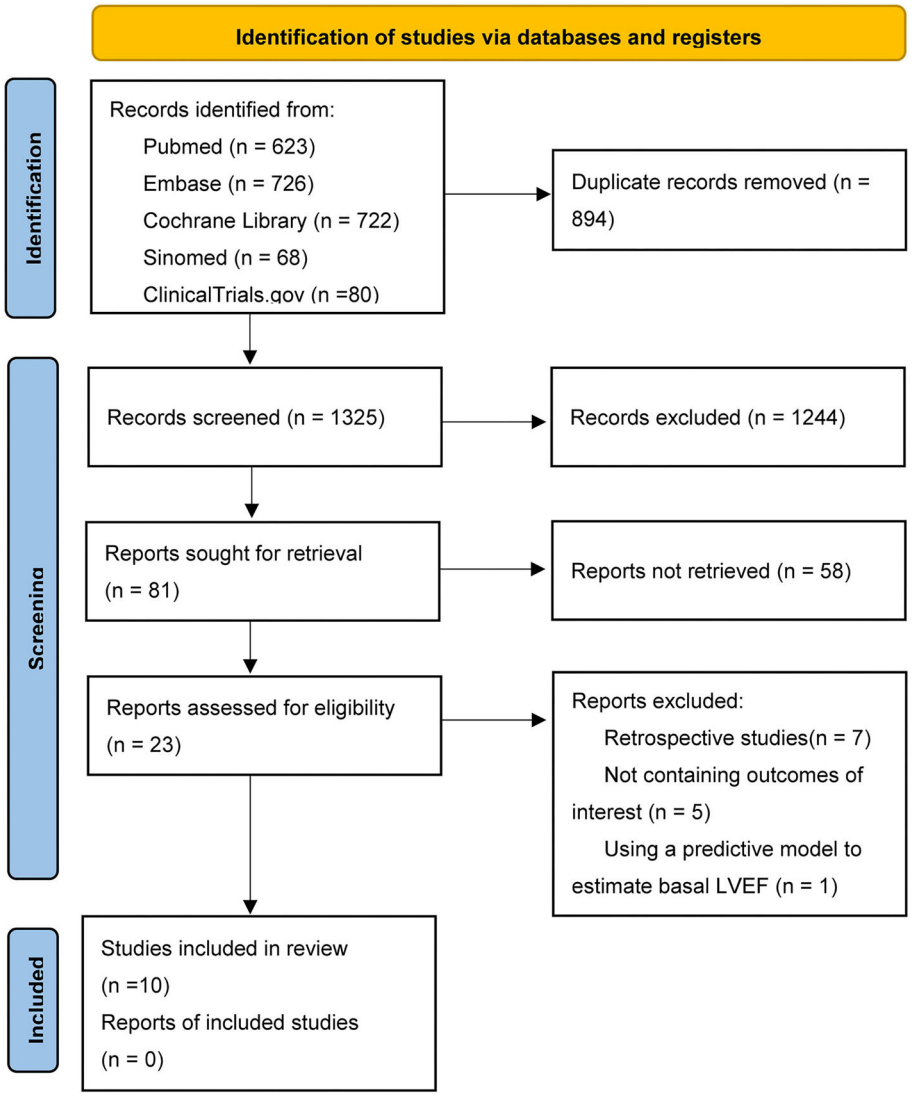

FIGURE 1
PRISMA flowchart of selection.

LVEF, NT-proBNP level, blood pressure, eGFR, and so on; (c) Study outcomes: A composite outcome of CV death and HHF, CV mortality, HHF, all-cause mortality, KCCQ score of subscales, 6MWT, and NT-proBNP level.

The quality of each study was assessed by two reviewers (DN.Y. and Y.Zh.) separately using the Cochrane Collaboration's tool for assessing risk of bias, which includes the following seven domains: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias (12). All disagreements were resolved with consensus by a third reviewer (J.Y.)

Statistical analysis and certainty of the evidence

This meta-analysis was performed using Stata16.0. For the composite outcome of CV death and HHF, we extracted hazard ratios (HRs) and corresponding 95% confidence intervals (95% CIs) to make a pooled estimate. For CV mortality, HHF, and all-cause mortality, we used odd ratios (ORs) to measure the intervention effects of dichotomous outcomes. For KCCQ and 6MWT, we extracted adjusted mean differences (MDs) and 95% CIs from original studies. The level of NT-proBNP was analyzed using SMD. The random-effects model was used to calculate the pooled effect, and only when $p < 0.05$, the results were considered to be statistically meaningful. Statistical

heterogeneity was assessed using the chi-squared test ($p < 0.10$ was considered statistically significant for heterogeneity) and was quantified using the I-squared statistic ($I^2 > 50\%$ was considered substantial heterogeneity). Sensitivity analysis was performed to examine the robustness of the results and the effect of potential effect modifiers.

Evidence summaries were prepared for each outcome using GRADEprofiler (Version 3.6). Certainty of the evidence was assessed using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) system. The confidence of the estimate effect can be categorized into four levels: very low, low, moderate, and high, and RCTs are classified into the highest grade. Reasons for downgrading the evidence included risk of bias, inconsistency, indirectness, imprecision, and publication bias (13).

Results

The selection process is shown in Figure 1. We searched a total of 2,219 literature, and among them, 1,325 studies were screened based on the abstracts and titles. Finally, 11 studies involving 10 RCTs were included in this meta-analysis (9, 14–23). It should be mentioned that among them, the DETERMINE-preserved trial has not been published yet, whereas we included the results published in ClinicalTrials.gov (23). About 10,334 patients who met our inclusion criteria were randomized to either the SGLT-2 inhibitor group or placebo group. There were four dapagliflozin trials (14, 20, 22, 23) (1,636 patients), two empagliflozin trials (9, 16, 17) (6,203 participants), two sotagliflozin trials (18, 19) (921 participants), one ertugliflozin trial (15) (1,007 participants), and one canagliflozin trial (21) (267 participants). The characteristics of included studies and patients are shown in Table 1.

Quality assessment is shown in Figure 2. Three of the 10 studies had a relatively greater risk of bias because of the possible mistakes during the trial. Given that no more than 10 studies were involved in each outcome, no funnel plot was conducted to detect publication bias.

Cardiovascular mortality and hospitalization for heart failure

Five studies (9, 14, 15, 18, 19), accessible for their hazard ratios (HRs) and corresponding 95% confidence intervals (CIs), were selected to evaluate the effects of SGLT-2 inhibitors on a composite of CV death and HHF. Meta-analysis showed that treating with SGLT-2 inhibitors decreased the incidence of the composite outcome (HR: 0.77, 95% CI: 0.65–0.91, $p = 0.00$; Figure 3A). No obvious statistical heterogeneity was observed between the studies ($I^2 = 31.6\%$, $p = 0.211$). When eliminating each study, the result kept stable. Subgroup analysis illustrated a consistency no matter whether patients had T2DM (Supplementary Figure 1).

Combination of three relevant trials (9, 14, 15) indicated that treatment with SGLT-2 inhibitors could lower incidence of hospitalization for heart failure (OR: 0.71, 95% CI: 0.61–0.83, $p = 0.00$; $I^2 = 0.00\%$, $p = 0.970$; Figure 3B). However, we did not observe significant difference in CV mortality (9, 14, 15) (OR: 1.02, 95% CI: 0.77–1.35, $p = 0.888$; $I^2 = 35.5\%$, $p = 0.212$; Figure 3C) when treating with SGLT2i. Seven studies (9, 14–16, 20, 22, 23) reported statistics to do with all-cause mortality. The results indicated that SGLT-2 inhibitors showed no advantage in reducing all-cause mortality (OR: 0.99, 95% CI: 0.87–1.13, $p = 0.936$; $I^2 = 0.00\%$, $p = 0.973$; Figure 3D). In consideration that the follow-up duration of included studies showed a great difference, we implemented a subgroup analysis which showed no difference (Supplementary Figure 2).

Health-related quality of life

Five studies (9, 16, 17, 20, 21, 23) reported the therapeutic effect of SGLT-2 inhibitors in HFpEF patients using health-related quality of life outcomes measured by the KCCQ-23 Scale. Given that we set KCCQ-TSS as our main observation subscale, after estimating, the SGLT2i group showed a greater improvement in KCCQ-TSS from baseline compared with placebo (MD:2.74, 95% CI: 1.30–4.18, $p = 0.00$; Figure 4A). Statistical heterogeneity between the studies was not present ($I^2 = 30.9\%$, $p = 0.215$). The result remained stabilized when we eliminated each study one by one.

We also measured other subscales of KCCQ reported in the incorporated trials, including KCCQ-PL, KCCQ-CSS, and KCCQ-OSS. Four studies reported statistics of KCCQ-PL. The mean treatment difference between the two groups was not significant (MD:1.66, 95% CI: −0.67 to 3.98, $p = 0.162$; $I^2 = 64.3\%$, $p = 0.038$; Figure 4B). Three studies reported KCCQ-CSS and KCCQ-OSS. However, meta-analysis also indicated that SGLT-2 inhibitors did not show significant effects in the two aspects compared with placebo (KCCQ-CSS: MD: 2.13, 95% CI: −0.65 to 4.90, $p = 0.133$; $I^2 = 72.6\%$, $p = 0.026$; Figure 4C; KCCQ-OSS: MD:1.66, 95% CI: −0.29 to 3.62, $p = 0.096$; $I^2 = 51.2\%$, $p = 0.129$; Figure 4D).

Exercise capacity outcomes

Three trials (16, 20, 23) worked on 6MWT. However, our research did not indicate that short-term treatment with SGLT-2 inhibitors would improve exercise capacity (MD: 6.70, 95% CI: −2.31 to 15.71, $p = 0.145$; Figure 5). Statistical heterogeneity between the studies was present ($p = 0.083$, $I^2 = 59.9\%$). When

TABLE 1A Basal characteristics of included studies.

Clinical trial	Publication time	Study design	Intervention	Follow-up duration	LVEF inclusion criteria	T2DM,%	Total amount	End points*
DECLARE-TIMI 58 NCT01730534	2019	Randomized Controlled Trial	Dapagliflozin/10 mg qd placebo	4.2 years	LVEF ≥ 45%	100%	808	1,2,3,4
VERTIS CV NCT01986881	2020	Randomized Controlled Trial	Ertugliflozin/5 mg qd or 15 mg qd placebo	3.5 years	LVEF > 45%	100%	1,007	1,2,3,4
EMPERIAL-Preserved NCT03448406	2020	Randomized Controlled Trial	Empagliflflozin/10 mg qd placebo	12 weeks	LVEF > 40%	51.10%	315	4,5,6,7
EMPEROR-Preserved NCT03057951	2021	Randomized Controlled Trial	Empagliflozin/10 mg qd placebo	26.2 months	LVEF > 40%	49.10%	5,988	1,2,3,4,5
SOLOIST-WHF NCT03521934	2021	Randomized Controlled Trial	Sotagliflozin/200 mg qd placebo	9 months	LVEF ≥ 50%	100%	256	1
SCORED NCT03315143	2021	Randomized Controlled Trial	Sotagliflozin/200 mg qd placebo	16 months	LVEF ≥ 50%	100%	665	1
PRESERVED-HF NCT03030235	2021	Randomized Controlled Trial	Dapagliflozin/10 mg qd placebo	12 weeks	LVEF ≥ 45%	55.90%	324	4,5,6,7
TANG Xiaodi, FAN Ying, etc.	2021	Randomized Controlled Trial	Dapagliflozin/10 mg qd conventional treatment	24 weeks	LVEF ≥ 50%	100%	200	4,7
CHIEF-HF NCT04252287	2022	Randomized Controlled Trial	Canagliflozin/100 mg qd placebo	12 weeks	LVEF ≥ 45%	Not 100%, NA	267	5
DETERMINE-preserved NCT03877224	No publication	Randomized Controlled Trial	Dapagliflozin/10 mg qd placebo	16 weeks	LVEF > 40%	Not 100%, NA	504	4,5,6

LVEF, left ventricular ejection fraction; T2DM, type 2 diabetes mellitus.
*Endpoints: 1. CV death and HHF; 2. hospitalization for heart failure; 3. cardiovascular mortality; 4. all-cause mortality; 5. KCCQ; 6. 6MWT; 7. NT-proBNP level.

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TABLE 1B Basal characteristics of included studies.

Clinical trial	Intervention	Patient size	Age, y	Male, %	Body mass index, kg/m²	LVEF, %	NYHA				Heart rate, bpm	Systolic pressure, mmHg	Median NT-proBNP concentration, pg/mL
							I	II	III	IV			
DECLARE-TIMI 58	Dapagliflozin	399	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Placebo	409	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
VERTIS CV	Ertugliflozin	680	63.8 ± 8.3	65.6	32.6 ± 5.3	NA	22.5	67.1	7.1	0.1	NA	NA	NA
	Placebo	327	64.7 ± 8.2	63.3	32.9 ± 5.3	NA	25.7	67.6	4.6	0	NA	NA	NA
EMPERIAL-Preserved	Empagliflflozin	157	73.6 ± 8.2	55.4	30.3 ± 5.8	51.9 ± 9.7	0.7	74.5	24.8	0	70.6 ± 12.7	127.6 ± 18.7	966 (572, 1,653)
	Placebo	158	74.6 ± 9.7	58.2	29.3 ± 5.0	52.6 ± 9.7	0	79.7	20.3	0	70.4 ± 12.7	132.1 ± 18.7	843 (407, 1,913)
EMPEROR-Preserved	Empagliflozin	2997	71.8 ± 9.3	55.4	29.8 ± 5.8	54.3 ± 8.8	0.1	81.1	18.4	0.3	70.4 ± 12.0	131.8 ± 15.6	994 (501, 1,740)
	Placebo	2991	71.9 ± 9.6	55.3	29.9 ± 5.9	54.3 ± 8.8	<0.1	81.9	17.8	0.3	70.3 ± 11.8	131.9 ± 15.7	946 (498, 1,725)
SOLOIST-WHF	Sotagliflozin	127	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Placebo	129	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SCORED	Sotagliflozin	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Placebo	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PRESERVED-HF	Dapagliflozin	162	70.1 ± 9.7	43.2	35.8 ± 8.5	60.0 ± 7.5	59.3	40.1		0.6	69.3 ± 12.0	135.4 ± 23.9	641 (373, 1,210)
	Placebo	162	70.6 ± 11.2	43.2	34.9 ± 8.0	59.6 ± 8.2	55.6	44.4		0	68.4 ± 9.7	132.7 ± 22.4	710 (329, 1,449)
TANG Xiaodi,etc.	Dapagliflozin	100	63.4 ± 11.0	61	25.0 ± 3.2	NA	0	62.0	38.0	0	NA	NA	938 (469, 1,407)
	Conventional treatment	100	63.6 ± 14.8	60	25.4 ± 3.1	NA	0	69.0	31.0	0	NA	NA	932 (466, 1,398)
CHIEF-HF	Canagliflozin	132	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	placebo	135	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
DETERMINE-preserved	Dapagliflozin	253	72.0 ± 9.1	64	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Placebo	251	71.7 ± 9.7	62	NA	NA	NA	NA	NA	NA	NA	NA	NA

Data reported as mean ± SD or median (interquartile range).
LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; bpm, beats/minute.

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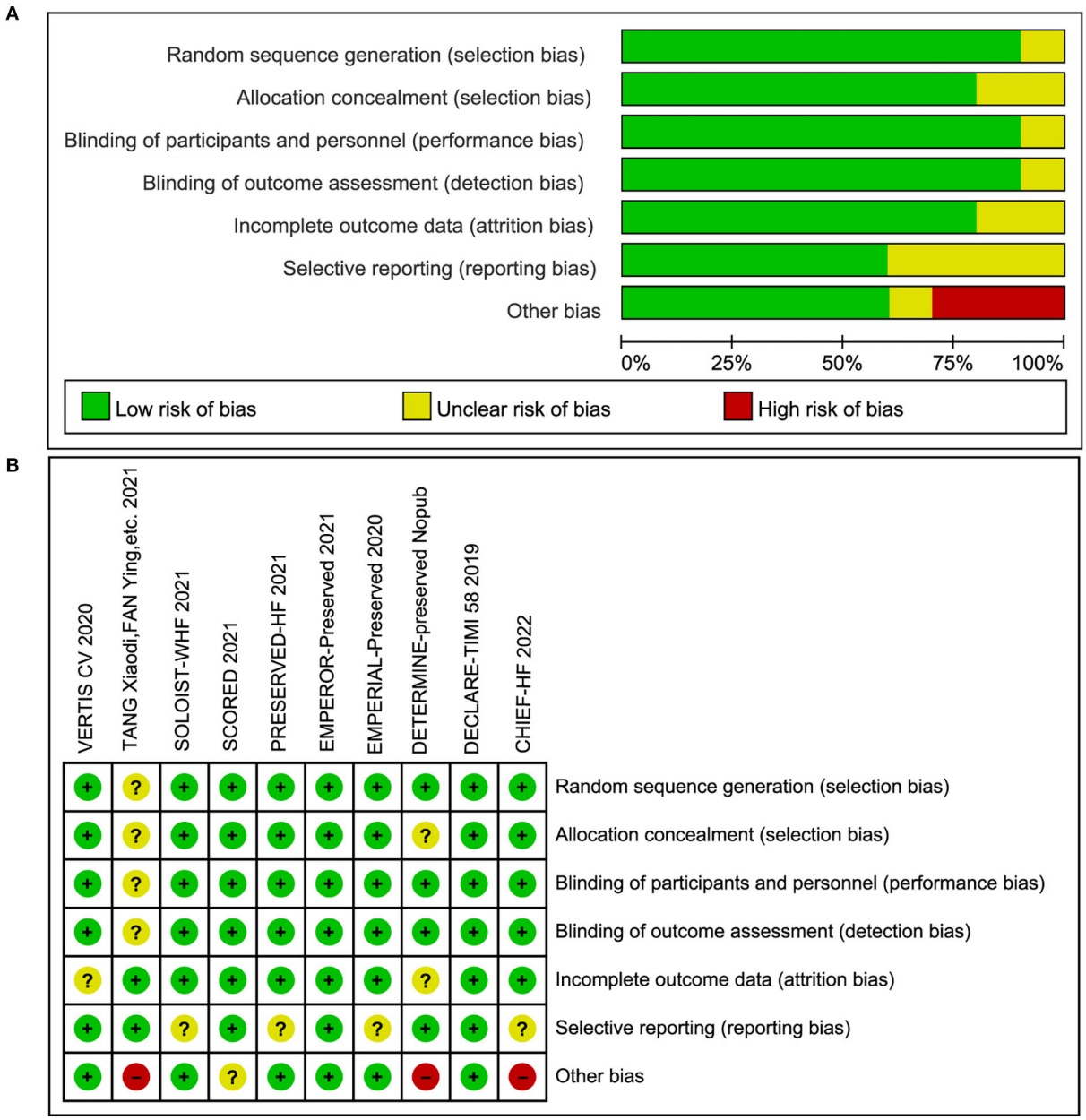

FIGURE 2
(A) Risk of bias graph. **(B)** Risk of bias summary.

we excluded the PRESERVED-HF trial, the overall MD was 2.58 m (95% CI −3.16 to 8.31, $p = 0.379$) and there was no significant heterogeneity ($p = 0.687$, $I^2 = 0\%$).

Serum NT-proBNP level

Three trials (16, 20, 22) provided accessible statistics associated with serum NT-proBNP levels. We could not observe a significantly statistical difference in SGLT2i therapy group compared with other treatments (SMD: −0.09, 95% CI: −0.30 to 0.12, $p = 0.388$; $I^2 = 55.5\%$, $p = 0.106$; Figure 6).

Certainty of evidence

Certainty of evidence, as shown in Table 2, was evaluated by GRADE methodology. All studies included were RCTs and thus

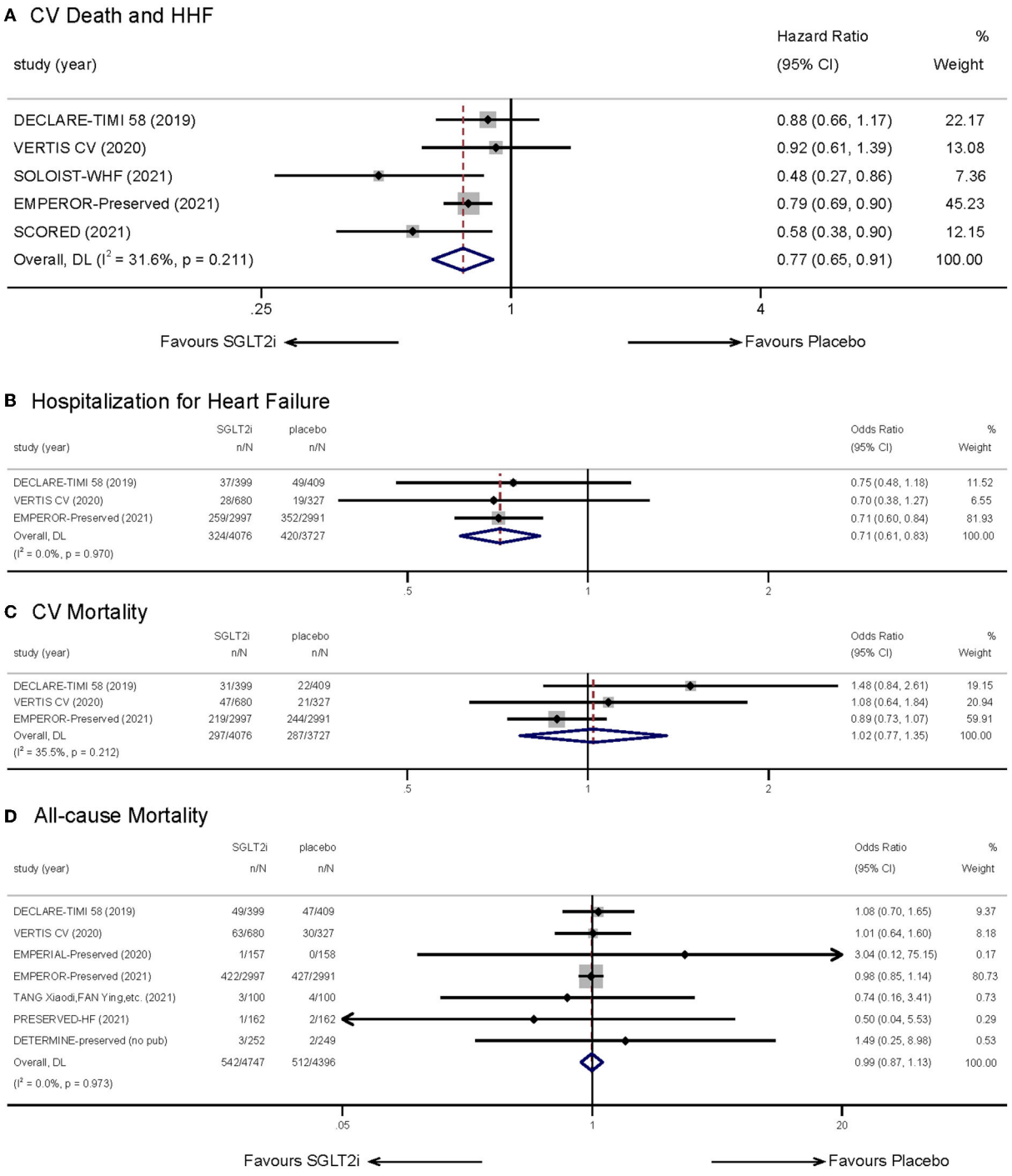

FIGURE 3
Effect of SGLT-2 inhibitors vs. placebo on the primary outcome of **(A)** cardiovascular death and hospitalization for heart failure; **(B)** hospitalization for heart failure; **(C)** cardiovascular mortality; **(D)** all-cause mortality.

were originally classified into the highest grade. After evaluation of primary and main secondary outcomes, exercise capacity was degraded to moderate evidence as a result of the presentation of heterogeneity.

Discussion

This meta-analysis, covering over 10,300 HFpEF patients in 10 prospective studies, explicitly demonstrated that treatment

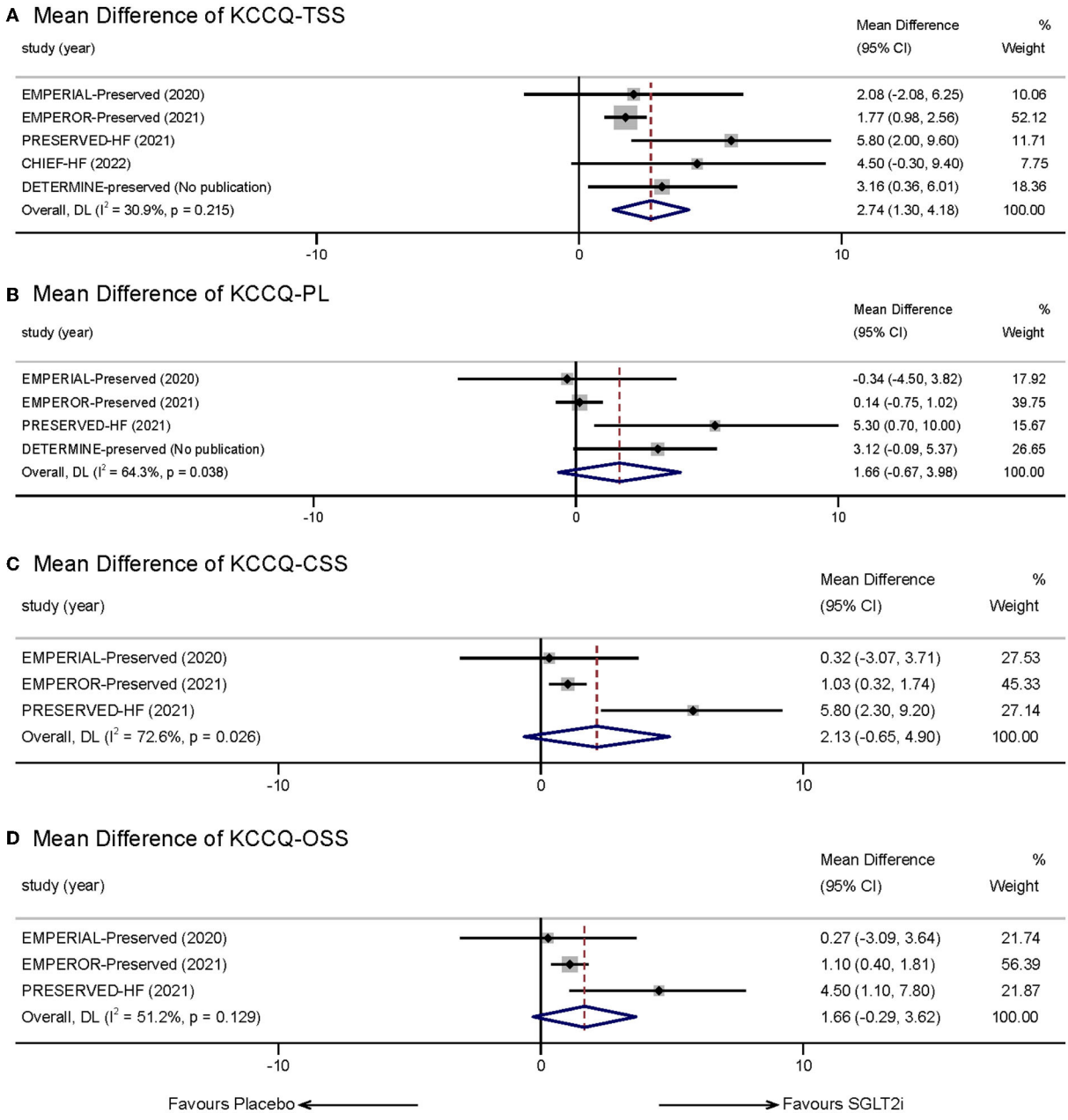

FIGURE 4
Effect of SGLT-2 inhibitors vs. placebo on KCCQ Subscales: **(A)** KCCQ-TSS; **(B)** KCCQ-PL; **(C)** KCCQ-CSS; **(D)** KCCQ-OSS.

with SGLT-2 inhibitors could lower the incidence of a composite outcome including CV death and HHF in the HFpEF population. In addition, SGLT-2 inhibitors showed a beneficial impact on decreasing events for HHF separately. We also made an analysis to illustrate the amelioration in health-related quality of life after SGLT2i therapy, which specified significant excellence in improving symptom-relevant KCCQ score.

For a long time, it was discouraged when referred to therapeutic medications dealing with HFpEF. It is now widely believed that originated from various risk factors such as overweight, hypertension, and diabetes mellitus (24), complicated physiology and molecular processes are involved in the onset and development of HFpEF, including systematic inflammation, LV structural remodeling, and abnormal hemodynamics (1, 25). Large-scale trials with several medications, including RAAS inhibitors, aldosterone antagonists (MRAs), and angiotensin receptor/neprilysin inhibitors (ARNIs), which had confirmed notable benefits to improve the prognosis of HFrEF patients, did not show superiority for their primary efficacy endpoints in HFpEF

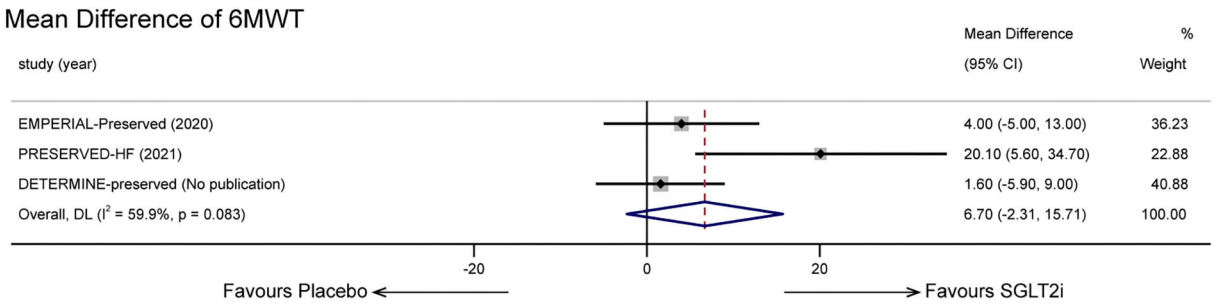

FIGURE 5
Effect of SGLT-2 inhibitors vs. placebo on 6-Minute Walking Test.

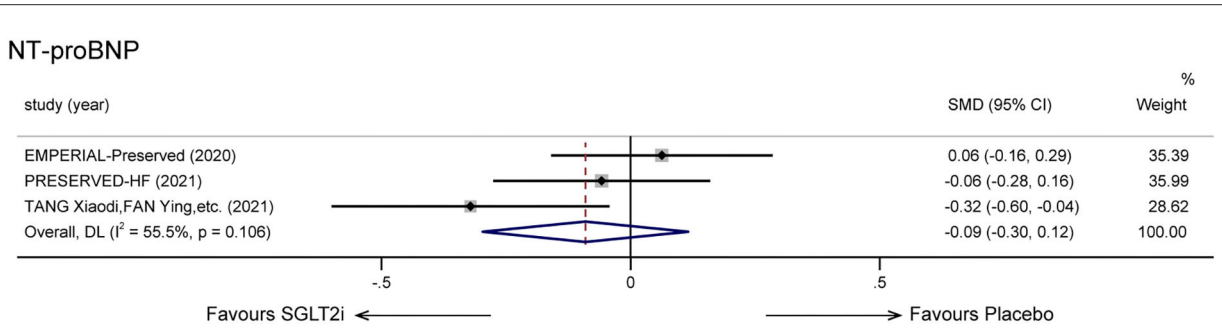

FIGURE 6
Effect of SGLT-2 inhibitors vs. placebo on NT-proBNP.

(26–28). Despite there existed studies showing merits in some aspects of HFpEF treatment, for example, lowering NT-proBNP levels (29, 30), and improving LV diastolic function (31), these data had not been widely endorsed by international guidelines (32). However, the situation seemed to start changing when the EMPEROR-Preserved group announced their results in 2021. A 21% lower relative risk on the primary outcome compared with placebo group made empagliflozin the first and only drug therapy significantly improving prognosis outcomes of HFpEF, no matter whether T2DM existed (9). In a *post-hoc* analysis of the EMPA-REG OUTCOME trial, empagliflozin similarly reduced the risk of HHF or CV mortality in predicted HFpEF patients with T2DM (33). Compared with some earlier subgroup analyses that proved ineffective in improving prognosis, the efficacy of SGLT-2 inhibitors seemed to be conflicted and needed further exploration. This meta-analysis, making a pooled estimate of several clinical trials related to HFpEF, indicated that (a) intervening with SGLT-2 inhibitors could decrease 23% relative risk of the composite outcome including cardiovascular death and hospitalization for heart failure; (b) the results of primary outcome remained consistent no matter T2DM existed; and (c) the incidence of hospitalization for heart failure was also reduced, whereas CV mortality and all-cause mortality remained unchanged. The strength of SGLT-2 inhibitors in improving prognosis outcomes in HFpEF patients was confirmed in our study, and this could provide direct evidence for the following therapeutic options.

Health-related quality of life (HRQoL) can also be improved in HFpEF patients after treatment with SGLT-2 inhibitors. Patient-reported HRQoL is considered standardized information reflecting a patient's current health status and prognostic implications. Poor HRQoL not only means aggravation of heart failure symptoms and emergency of adverse events but also gives an implication of all-cause death and the composite of death or HF hospitalization, especially in HF patients with a preserved fraction (34, 35). Thus, we evaluated the improvement of HRQoL by making a pooled estimate of KCCQ-23, which has been qualified by the U.S. Food and Drug Administration as a patient-reported clinical assessment tool in heart failure (36). KCCQ Scale, comprising seven domains and 23 items, gives different manifestations of heart failure, including symptoms, functional limitation, and quality of life, by dividing different domains into four subscales—KCCQ-TSS, KCCQ-PL, KCCQ-CSS, and KCCQ-OSS (36). Our meta-analysis put KCCQ-TSS as a main observe indicator as we were more interested in patient-reported improvement in symptoms. In this meta-analysis, treating with SGLT-2 inhibitors could lead to significant 2.74 points higher KCCQ-TSS than the placebo

TABLE 2 Summary of findings.

Outcome	Quality assessment							Effect		Quality
	No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Relative (95% CI)	Absolute	
CV Death and HHF	5	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	HR 0.78 (0.69 to 0.88)	22 fewer per 1000 (from 12 fewer to 31 fewer)	⊕⊕⊕⊕ HIGH[2]
HHF	3	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	OR 0.71 (0.61 to 0.83)	30 fewer per 1000 (from 17 fewer to 41 fewer)	⊕⊕⊕⊕ HIGH[2]
CV Mortality	3	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	RR 1.02 (0.77 to 1.35)	2 more per 1000 (from 18 fewer to 27 more)	⊕⊕⊕⊕ HIGH[2]
All-cause Mortality	7	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	OR 0.99 (0.87 to 1.13)	1 fewer per 1000 (from 14 fewer to 13 more)	⊕⊕⊕⊕ HIGH[2]
KCCQ-TSS	5	randomised trials	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	none	MD 2.74(1.30 to 4.18)	MD 2.74 higher (1.3 to 4.18 higher)	⊕⊕⊕⊕ HIGH[2]
6-Minute Walking Test	3	randomised trials	no serious risk of bias	serious[1]	no serious indirectness	no serious imprecision	none	MD 6.70(-2.31 to 15.71)	MD 6.70 higher (2.01 lower to 15.71 higher)	⊕⊕⊕ MODERATE[2]

CV, Cardiovascular; HHF, Hospitalization for heart failure; KCCQ, the Kansas City Cardiomyopathy Questionnaire; TSS, Total symptom score; HR, Hazard ratio; OR, Odd ratio; MD, Mean Difference.

[1]Statistical heterogeneity showed $P<0.1$, $I^2>50\%$.

[2]High quality means further research is very unlikely to change our confidence in the estimate of effect, and moderate quality means further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

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group in total and that means patients are more likely to get remission for heart failure symptoms, such as fatigue, dyspnea, and edema after a same course of treatment. Given that the follow-up duration of the involved studies is relatively short (12 weeks in four trials and 16 weeks in one trial), we can probably infer that short-term treatment with SGLT-2 inhibitors can still improve health status. Another publication of the EMPEROR-Preserved trial revealed Weeks 32 and 52 results of KCCQ score, and a significant increase was observed across all domains of the KCCQ Scale except physical limitation (17). Earlier studies of other medications showed limited evidence in promoting health-related quality of life of HFpEF patients; therefore, our findings of SGLT2i demonstrated the ability on remitting patient-reported HF symptoms. KCCQ-CSS and KCCQ-OSS, with relatively greater heterogeneity, did not show significant differences in our study. The lack of relevant literature and statistics may account for the results. Ongoing clinical trials may provide us with more evidence to clarify the improvement of KCCQ Scales in the coming days.

As for exercise capacity outcomes, no statistically meaningful results were observed. It is generally believed that performance in 6MWT is associated with the health status and prognosis of HF patients (37). Several explanations may explicate the controversial result. First, studies related to 6MWT were limited. The involved patient size was small, and the follow-up duration was relatively short. Second, the heterogeneity between groups was great, and the initial NYHA class was unbalanced. More than 55% of patients in PRESERVED-HF were initially divided into class I, which could explain the greater improvement in the PRESERVED-HF trial. Third, there may be a dissociation between exercise capacity and heart failure, as it can also be influenced by respiratory and musculoskeletal systems (38). Assessing peak oxygen consumption (peak VO_2) during cardiopulmonary exercise testing (CPET), recommended in recent years, provides more precise information on functional capacity from a cardiovascular intervention compared with 6MWT (39). In the field of HFrEF, there have been studies assessing the effective parameter (40). Regretfully, clinical trials relevant to HFpEF and SGLT2i have yet involved the parameter, and it may be another direction to clarify the functional capacity effect. We did not observe a significant disparity in NT-proBNP levels compared to SGLT-2 inhibitors with placebo as well. In the EMPEROR-Preserved trial, mean differences emerged after a follow-up period of 52 weeks (9). Taking follow-up duration into account, we did not enroll in this trial as other trials had a relatively short duration time. However, SGLT-2 inhibitors did not show a better effect in lowering NT-proBNP levels in our meta-analysis. Trials with shorter-term or longer-term duration time are in need to make clear the effect on new-onset or chronic HFpEF.

Our finding shows the prospect of SGLT2i in the management of HFpEF. Previous studies and reviews of the SGLT-2 inhibitors in HF models illustrated some potential mechanisms of the cardioprotective effects which might help us understand how it works (41). First, SGLT-2 inhibitors show unique diuretic and natriuretic effects without neurohormonal activation or electrolyte disturbance (42, 43). This offers advantages in the management of volume status in patients with heart failure. Second, after treatment with SGLT2i, a metabolic switch occurs through reducing cardiac glucose oxidation and increasing utilization of ketone bodies and fatty acids (44, 45). Improvement of myocardial metabolism brings more efficient supply of energy and less accumulation of toxic products. An improved mitochondrial function may also be involved in the process (46). Third, LV diastolic function can be significantly ameliorated on account of reducing interstitial myocardial fibrosis and decreasing cardiomyocytes' stiffness (47). Besides, inhibition of myocardial Na+/H+ exchanger (NHE) and reduction of epicardial adipose can also make sense in the process (41). However, it is becoming more and more widely believed that rather than a simple glucose-lowering effect, the cardioprotective effect of SGLT2i is a combined pathophysiological process involving heart, kidney, vasculature, and even the whole body (48).

To sum up, treatment with SGLT-2 inhibitors makes a positive impact on prognosis outcomes in the HFpEF population and that may arise from the decrease of HHF. Although the incidence of CV death and all-cause death has not been proven to have a reduction, fewer events of rehospitalization indicate better quality of life and greater healthy status. Our finding of improvement in KCCQ-TSS conveys similar information. A higher score of KCCQ-TSS illustrated a present relief, at least symptomatically, after a short period of remedy of SGLT-2 inhibitors. What we mentioned above is consistent with the present therapeutic goal of reducing symptoms in HFpEF patients (3).

Based on the available results, SGLT-2 inhibitors could become a preferred choice in the following days when it comes to medications for HFpEF. In the 2022 AHA Guideline for the management of heart failure, the application of SGLT-2 inhibitors in HFpEF patients was first put forward and given a 2a class recommendation (49). As yet, there are still many ongoing or unpublished trials committing to clarifying the therapeutic effect of SGLT2i in the HFpEF population, and among them, the most compelling one may be the DELIVER trial, which is the largest and broadest trial in patients with HFmrEF or HFpEF intervening with SGLT2i (50). According to an internal announcement released by AstraZeneca, DELIVER confirmed that dapagliflozin reached a statistically significant and clinically meaningful reduction in the primary composite endpoint of CV death or worsening HF, and this is consistent with our conclusion. Complete results of DELIVER will be published in 2022 ESC Congress, and the efficacy of SGLT-2 inhibitors in HFpEF population will be further confirmed.

The limitations of this meta-analysis are as follows. First, the follow-up duration of included studies was diverse, from 12 weeks to 4.2 years, and that led to some selection when discussing certain outcomes to eliminate heterogeneity. Second, the LVEF cutoffs of included studies were variable. 2021 ESC Guideline and 2022 AHA Guideline all set LVEF $\geq$ 50% as a diagnostic criterion for HFpEF, but the number of studies completely met this standard was limited as LVEF $>$ 40% or LVEF $>$ 45% was thought general inclusion criteria in previous studies. Third, included statistics of reported outcomes were restricted because of the finite amount of original studies. Further subgroup analysis or regression analysis was not performed in this meta-analysis as well. Fourth, the majority of patients involved in our meta-analysis had diabetes. More evidence is needed to confirm the therapeutic effect in HFpEF patients without diabetes.

Conclusion

As far as we know, our meta-analysis first illustrates remission of symptoms and improvement of prognosis at the same time in HFpEF patients using SGLT-2 inhibitors. Significant improvement in cardiovascular outcomes and health-related quality of life in HFpEF patients are explicated after the pooled estimate, and our results may provide support in therapeutic options and guideline development of HFpEF in the coming day.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

DY: methodology, statistical analysis, quality assessment, and write and polish the draft. YZ: study selection, data collection, bias assessment, and write the draft. JY and ML: data visualization and consultation. FA: review and edit the manuscript. All authors reviewed the manuscript, gave their final approval, and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

Funding

This study was supported by the National Natural Science Foundation of China (Nos. 81670325 and 81970368) and the Key Research and Development Project of Shandong Provence (No. 2019GSF108124).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.942125/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 29 September 2022
DOI 10.3389/fcvm.2022.984092

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 01 July 2022
ACCEPTED 07 September 2022
PUBLISHED 29 September 2022

CITATION
Katsiadas N, Xanthopoulos A, Giamouzis G, Skoularigkis S, Skopeliti N, Moustaferi E, Ioannidis I, Patsilinakos S, Triposkiadis F and Skoularigis J (2022) The effect of SGLT-2i administration on red blood cell distribution width in patients with heart failure and type 2 diabetes mellitus: A randomized study. *Front. Cardiovasc. Med.* 9:984092. doi: 10.3389/fcvm.2022.984092

The effect of SGLT-2i administration on red blood cell distribution width in patients with heart failure and type 2 diabetes mellitus: A randomized study

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Background: Recent studies suggest that the pivotal mechanism of sodium glucose co-transporter-2 inhibitors (SGLT-2i) favorable action in patients with heart failure (HF) and type 2 diabetes mellitus (DM) is the stimulation of erythropoiesis *via* an early increase in erythropoietin (EPO) production which leads to hematocrit rise. Red blood cell distribution width (RDW) is a simple hematological parameter which reflects the heterogeneity of the red blood cell size (anisocytosis). Since, EPO has been also implicated in the pathophysiology of RDW increase, the current mechanistic study examined the effect of SGLT-2i administration on red blood cells size (RDW) in patients with HF and DM.

Methods: The present was a prospective single-center study. Patients (N=110) were randomly assigned to dapagliflozin (10 mg a day on top of antidiabetic treatment) or the control group. Inclusion criteria were: (a) age > 18 years, (b) history of type 2 DM and hospitalization for HF exacerbation within 6 months. The evaluation of patients (at baseline, 6 and 12 months) included clinical assessment, laboratory blood tests, and echocardiography. Data were modeled using mixed linear models with dependent variable the RDW index. In order to find factors independently associated with prognosis (1-year death or HF rehospitalization), multiple logistic regression was conducted with death or HF rehospitalization as dependent variable.

Results: An RDW increase both after 6 and after 12 months was observed in the SGLT-2i (dapagliflozin) group ($p < 0.001$ for all time comparisons), whereas RDW didn't change significantly in the control group. The increase in RDW was positively correlated with EPO, while negatively correlated with ferritin and folic acid ($p < 0.005$ for all). Baseline RDW was significantly associated with 1-year death or rehospitalization, after adjusting for group (SGLT-2i vs. control), age, gender, smoking and BMI at baseline.

Conclusion: RDW increased with time in patients with HF and DM who received SGLT-2i (dapagliflozin). The increased RDW rates in these patients may stem from the induction of hemopoiesis from dapagliflozin. Baseline RDW was found to be independently associated with outcome in patients with HF and DM.

KEYWORDS

sodium glucose co-transporter-2 inhibitors, red blood cell distribution width, mechanisms, aging, oxidative stress, erythropoietin

Introduction

Red blood cell distribution width is a simple parameter of the complete blood count (CBC) which reflects the heterogeneity of the red blood cell size (anisocytosis) and has been traditionally used for the classification of several types of anemia (1, 2). Over the last decade, high RDW values have been associated with adverse outcomes in patients with cardiovascular disease including stable coronary artery disease, acute coronary syndromes, stroke, diabetes mellitus (DM), and heart failure (HF) (3–7). DM is a major risk factor for new-onset HF and vice versa (8). An increase in Hemoglobin A1C (HbA1C) of 1% correlates to an increment of 8% in HF, whereas diabetic patients have an almost twofold increased risk of HF (9, 10). Interestingly, it has been reported that red blood cell distribution width (RDW) is a good prognostic marker in patients with HF and DM (11). Reduced iron mobilization, oxidative stress, renal failure as well as ineffective erythropoiesis, have been implicated in the pathophysiology of RDW increase (12). However, the exact pathophysiological mechanism of RDW increase in HF, DM, and other pathological states, remain unclear (11).

Sodium glucose co-transporter-2 inhibitors (SGLT-2i) are a new class of antidiabetic drugs which reduce hyperglycemia through inhibition of glucose reabsorption in the renal proximal tubules (8). SGLT-2i administration has been associated with favorable cardiovascular outcomes (13, 14). Although several hypotheses have been proposed (including diuresis and natriuresis, reduction in blood pressure and afterload, direct effects on myocardial sodium and calcium handling, alterations in myocardial energetics, and improved progenitor cell response), recent studies in patients with type 2 DM suggest that the pivotal mechanism of SGLT-2i favorable action is the stimulation of erythropoiesis *via* an early increase in erythropoietin (EPO) production which leads to hematocrit (Ht) rise (15, 16). Since erythropoietin has been implicated in the pathophysiology of RDW increase as well, the current mechanistic study examined the effect of SGLT-2i administration on red blood cells size (RDW) in patients with HF and DM.

Methods

Study patients

The present was a prospective single-center study which took place during the period from 4-2020 to 7-2021 on the Cardiology Department of Konstantopoulio General Hospital (Greece). Patients were randomly assigned *(Random allocation software)* (17) to dapagliflozin (10 mg a day on top of antidiabetic treatment) or the control group (no change in antidiabetic treatment) (1:1) in an open-label fashion. Inclusion criteria were: (a) age > 18 years, (b) history of type 2 DM and hospitalization for HF exacerbation within 6 months. Exclusion criteria were: (a) current or prior treatment with SGLT-2i or gucagon-like peptide 1 (GLP1) agonists, (b) blood transfusions or ferrum or folic acid or vitamin B12 administration the last 6 months, (c) glomerular filtration rate (GFR) < 30 mL/min/1.73 m^2, (d) active cancer, or (e) predicted survival <1-year. NK and EM generated the random allocation sequence, enrolled participants, and assigned participants to the study groups.

The evaluation of patients (at baseline, 6 and 12 months) included clinical assessment, laboratory blood tests, and echocardiography. Levels of Ht and RDW were measured with the use of the Unicel DxH 600, Beckman USA analyzer on samples obtained for standard of care evaluation. The normal reference range for age, sex, and ethnicity using NHANES III normal range data for RDW in the hospital laboratory was 11.5–15% with intra-assay variation of 2.6% and inter-assay variation of 1.5% (18). Ferrum, ferritin, vitamin B12, folic acid and EPO measured with the use of Access 2, Beckman USA analyzer, while HbA1C, glucose, urea, creatinine, electrolytes, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), brain natriuretic peptide (BNP), uric acid, and troponin with Dimension EXL, Siemens analyzer. Echocardiography was performed and reviewed by two independent echocardiographers, with the use of GE, HealthcareVivid e95.

This study conforms to the principles outlined in the Declaration of Helsinki and was approved by the institutional

review committee. All patients provided written their informed consent.

Definitions

Diabetes mellitus was defined as HbA1C $\geq$ 6.5% and history of antidiabetic treatment (19–22). Heart failure was defined as a clinical syndrome consisting of cardinal symptoms (e.g., breathlessness, ankle swelling, and fatigue) that may be accompanied by signs (e.g., elevated jugular venous pressure, pulmonary crackles, and peripheral oedema). It is due to a structural and/or functional abnormality of the heart that results in elevated intracardiac pressures and/or inadequate cardiac output at rest and/or during exercise (23, 24).

Outcomes and follow up

The aim of this study was to compare the RDW longitudinal changes between the group of patients who received SGLT-2i (dapagliflozin) and the control group. Furthermore, we examined the association between RDW changes with time and clinical parameters that were known from the literature to be associated with RDW. Lastly, we investigated whether baseline RDW was independently associated with the combined endpoint of death or HF rehospitalization at 12 months. The study follow up was 1-year.

Statistical analysis

Quantitative variables were expressed as mean (Standard Deviation) or as median (interquantile range). Qualitative variables were expressed as absolute and relative frequencies. For the comparison of proportions chi-square tests were used. Independent samples Student's *t*-tests and Mann-Whitney tests were used for the comparison of quantitative variables between the two groups. Wilcoxon test was used for the time comparisons of RDW, in each group separately. Data were modeled using mixed linear models with dependent variable the RDW index. Primarily, the regression equation included terms for time, group, the interaction term of time and group as well as all characteristics that differed significantly between the two groups at baseline (Model 1). Secondly, another model was constructed with terms for time, group, the interaction term of time and group as well as all characteristics that were known from literature to be associated with RDW (Model 2). Adjusted regression coefficients (β) with standard errors (SE) were computed from the results of the mixed models. Hypothesized interactions of variables in the models were checked. Log transformations were used in RDW due to lack of normal distribution. In order to find factors independently associated with prognosis, multiple logistic regression was conducted with 1-year death or HF rehospitalization as dependent variable. As independent variables RDW (at baseline), group (SGLT-2i vs. control), gender, age, smoking and body mass index (BMI) (at baseline) were used. Adjusted odds ratios (OR) with

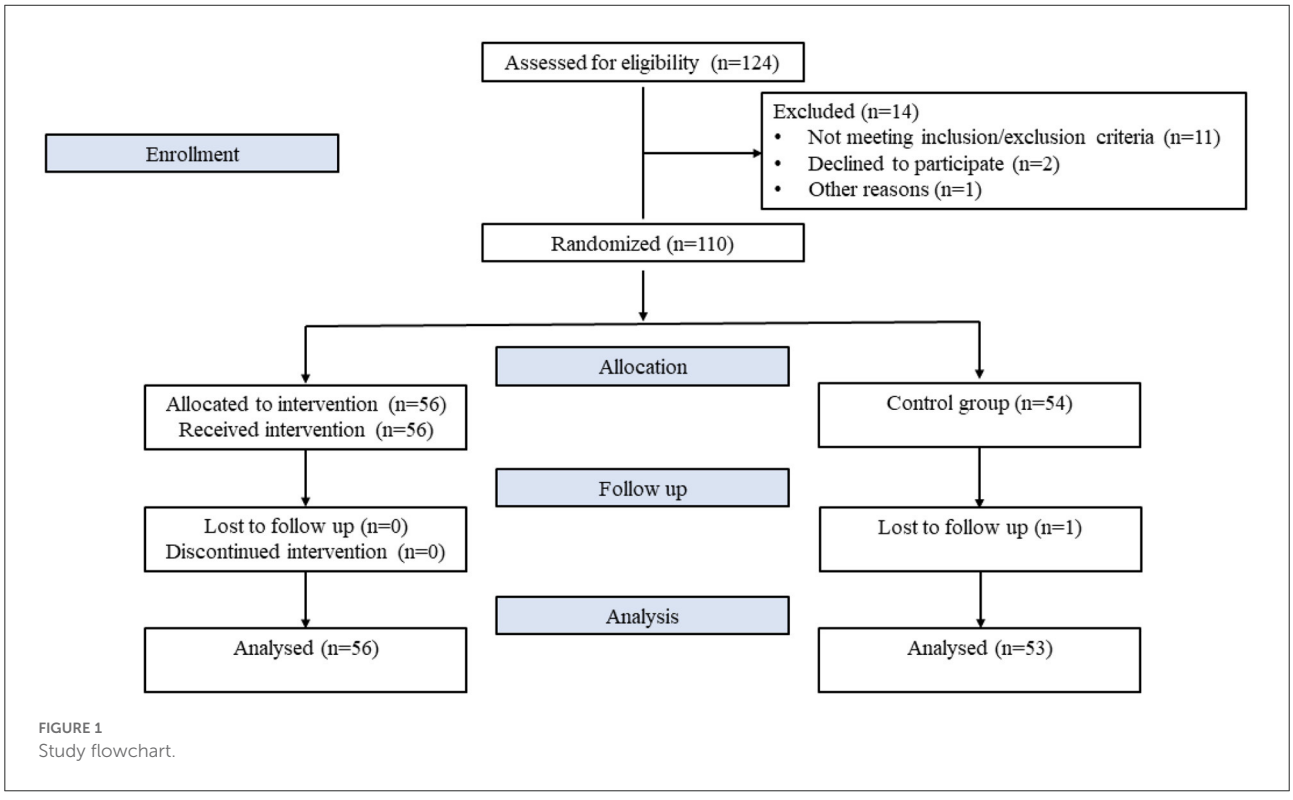

FIGURE 1
Study flowchart.

95% confidence intervals (95% CI) were computed from the results of the logistic regression analyses. ROC curves (Receiver operating characteristic curves) were used in order to estimate the prognostic value of RDW regarding poor prognosis (1-year death or HF rehospitalization), for each group separately. Sensitivity and specificity were calculated for optimal cut-offs. The area under the curve (AUC) was also calculated. The association of ferritin with C-reactive protein (CRP), white blood cells (WBC), ferrum and soluble transferrin receptor (STFR) was investigated *via* mixed linear regression models having ferritin as dependent variable. All reported *p*-values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 22.0).

Results

Patient characteristics

This study randomized 110 patients (124 patients were screened, but 14 patients were excluded, mainly due to inclusion/exclusion criteria) (Figure 1; Supplementary material, CONSORT 2010 Checklist). The overall baseline patient characteristics, as well as baseline characteristics split by the group type (dapagliflozin vs. control group), are presented in Table 1. Patients in the control group were older, had higher heart rate, urea, creatinine and uric acid compared to those on dapagliflozin. On the other hand, patients on dapagliflozin exhibited higher platelet, HbA1C and GFR values than the control group. The other baseline characteristics were not different between the 2 study groups. One patient from the control group was lost to follow up and there were no missing values.

RDW longitudinal changes

RDW was similar at baseline for both groups ($p = 0.974$). At 6 and at 12 months the SGLT-2i (dapagliflozin) group had significantly greater values compared to the control group ($p = 0.018$ and $p = 0.001$; Figure 2). Also, it was found that in the control group RDW was similar throughout the follow-up period, while in the SGLT-2i group there were significant increases between the consecutive measures as well as between baseline and last follow-up measurement ($p < 0.001$ for all time comparisons) (Figure 2).

After adjusting for all characteristics that differed significantly between the two groups at baseline ***(Model 1)***, RDW still increased significantly only in the SGLT-2i group over the follow-up period (Table 2). Age was positively correlated with RDW index. All other parameters included in the analysis were not significantly associated with RDW.

The analysis was repeated including parameters known from the literature that could be associated with RDW (Model 2, Table 3). It was found that RDW continued to increase significantly only in the SGLT-2i group over the follow-up period. Also, EPO was significantly and positively associated with RDW. On the other hand, ferritin and folic acid were significantly and negatively associated with RDW. Age, gender, hemoglobin, B12, uric acid, BMI, GFR, antiplatelets, and HbA1C were not significantly associated with RDW.

Outcomes

Altough, the percentage of 1-year death or HF rehospitalization was numerically lower in the SGLT-2i group (8.9% for the dapagliflozin group vs. 16.7% for the control group), there was no significant difference between the two groups ($p = 0.223$; Supplementary Table 1).

Baseline RDW and outcome

RDW was significantly and independently associated with 1-year death or HF rehospitalization, after adjusting for age, group, gender, smoking and BMI at baseline. More specifically, greater RDW values at baseline were significantly associated with greater probability of death or rehospitalization, i.e., worse prognosis. All other independent factors were not found to be significantly associated with prognosis (Table 4).

The prognostic value of RDW is listed in the Supplementary Figure 1. Baseline RDW was of prognostic significance regarding the combined endpoint of 1-year death/rehospitalization in both groups [AUC 0.82, 95% CI (0.68–0.96), $p = 0.019$ in the dapagliflozin group and AUC 0.72, 95% CI (0.56–0.87), $p = 0.042$ in the control group]. No significant difference was observed between the aforementioned AUCs ($p = 0.349$). Optimal RDW cut-off for the dapagliflozin group was 15.7%, with 80% sensitivity and 70.6% specificity. For the control group, optimal RDW cut-off value was 14.8%, with 77.8% sensitivity and 57.8% specificity.

Association of ferritin with markers of iron overload and inflammation

Greater WBC and ferrum values were significantly associated with greater ferritin values. On the contrary, soluble transferrin receptor (STFR) was significantly negatively associated with ferritin. CRP was not significantly associated with ferritin (Supplementary Table 2).

TABLE 1 Baseline characteristics of the study population.

Baseline characteristics	Overall ($N = 110$)	Dapagliflozin group ($N = 56$)	Control group ($N = 54$)	*P*-value
Age (years), mean ± SD	69.95 ± 9.33	68.11 ± 9.25	71.87 ± 9.10	**0.034**
Male sex, *N* (%)	90 (81.8)	47 (83.9)	43 (79.6)	0.559
Systolic blood pressure (mm Hg), median (IQR)	125.50 (28)	124.50 (26)	130 (31)	0.578
Diastolic blood pressure (mm Hg), mean ± SD	75.52 ± 11.88	75.96 ± 12.51	75.06 ± 11.29	0.690
Heart rate (bpm), median (IQR)	72 (14)	69 (11)	74.50 (17)	**0.014**
Left ventricular ejection fraction (%), median (IQR)	35 (10)	35 (15)	35 (10)	0.619
New York Heart Association, *N* (%)				
II, *N* (%)	53 (48.2)	24 (42.9)	29 (53.7)	0.255
III, *N* (%)	57 (51.8)	32 (57.1)	25 (46.3)	
Body weight (kg), median (IQR)	85 (25)	85 (27)	85.5 (25)	0.895
Body mass index (BMI), median (IQR)	27.7 (4.5)	27.6 (5.8)	27.9 (3.9)	0.654
Comorbidities/Risk factors				
Hypertension, *N* (%)	98 (89.1)	48 (85.7)	50 (92.6)	0.247
Atrial fibrillation, *N* (%)	36 (32.7)	15 (26.8)	21 (38.9)	0.176
Coronary artery disease, *N* (%)	103 (93.6)	51 (91.1)	52 (96.3)	0.438
Valvular disease, *N* (%)	11 (10)	5 (8.9)	6 (11.1)	0.703
Dyslipidemia, *N* (%)	104 (94.5)	52 (92.9)	52 (96.3)	0.679
Peripheral arterial disease, *N* (%)	25 (22.7)	12 (21.4)	13 (24.1)	0.741
Stroke, *N* (%)	17 (15.5)	7 (12.5)	10 (18.5)	0.383
Smoking, *N* (%)	36 (32.7)	21 (37.5)	15 (27.8)	0.277
Laboratory blood values				
Hematocrit (%), mean ± SD	39.98 ± 5.33	40.68 ± 5.90	39.25 ± 4.61	0.161
Hemoglobin (g/dl), mean ± SD	12.99 ± 1.93	13.16 ± 2.10	12.82 ± 1.71	0.352
MCV (fl), mean ± SD	85.88 ± 6.91	86.42 ± 6.87	85.32 ± 6.97	0.406
MCH (pg), mean ± SD	28.19 ± 2.86	28.31 ± 2.99	28.07 ± 2.73	0.667
MCHC (g/dl), median (IQR)	32.40 (1.6)	32.55 (1.4)	32.35 (1.8)	
White blood cells (cells/μl), mean ± SD	8,112.73 ± 1,858.629	8,240.36 ± 2,020.424	7,980.37 ± 1,683.248	0.466
Red blood cell distribution width (%), median (IQR)	14.60 (3.4)	14.60 (3.5)	14.60 (3.2)	0.974
Platelets (cells/μl), median (IQR)	219,500 (85,750)	238,000 (80,250)	210,500 (73,250)	**0.049**
Erythrocyte sedimentation rate (mm/h), median (IQR)	25.50 (28)	26 (27)	23 (30)	0.428
C-Reactive protein (mg/l), median (IQR)	6.25 (13.55)	6.03 (13.98)	7.98 (13.53)	0.421
Glucose (mg/dl), median (IQR)	153 (57)	146 (67)	156 (49)	0.724
HbA1C (%), median (IQR)	7.20 (1.4)	7.45 (1.3)	6.8 (1.3)	**<0.0001**
Urea (mg/dl), median (IQR)	48.50 (31)	45 (21)	55 (39)	**0.030**
Glomerular Filtration Rate (GFR)	68.8 (36.6)	72.0 (32.5)	61.1 (29.9)	**0.032**
Chronic kidney disease stage, *N* (%)				
G1	22 (20.0)	15 (26.8)	7 (13.0)	0.059
G2	48 (43.6)	26 (46.4)	22 (40.7)	
G3a	24 (21.8)	10 (17.9)	14 (25.9)	
G3b	11 (10.0)	5 (8.9)	6 (11.1)	
G4	5 (4.5)	0 (0.0)	5 (9.3)	

(Continued)

TABLE 1 (Continued)

Baseline characteristics	Overall ($N = 110$)	Dapagliflozin group ($N = 56$)	Control group ($N = 54$)	*P*-value
Creatinine (mg/dl), median (IQR)	1.10 (0.5)	1.03 (0.41)	1.16 (0.6)	**0.039**
SGOT (IU/l), median (IQR)	17.50 (10)	18 (10)	17 (10)	0.697
SGPT (IU/l), median (IQR)	28 (20)	27.50 (21)	28.50 (20)	0.592
Troponin (ng/ml), median (IQR)	0.04 (0.00)	0.04 (0.01)	0.04 (0.00)	0.492
K^+ (mmol/l), mean ± SD	4.20 ± 0.46	4.25 ± 0.43	4.14 ± 0.49	0.222
Na^+ (mmol/l), mean ± SD	139.73 ± 3.10	139.82 ± 2.91	139.63 ± 3.32	0.748
T3 (ng/ml), mean ± SD	0.92 ± 0.18	0.93 ± 0.17	0.91 ± 0.19	0.470
Ferrum (μg/dl), median (IQR)	60 (43)	62 (44)	60 (42)	0.879
Ferritin (ng/ml), median (IQR)	53.20 (87.6)	62.9 (99.3)	43.1 (76)	0.349
B12 (pg/ml), median (IQR)	228 (158)	241.5 (183)	217.5 (120)	0.274
Fil acid (ng/ml), median (IQR)	7.55 (3.9)	7.75 (4.2)	7.45 (3.8)	0.917
STFR (mg/l), median (IQR)	18.78 (11.14)	19.26 (10.63)	18.37 (11.63)	0.220
Brain natriuretic peptide (pg/ml), median (IQR)	199.95 (396)	183.95 (355)	211.15 (532.75)	0.462
Uric acid (mg/dl), median (IQR)	6.7 (2.5)	6 (2.3)	7.45 (2.8)	**0.004**
Erythropoietin (mIU/ml), median (IQR)	13.53 (12.06)	12.71 (12.59)	14.26 (11.78)	0.650
Medical treatment				
b-blocker, *N* (%)	102 (92.7)	52 (92.9)	50 (92.6)	1.000
ACE-i/ARB, *N* (%)	82 (74.5)	42 (75)	40 (74.1)	0.911
Sacubitril-Valsartan, *N* (%)	23 (20.9)	12 (21.4)	11 (20.4)	0.891
Mineralocorticoid antagonists, *N* (%)	71 (64.5)	37 (66.1)	34 (63)	0.733
Loop diuretics, *N* (%)	73 (66.4)	37 (66.1)	36 (66.7)	0.947
Antiplatelets, *N* (%)	69 (62.7)	38 (67.9)	31 (57.4)	0.257
Metformin, *N* (%)	97 (88.2)	50 (89.3)	47 (87)	0.715
Dipeptidyl peptidase-4-inhibitors, *N* (%)	50 (45.5)	22 (39.3)	28 (51.9)	0.250
Thiazolidinediones, *N* (%)	3 (2.7)	1 (1.8)	2 (3.7)	0.615
Sulfonylureas, *N* (%)	8 (7.3)	3 (5.4)	5 (9.3)	0.485
Insulin, *N* (%)	50 (45.5)	19 (33.9)	22 (40.7)	0.460

The bold values represent the p values that are < 0.05 (meaning that there is statistical significance).

Adverse events

No serious adverse events (diabetic ketoacidosis, symptoms of volume depletion, renal events, major hypoglycemia, fracture, lower limb amputations, Fournier's gangrene), and adverse events leading to discontinuation of the study drug were reported. Urinary tract infection was reported in two patients (one in the dapagliflozin group and 1 in the control group).

Discussion

The present study demonstrated a correlation between the SGLT-2i (dapagliflozin) use, and an increase in the rates of RDW in patients with HF and DM both after 6 and after 12 months against those not on SGLT-2i (control group). It was equally noted that the increase in RDW was positively correlated with factors such as EPO, while negatively correlated with ferritin and folic acid. Lastly, baseline RDW proved to be independently associated with outcomes (death or HF rehospitalization) at 1-year, in both groups.

Potential mechanisms of RDW increase

HF and DM often coexist and share common pathophysiological routes such as oxidative stress, inflammation and the disruption in normal hematopoiesis, conditions which can lead patients in developing anemia of chronic disease (8). EPO is a hormone produced by the kidneys and secreted in situations of hypoxia; its principal function is to induce hematopoiesis (25, 26). The increase in hematopoiesis which happens through erythropoietin results in an increase of the size

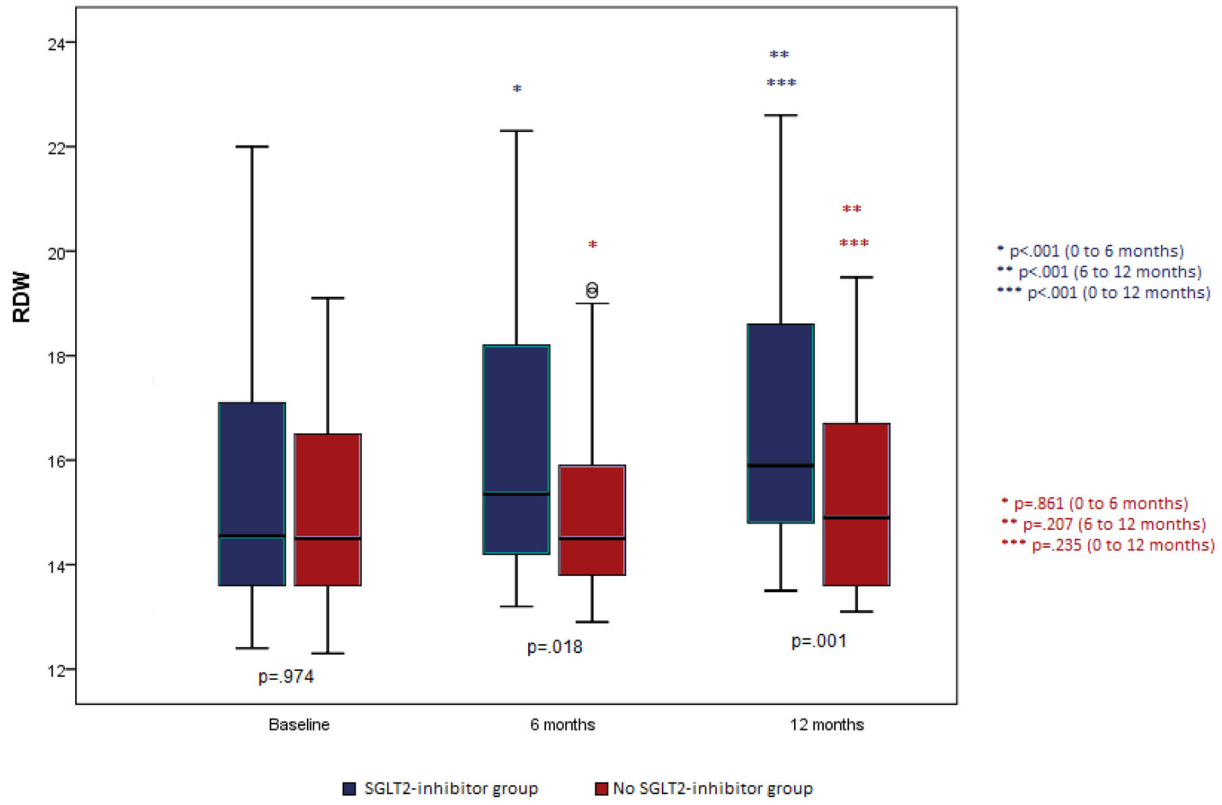

FIGURE 2
RDW change during the follow-up period for each group separately.

TABLE 2 Mixed linear regression results with RDW as dependent variable (after logarithmic transformation) and group, time, interaction term of group and time, and all characteristics differed significantly at baseline as independent variables (Model 1).

	β+	SE++	*P*
Time	0.0008	0.0010	0.381
Group			
No SGLT-2i group (reference)			
SGLT-2i group	0.0445	0.0249	0.074
Time*group	0.0061	0.0013	**<0.001**
Age	0.0044	0.0013	**0.001**
Heart rate	0.0001	0.0005	0.783
Platelets	0.0000	0.0000	0.496
HbA1C	−0.0076	0.0063	0.227
Urea	0.0002	0.0004	0.597
Creatinine	0.0580	0.0395	0.142
Uric acid	−0.0013	0.0041	0.752
GFR	0.0002	0.0005	0.635

+regression coefficient, ++standard error. The bold values represent the p values that are < 0.05 (meaning that there is statistical significance).

of the circulating RBCs and consequently in an increase of the rate of RDW (anisocytosis) (27).

SGLT-2i are antidiabetic medications with pleiotropic effects in multiple organs and proven cardiovascular benefits, especially in patients with HF and DM (28). One of their favorable actions is the increase in the levels of Ht although the exact mechanism through which this happens is unknown (29). A sub-analysis of EMPA-HEART CardioLink-6 (Effects of Empagliflozin on Cardiac Structure in Patients With Type 2 Diabetes) randomized clinical trial, including 82 patients with DM and coronary artery disease showed that the administration of empagliflozin was associated with an increase in the EPO levels, a change in the morphology of RBCs (increase in RDW), a decrease in ferritin reserves and an increase in Ht concluding that SGLT-2i induce hematopoiesis through the increase in the secretion of EPO (15). Another randomized study investigated the effect of dapagliflozin on the levels of Ht and hepcidin (a suppressive hormone for hemopoiesis which increases in pre-inflammatory conditions such as DM or HF) in 52 patients with type 2 DM (30). Patients who were on dapagliflozin presented an increase in Ht and EPO, but a decrease in the rate of hepcidin and ferritin. The investigators concluded that dapagliflozin induces hematopoiesis through the mobilization of iron reserves, increase in EPO and suppression of hepcidin (30). The present study demonstrated that dapagliflozin administration increases

TABLE 3 Mixed linear regression results with RDW as dependent variable (after logarithmic transformation) and group, time, interaction term of group and time, age, EPO, Hb, Ferritin, B12, folic acid, uric acid, gender, BMI, GFR, antiplatelets, and HbA1C as independent variables (Model 2).

	β+	SE++	*P*
Time	0.0003	0.0010	0.721
Group			
No SGLT-2i group (reference)			
SGLT-2i group	0.0395	0.0217	0.069
Time*group	0.0056	0.0014	**<0.001**
Age	0.0024	0.0013	0.063
EPO	0.0007	0.0002	**0.001**
Hemoglobin	−0.0029	0.0049	0.544
Ferritin	−0.0003	0.0001	**<0.001**
B12	0.0001	0.0001	0.275
Folic acid	−0.0050	0.0019	**0.008**
Uric acid	0.0057	0.0039	0.140
Gender			
Women (reference)			
Men	0.0107	0.0278	0.701
BMI	−0.0008	0.0016	0.616
GFR	−0.0005	0.0003	0.055
Antiplatelets			
No (reference)			
Yes	−0.0311	0.0222	0.162
HbA1C	−0.0024	0.0060	0.691

+regression coefficient, ++standard error. The bold values represent the p values that are < 0.05 (meaning that there is statistical significance).

TABLE 4 Multiple logistic regression results with 1-year death or HF rehospitalization as dependent variable.

	OR (95% CI)+	*P*
RDW (at baseline)	**1.40 (1.07–1.83)**	**0.014**
Group		
No SGLT-2i group (reference)		
SGLT-2i group	0.42 (0.12–1.56)	0.196
Gender		
Women (reference)		
Men	0.54 (0.10–2.89)	0.468
Age	1.04 (0.96–1.13)	0.292
Smoking		
No (reference)		
Yes	0.89 (0.22–3.64)	0.870
BMI (at baseline)	1.00 (0.90–1.11)	0.989

+Odds ratio (95% Confidence Interval). The bold values represent the p values that are < 0.05 (meaning that there is statistical significance).

RDW values in patients with HF and DM and that this increase is associated with the EPO rates. Taking into consideration that the induction of hemopoiesis through the increase of endogenous EPO leads to anisocytosis (and by extension to an increase in RDW), it is possible that these observations suggest the stimulation of hemopoiesis from dapagliflozin in patients with HF and DM. This supposition is reinforced by the fact that a negative correlation between RDW and ferritin as well as folic acid was noted, which suggests a potential mobilization of the reserves of endogenous iron and folic acid for the induction of hemopoiesis as necessary ingredients in the composition of RBCs. Furthermore, the present study revealed a positive association between ferritin values and ferrum as well as WBC (markers of iron and inflammation, respectively), and a negative association between ferritin and STFR (marker of erythropoiesis). Potential mechanisms through which a stimulation of hemopoiesis through SGLT-2i can occur have not yet been clarified (30–32).

Another parameter which has been correlated with higher RDW rates is increased age. In a retrospective study of 1907 healthy subjects, RDW rates increased according to the age group ($p < 0.001$) while the average rate of RDW was 11% higher in persons aged over 60 years compared to persons aged under 60 years (14.6 vs. 13.2%; $p < 0.001$) (33). Similar findings were reported in a study utilizing data from 8,089 unique individuals, reporting an RDW increase of 6% from the youngest to oldest age class (34). In the present study, RDW rates were positively correlated with aging in Model 1. Taking into consideration that increase in age parallels an increase in inflammation, oxidative stress as well as deficiencies in basic ingredients for the maturing RBCs such as folic acid, vitamin B12 and iron, it can be suggested that all of the above pathologies lead to disorders in normal hemopoiesis and by extension to presenting anisocytosis (35, 36).

Based on all the above, it could be proposed that the administration of SGLT-2i appears to result in the induction of hemopoiesis in patients with HF and DM (Supplementary Figures 2, 3) resulting in RDW increase. Notably in the present study, the abovementioned cardioprotective mechanism of SGLT-2i didn't result in better outcome in the dapagliflozin group vs. the control group. However, this may be due to the small number of adverse events observed in both groups.

RDW is an important marker of prognosis in HF patients (37, 38). In the present study, the administration of SGLT-2i (dapagliflozin) was associated with the increase of RDW over time. Having in mind, that the cardioprotective actions of SGLT-2i in HF patients are established (39–42), this beneficial effect on prognosis might be mediated by mechanistic actions not comprehensively investigated in the present study, such as the reduction in oxidative stress involving the xanthine oxidase pathway (43, 44). Oxidative stress has also been suggested to increase RDW rates (45, 46). One of the endogenous sources of reactive oxygen species (ROS) is the enzyme xanthine oxidase which catalyzes hypoxanthine to xanthine and xanthine to uric acid. Oxidative stress has an important role in the onset

and development of HF and DM (47, 48) while studies have highlighted the prognostic value of uric acid as a potential indicator of increased oxidative stress in both conditions (49–52). However, uric acid may be caused not only by oxidative stress but also by decreased uric acid excretion from the kidneys and high purine diets (53). The present study was not able to demonstrate any association between uric acid and RDW.

RDW as a prognostic indicator

The value of RDW as a prognostic indicator in patients with HF and DM has been previously reported (11, 54). The present study adds to the current knowledge by showing that the prognostic value of baseline RDW in patients with HF and Type 2 DM was significant both in patients who started receiving SGLT-2i (dapagliflozin) or not. Therefore, in the era of novel life saving therapies (SGLT-2i) in HF, baseline RDW remains a simple, inexpensive and reliable prognostic marker.

It is well-known from the literature, that a high RDW value in patients with DM and HF is likely a consequence of a chronic inflammatory state involving a reduced use of iron reserves and reduced production of erythropoietin and correlates negatively with prognosis (11, 54). However, the pathophysiological significance of an increased RDW at follow-up in patients treated with SGLT-2i (dapagliflozin) could be different, and likely linked to a greater use of iron reserves and increase in erythropoiesis as demonstrated in the present work (Supplementary Table 3). Large, prospective clinical studies are urgently needed.

Study limitations

The sample size was not large (110 patients), however each patient completing the follow-up had 3 measurements (baseline, 6 and 12 months). Another limitation was the lack of double blind and placebo group design, although patients were treated on top of optimized medical therapy. Consequently, the results of this study should be evaluated with caution and used as a basis for larger studies. Furthermore, the SGLT-2i used in the current study was dapagliflozin and therefore the current observations may not apply to other SGLT-2i. However, the possibility that those mechanisms reflect a "class effect" can't be excluded.

Conclusion

RDW, a simple parameter of a blood count which indicates anisocytosis, has been found in this study to increase in patients with HF and DM who received SGLT-2i (dapagliflozin). The increased RDW rates in these patients may be due to the induction of hemopoiesis from dapagliflozin. RDW was independently associated with 1-year death or HF rehospitalization, in patients with DM and HF.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Committee of the Konstantopoulio General Hospital (email: grammateia.ep.symvouliou@konstantopouleio.gr). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NK and EM collected the data. AX, GG, II, SP, FT, and JS conceived and designed the study. SP, II, and JS were responsible for the project administration. II, FT, and JS were responsible for the supervision of the study. NK, AX, and JS wrote the main manuscript text. GG, SS, and EM prepared figures. NK (biostatistician) performed the statistical analysis. II, SP, and FT revised the manuscript critically for important intellectual content. All authors reviewed the manuscript, discussed the results, and commented on the manuscript.

Acknowledgments

The authors thank the participants of the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.984092/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 09 November 2022
DOI 10.3389/fcvm.2022.1019797

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Heart Failure and Transplantation, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 15 August 2022
ACCEPTED 19 October 2022
PUBLISHED 09 November 2022

CITATION
Fang Z, Wang Z, Cao X, Wang Z-M, Yu C, Ju W and Li D (2022) Association between energy intake patterns and outcome in US heart failure patients.
Front. Cardiovasc. Med. 9:1019797.
doi: 10.3389/fcvm.2022.1019797

Association between energy intake patterns and outcome in US heart failure patients

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Background: The association between dietary energy patterns, calories, and the outcomes of heart failure (HF) is still unclear.

Objectives: To evaluate the proper energy intake patterns and daily calorie intake in patients with heart failure among US adults.

Methods: The data were derived from the 2001–2014 National Health and Nutrition Examination Survey (NHANES). A calorie intake pattern variable was created using latent class analysis (LCA) based on the calorie ratio of three major nutrients. Cox proportional hazard regression models were used to evaluate the hazard ratios (HR) and 95% confidence intervals (CI) of the association between calorie intake and energy patterns. The primary endpoint was all-cause mortality.

Results: Among 991 participants (mean age 67.3 ± 12.9 years; 55.7% men) who suffered from heart failure; the median calorie intake was 1,617 kcal/day [interquartile range (IQR): 1,222–2,154 kcal/day]. In the multivariable-adjusted model, moderate malnutrition was more frequent to death (HR: 2.15; 95% CI: 1.29–3.56). Low-carbohydrate pattern (LCP) and median-carbohydrate pattern (MCP) had lower risks of death compared to high-carbohydrate pattern (HCP) (LCP: HR: 0.76; 95% CI: 0.59–0.97; MCP: HR: 0.77; 95% CI: 0.60–0.98). No association between different amounts of calorie intake and all-cause mortality was found. There was an adjusted significant interaction between calorie intake and energy intake patterns (p = 0.019). There was a linear relationship between energy intake through HCP and all-cause mortality (p for non-linear = 0.557). A non-linear relationship between energy intake through MCP and all-cause mortality (p for non-linear = 0.008) was observed.

Conclusion: Both LCP and MCP, compared to HCP, were associated with better outcomes in the HF population. The relationship between energy intake and all-cause death may be influenced by energy intake patterns in HF patients.

KEYWORDS

heart failure, nutrient, dietary patterns, all-cause mortality, National Health and Nutrition Examination Survey

Highlights

- Malnutrition is associated with poor outcomes in patients with heart failure (HF).
- No previous data exists about the dietary energy patterns and calorie intake in all-cause mortality of HF.
- Contrary to common belief, increasing the proportion of fat calories in the diet was associated with better outcomes in heart failure.
- This relationship between dietary energy patterns and the outcome was investigated further in the HF subgroup with different comorbidities.
- Daily calorie consumption affects heart failure outcomes based on dietary energy patterns of macro-nutrients.

Introduction

Heart failure (HF) is currently a global public health problem, with high morbidity and mortality (1). In the United States, investigators estimated the prevalence of HF to be approximately 2.5% based on data reported in questionnaires, affecting nearly 6.5 million American adults and the prevalence of the disease continues rise (2). Despite promising advances in pharmacological treatment of HF (3), the outcome for HF patients remains unsatisfactory and its treatment is a long-term and expensive process. People with HF tend to have a poorer quality of life, and the disease itself can make patients frailer and more present with severe malnutrition, shortening survival times.

Nutrition is one of the modifiable factors of lifestyle and plays an important role in ensuring normal cardiac ejection fraction and maintaining favorable cardiac function (4). However, malnutrition in HF patients has been a common phenomenon partially because fluid and sodium restriction, which is an essential part of HF treatment, often leads to artificial reductions in active feeding and thus causes malnutrition, which is detrimental to patients with HF (5). There is currently limited evidence on the associated effects of nutritional interventions in patients with HF as evidence-based nutritional recommendations are lacking in major HF guidelines (3). In recent years, diet-related topics including calorie restriction (CR), dietary patterns, protein or amino acids supplementation and dietary fat intake have attracted extensive attention, such as the preventing and improving HF patient outcomes (6) and extending life expectancy (7) by calorie restriction. Mediterranean Diet (MedDiet) and the Dietary Approaches to Prevention of Hypertension (DASH) are the most widely studied dietary patterns in HF patients (8), but their focus is only on the intake of certain foods and nutrients (9), while lack of comprehensive consideration of calorie and associated energy intake patterns. Previous studies have shown that insufficient calorie intake was associated with poorer quality of life and greater burden of readmission in patients with HF (10) and adequate nutritional intake can delay the progression of HF (11). Therefore, nutritional assessment and related dietary interventions for HF patients are very necessary.

Considering that generally accepted nutritional strategies to improve quality of life and outcome in HF patients remain unmet, we designed this study to investigate the relationship between daily energy intake, different ratios of nutrient consumption, and all-cause mortality in HF patients, and explored the optimal calorie patterns for them.

Abbreviations: BMI, body mass index; CR, calorie restriction; DII, dietary inflammation index; HCP, high-carbohydrate pattern; HF, heart failure; LCP, low-carbohydrate pattern; MCP, median-carbohydrate pattern; NRI, Nutritional Risk Index.

Materials and methods

Study design

The data we examined were from the National Health and Nutrition Examination Survey (NHANES) 2001–2014 which is ongoing surveys of health status performed in 2-year cycles by the National Center for Health Statistics, Centers for Disease Control and Prevention. Its data were designed to determine the risk factors of diseases and to provide critical information on the health and nutritional status of the US population. The detailed survey operations manuals, consent documents, and brochures of NHANES can be viewed on the NHANES website. All data of this study are also publicly available at http://www.cdc.gov/nchs/nhanes.htm.

Population

Our analyses were limited to NHANES 2001–2014 participants considering the consistency of variables. The flowchart of patient inclusion is shown in **Figure 1**. Participants aged 18 years and older who self-reported congestive HF and participated in a 24-h dietary recall assessment were included (n = 1,124). Further exclusions were made for those who had missing data on height and weight (n = 66), had missing serum albumin data needed to define nutritional risk index (NRI) (n = 66), and were pregnant (n = 1). After exclusions, 991 were used for this analysis. Data collection was reviewed and approved by the National Center for Health Statistics Research Ethics Review Board and signed informed consent forms were obtained from participants enrolled in the study.

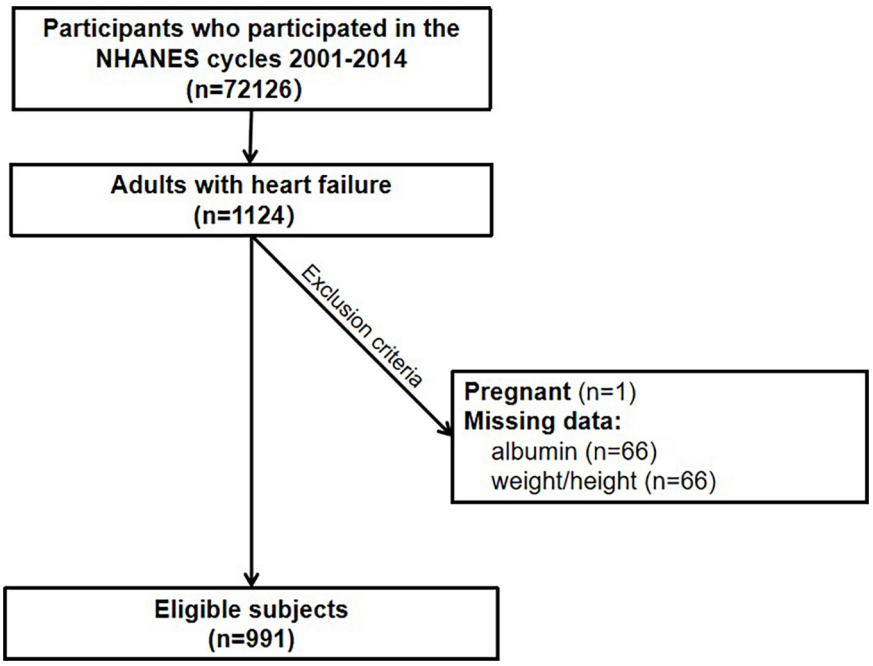

FIGURE 1
Flow diagram.

Dietary assessment

Daily dietary assessment was an NHANES-derived variable from Total Nutrient Intakes File, whose data were obtained through a 24-h Dietary Recall interview. The respondents needed to report all foods and beverages consumed during the previous 24 h. In the interview. A set of 3-dimensional measuring guides were used to help the respondent estimate the portion size. Information collected from the interview will be coded and linked to a database of nutrient composition of foods. The energy intake ratio of the three nutrients (fat, protein, and carbohydrate) was calculated by their intake. Diets were assessed in terms of total energy intake and calorie patterns. An overall calorie pattern variable was created using latent class analysis (LCA) based on proportion of energy of three major nutrients in total energy intake (each factor had three levels: lowest tertile, middle tertile, and highest tertile).

Covariates

Demographic data were obtained through relevant questionnaires including gender, age, race, education, marital status, income, occupation, and type of health insurance. Participants were divided into three groups based on their poverty-to-income ratios: low ($\leq$ 1), midrange (1–4), and high ($\geq$ 4) (12). Less than a high school diploma, a high school graduate or its equivalent, and a college degree or more were the three categories for education. According to the commonly used socioeconomic index in the US, occupations were grouped into upper-skilled jobs (socioeconomic index 50), lower-skilled jobs (socioeconomic index 50, including retirees16 and students), and unemployed jobs (13). Health insurance was separated into three categories: private health insurance, public health insurance only, and no health insurance (14). We divided smoking status into former, current, and never. We also considered the exercise factor, but since the variables measuring exercise also varied in a different cycle, we calculated the metabolic equivalent at each cycle and divided the population into three groups based on the metabolic equivalent (15). The body mass index (BMI) is obtained through Body Measures File. We used the NRI to assess nutritional status. The NRI was calculated as NRI = (1.519 $\times$ serum albumin, g/dL) + [41.7 $\times$ weight (kg)/ideal body weight (IBW; kg)] (16). The IBW was calculated with the Lorentz equations (For men: H=100–[(H–150)/4], For women: H=100–[(H–150)/2.5]. NRI scores of $>$ 100, 97.5–100, 83.5–97.5, and $<$ 83.5 indicate no, mild, moderate, and severe risk of nutrition-related complications, respectively. We also included several comorbidities that may affect the outcome of HF, including hypertension, diabetes, stroke, myocardial infarction (MI), and chronic kidney disease (CKD). Diabetes was defined as fasting glucose of at least 7.0 mmol/L, non-fasting glucose of at least 11.1 mmol/L, glycated hemoglobin of at least 6.5%, use of glucose-lowering drugs, or self-reported

diabetes. The history of the other above-mentioned diseases was obtained in the form of a self-report. For example, we defined CKD based on the answer to this question, "Have you ever been told by a doctor or other health professional that you had weak or failing kidneys? Do not include kidney stones, bladder infections, or incontinence?" To the question "Have you had shortness of breath either when hurrying on the level or walking up a slight hill?" Those who answered "yes" were defined as New York Classification of Cardiac Function (NYHA) III-IV of cardiac function. Meanwhile, the dietary inflammation index (DII) was calculated by dietary questionnaire (17).

Ascertainment of mortality outcomes

The primary outcome was all-causes mortality, which was identified through linkage to the National Death Index (NDI) through December 31, 2015. The NDI is a centralized NCHS database of all deaths in the United States. Eligible participants were matched to this database to determine mortality status.

Statistical analyses

Baseline characteristics for both groups were compared using the Wilcoxon rank sum test and Student's *t*-test for continuous variables and the chi-square test for categorical variables. Multiple interpolations were used to deal with missing data on covariables. We used Cox proportional hazard regression models to estimate the hazard ratios (HR) and 95% confidence intervals (CI) associated with all-cause death and calorie intake pattern. We adjusted for sex, age, race, education, income, occupation, type of health insurance, marital status, smoking status, exercise, sodium intake, BMI, NRI, DII, comorbidities (hypertension, diabetes, MI, stroke, CKD), and NYHA Classification. We additionally fitted the restricted cubic spline with four knots at the 5th, 35th, 65th, and 95th to examine a linear relation between calorie intake and all-cause mortality in different patterns to explore the relevance. We performed a subgroup analysis based on comorbidities and calorie intake patterns. For database management and statistical analysis, we used R software, Version 4.1.1, and considered both two-sided *P*-values and P-interaction values < 0.05 to be significant.

Results

Population characteristics

The baseline characteristics of the study are presented in **Table 1**. Among 991 participants from NHANES 2001–2014 (mean age 67.3 years, SE ± 12.9; 55.7% men), 590 (59.5%) were Non-Hispanic White. The median calorie intake was 1,617 kcal/day (IQR: 1,222–2,154 kcal/day). The number of HF patients with the underlying disease is shown in **Figure 2**. 88 patients with HF had no comorbidities 0.427 deaths were recorded during a mean follow-up of 67.5 months. There was no difference in HF death rates between men and women ($p = 0.264$). Living HF patients had higher BMI ($p < 0.001$), lower risk of malnutrition ($p < 0.001$), and more energy intake ($p = 0.019$) than those with primary outcomes. In terms of diet, the survivors ate more carbohydrates ($p = 0.042$) and sodium ($p = 0.014$).

Calorie intake pattern

We divided calorie intake patterns into three categories by LCA. The results of LCA were consistent with the proportion of carbohydrate energy classification by tertile. Patterns 1, 2, and 3 correspond to the lowest tertile (≤ 46.0%), medium tertile (46.0–54.4), and highest tertile (> 54.4%) groups of carbohydrate intake, respectively. The proportion of fat energy in pattern 1 exceeding 37.2 was 75.5%. In pattern 3, the majority (78.5%) of the population received less than 30.5% of their total energy from fat. Therefore, we defined patterns 1, 2, and 3 as, respectively, low-carbohydrate pattern (LCP), median-carbohydrate pattern (MCP), and high-carbohydrate pattern (HCP). LCP and MCP had lower risks of death compared to HCP (LCP: HR: 0.76; 95% CI: 0.59–0.97; MCP: HR: 0.77; 95% CI: 0.60–0.98) in Model 1. After we did a sensitivity analysis that excluded people with cancer, LCP was associated with a lower risk of death (HR: 0.72; 95% CI: 0.54–0.97). We examined interactions and performed subgroup analyses in subgroups with other diseases, and the results are shown in **Figure 3** and **Supplementary Table 1**. Among people with a history of MI, diabetes, or hypertension, MCP showed better outcomes, the same as people without stroke. However, LCP was associated with a lower risk in people without comorbidities. There was no interaction between calorie intake patterns and diseases.

Calorie intake

We found that poorer nutritional status, assessed by NRI, was associated with all-cause mortality in HF in Model 1 (Mild malnutrition: HR: 2.01; 95% CI: 1.03–3.90; Moderate malnutrition: HR: 2.15; 95% CI: 1.29–3.56) as seem in **Table 2**, After multivariable adjustment. We did not observe any association between different amounts of calorie intake and all-cause mortality. There was an adjusted significant interaction between calorie intake and energy intake patterns

TABLE 1 Baseline characteristics of the patients.

Patient characteristics	All $N = 991$	Missing	No event $N = 564$	Event $N = 427$	*P*-value
Age, years	67.3 ± 12.9	0/991	62.9 ± 12.8	73.1 ± 10.5	<0.001
Gender, %		**0/991**			**0.264**
Men	552 (55.7)		305 (54.1)	247 (57.8)	
Women	439 (44.3)		259 (45.9)	180 (42.2)	
Race, %		**0/991**			**<0.001**
Mexican American	91 (9.18)		55 (9.8)	36 (8.4)	
Other Hispanic	51 (5.15)		39 (6.9)	12 (2.8)	
Non-Hispanic White	590 (59.5)		297 (52.7)	293 (68.6)	
Non-Hispanic Black	222 (22.4)		153 (27.1)	69 (16.2)	
Other race—including multi-racial	37 (3.73)		20 (3.5)	17 (4.0)	
Marital status, %		**0/991**			**<0.001**
Married	477 (48.1)		294 (52.1)	183 (42.9)	
Widowed/divorced/separated	414 (41.8)		198 (35.1)	216 (50.6)	
Not married	100 (10.1)		72 (12.8)	28 (6.6)	
Income, %		**67/991**			**0.008**
≤1.0	229 (24.8)		143 (27.1)	86 (21.7)	
1.0–4.0	574 (62.1)		305 (57.9)	269 (67.8)	
≥4.0	121 (13.1)		79 (15.0)	42 (10.6)	
Medical insurance, %		**2/991**			**<0.001**
No insurance	76 (7.68)		60 (10.7)	16 (3.7)	
Public insurance	800 (80.9)		424 (75.4)	376 (88.1)	
Private insurance	113 (11.4)		78 (13.9)	35 (8.2)	
Occupation, %		**45/991**			**<0.001**
Unemployment	327 (34.6)		210 (38.9)	117 (28.8)	
Lower-skilled	598 (63.2)		312 (57.8)	286 (70.4)	
Upper-skilled	21 (2.22)		18 (3.3)	3 (0.7)	
Education, %		**1/991**			**0.022**
No high school graduate	394 (39.8)		211 (37.4)	183 (43.0)	
High school graduate	239 (24.1)		129 (22.9)	110 (25.8)	
College or above	357 (36.1)		224 (39.7)	133 (31.2)	
Exercise, %		**0/991**			**<0.001**
Inactive	557 (56.2)		264 (46.8)	293 (68.6)	
Median	258 (26.0)		173 (30.7)	85 (19.9)	
Active	176 (17.8)		127 (22.5)	49 (11.5)	
Smoke, %		**0/991**			**0.006**
Never	382 (38.5)		220 (39.0)	162 (37.9)	
Former	411 (41.5)		214 (37.9)	197 (46.1)	
Current	198 (20.0)		130 (23.0)	68 (15.9)	
BMI, kg/m^2	31.3 ± 7.8	0/991	32.3 ± 8.3	29.9 ± 7.0	<0.001
Current height, cm	167.0 ± 10.3	0/991	167.0 ± 10.5	166.1 ± 10.0	0.206
Current weight, kg	87.1 ± 24.0	0/991	90.3 ± 25.2	82.9 ± 21.5	<0.001
Myocardial infarction, %		**5/991**			**0.228**
No	553 (56.1)		325 (57.8)	228 (53.8)	
Yes	433 (43.9)		237 (42.2)	196 (46.2)	
Stroke, %		**3/991**			**0.042**
No	793 (80.3)		465 (82.6)	328 (77.2)	
Yes	195 (19.7)		98 (17.4)	97 (22.8)	
Hypertension, %		**1/991**			**0.324**

(*Continued*)

TABLE 1 (Continued)

Patient characteristics	All N = 991	Missing	No event N = 564	Event N = 427	*P*-value
No	221 (22.3)		119 (21.1)	102 (23.9)	
Yes	769 (77.7)		445 (78.9)	324 (76.1)	
Diabetes mellitus, %		**33/991**			**0.04**
No	581 (60.6)		347 (63.6)	234 (56.8)	
Yes	377 (39.4)		199 (36.4)	178 (43.2)	
CKD, %		**2/991**			**0.172**
No	834 (84.3)		483 (85.8)	351 (82.4)	
Yes	155 (15.7)		80 (14.2)	75 (17.6)	
Albumin, g/L	40.6 (3.48)	0/991	40.94 (3.33)	40.07 (3.61)	<0.001
NYHA, %		**38/991**			**0.058**
1–2	262 (27.5)		160 (30.0)	102 (24.3)	
3–4	691 (72.5)		373 (70.0)	318 (75.7)	
Malnutrition, %		**0/991**			**<0.001**
No	945 (95.4)		551 (97.7)	394 (92.3)	
Mild,	18 (1.82)		7 (1.2)	11 (2.6)	
Moderate	28 (2.83)		6 (1.1)	22 (5.2)	
Total energy intake, kcal/day	1617.0 [1222.0, 2154.0]	0/991	1665.00 [1253.0, 2270.5]	1572.00 [1208.0, 2024.5]	0.019
Protein intake, g	62.9 [45.9, 86.2]	0/991	64.9 [46.3, 88.4]	61.1 [44.9, 82.6]	0.069
Carbohydrate intake, g	203.3 [143.0, 264.3]	0/991	209.5 [144.7, 280.3]	194.9 [141.7, 250.9]	0.042
Fat intake, g	60.2 [40.2, 86.7]	0/991	62.1 [40.7, 90.0]	57.9 [39.7, 80.6]	0.056
Sodium, mg	2565.0 [1874.5, 3637.0]	0/991	2689.5 [1926.5, 3781.0]	2508.0 [1809.0, 3400.5]	0.014
Dietary inflammation index	0.2 [-0.7, 1.6]	0/991	0.4 [-0.9, 1.7]	0.0 [-0.5, 1.3]	0.438

Values are mean ± SD, median (interquartile range) or *n* (%). CKD, chronic kidney diseases; NYHA, New York Heart Association; DII, dietary inflammation index.

(p = 0.019). We performed a subgroup analysis based on calorie intake patterns shown in Table 3, a higher risk of death was shown in the third and fourth quartile compared with the second quartile (> 1222.0 to ≤1627.0 kcal/d) in HF patients with HCP. As shown in Figure 4, We found a linear relationship between energy intake through HCP and all-cause mortality (p for non-linear = 0.557), We also found a non-linear relationship between energy intake through MCP and all-cause mortality (p for non-linear = 0.008), with the lowest risk occurring at 2564.9 kcal. In MCP, the fourth quartiles (HR: 0.29; 95% CI: 0.14–0.61) were linked to lower risk of all-cause death. We observed no significant correlation between calorie intake and death in LCP (p for overall association = 0.446).

Discussion

Malnutrition was associated with higher all-cause mortality in HF patients. Our study found that different energy intake patterns were associated with the outcome of HF patients. LCP (≤ 46.0% of energy from carbohydrate) or MCP (46.0–54.4% of energy from carbohydrate) have a better outcome and HCP (> 54.4% of energy from carbohydrate) has a more unfavorable outcome among the patients with HF. We also found that the relationship between energy intake and all-cause death may be influenced by energy intake patterns in HF patients.

A recent analysis of calorie intake patterns and adverse outcomes in the general population showed a U-shaped curve, with the lowest risk of all-cause mortality in the population consuming calorie patterns that intake 50–55% of carbohydrates, while the low (< 40%) and high (> 70%) carbohydrate calorie patterns were all associated with an increased risk of death (18). However, several studies have shown that low-carbohydrate-diet scores not associated with increased risks of coronary heart disease or total mortality, which depended on the quality and food sources of macronutrients (19, 20). However, there is little evidence that similar result can be generalized to patients with HF, because such patients often have intestinal function changes (21) and metabolic impairment (22), which may disorganize the absorption and utilization of energy. In our study, LCP with high fat and protein proportion was more likely to offer a better outcome for HF patients when using HCP as a reference. These findings suggested that patients with HF might benefit from increased fat intake. Previous studies have also shown that low-fat diets didn't reduce morbidity or mortality from cardiometabolic

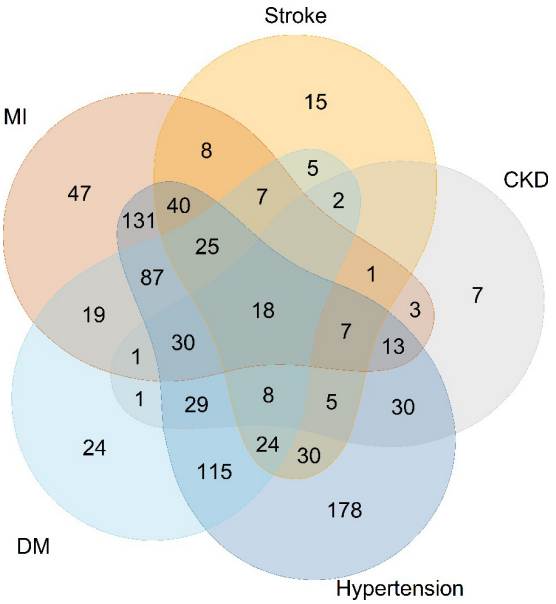

FIGURE 2
Numbers of heart-failure patients with different comorbidities. MI, myocardial infarction; CKD, chronic kidney diseases; DM, diabetes mellitus.

diseases and might not be used for the prevention of these diseases (23, 24). And chronic heart failure patients with low cholesterol were instead associated with increased mortality (25, 26). What's worse, low-fat diets are often accompanied by a high intake of carbohydrates to make up for the loss of energy. While the pro-inflammatory effects of carbohydrates may lead to a systemic inflammatory response (27).

HF is precisely a metabolic disease and a systemic inflammatory response (28). This may be related to the propensity of the heart to consume substrates for energy under pathological conditions, influenced by the etiology of HF and other comorbidities (29). As more efficient substrates in cardiometabolic processes, ketone bodies are elevated in HF patients and serve as an alternative fuel for increased "productivity" (30–33). We advocate low-carb diets because low carbohydrate intake favors ketone body production and utilization to compensate for the reduction in cardiac glucose and fatty acid oxidation (34), rather than increased cardiomyocyte oxidation of fatty acids (35). Moreover, recent researches suggested that SGLT-2 inhibitors might help maintain higher ketone body levels in the body (31, 36–38), which might be related to its cardioprotective effects. In addition, ischemic heart disease and hypertension are major contributors to HF, in which ketone body utilization-related enzymes increased as well (33, 34, 39). Our study found that the MCP was indeed beneficial in patients with hypertension and coronary heart disease, while the advantages of the LCP seemed to be attenuated, which might be due to the adverse effects of high fat intake on comorbidities. Further studies are still

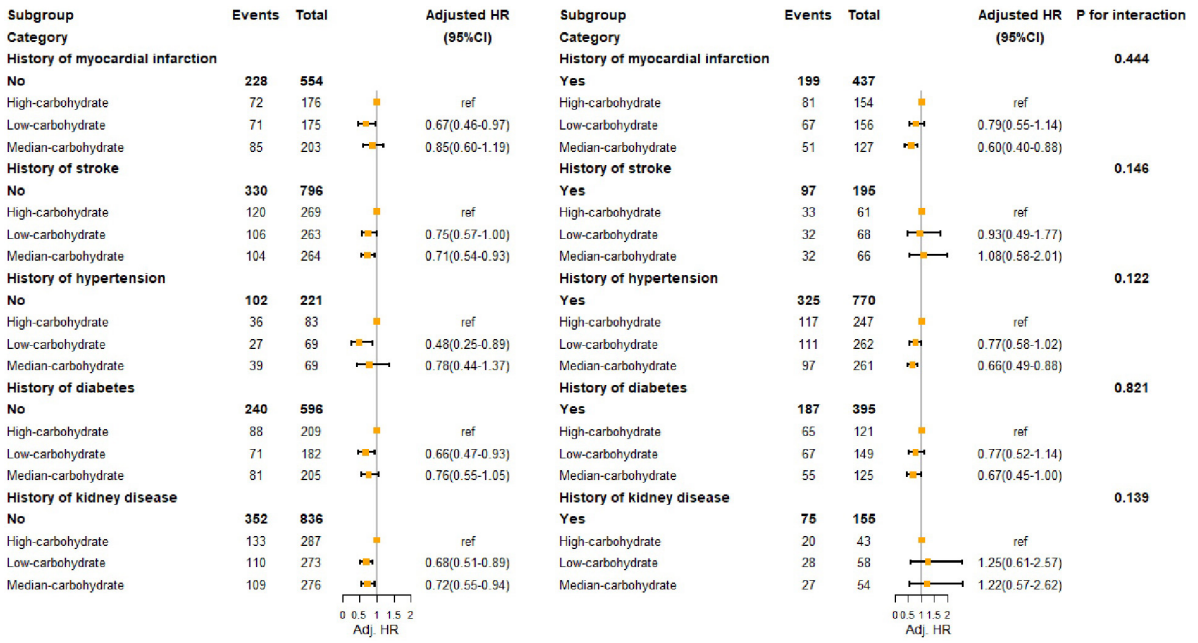

FIGURE 3
Hazard ratio of all-cause death of HF population with different comorbidities. MI, myocardial infarction; CKD, chronic kidney diseases.

TABLE 2 Hazard ratio of outcomes by adjusted analysis.

	Model 1	
	HR (95%CI)	*P*-value
Age, years	1.07 (1.06–1.09)	<0.001
Women	0.54 (0.42–0.70)	<0.001
Race		
Mexican American	Ref	
Other Hispanic	0.72 (0.37–1.42)	0.347
Non-Hispanic White	1.01 (0.69–1.49)	0.957
Non-Hispanic Black	0.85 (0.55–1.30)	0.454
Other race—including multi-racial	1.22 (0.66–2.23)	0.529
Marital status		
Married	Ref	
Widowed/divorced/separated	1.37 (1.10–1.72)	0.006
Not married	1.91 (1.23–2.96)	0.004
Income		
≤1.0	Ref	
1.0–4.0	1.06 (0.82–1.37)	0.671
≥4.0	0.68 (0.45–1.04)	0.073
Medical insurance		
No insurance	Ref	
Public insurance	1.48 (0.87–2.5)	0.149
Private insurance	1.80 (0.97–3.35)	0.063
Occupation		
Unemployment	Ref	
Lower-skilled	0.80 (0.63–1.02)	0.075
Upper-skilled	0.84 (0.36–1.99)	0.698
Education		
No high school graduate	Ref	
High school graduate	1.43 (1.11–1.83)	0.005
College or above	1.07 (0.84–1.36)	0.602
Smoke		
Never	Ref	
Former	0.95 (0.75–1.20)	0.671
Current	1.00 (0.72–1.39)	0.987
Exercise		
Low	Ref	
Median	0.61 (0.48–0.79)	<0.001
High	0.58 (0.42–0.80)	0.001
Myocardial infarction	0.91 (0.75–1.12)	0.384
Diabetes mellitus	1.45 (1.17–1.81)	0.001
Stroke	1.08 (0.85–1.38)	0.528
Hypertension	0.86 (0.67–1.09)	0.202
Kidney failure	1.29 (0.98–1.68)	0.066
NYHAIII-IV	1.45 (1.15–1.85)	0.002
BMI		
<25	Ref	
25–30	0.72 (0.54–0.98)	0.033
30–40	0.85 (0.63–1.16)	0.305
≥40	0.89 (0.58–1.37)	0.591
Malnutrition		
No	Ref	
Mild	2.01 (1.03–3.90)	0.040
Moderate	2.15 (1.29–3.56)	0.003
DII	1.02 (0.96–1.09)	0.448
Dietary pattern		
High-carbohydrate pattern	Ref	
Low-carbohydrate pattern	0.76 (0.59–0.97)	0.028
Median-carbohydrate pattern	0.77 (0.60–0.98)	0.035
Total energy intake		
Q1	0.94 (0.72–1.24)	0.683
Q2	Ref	
Q3	1.07 (0.81–1.42)	0.640
Q4	0.98 (0.68–1.40)	0.910

Values are *n* or HR (95% CI). CI, confidence interval; HR, hazard ratio; other abbreviations as in Table 1.

TABLE 3 Hazard ratio of all-cause death based on dietary patterns.

Dietary pattern	Total energy intake	HR (95%CI)	*P*-value
Low-carbohydrate	Q1	1.44 (0.85–2.44)	0.172
	Q2	Ref	0.172
	Q3	0.65 (0.38–1.11)	0.111
	Q4	0.29 (0.14–0.61)	0.001
Median-carbohydrate	Q1	0.60 (0.35–1.02)	0.057
	Q2	Ref	
	Q3	0.77 (0.44–1.35)	0.363
	Q4	0.93 (0.48–1.78)	0.819
High-carbohydrate	Q1	1.37 (0.82–2.29)	0.224
	Q2	Ref	
	Q3	2.22 (1.32–3.74)	0.003
	Q4	2.14 (1.09–4.20)	0.026

Subgroup analysis of adjusting for sex, age, race, socioeconomic status, marital status, smoking status, exercise, body mass index, nutritional risk index, dietary inflammation index, comorbidities (hypertension, diabetes, myocardial infarction, stroke, chronic kidney diseases) and New York Classification of Cardiac Function.

needed to understand the underlying molecular mechanisms of these effects.

In addition, we explored the relationship between total energy intake and outcome in patients with HF based on different energy intake distributions. We found that this relationship seemed to be affected by the form of energy supplements, which required us to consider the allocation of energy sources when providing energy supplements to the population with heart failure. Calorie restriction (CR) is a widely studied dietary intervention in the field of HF treatment with many positive effects (40), including reduction of left ventricular hypertrophy (41), reduction of myocardial ischemic injury to improvement variable reserve (42), and improving cardiac function (6). Our research

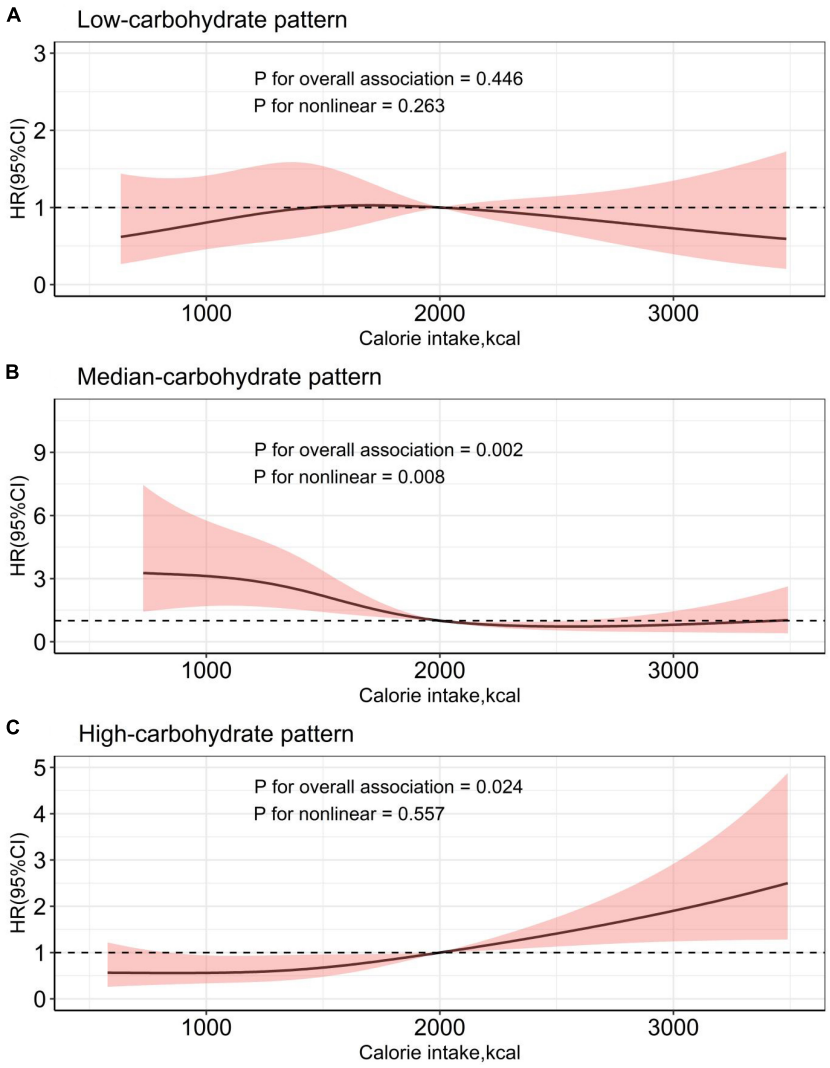

FIGURE 4
Calorie intake and risk for all-cause mortality of heart failure in different dietary patterns.

shed light on the exact energy intake pattern of nutrient proportion that might benefit from CR. Additionally, more calorie intake seemed to have better outcomes in MCP, compensating for the advantage of CR. And more notably, more than one-fifth of our study population suffered from the complications of malnutrition. Insufficient calorie intake may cause poorer quality of life and increased risks of readmission in this group of people. We provide potential evidence for the calorie intake of HF people who are malnourished or who need to consume more energy.

Study limitations

Dietary alterations may exist after assessment. Different dietary treatments may be required for the diverse etiology of HF, which is not exactly clear in our study. Also, our failure to classify HF in line with ejection fraction may need to be remedied in future studies.

Conclusion

In this study, low carbohydrate patterns and median carbohydrate patterns were associated with lower total mortality. We observed a U-shaped relationship between energy intake and mortality, under the median carbohydrate pattern. While in the high carbohydrate patterns, the more the intake of energy was, the worse the outcome was. These findings

suggested that the associations of energy intake with mortality may depend on the energy intake patterns in HF patients.

Perspectives

Competency in medical knowledge

The outcome of HF is influenced by nutritional status and energy intake patterns, and low-carb proportion diets may assist improve the outcome of HF. Whether increase daily calorie intake or reduce it depends on different percentages of nutrients consumption. Nutritional assessment and dietary therapy have potential prognostic value in HF patients.

Translational outlook

Possible approaches were provided for selecting suitable patients for dietary intervention, which have significant implications for ameliorating adverse outcomes of HF. Additional researches are needed to expose the pathophysiological mechanisms underlying the relationship between various energy patterns, calorie consumption, and outcome of HF.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the National Center for Health Statistics, Centers for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DL conceived and designed the study. WJ conceived the study. ZW and CY analyzed the data. ZF, XC, and Z-MW wrote the manuscript. All authors provided critical revisions of the manuscript and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1019797/full#supplementary-material

SUPPLEMENTARY TABLE 1
Hazard ratio of all-cause death of HF population with different comorbidities. MI, myocardial infarction; CKD, chronic kidney diseases.

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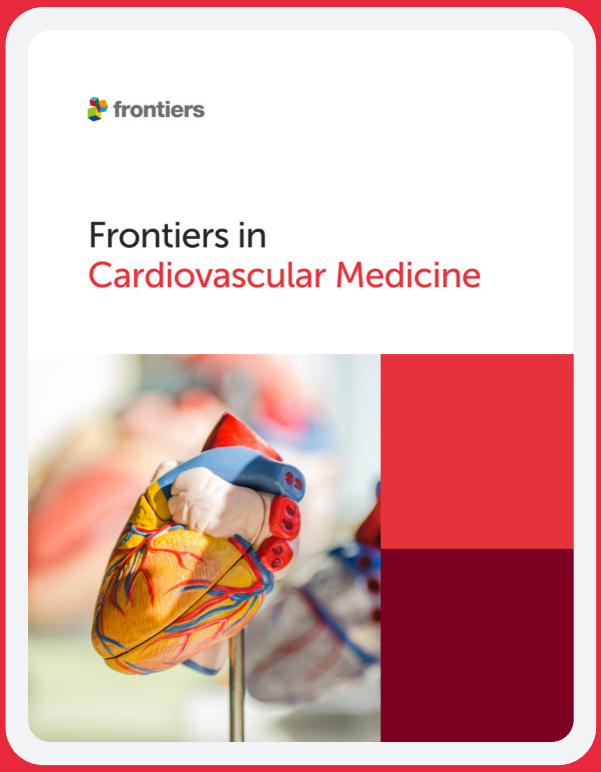

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