HFPEF AND HFMREF: DIFFERENT SIDES OF THE SAME COIN?

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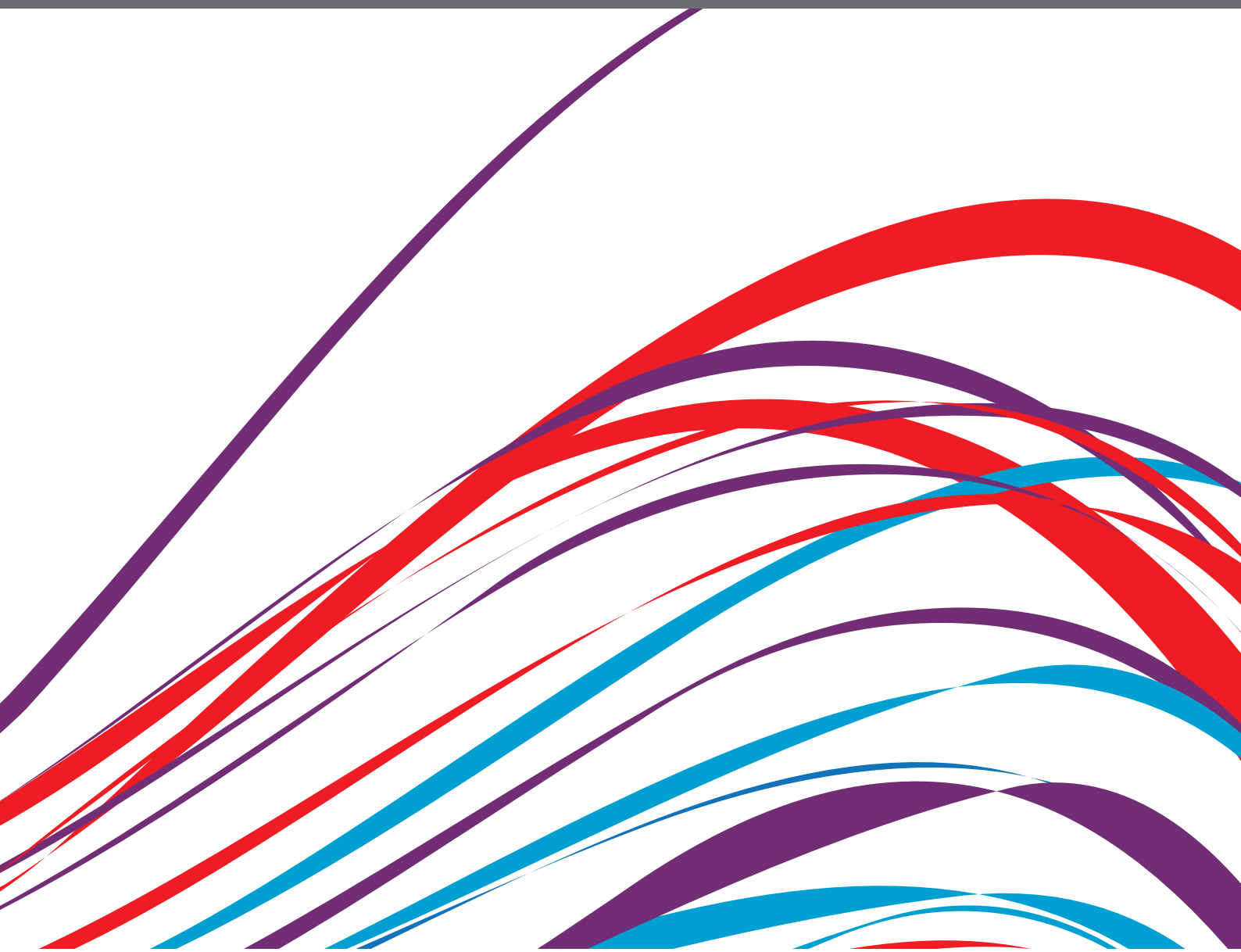

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HFPEF AND HFMREF: DIFFERENT SIDES OF THE SAME COIN?

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EDITORIAL
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Editorial: HFpEF and HFmrEF: Different Sides of the Same Coin?

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Keywords: heart failure, elderly, ejection fraction, prognosis, pathophysiology

Editorial on the Research Topic

HFpEF and HFmrEF: Different Sides of the Same Coin?

Heart failure (HF) has traditionally been divided into distinct phenotypes based on left ventricular ejection fraction (LVEF). The most common way to evaluate LVEF is echocardiography, yet its measurements are subject to substantial variability associated with the technique itself as well as hemodynamic conditions of the patient. In any case, as clinical trials have used specific cut-offs for LVEF, some treatment benefits have only been proven below a certain LVEF value. This is the main reason that explains the recommendation of the European Society of Cardiology in the use of the following three categories (1): HF with reduced ejection fraction (HFrEF, LVEF ≤40%), HF with mildly reduced ejection fraction (HFmrEF, LVEF 41–49%), and HF with preserved ejection fraction (HFpEF, LVEF>50%). In any case, most studies that have included patients with HFmrEF suggest that they may benefit from similar therapies to those with HFrEF. This was the main reason for the recent change of the name in the group of patients with LVEF 41–49% that was previously named "heart failure with mid-range ejection fraction."

This Research Topic aims to focus on patients with HFpEF and HFmrEF, highlighting their similarities and differences. The clinical profile of these patients has particularities that differentiate them from HFrEF, including a more advanced age and a higher prevalence in women (2, 3). In addition, biomarkers and ionic parameters have also a different impact according to LVEF and their role, levels and thresholds in HFpEF and HFmrEF are different from the ones observed in HFrEF (4).

HFPEF

In this special volume, Chi et al. review the role of arterial stiffness and its current treatment strategies. Several original clinical studies are also presented. Bai et al. evaluate the interrelation between neutrophil to lymphocyte ratio and diastolic dysfunction, showing that a high neutrophil to lymphocyte ratio coupled with transcriptional activation of neutrophils correlates with systemic inflammation and functional impairment. Liang et al. present a *post-hoc* analysis of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial (TOPCAT) focusing on liver function. The authors found that elevated serum cholestasis markers such as total

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bilirubin and alkaline phosphatase were associated with a poor clinical outcome. Wang et al. show that the MELD-XI score is associated with short-term adverse events in these patients and provides additional discriminatory capacity to risk stratification models in hospitalized patients. Huang et al. describe the association of weight change with mortality risk in patients from the Americas from the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist study, showing that weight loss is related with all-cause mortality, while weight gain is not associated with better survival.

Animal studies are also presented whereby, Zhang et al. describe the alteration of N6-methyladenosine RNA methylation in patients and in a mouse model of HFpEF, suggesting that the modulation of epitranscriptomic processes might be an interesting target for therapeutic interventions. Zhao W. et al. demonstrate how cardiomyocyte-specific deletion of STAT3 results in a murine HFpEF model, an interesting model that could help us to better understand this condition and to test new therapies.

HFMREF

With regards to HFmrEF. Zhu et al. summarize the current knowledge regarding clinical epidemiology, pathophysiology, and prognosis of HFmrEF. Ma T. et al. review the treatment regime, showing data that support a similar approach to HFrEF. Palazzuoli and Beltrami review the (few) differences of HFmrEF and HFpEF and emphasize that a same patient evaluated in different periods or by different physicians could lead to varying classification from HFmrEF to HFpEF. Zhou et al. suggest that HFmrEF may represent a transitional stage. Maeder et al. describe the important role of pulmonary hypertension in mediating HFmrEF.

HF PATHOPHYSIOLOGY

Two reviews from this Research Topic focus on HF pathophysiology. Zhao Y-L. et al. perform a systematic review and meta-analysis to compare the effectiveness of exercise training for patients with chronic thromboembolic pulmonary hypertension after pulmonary endarterectomy, concluding that exercise training may be associated with a significant improvement in the exercise capacity and quality of life. Bingel et al. describe the hemodynamic changes during physiological and pharmacological stress testing in HF patients presenting reference values that can help to estimate the expected hemodynamic responses.

Several original studies report interesting finding on HF patients. Qin et al. demonstrate how epicardial adipose tissue measured from computed tomography predicts cardiac resynchronization therapy response in patients with non-ischemic HFrEF. Ma Z. et al. describe a new biomarker, elabela, and show how low plasma levels in hypertensive HF may predict the occurrence of major adverse cardiac events. Pang et al. demonstrate how TRAF family member associated NF-κB accelerates the progression of pathological cardiac hypertrophy and is a potential therapeutic target. Ma M. et al. use a single-cell transcriptome analysis to decipher new potential regulation mechanism of angiotensin-converting enzyme 2 and NPs signaling among HF patients infected with SARS-CoV-2, suggesting that in the failing heart, the upregulation of ACE2 and virus-associated genes could potentially facilitate SARS-CoV-2 virus entry and replication in vulnerable cardiomyocytes. Weijing et al. present the results of a randomized trial showing how cardiac shock wave therapy can ameliorate myocardial ischemia in patients with chronic refractory angina pectoris, an important finding as ischemic heart disease is a common cause of HF.

In summary, this Research Topic highlights the importance of distinguishing between HFpEF and HFmrEF. The prevalence of HF with LVEF <40% is similar or even higher than the prevalence of HFrEF, but the amount of data regarding these conditions is quite scarce when compared against the number of clinical trials that have shown important benefits of HFrEF treatments. Further studies specifically focused on these patients may help to clarify their pathophysiology and to provide new therapeutic tools.

AUTHOR CONTRIBUTIONS

MM-S designed the manuscript and wrote the first draft. All authors contributed and approved the final draft.

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ORIGINAL RESEARCH
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Effects of Cardiomyocyte-Specific Deletion of STAT3–A Murine Model of Heart Failure With Preserved Ejection Fraction

Weilin Zhao[1,2†], Yanjia Chen[1†], Wenbo Yang[1], Yanxin Han[1], Zhiyan Wang[1,2], Fanyi Huang[1,2], Zeping Qiu[1,2], Ke Yang[1] and Wei Jin[1,2]*

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Aims: There is a high incidence of heart failure with preserved ejection fraction (HFpEF), but the options of treatment are limited. A new animal model of HFpEF is urgently needed for in-depth research on HFpEF. Signal transducer and activator of transcription 3 (STAT3) may affect the passive stiffness of myocardium, which determines cardiac diastolic function. We hypothesized that cardiomyocyte-specific deletion of STAT3 increases cardiac passive stiffness, which results the murine features of HFpEF.

Methods and Results: Cardiomyocyte-specific deletion of STAT3 (STAT3cKO) mice was generated by the Cre/FLOXp method. The STAT3cKO mice showed heavier cardiac fibrosis and cardiac hypertrophy comparing with wild-type (WT) mice. Furthermore, STAT3cKO mice showed increased serum brain natriuretic peptide (BNP) level, and growth stimulation expressed gene 2 (ST2) level. Other indicators reflecting cardiac passive stiffness and diastolic function, including end diastolic pressure volume relation, MV A value, MV E value, E/A and E/E' had different fold changes. All these changes were accompanied by decreasing levels of protein kinase G (PKG). Bioinformatic analysis of STAT3cKO mice hearts suggested cGMP-PKG signaling pathway might participate in the pathogenesis of HFpEF by means of adjusting different biological functions.

Conclusions: Cardiomyocyte-specific deletion of STAT3 results in a murine HFpEF model which imitates the clinical characteristics partly by affecting cardiac PKG levels. Better understanding of the factors influencing HFpEF may finally provided innovative therapies.

Keywords: HFPEF, stat3, fibrosis, cardiac hypertrophy, passive stiffness

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INTRODUCTION

Heart failure (HF) is a complex clinical syndrome caused by various etiologies and can be classified as preserved, mild-range and reduced ejection fraction (EF) (1). According to reports, heart failure with preserved ejection fraction (HFpEF) accounts for more than 50% in all HF patients, and there is no doubt that HFpEF will become the commonest type of HF around the world in the future (2–4). HFpEF is a complex syndrome with high morbidity and mortality. Despite many efforts, so far, there are no evidence-based therapies (5, 6).

In the past few decades, plenty of murine models were developed to simulate diverse pathological mechanisms administering to HFpEF. The most common HFpEF models focus on investigating classic risk factors including hypertension, obesity, diabetes mellitus, and aging (7–11). In addition to these limitations, these classic animal models are highly heterogeneous and do not meet the commonality seen with any specific HFpEF population. Clinical trials have suggested that the cardiac passive stiffness in HFpEF patients increase obviously (12, 13), which is the main characteristic of HFpEF and has been ignored in other animal models. So there is an urgent need to produce new animal models for further resolving these problems.

Signal transducer and activator of transcription 3 (STAT3) can be activated by various cytokines to exert a variety of biological effects (14, 15). Previous studies showed the loss of STAT3 was prone to fibrosis development and other pathogenesis in heart (16). Significantly, cardiomyocyte-specific deletion of STAT3 in mice induced deep reduction of PKG (17), which involved in interstitial fibrosis and cardiomyocyte hypertrophy (18, 19). In diastole, collagen as the major constituent of extracellular matrix contributes mostly to cardiac passive stiffness (19–21). Thus, we aimed to determine if cardiomyocyte-specific deletion of STAT3 could impair cardiac diastolic function in this model. Our data established that cardiomyocyte-specific deletion of STAT3 in mice would lead to cardiac fibrosis, decreased capillary density, cardiac hypertrophy, and eventually impaired cardiac diastolic function partly by reducing the levels of PKG.

METHODS AND MATERIALS

Animal Models

The experimental protocols of all animals were ratified by the Committee on the Ethics of Animal Experiments of Ruijin Hospital. Cardiomyocyte-specific αCre mice and male STAT3 ($flox^{+/+}$) mice at 4 weeks were purchased from the Jackson Laboratory. All of the experimental mice were kept in the Animal Experiment Center of RuiJin Hospital. Flox/flox mice were mated with the tamoxifen-inducible αCre mice. The 8-week-old flox/flox Cre+ mice subjected to intraperitoneal injection of tamoxifen (T5648, Sigma) at a dose of 50 mg/kg/day for 5 consecutive days. When cardiomyocyte-specific deletion of STAT3 had been performed and mice had grown to 4 months old, we tested all the indicators described below.

Echocardiography

The echocardiology parameters such as left ventricular eject fraction (LVEF), fractional shortening, interventricular septum thickness at end-systole (IVS; s) and end-diastole (IVS; d), the left ventricular posterior wall thickness at end-systole (LVPW; s) and end-diastole (LVPW; d), trans-mitral valve velocity E peak (MV E) and A peak (MV A), E/A and E/E' were all performed by VisualSonics Vero2100 system and achieved from M-mode long-axis views or apical four-chamber views.

Blood Pressure and Pressure-Volume Measurements

The CODA apparatus and tail-cuff method (Kent Scientific) were used to measure mice systolic blood pressure. All the mice were tested at least eight times. We calculated the mean systolic blood pressure value of repeated measurements. The pressure-volume measurements were achieved by SciSense Advantage Admittance Derived Volume Measurement System and 1.2F catheters (SciSense). Data were captured and analyzed by LabScribe2.

Western Blot Analysis

The protein samples were achieved from heart tissue. The prepared protein samples with equal amounts were separated by SDS-PAGE and blotted onto polyvinylidene fluoride membranes. The membranes were then incubated with antibodies against STAT3 (1:1000) (Cell signaling technology, cat# 9139; RRID:AB_331757), collagen 1 (1:1000) (Abcam, cat# ab21286; RRID:AB_446161), collagen 3 (1:1000) (Antibodies-Online, cat# ABIN285714; RRID:AB_10789249), fibronection (1:1000) (Abcam, cat# ab2413; RRID:AB_2262874), CD31 (1:1000) (Cell signaling technology, cat# 77699; RRID:AB_2722705), p-phospholamban (1:1000) (Cell signaling technology, cat# 14562; RRID:AB_2798511), p-troponin I (1:1000) (Cell signaling technology, cat# 4004; RRID:AB_2206275), PKG (1:1000) (Cell signaling technology, cat# 3248; RRID:AB_2067450) and GAPDH (1:10000) (MBL International, cat# M171-3; RRID:AB_10597731) at 4°Covernight. The horseradish peroxidase (HRP)-conjugated secondary antibodies were incubated with polyvinylidene fluoride membranes for 1 h at room temperature. Membranes were detected using an enhanced chemiluminescence (ECL) system. Image-Pro Plus 6 was applied to quantify the density of immunoreactive bands.

Immunohistochemistry and Immunofluorescence

Hearts were fixed with 4% paraformaldehyde, embedded in paraffin, and dissected into 5-μm-thick sections. Hematoxylin and eosin (H&E) staining was used to observe heart tissue morphology. MASSON staining was used to observe heart tissue fibrosis. Immunohistochemical staining was achieved by using the anti-collagen 1 antibody (1:100) (Abcam, cat# ab21286; RRID:AB_446161), anti-collagen 3 antibody (1:100) (Antibodies-Online, cat# ABIN285714; RRID:AB_10789249), anti-fibronectin antibody (1:100) (Abcam, cat# ab2413; RRID:AB_2262874), and anti-CD31 antibody (1:100) (Cell signaling technology, cat# 77699; RRID:AB_2722705) for 24 h at 4°C, and then HRP-conjugated anti-rabbit antibody for 1 h at room temperature. Then, the glass slides were incubated with 3, 3′-diaminobenzidine and counter-stained with hematoxylin. The following antibodies were used for co-localization immunohistochemistry analysis: anti-STAT3 (1:50) (Cell signaling technology, cat# 9139; RRID:AB_331757) co-labeled with anti–a-actinin (1:50) (Cell signaling technology, cat# 6487; RRID:AB_11179206). After incubation with Alexa 555– or Alexa 488–conjugated secondary antibodies (1:1000) (Thermo Fisher Scientific, cat# 21833;

RRID:AB_2532155), all sections were observed with a laser confocal microscope (Zeiss LSM 710 system). Detection of the cell membrane was performed using fluorescein isothiocyanate–conjugated wheat germ agglutinin (Sigma-Aldrich).

Plasma Biomarker

The blood samples of mice were collected and centrifuged at 2000rpm for 15 min. Then the serum were collected and stored frozen at −80°C in multiple aliquots until analysis. Biomarkers that reflect heart failure and a proinflammatory and profibrotic state, specifically brain natriuretic peptide (BNP), growth Stimulation expressed gene 2 (ST2) and interleukin 6 (IL-6) were chosen. The serum levels of BNP, ST2 and IL-6 were tested by mouse BNP enzyme-linked immunosorbent assay (ELISA) kit (Bio Tech Senxiong, catalog# Sxm117), mouse ST2 ELISA kit (Bio Tech Senxiong, catalog# Sxm106) and mouse IL-6 ELISA kit (Bio Tech Senxiong, catalog# Sxm032) following the manufacture's protocols.

RNA-Sequence

The Cloud-seq biotechnology (Shanghai, China) helped to perform the RNA-sequencing. Data were analyzed by using R software on the Novelbrain platform (https://cloud.novelbrain.com). Under the guidance of Ensembl Gff gene annotation file, the HISAT2 method was used to compare the high-quality modified read with the reference genome (MiRbase v22). The DESeq2 method was used to calculate the fold change and P value based on the RNA count and RNAs expressed differently between the STAT3cKO mice hearts and WT mice hearts were finally placed. The standard between two groups was set as fold-change $\geq$2 or $\leq$-2 and P-value <0.01.

Statistical Analysis

All data were expressed as mean values ± standard error of the mean (SEM). Student's t test was performed to compare the difference between WT and STA3cKO groups, P-values <0.05 were considered statistically significant.

RESULTS

Cardiomyocyte-Specific Deletion of STAT3 Mice Were Generated by the Cre/FloxP Method

The α-MyHC-Cre transgenic mice were crossed with mice having a loxP-flanked allele targeted STAT3 exons 3–4. The α-MyHC-Cre transgenic mice expressed Cre-recombinase in cardiomyocytes which was under the control of the α-myosin heavy chain (α-MyHC) gene promoter. Tamoxifen was administered for 8-wk-old flox/flox-Cre+ mice. All the indicators described below were tested when mice had grown to 4 months old (**Figure 1A**). Disruption of the STAT3 gene in cardiomyocytes was confirmed by immunofluorescence (**Figure 1B**) and western blot analysis (**Figure 1C**), and the STAT3 protein levels were significantly decreased in the heart of STAT3cKO mouse. In addition, we also demonstrated that there was no difference in the expression levels of STAT3 in the gastrocnemius, liver, and kidney of the STAT3cKO and WT mice (**Figure 1C**).

Cardiomyocyte-Specific STAT3 Ablation Promoted Cardiac Fibrosis and Decreased Capillary Density

Next, we tried to confirm whether STAT3cKO hearts had an altered response in cardiac fibrosis. Western blot analysis (**Figure 2A**) and histological analysis (**Figures 2B,C** and **Supplementary Figure 1a**) showed that cardiomyocyte-specific Stat3 ablation dramatically promoted cardiac fibrosis, as shown by collagen 1, collagen 3 and fibronectin. By histological and western blot analysis, we also found that the levels of CD31, a marker of capillary density were also significantly decreased in STAT3cKO mice (**Figures 2B,C** and **Supplementary Figure 1b**).

Cardiomyocyte-Specific STAT3 Ablation Induced Cardiac Hypertrophy Without Affecting Blood Pressure

The STAT3cKO mice exhibit cardiac hypertrophy, as demonstrated by increased heart weight, heart weight to tibial length ratios and heart weight to body weight ratios (**Figures 3C–E**). These data were consistent with the observations seen in the photo shown in **Figures 3A,B**. Cardiac hypertrophic growth was accompanied by a larger cardiomyocyte size (**Figure 3G**). To assess cardiac hypertrophy further, 4-month-old STAT3cKO and WT mice were subjected to echocardiogram analysis. Compared with WT mice, STAT3cKO mice had increased interventricular septum thickness at end-systole (IVS; s) (**Figure 3I**) and end-diastole (IVS; d) (**Figure 3J**). Additionally, the left ventricular posterior wall thickness at end-systole (LVPW; s) and end-diastole (LVPW; d) significantly increased in STAT3cKO mice compared to WT mice (**Figures 3K,L**). Additionally, the blood pressures of all kinds of mice were similar (**Figure 3H**). Taken together, these findings suggested that cardiomyocyte-specific STAT3 ablation made mice developed cardiac hypertrophy without affecting blood pressure.

Cardiomyocyte-Specific STAT3 Ablation Impaired Cardiac Diastolic Function

We next tested some indicators of heart failure. The levels of serum brain natrium peptide (BNP), growth stimulation expression gene 2 (ST2) and interleukin 6 (IL-6) all increased heavily in STAT3cKO mice, which were the key biomarkers of heart failure (**Figures 4A,B** and **Supplementary Figure 1f**). Trans-mitral Doppler flow velocity showed higher trans-mitral valve velocity E peak (MV E) and A peak (MV A) in STAT3cKO mice than in WT mice, suggesting an increase in left ventricular (LV) chamber stiffness (**Figures 4H,I**). The ratio of mitral E velocity to mitral annular E' velocity (E/E'), a reliable predictor of LV end diastolic pressure, was elevated in STAT3cKO mice relative to WT mice (**Figure 4K**). The ratio of mitral E/A velocity was increased, also suggesting a restrictive LV filling pattern (**Figure 4J**), while the ejection fraction (EF) and fractional shortening were preserved (**Figures 4E–G**).

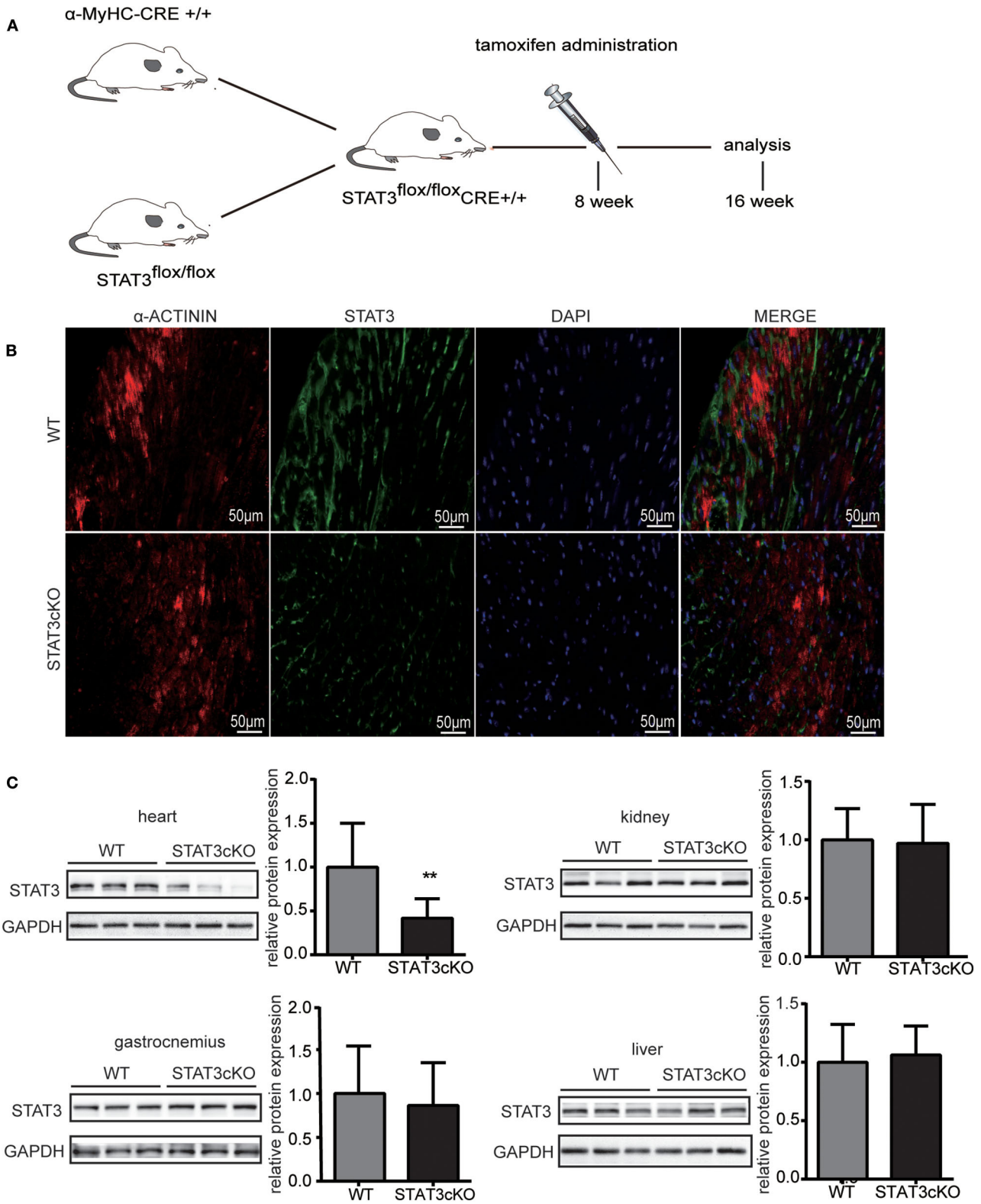

FIGURE 1 | The stat3 gene was actually knock out in STATcKO mice. **(A)** Experimental design. $STAT3^{flox/flox}$ Cre+ mice were administrated by tamoxifen at 8 weeks and analysis followed indicators at 16 weeks. **(B)** Representative mouse heart sections immunostained for α-ACTININ (red), STAT3 (green) and DAPI (blue) **(C)** Western blot analysis showed the levels of STAT3 in WT mice hearts, livers, kidneys and gastrocnemius ($n = 3$) and STAT3cKO mice hearts, livers, kidneys and gastrocnemius ($n = 3$). Compared with WT group, $^{*}P < 0.05$ by Student's unpaired *t*-test. $^{**}P < 0.01$ by Student's unpaired *t*-test.

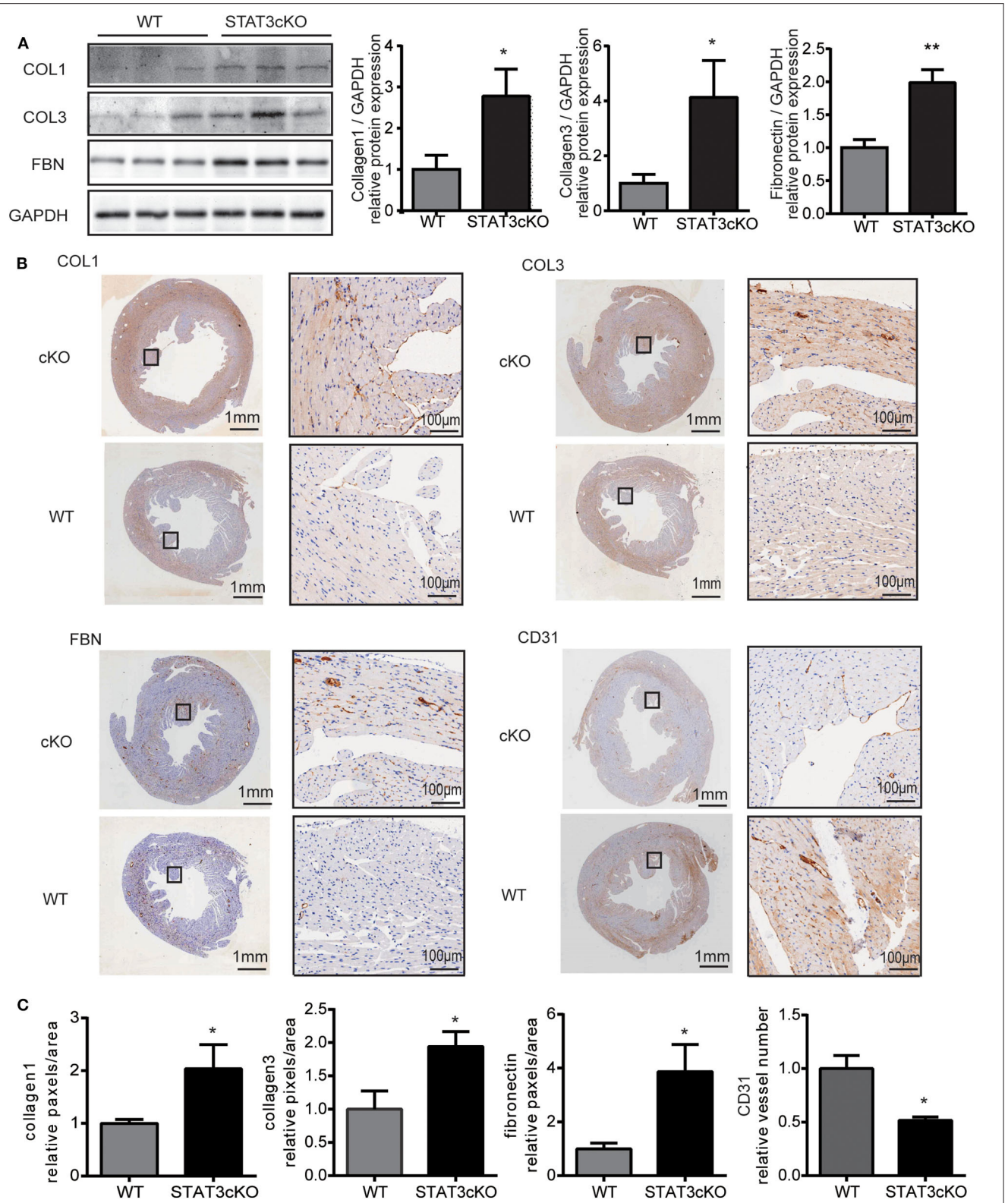

FIGURE 2 | Cardiomyocyte-specific STAT3 ablation promotes cardiac fibrosis and reduces capillary density. **(A)** Western blot analysis showed the levels of collagen 1, collagen 3 and fibronectin in WT mice hearts ($n = 3$) and STAT3cKO mice hearts ($n = 3$). **(B)** Representative images of immunohistochemical staining of COL1, COL3, FBN, and CD31 in cardiac left ventricular tissue of WT mice and STAT3cKO mice. (Left panel of every image is original magnification. Right panel of every image is original magnification x10.) **(C)** Fixed quantity of immunohistochemical staining of COL1, COL3, FBN, and CD31 in cardiac left ventricular tissue of WT mice and STAT3cKO mice ($n = 3$). Compared with WT group, $^{*}P < 0.05$ by Student's unpaired *t*-test. $^{**}P < 0.01$ by Student's unpaired *t*-test.

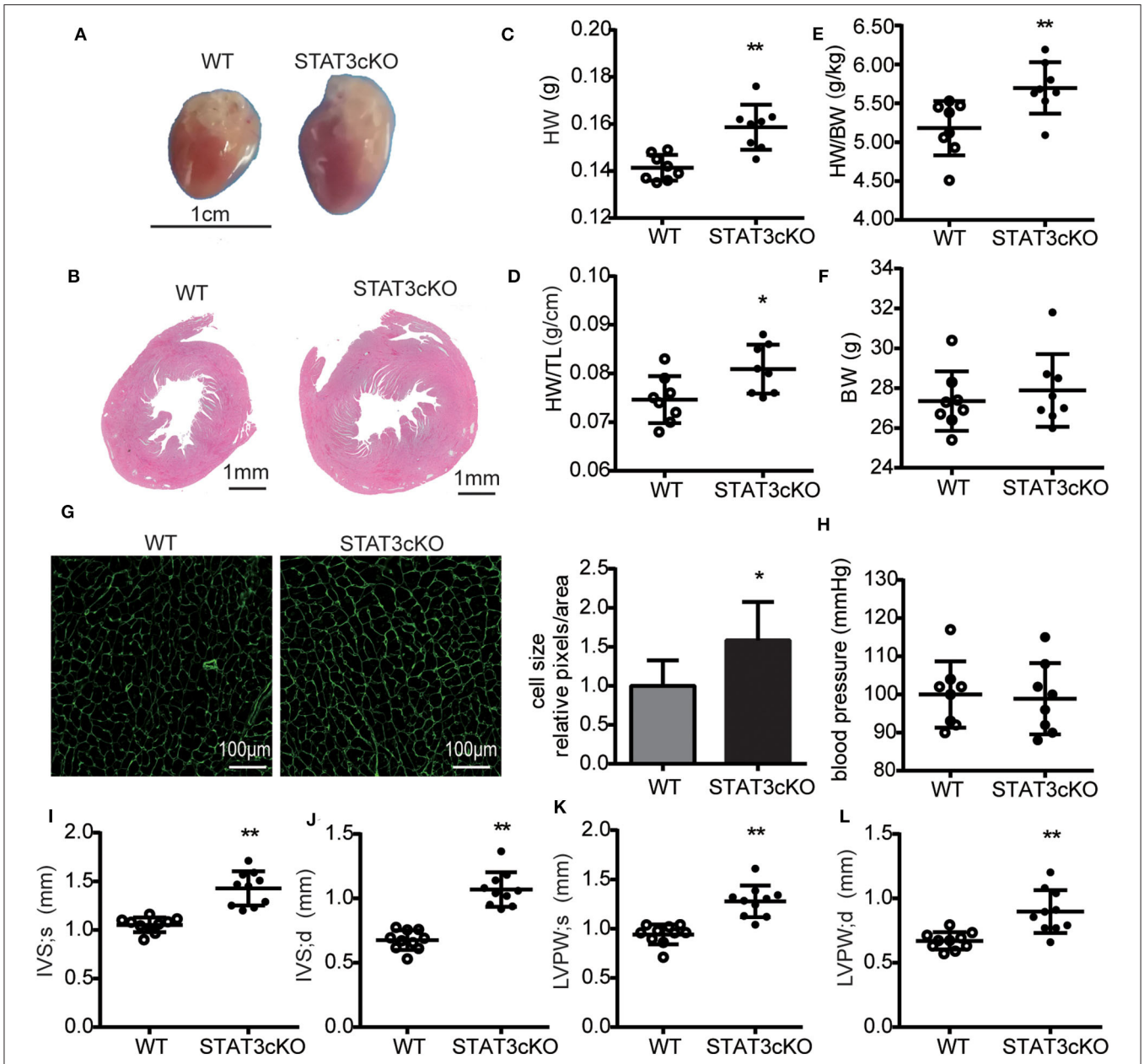

FIGURE 3 | Cardiomyocyte-specific STAT3 ablation induces cardiac hypertrophy without affecting blood pressure. **(A)** Representative pictures of hearts from a WT mouse and STAT3cKO mouse. **(B)** Representative images of HE staining in cardiac left ventricular tissue of WT mice and STAT3cKO mice. **(C–F)** Heart weight (HW), ratio of heart weight to tibia length (HW/TL), ratio of heart weight to body weight (HW/BW), body weight in WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). **(G)** Cardiomyocyte size as assessed by WGA staining in WT mice and STAT3cKO mice. **(H)** Blood pressure of WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). **(I–L)** Levels of IVS;s, IVS;d, LVPW;s and LVPW;d in echocardiographic observations of WT mice ($n = 10$) and STAT3cKO mice ($n = 10$). Compared with WT group, $^{*}P < 0.05$ by Student's unpaired t-test. $^{**}P < 0.01$ by Student's unpaired t-test.

These echo parameters were supported by a pressure volume analysis that revealed an increase in diastolic stiffness coefficient of the end diastolic pressure volume relation (EDPVR) in STAT3cKO mice (**Figures 4C,D** and **Supplementary Figure 1e**). In order to understand the condition of peripheral tissue edema, we tested the lung weight and HE staining of lung for all the mice. As a result, the HE staining showed intimal thickening of pulmonary capillaries in STAT3cKO mice and lung weight of STAT3cKO mice was heavier than that of WT mice (**Supplementary Figures 1c,d**). These data suggested that STAT3cKO mice have pulmonary edema to a certain extent. In summary, the STAT3cKO mice developed a deep degree of diastolic dysfunction while systolic function was preserved.

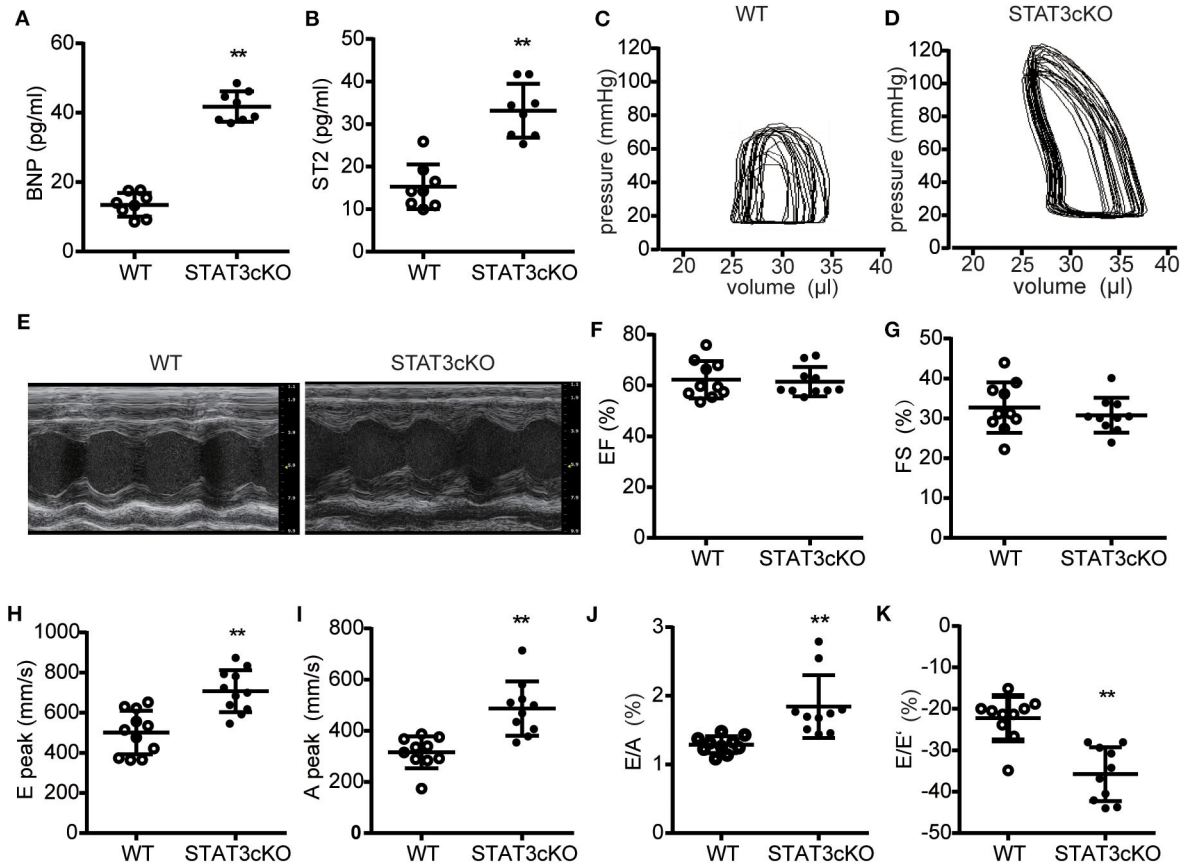

FIGURE 4 | Cardiomyocyte-specific STAT3 ablation impaired cardiac diastolic function. **(A)** The levels of BNP concentration in WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). **(B)** The levels of ST2 concentration in WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). **(C,D)** Representative pictures of pressure-volume loop from a WT mouse and STAT3cKO mouse. **(E)** Representative left ventricular M-mode echocardiographic tracings of WT mice ($n = 10$) and STATcKO mice ($n = 10$). **(F,G)** Percentages of LVEF and FS in WT mice ($n = 10$) and STAT3cKO mice ($n = 10$). **(H–K)** Levels of E peak, A peak, E/A, E/E' in WT mice ($n = 10$) and STAT3cKO mice ($n = 10$). Compared with WT group, $^{*}P < 0.05$ by Student's unpaired t-test. $^{**}P < 0.01$ by Student's unpaired t-test.

Cardiomyocyte-Specific Deletion of STAT3 Reduced Myocardial PKG Levels and Eventually Impaired Cardiac Diastolic Function

Based on the data above, we tried to explore the possible mechanisms of cardiac diastolic dysfunction in STAT3cKO mice. RNA sequence of the heart tissue of STAT3cKO mice was tested. Bioinformatic analysis [including Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and Gene Set Enrichment Analysis (GSEA)] were performed to identify potential effects of differential genes in STAT3cKO mice hearts. The hierarchical clustering method was used to confirm the consistency of dysregulated mRNAs in STAT3cKO mice ($N = 3$) and WT mice ($N = 3$) hearts (**Figure 5A**). A total of 508 dysregulated genes (including 223 up-regulated genes and 285 down-regulated genes) were distinguished via the expression analysis, (**Figure 5B**). KEGG analysis were done to identify the relevant pathways for predicting target mRNAs (**Figure 5C**). The top five associated pathways were ECM-receptor interaction, Vascular smooth muscle contraction, Focal adhesion, PI3K-Akt signaling pathway and cGMP-PKG signaling pathway. Studies showed that cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) signaling pathway has been provided novel perspectives on HFpEF (13, 19). ECM-receptor interaction and PI3K-Akt signaling pathway may influnce cardiac function (1). So GSEA analysis was applied to further explore the hub pathway. The GSEA analysis showed that cGMP-PKG signaling pathway had a better enrichment score and gene original size comparing with ECM-receptor interaction and PI3K-Akt signaling pathway (**Figure 5D**). So we focused on cGMP-PKG signaling pathway. Through KEGG map of cGMP-PKG signaling pathway, we found that there were 15 downregulated genes (including PKG, βAR, ATPase and so on) and two upregulated genes (ROS and βMHC) in STAT3cKO mice hearts (**Supplementary Figure 2**). As a result, the 17 differential gens in cGMP-PKG signaling pathway affected cardiac systolic function by increasing cardiac hyprertrophy, increasing intracellular free calcium, decreasing cardioprotection of mitochondria, inducing endothelial dysfunction and so on.

Consistent with the results of RNA sequence, the protein levels of PKG were significantly down-regulated in STAT3cKO

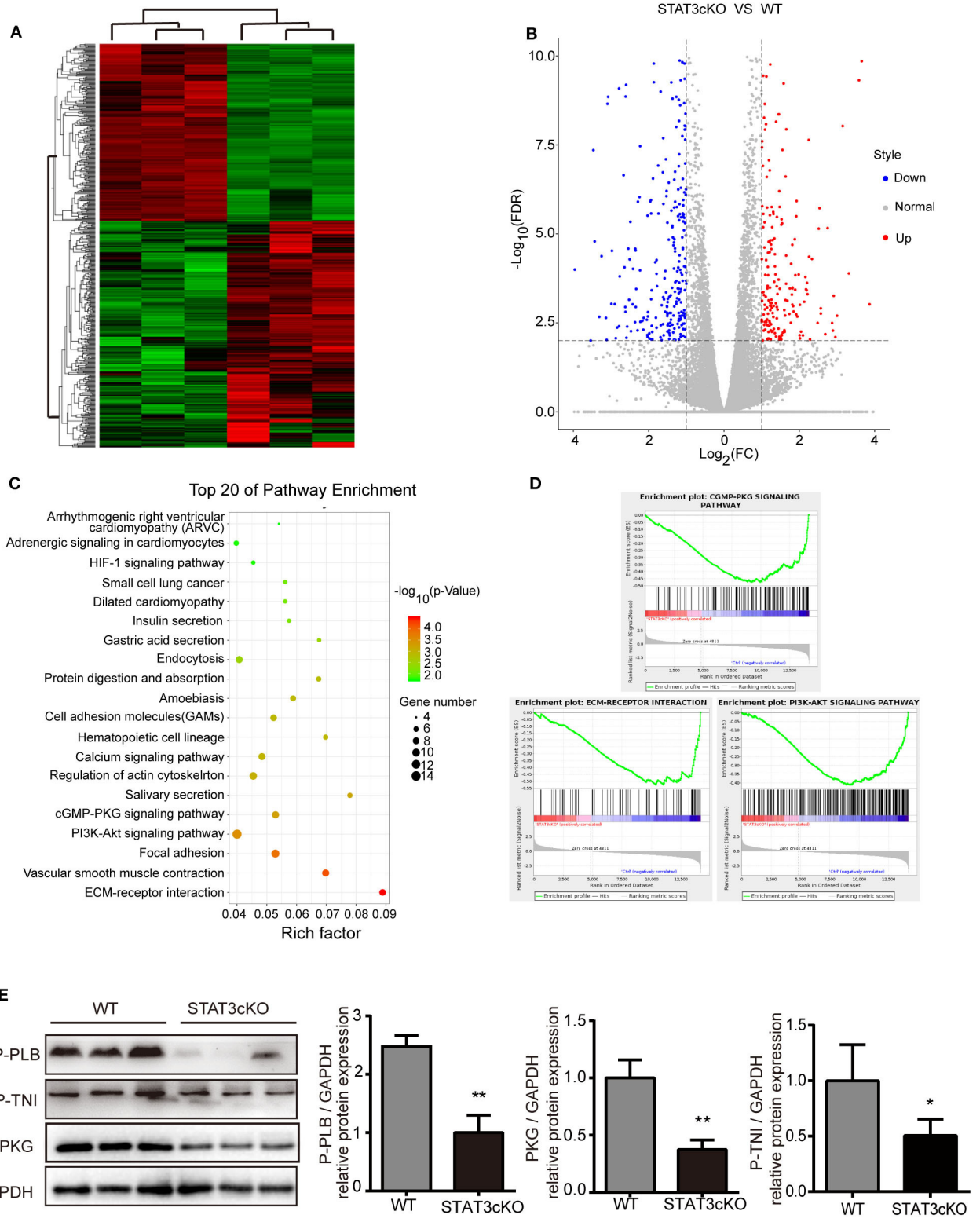

FIGURE 5 | Cardiomyocyte-specific deletion of Stat3 reduced myocardial PKG levels and RNA-sequencing of STAT3cKO mice hearts suggested potential pathogenesis of HFpEF. **(A)** The hierarchical clustering method was used to identify the consistency of dysregulated mRNAs in STAT3cKO ($n = 3$) and WT ($n = 3$) mice hearts. **(B)** The colcano map showed the 223 up-regulated gens and 285 down-regulated gens in STAT3cKO and WT mice hearts (Fold change≥2 and

(Continued)

FIGURE 5 | $P < 0.01$). **(C)** The top 20 of pathway enrichment were ananlyzed by Kyoto Encyclopedia of Genes and Genomes analysis. **(D)** The enrichment plot of cGMP-PKG signaling pathway, ECM-RECEPTOR interaction, and PI3K-AKT signaling pathway enrichment were ananlyzed by Gene Set Enrichment Analysis. **(E)** Western blot analysis showed the levels of PKG, P-PLB and P-TNI in WT mice hearts ($n = 3$) and STAT3cKO mice hearts ($n = 3$). Compared with WT group, $^{*}P < 0.05$ by Student's unpaired *t*-test. $^{**}P < 0.01$ by Student's unpaired *t*-test.

hearts. Additionally, the levels of phosphorylation of troponin I [p(S23/24)-TNI] and phospholamban [p(S16)-PLB] which were two of the major events underlying β-adrenergic–mediated signaling (17, 22), also decreased obviously in STAT3cKO mice hearts (**Figure 5E**). These results indicated that loss of STAT3 in cardiomyocyte ultimately promoted cardiac diastolic dysfunction partly due to the reduced myocardial PKG levels.

DISCUSSION

HFpEF is a fetal disease and there is not enough effective clinical therapies (23). Considering the limitations of present animal models, we developed a murine model of cardiomyocyte-specific deletion of STAT3 that recapitulated the clinical characteristics of HFpEF.

The disruptions which we performed to duplicate the clinical characterization are on the basis of pathophysiological observations of human condition. Changes in cardiac passive stiffness have been confirmed in HFpEF patients, and our murine model takes full advantage of this feature to manage increased cardiac passive stiffness from the start. By contrast, the traditional HFpEF animal models imitate specific characteristics matching particular HFpEF populations such as hypertension, obesity, diabetes mellitus, and aging. For example, a typical hypertension animal model imitated HFpEF-the Dahl salt-sensitive rat (8). Actually, this kind of model can implicate the renin–angiotensin–aldosterone system in HFpEF. However, with increasing HFpEF research, therapies targeted RAAS system have little clinical value for HFpEF patients (23, 24). In addition to this, murine diabetes mellitus and obesity models such as the Akita mouse (Ins2 Akitaþ/-) (9, 10), glucosamine-nitrosourea streptozotocin (STZ) mice (9, 10), ob/ob (11), and db/db (25) mice all exhibited some phenotypes of HFpEF (26–29). In fact, these are more suitable as metabolic syndrome animal models and give little help for research on preclinical evaluation of potentially novel therapeutic strategies.

In the present study, we demonstrated that animals lacking cardiomyocyte expression of STAT3 were more likely to develop HFpEF (**Figure 4**). More significantly, we found that cardiac deletion of STAT3 led to cardiac passive stiffness. More and more studies have shown that cardiac diastolic function can be affected by LV stiffness, which includes ECM-based and titin-based passive stiffness (30, 31). Our study focused on the former. Cardiomyocyte-specific deletion of STAT3 dramatically increased the levels of the collagen 1 and collagen 3 which were the major ECM components contributing to cardiac passive stiffness (**Figure 2**) These results were completely in line with the characteristic of increased ECM-based cardiac stiffness in HFpEF (19, 20). Additionally, previous studies showed that the release of catecholamines activated the PKA pathway through β-adrenergic receptors (β ARs) and induces the phosphorylation of the spring-like domains of titin. Subsequently, increased flexibility and compliance of titin extends the physiological length of the sarcomere and improves the diastolic function of the heart (12, 13, 21, 32–34). We found cardiomyocyte-specific deletion of STAT3 down regulated β-adrenergic–mediated signaling (**Figure 5**). However, the phosphorylational state of titin remains unclear in STAT3cKO mice, which need to be further investigated.

Reporters suggested that HFpEF patients showed reduced myocardial PKG levels and lower cGMP concentration comparing with HFrEF patients (13). As the main protein kinases, PKG phosphorylates a great deal of proteins, showing a variety of downstream effects such as enhancing intracellular diastolic calcium reuptake by phosphorylation of phospholamban, inhibiting hypertrophic signaling via inhibition of G-protein coupled receptors, and stimulation of left ventricular relaxation and distensibility by phosphorylation of troponin I (TnI) and titin, and so on (35). These alterations were clearly consistent with our results (**Figure 5** and **Supplementary Figure 2**). Alterations in cardiomyocyte cGMP-PKG pathway ultimately increased interstitial fibrosis, cardiomyocyte hypertrophy and finally weaken cardiac diastolic function through associated downstream effects explaining above.

Early studies showed that global ablation of the STAT3 gene in mice results in embryonic lethality during embryonic development (36). Other studies have shown that cardiomyocyte-specific deletion of mouse STAT3 gene did not affect the heart structure and function of young mice (16). So we generated cardiomyocyte-specific STAT3 knock-out models in 8-wk-old mice and tested all the data when the mice were 16–wk old. In addition to this, as a temporary cardiomyopathy caused by Cre expression existed about 4 weeks, all mice should be permitted to recover for 6 weeks after the last tamoxifen injection (37). In our study, tamoxifen had no effect on the 16-wk old STAT3cKO mice. Although the STAT3cKO mice model imitated one of the most common comorbidity in the human setting, there still exerts some limitations in the model. The main limitation is that as the main member of signal transducer and activator of transcription family, STAT3 regulates a large number of biological functions primarily in response to extracellular signaling molecules such as cytokines and growth factors (**Figure 5**). As STAT3 is the key cellular molecule, it is not certain whether other impaired signaling pathways would affect cardiac diastolic function in a STAT3cKO mouse model. Another important limitation is that we did not exam the advanced performance in STAT3cKO mice. In our study, the cardiac systolic function remained normal in 16-wk-old

STAT3cKO mice. Previous studies suggested that adult Stat3cKO mice spontaneously developed heavily myocardial fibrosis and eventually HFrEF at 36 weeks (16). Actually, the transformation of HF phenotype in STAT3cKO mice with aging is consistent with clinical HF patients. Under the physiological stresses or other factors, HFpEF patients may develop to heart failiure with reduced eject fraction.

In summary, we have shown that cardiomyocyte-specific deletion of STAT3 caused cardiac fibrosis, and hypertrophy. As a result, in mice with myocardial-specific deletion of STAT3, cardiac diastolic functions were impaired, while systolic function remained normal. Moreover, we have revealed that STAT3 regulates the levels of PKG, that affects the cardiac ECM-based passive stiffness. Together, these data clearly have demonstrated that mice with cardiomyocyte-specific deletion of STAT3 are a successful HFpEF animal model, which will contribute to the development of HFpEF research on treatment in the future.

DATA AVAILABILITY STATEMENT

The datasets generated for 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**.

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee on the Ethics of Animal Experiments of the Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

WZ, YC, KY, and WJ were responsible for the design of the study and the writing the manuscript. WY and YH were responsible for data analysiswork. ZW, FH, and ZQ were responsible for the edit of the manuscript. All authors read and approved the final manuscript.

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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.2020.613123/full#supplementary-material

Supplementary Figure 1 | Cardiomyocyte-specific STAT3 ablation impaired cardiac diastolic function. **(a)** Representative images of MASSON staining in heart of WT mice and STAT3cKO mice. **(b)** Western blot analysis showed the levels of CD31 in WT mice hearts ($n = 3$) and STAT3cKO mice hearts ($n = 3$) **(c)** Representative images of HE staining in lung of WT mice and STAT3cKO mice. **(d)** Lung weight of WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). **(e)** The diastolic stiffness coefficient of the end diastolic pressure volume relation (EDPVR) by pressure-volum analysis in STAT3cKO mice ($n = 7$) and WT mice ($n = 7$). **(f)** The levels of IL-6 concentration in WT mice ($n = 8$) and STAT3cKO mice ($n = 8$). $^{*}P < 0.05$ by Student's unpaired *t*-test. $^{**}P < 0.01$ by Student's unpaired *t*-test.

Supplementary Figure 2 | The cGMP-PKG signaling pathway might be involved in the pathogenesis of HFpEF via regulating different biological functions. The cGMP-PKG signaling pathway KEGG map showed the 15 downregulated genes and two upregulated genes.

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
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Single-Cell Transcriptome Analysis Decipher New Potential Regulation Mechanism of ACE2 and NPs Signaling Among Heart Failure Patients Infected With SARS-CoV-2

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Aims: COVID-19 patients with comorbidities such as hypertension or heart failure (HF) are associated with poor clinical outcomes. The cellular distribution of Angiotensin-converting enzyme 2 (ACE2), the critical enzyme for SARS-CoV-2 infection, in the human heart is unknown. We explore the underlying mechanism that leads to increased susceptibility to SARS-CoV-2 in patients with cardiovascular diseases and patients of cardiac dysfunction have increased risk of multi-organ injury compared with patients of normal cardiac function.

Methods and Results: We analyzed single-cell RNA sequencing (scRNA-seq) data in both normal and failing hearts. The results demonstrated that ACE2 is present in cardiomyocytes (CMs) and non-CMs, while the number of ACE2-postive (ACE2+) CMs and ACE2 gene expression in these CMs are significantly increased in the failing hearts. Interestingly, both brain natriuretic peptides (BNP) and atrial natriuretic peptide (ANP) are significantly up-regulated in the ACE2+ CMs, which is consistent with other studies that ACE2, ANP, and BNP increased in HF patients. We found that genes related to virus entry, virus replication and suppression of interferon-gamma signaling are all up-regulated in failing CMs, and the increase was significantly higher in ACE2+ CMs, suggesting that these CMs may be more vulnerable to virus infection. As the level of expression of both ACE2 and BNP in CMs were up-regulated, we further performed retrospective analysis of the plasma BNP levels and clinical outcomes of 91 COVID-19 patients from a

single-center. Patients with higher plasma BNP were associated with significantly higher mortality and expression levels of inflammatory and infective markers.

Conclusion: In the failing heart, the upregulation of ACE2 and virus infection associated genes could potentially facilitate SARS-CoV-2 virus entry and replication in these vulnerable cardiomyocyte subsets. COVID-19 patients with higher plasma BNP levels had poorer clinical outcomes. These observations may allude to a potential regulatory association between ACE2 and BNP in mediating myocarditis associated with COVID-19.

Keywords: COVID-19, SARS-CoV-2, heart failure, angiotensin converting enzyme 2, single-cell RNA sequencing

INTRODUCTION

Novel coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). As of January 2021, more than 102 million cases of COVID-19 and more than 1 million deaths have been reported worldwide (2). In addition to the severe lung infection, the SARS-CoV-2 virus also causes myocarditis, cardiac dysfunction, and heart failure (HF) (1, 3–5). Conversely, COVID-19 patients with pre-existing conditions, such as hypertension and coronary heart disease, have worse clinical outcomes than those without these comorbidities (5–7). In this regard, these findings may point towards the presence of a vicious cycle between SARS-CoV-2 infection and cardiac dysfunction or HF (6).

Angiotensin-converting enzyme 2 (ACE2) is the critical enzyme degrading the pro-inflammatory angiotensin-II (Ang II) to the anti-inflammatory Ang 1–7 (8, 9). Unfortunately, ACE2 also facilitates SARS-CoV-2 entry into host cells by binding its surface "spike" protein. ACE2 is highly expressed in the nose, kidney, intestine, colon, brain, endothelium, testis, and heart (10–15). A recent study from Zou et al. reported that ∼7% cardiomyocytes (CMs) express ACE2 in normal human cardiac tissues (12), suggesting that some CMs can be directly infected by SARS-CoV-2. However, ACE2 gene expression in different cardiomyocyte subsets, as well as its dynamic changes in failing human hearts at the single cell level, are totally unknown.

Since ACE2 plays an important role in SARS-CoV-2 infection and cardiac function, it is critically important to understand its distribution and the biological changes associated with its altered expression in normal and failing hearts. Therefore, we investigated the ACE2 gene expression profiles by analyzing the single-cell RNA sequencing (scRNA-seq) dataset derived from both normal and failing human hearts (16). We found that ACE2 was expressed in CMs, vascular endothelial cells, fibroblasts, smooth muscle cells and immune cells in normal hearts, and its expression was further increased in several cell subsets in the failing hearts. Importantly, we found that brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) (NPs) transcripts are co-upregulated in ACE2-postive (ACE2+) CMs. NPs and ACE2 may form a feedback loop associated with the rein-angiotensin-aldosterone-system (RAAS)/Ang II signaling pathway. Furthermore, ACE2 expression was also associated with the dynamic changes of a group of genes related to viral infection and acquired immunity. Since there is a positive correlation between the expressions of BNP and ACE2, we further analyzed the clinical outcome, inflammation markers, and blood BNP levels in COVID-19 patients retrospectively. Together, these findings provide important insights to advance our understanding of the interplay between ACE2, viral infection and inflammation, as well as cardiac injury and failure.

MATERIALS AND METHODS

Clinical Study

Design, Participants, and Data Collection

This is a retrospective, single-center study including 91 patients with laboratory-confirmed COVID-19 admitted to Ezhou Central Hospital, Ezhou, China from January 25, 2020 and March 30, 2020. All procedures were followed in accordance with institutional guidelines. PCR-Fluorescence probing based kit (Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit, Sansure Biotech, China) was used to extract nucleic acids from clinical samples and to detect the ORF1ab gene (nCovORF1ab) and the N gene (nCoV-NP) according to the manufacturer's instructions. SARS-CoV-2 infection was laboratory-confirmed if the nCovORF1ab and nCoV-NP tests were both positive results. The study protocol was approved by the ethics committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine (Shanghai, China) (Approval No. SHSY-IEC-4.1/20-63/02) and the ethics committee of Ezhou Central Hospital (Hubei, China) (Approval No. L2020-Y-013). Patient informed consent was waived by each ethics committee due to the COVID-19 pandemic.

COVID-19 was diagnosed by meeting at least one of these two criteria: (i) chest computerized tomography (CT) manifestations of viral pneumonia; and/or (ii) reverse transcription-polymerase chain reaction (RT-PCR) according to the New Coronavirus Pneumonia Prevention and Control Program (5th edition) published by the National Health Commission of China (New Coronavirus Pneumonia Prevention and Control Program, 2020) and WHO interim guidance (17). We used the following inclusion and exclusion criteria to determine the study cohort. The inclusion criteria were confirmed COVID19, valid BNP level and age above 18 years. The exclusion criteria were incomplete

medical records, pregnancy, preexisting valvular heart disease, congenital heart disease, acute myocardial infarction, acute pulmonary embolism, acute stroke, HIV infection or conformed other virus infection, and end-stage cancer.

The demographic characteristics and clinical data (comorbidities, laboratory findings, and outcomes) for participants during hospitalization were collected from electronic medical records. Cardiac biomarkers measured on admission were collected, including Troponin I (TNI), creatine kinase-MB (CK-MB), and BNP. All data were independently reviewed and entered into the computer database by three analysts. Since the echocardiography data were unavailable for most patients, patients were categorized according to the BNP level. Acute HF was defined as a blood BNP level ≥100 pg/ml. The clinical outcomes (i.e., discharges and mortality) were monitored up to 30 days.

Statistical Analysis

Descriptive statistics were obtained for all study variables. Continuous data were expressed as mean [standard deviation (SD)] or median [interquartile (IQR)] values. Categorical data were expressed as proportions. All continuous variables were compared using the *t*-test or the Mann–Whitney *U*-test if appropriate. In contrast, categorical variables were analyzed for the study outcome by Fisher exact test or χ^2 test. The Pearson correlation coefficient and Spearman rank correlation coefficient were used for linear correlation analysis. Survival analysis between patients with BNP<100 pg/mL and ≥100 pg/mL was conducted by the Kaplan-Meier estimate with *p*-value generated by the log-rank test. Data were analyzed using SPSS version 25.0 (IBM Corp) or Graphpad Prism 8.0.1 (GraphPad Software, San Diego, CA). For all the statistical analyses, 2-sided $p < 0.05$ was considered significant.

scRNA-Seq Analysis

Data Sources

Adult human heart scRNA-seq datasets were obtained from Gene Expression Omnibus (GEO) under accession codes GSE109816 and GSE121893. Briefly, samples from twelve healthy donors and samples from six patients with HF were collected at the time of heart transplantation. The range of donor ages was 21–52 year, with a median age of 45.5 years.

Sequencing Data Processing

The processed read count matrix was retrieved from existing sources based on previously published data as specified explicitly in the reference. Briefly, Raw reads were processed using the Perl pipeline script supplied by Takara.

Single-Cell Clustering and Identified Cell Types

The processed read count matrix was imported into R (Version 3.6.2) and converted to a Seurat object using the Seurat R package (Version 3.1.2). Cells that had over 75% UMIs derived from the mitochondrial genome were discarded. For the remaining cells, gene expression matrices were normalized to total cellular read count using the negative binomial regression method implemented in Seurat *SCTransform* function. Cell-cycle scores were calculated using Seurat *CellCycleScoring* function. The Seurat *RunPCA* functions were performed to calculate principal components (PCs). We further corrected the batch effect using Harmony because batch effects among the human heart samples were observed. The *RunUMAP* function with default setting was applied to visualize the first 35 Harmony aligned coordinates. The *FindClusters* function with resolution=0.2 parameter was carried out to cluster cells into different groups. Canonical marker genes were applied to annotate cell clusters into known biological cell types. Monocle 3 as used to perform trajectory and pseudotime analysis.

Identification of Differential Expression Genes (DEGs)

To identify DEG between two groups, we applied the Seurat *FindMarkers* function with the default parameter of method "MAST" and cells ID from each defined group (e.g., ACE2+ cells versus ACE2 negative (ACE2-) cells in CM1) as input.

Gene Function Analysis

GSEA (Version 4.03) was used to perform gene ontology (GO) term and pathway enrichment analysis with the Molecular Signatures Database (MSigDB, C2 and C5, Version 7.01).

RESULTS

Integrated Analysis of Normal and HF Conditions at Single-Cell Resolution

To detect the discrepancy between normal and HF patients, we utilized the scRNA-seq data by Wang et al. (16). Briefly, twelve control samples were collected from healthy donor hearts (hereinafter called normals). Samples from six HF patients were collected at the time of heart transplantation. Thirteen thousand nine hundred eighty-six out of 15,215 cells passed standard quality control and were retained for subsequent analyses. After UMAP and clustering analysis the entire cell population were grouped into nine subsets (**Figure 1A**). Dot plot showed the expression of known markers for nine clusters, which included: (1) endothelial cells (PECAM1 and VWF); (2) fibroblasts (LUM and DCN); (3) smooth muscle cells (MYH11); (4) NK-T/monocytes (CD3G and CD163); (5) granulocytes (HP and ITLN1); (6) CM2 and 3 subsets (MYH6 and NPPA); (7) CM1 and 4 subsets (MYH7 and MYL2) (**Figure 1B**). UMAP for individual sample exhibited the differential distribution of subsets between normal and HF patients (**Figure 1C**, **Supplementary Table 1**). As shown in **Figure 1C**, all nine subsets were detected in both normal and patient groups. However, the percentage of CM1 was dramatically decreased ($p < 0.0001$), while the percentage of CM4 was significantly increased ($p < 0.0001$) in HF samples. In addition, the percentages of CM2 ($p > 0.05$) and CM3 ($p > 0.05$) were not changed significantly. The percentages of vascular endothelial cells ($p < 0.0001$) and fibroblasts ($p < 0.0001$) were also significantly increased in the failing hearts (**Figure 1D**).

For each cluster, we calculated the cluster-specific genes (marker genes). Left ventricle (LV) marker genes MYL2 and MYL3 were highly expressed in CM1 and CM4; these subsets

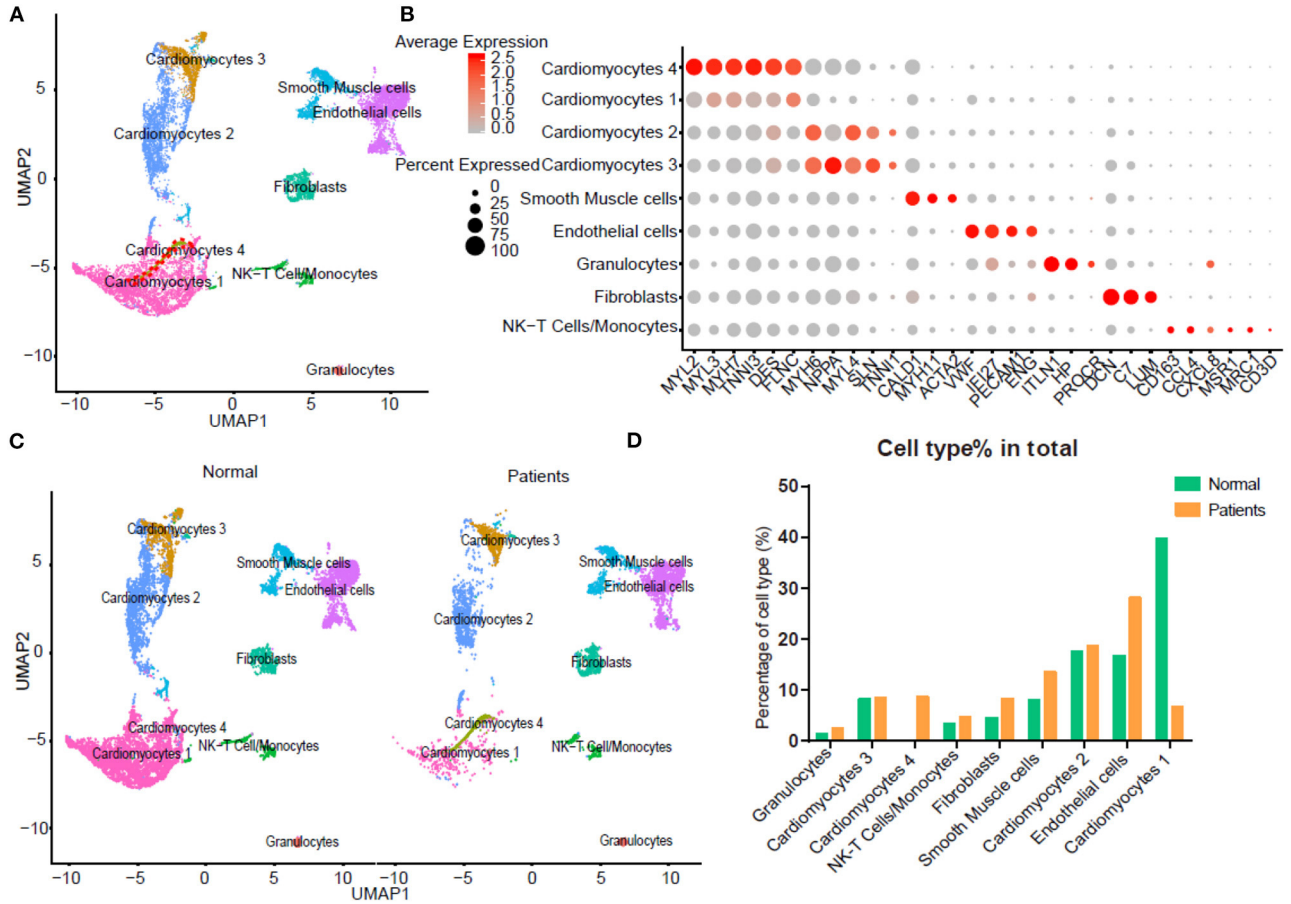

FIGURE 1 | Integrated analysis of normal and heart failure (HF) conditions at single-cell resolution. **(A)** Uniform manifold approximation and projection (UAMP) clustering of 14,698 cells isolated from normal and heart failure patients. Each dot represents a single cell. Cell type was annotated by the expression of known marker genes. **(B)** Dot plotting showing gene signature among different clusters, the shadings denotes average expression levels and the sizes of dots denote fractional expression. **(C)** Split views show the 9 subsets in normal and patient group. **(D)** The percentage of cell number for different cell types in normal and patient group.

were thus termed ventricular cardiomyocytes. Since the left atrial (LA) marker genes MYH6 and MYH7 were highly expressed in CM2 and CM3 subsets, they were termed atrial CMs (18).

Both CMs and Non-CMs (NCMs) Show Different Characteristics Between Normal and HF Patients

We compared gene expression of atrial CMs (CM2&3) and NCMs between normal and patients. We observed that GO term viral gene expression was up-regulated in all atrial CMs and NCMs in HF (**Supplementary Figures 1A–F**). These findings suggested that some CMs and NCMs in the heart may be liable to SARS-CoV-2 infection. In addition, GO results showed that genes related to the mitochondrial respiratory complexes and ATP synthesis were up-regulated, while genes related to the response to interferon-gamma and defense against pathogens were downregulated in atrial CMs in HF patients resulting in an increased sensitivity to SARS-CoV-2 virus infection in these atrial CMs (**Supplementary Figure 1A**).

To further characterize this unusual CM4 subset observed in failing hearts, we performed trajectories analysis of the integrated clusters to show the pseudotime of CMs and NCMs indicating that CM4 originated from CM1 (**Figure 2A**). We then conducted GSEA analysis (GO and Pathway) on DEG between CM4 and CM1. GO term "Viral Gene Expression", as well as pathways related to influenza infection were upregulated in CM4 (**Figures 2B,C**); while response to virus, response to interferon gamma and innate immune response, pathway of the adaptive immune response and interferon signaling were significantly down-regulated in CM4 (**Figures 2D,E**). Together, these results suggest that the CM4 subset predominantly observed in HF tissues would be more vulnerable to virus infection than the CM1 subset.

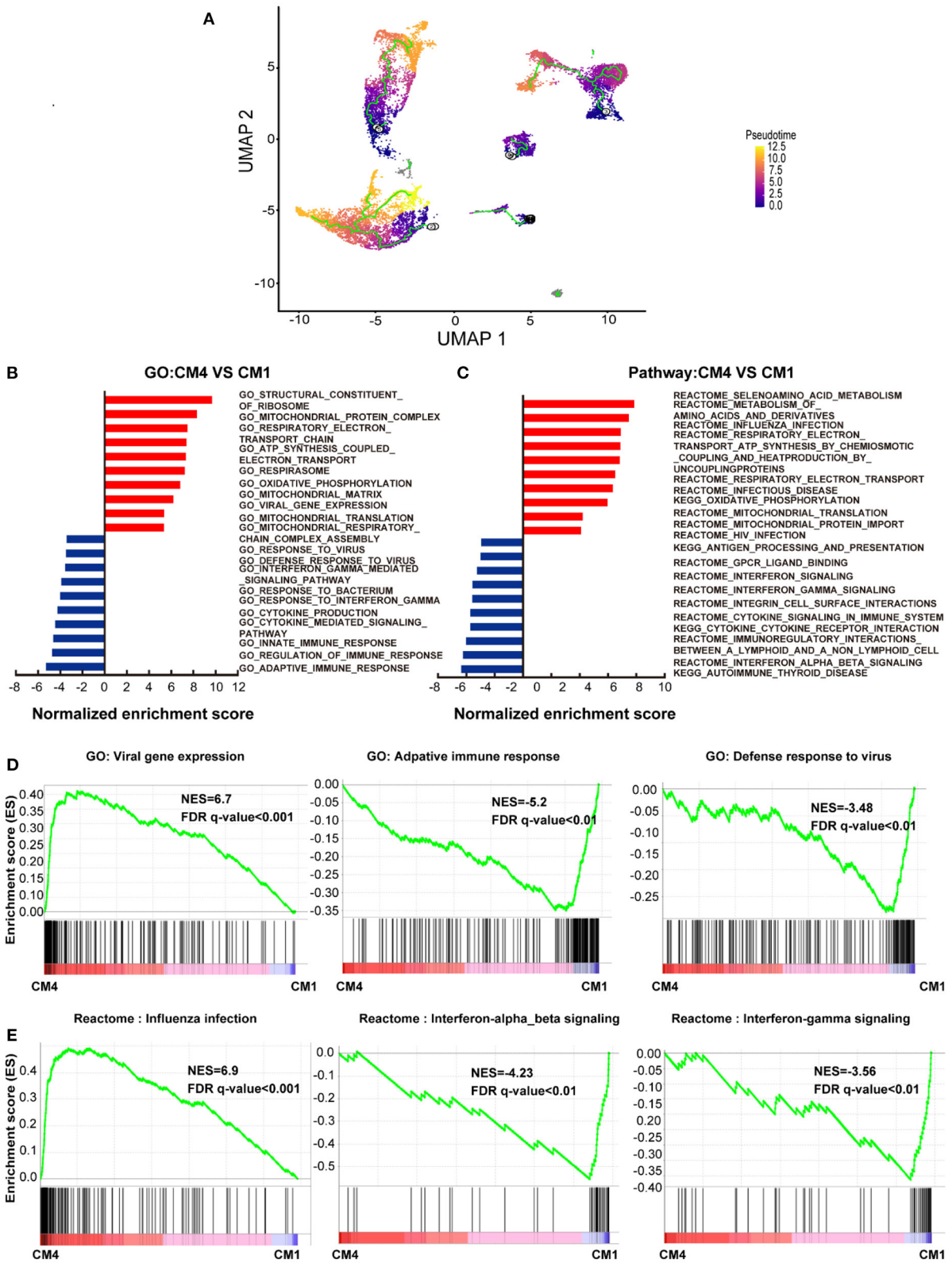

FIGURE 2 | Cardiomyocytes 4 (CM4) shows different characteristics with Cardiomyocytes 1(CM1). **(A)** Pseudotime analysis of the nine clusters, the color from purple to yellow denote the different developing stage, and the simultaneous principal curve indicates the pseudo-time stage. **(B,C)** GSEA analysis revealed significant enrichment of GO and pathways for CM4 compared with CM1. **(D)** GO enrichment showing GO terms of increased viral gene expression, decreased adaptive

(Continued)

FIGURE 2 | immune response, and defense response to virus. **(E)** Influenza infection signaling pathway is up-regulated, both interferon-alpha-beta signaling and interferon-gamma signaling are down-regulated.

Both CMs and NCMs Have Different ACE2 Expression Pattern After HF

We further investigated the frequency of ACE2+ cells in CMs and NCMs in normal and failing hearts. **Figure 3A** showed the overall distribution of ACE2+ cells in different subsets. The frequency of ACE2+ cells increased significantly in three of four CMs subsets in HF patients, especially in CM1($p < 0.0001$) and CM4 ($p < 0.0001$), while its frequency in CM2 subset did not change significantly ($p > 0.05$; **Figure 3B**). Moreover, the percentages of ACE2+ cells in fibroblasts ($p < 0.0001$) and smooth muscle cells ($p = 0.0104$) were both significantly decreased. The frequency of ACE2+ cells in NK-T Cell/Monocytes and granulocytes was insignificantly changed ($p > 0.05$; **Supplementary Table 2**).

Taken together, scRNA-seq results demonstrated that the ACE2+ CMs dramatically increased during HF, suggesting that CMs in HF patients may be more susceptible to SARS-CoV-2 virus infection than the normal subjects. In addition, ventricular myocytes had a higher percentage of ACE2+ cells than that of atrial myocytes, indicating that these cardiomyocyte subsets may have different responses to SARS-CoV-2 infection.

Virus Infection-Related Genes Are Upregulated in CMs in HF Patients

We then focused on gene expression dynamics of the SARS-CoV-2 entry receptor ACE2. To further examine the potential role of ACE2+ cells, we separated each cardiomyocyte subset into two sub-groups according to the expression of ACE2 (ACE2+ and ACE2-) and called DEGs between these two groups.

One of the most interesting findings was that *NPs* (*NPPA* and *NPPB)* were the top two upregulated genes in ACE2+ cells as compared to ACE2- cells. Previous studies reported that ACE2, NPs, TnT and TnI could make a feedback loop to preserve ejection fraction in HF patients (19–22). Interestingly, most of the ejection fraction preservation genes were significantly upregulated during HF, especially in ACE2+ CMs cells (**Figure 3C**). We used the top 100 DEGs of ACE2+ and ACE2- in CM1,4 to build a gene regulatory network (GRN) using IPA (Ingenuity Pathway Analysis, QIAGEN, CA, USA). GRN showed that *ACE2, NPs, AGT, TNNT1, TNNT2,* and *TNNT3* were well connected and shared the same upstream binding transcription factors HAND2, MYOCD, MEF2C, TBX5 which are the well-known transcription factors that can control the reprogramming of fibroblasts into CMs (**Figure 3D**) (23, 24). The above findings suggest that SARS-CoV-2 infection could damage CM, which in turn leads to feedback upregulation of cardio-differentiation.

We further studied the expression dynamics of *ACE2* and *NPs* in CMs and NCMs in normal and HF patients. Both *NPPB* and *NPPA* were co-expressed with *ACE2* and significantly up-regulated in CMs in HF samples (**Figures 4A,B**), but *NPPB* and *NPPA* showed different expression patterns. Specifically, *NPPA* was expressed only in CM2, 3 and NCMs in normal heart. *NPPA* was expressed in all CMs and NCMs and its expression was significantly upregulated in all cardiomyocyte subsets after HF (**Figure 4B**). *NPPB* was only expressed in CM2 and CM3 subsets in normal heart, and its expression was significantly upregulated in CMs except CM4 after HF (**Figure 4A**). Pro-ANP can be processed by corin and pro-BNP by corin and intracellular endoprotease furin in *in vitro* experiments to form active ANP and BNP (25, 26). We found that in HF patients, corin expression increased significantly in CMs while the change for furin was insignificant (**Supplementary Figure 2A**), which is consistent with the observation that furin activity, but not its concentration, increased (27). Importantly, at the S1/S2 boundary of SARS-CoV-2, a furin cleavage site has been identified, which can enhance the binding of spike protein and host cells (28). It was reported that Polypeptide N-Acetylgalactosaminyl transferase, such as B3GALNT1, GALNT1 can mediate the glycosylation of pro-BNP and increase pro-BNP secretion in human cardiac during HF (29). Both B3GALNT1 and GALNT1 transcription increased in HF patients (**Supplementary Figure 2B**). We then assessed other virus infection-related genes, and found that genes contributing to virus entry (BSG, CAV2, CHMP3, CHMP5, and STOML2, **Figure 4C**, **Supplementary Figures 2C,D**), cysteine proteases cathepsins (CSTB, CSTD, and CSTL, **Figure 4D**), suppression of IFN-γ signaling (LARP1, RBX1, and TIMM8B, **Figure 4E**), and virus replication (AKAP9, RDX, and MTCH1, **Figure 4F**) were all up-regulated in CMs in failing hearts.

It was reported that SARS-CoV-2 enters host cells through the binding of its spike protein with ACE2 and subsequent S protein priming by host cell protease TMPRSS2 (30, 31).We barely detected any expression of TMPRSS2 in both normal and HF samples (**Supplementary Figure 2E**). Since it is reported that in the absence of cell surface protease TMPRSS2, SARS-CoV can achieve cell entry via an endosomal pathway in which it can be activated by other proteases such as cathepsin L (31), we further investigated gene expression dynamics of the endosomal cysteine proteases, cathepsins and found out that CTSB, CTSD, and CTSL were up-regulated significantly in CMs during HF (**Figure 4D**). Also, some inflammatory cytokines were detected and found increased in several subsets in the HF patients, such as CXCL8 which was significantly increased in the subset of granulocytes and NK-T cell/Monocytes as well as IL-32 which was increased in the subsets of NK-T cell/Monocytes and endothelial cells, respectively (**Supplementary Figure 2F**). Thus, we speculate that SARS-CoV-2 may use the ACE2-CTSB/L axis for cell entry in cardiac tissues. Together, these findings suggest that failing hearts might be more vulnerable to SARS-CoV-2 infection.

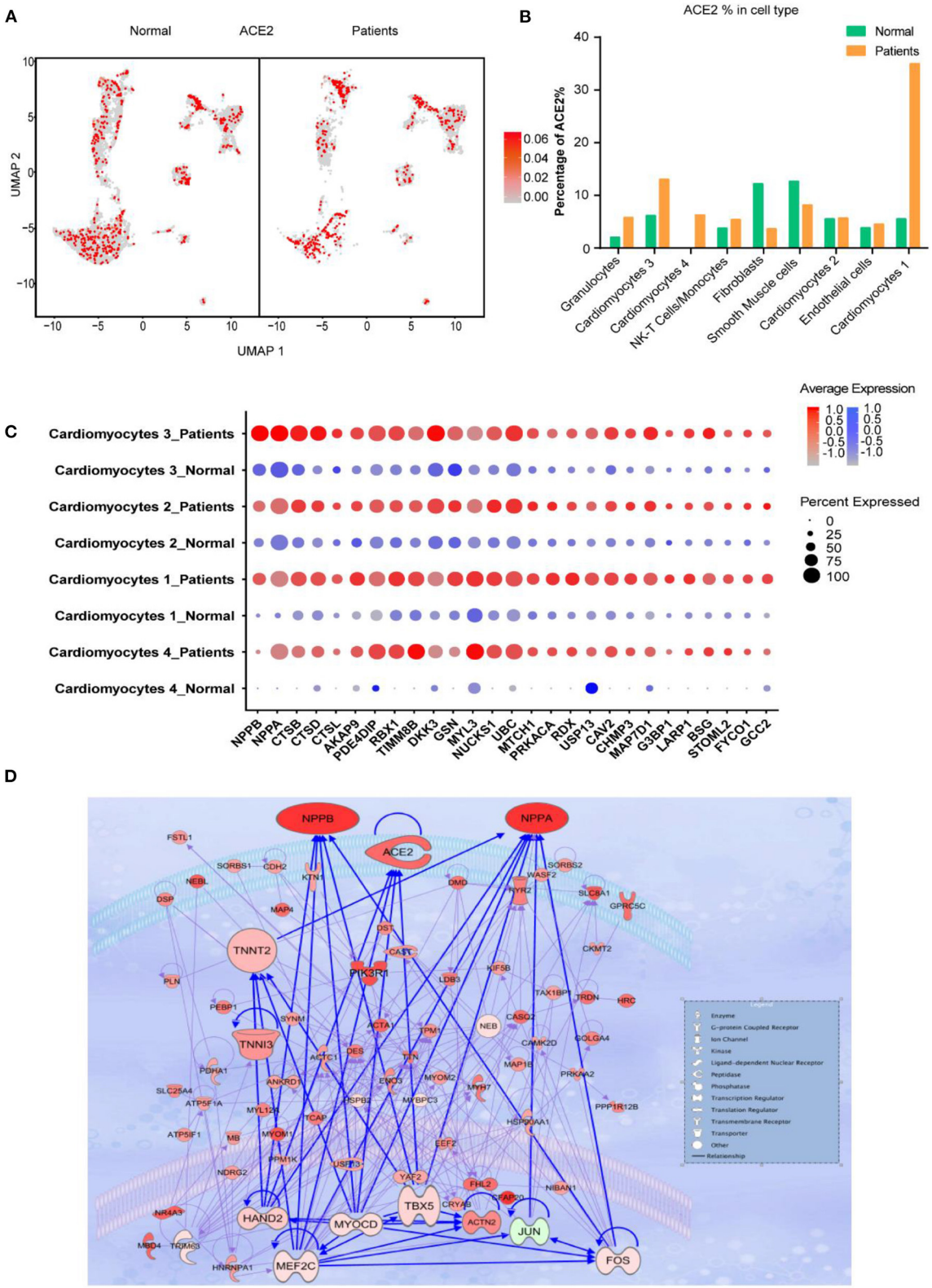

FIGURE 3 | Cardiomyocytes (CMs) and Non-CMs (NCMs) have different ACE2 expression pattern. **(A)** UAMP of the CMs and NCMs subsets in normal and HF patients. **(B)** Frequency of ACE2+ cells in different cell types. **(C)** Gene expression pattern of virus infection-related genes in different subsets of CMs during HF. **(D)** Gene regulatory network of ACE2, NPPA, NPPB, and TNNT1,2,3 and their upstream binding transcription factor of HAND2, MYOCD, MEF2C, and TBX5.

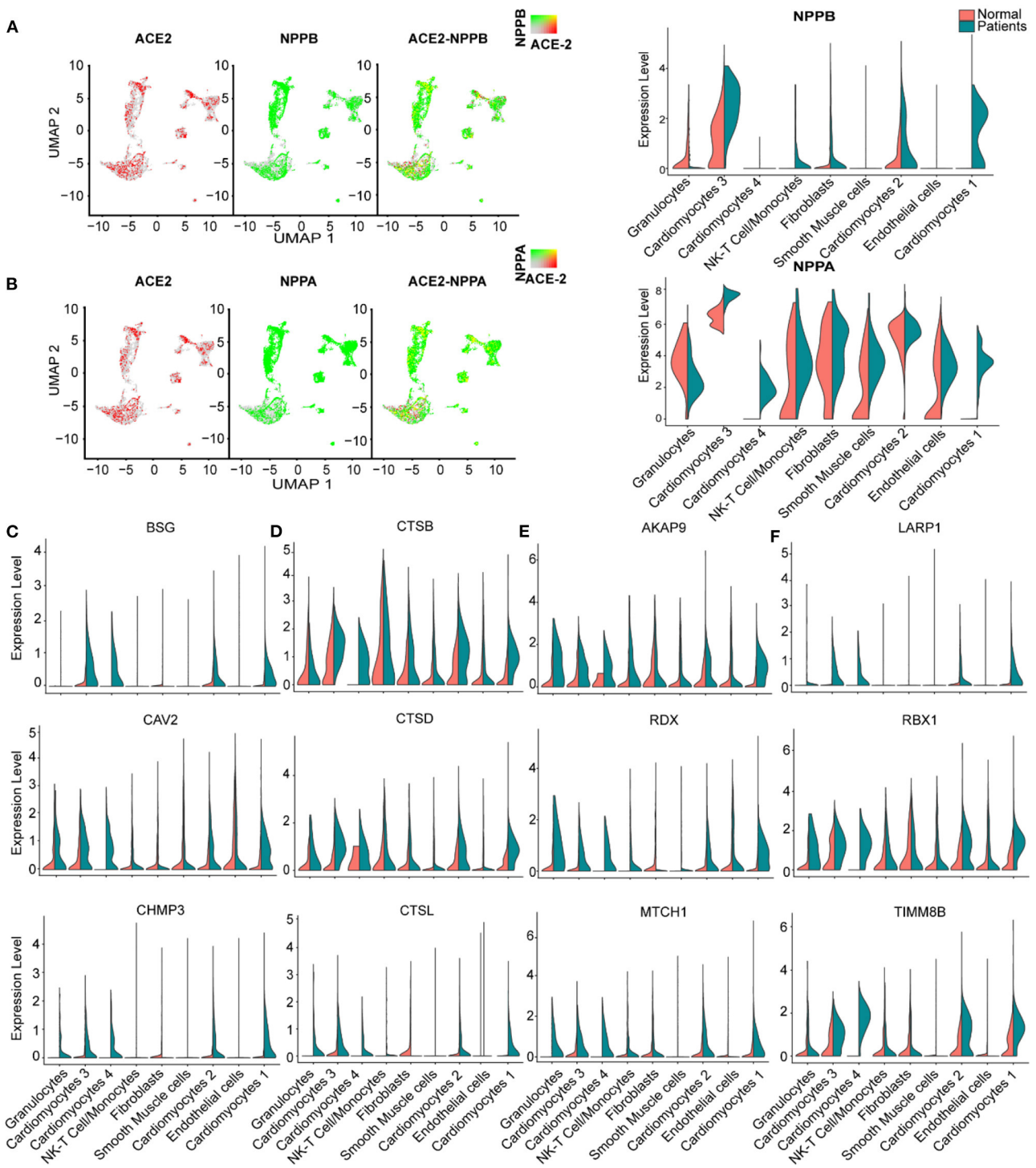

FIGURE 4 | Virus related genes are upregulated in heart failure (HF) patients compared with normal. **(A)**, Expression level of ACE2 (red dots), NPPB (green dots) in different clusters, overlapping is shown in the right panel, and the co-expression is shown in yellow dots. Violin plots of the distribution of NPPB between normal and HF patients in different subsets. **(B)** Expression level of ACE2 (red dot), NPPA (green dot) in different subsets, overlapping is shown in the right panel, and the co-expression is shown in yellow dots. Violin plots of the distribution of NPPA between normal and HF patients in different subsets. **(C)** Violin plots of the distribution of genes (from top to bottom BSG, CAV2, CHMP3) related to viral infection. **(D)** Violin plots of the gene expression pattern of CST B/L. **(E)** Violin plots of the distribution of genes (from top to bottom AKAP9, RDX, MTCH1) related to IFN-γ signaling pathway. **(F)** Violin plots of the distribution of genes (from top to bottom LARP1, RBX1, and TIMM8B) on viral replication.

Thrombosis is commonly observed in severe COVID-19 patients (32). Tissue factor (TF/CD142) activation causes thrombus formation on atherosclerotic plaques coded by F3 (33). We found that blood clotting- related gene F3 was co-expressed with ACE2 and significantly up-regulated in CM3 and CM1 during HF (**Supplementary Figures 2G,H**), suggesting that

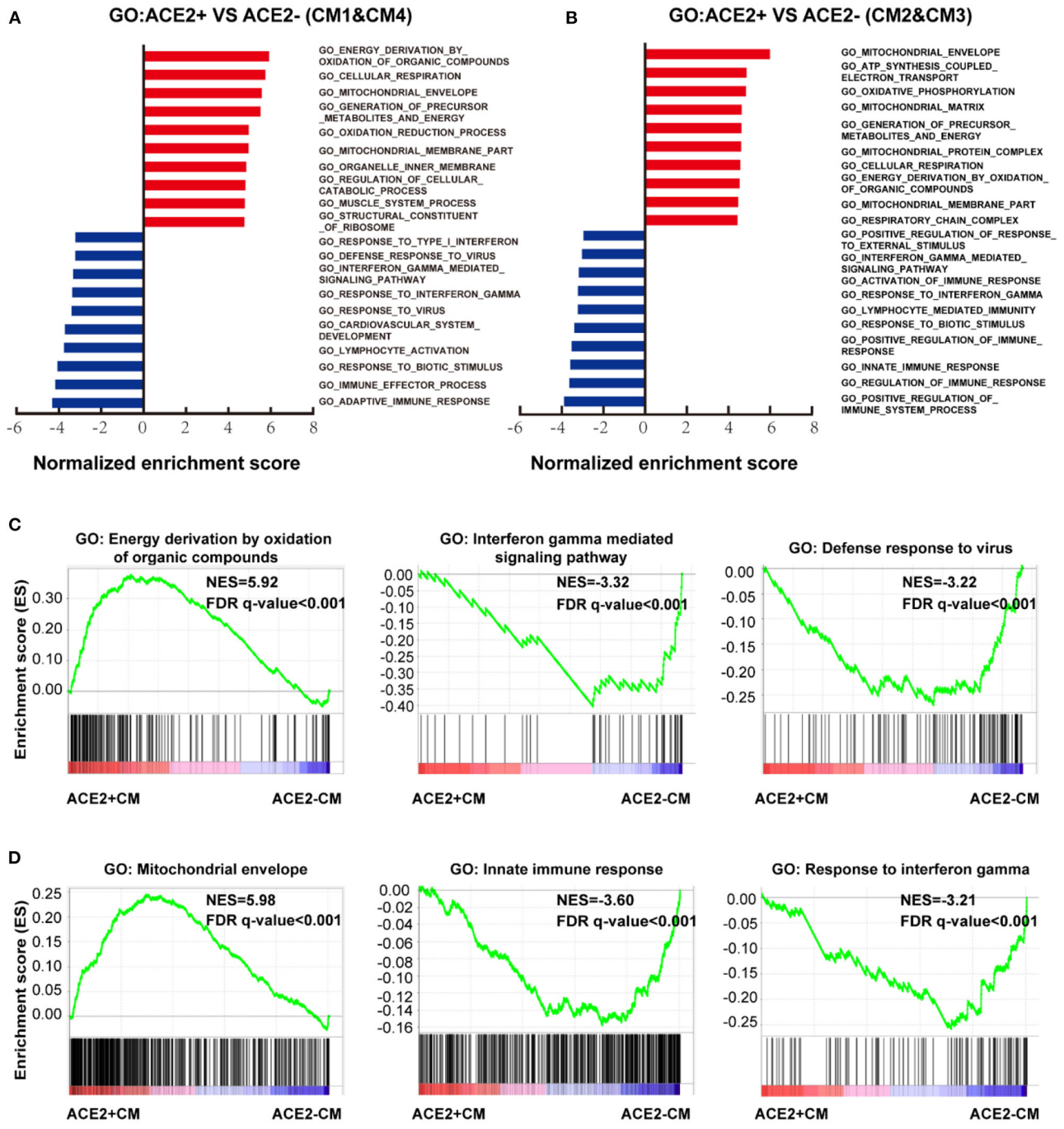

FIGURE 5 | Characteristics of ACE2+ ventricular and atrial myocytes. **(A)** GO analysis revealed significant enrichment of biological pathways for ACE2+ compared with ACE2- in ventricular myocytes. **(B)** GO analysis revealed significant enrichment of biological pathways for ACE2+ compared with ACE2- in atrial myocytes. **(C)** GO plots showing GO terms of increased energy derivation by oxidation of organic compounds (left), decreased interferon gamma mediated signaling pathway (median), and down-regulated defense response to virus (right). **(D)** GO enrichment plots showing GO terms of increased mitochondrial envelope (left), decreased innate immune response (median), and down-regulated innate immune response (right). The NES and false discovery rate (FDR) were showed in panel.

increased F3 and ACE2 may contribute to the increased risk of thrombosis in HF patients.

Characteristics of ACE2-Positive Ventricular and Atrial CMs, and NCMs

We further conducted GSEA analysis on DEGs of cells between ACE2+ and ACE2- in all CMs (**Figures 5A,B**, **Supplementary Figures 3A,B**). GO terms associated with energy consumption (**Figure 5A**), energy derivation by oxidation (**Figure 5C**), and pathway influenza infection (**Supplementary Figure 3C**) were positively enriched in ACE2+ CM1&4; GO terms associated with energy consumption, mitochondrial envelope (**Figure 5D**), and pathway respiratory electron transport (**Supplementary Figure 3D**) were positively enriched in CM2&3. In contrast, GO terms associated with interferon gamma-mediated signaling pathway, defense response to virus, innate immune response and pathway interferon signaling were negatively enriched in ACE2+ CMs (**Figures 5C,D**, **Supplementary Figures 3C,D**).

Moreover, we also identified DEGs between ACE2+ NCMs and ACE2- NCMs and performed GSEA analysis

TABLE 1 | Comparison of COVID-19 patient characteristics between BNP groups.

Parameters	Total ($N = 91$)	BNP<100 ($N = 45$)	BNP ≥ 100 ($N = 46$)	*p*-value
Age, yrs, median [min, max]	66 (27–89)	62 (27–79)	71 (44–89)	**<0.0001***
Male, *n* (%)	54 (59.3)	23 (51.1)	31 (67.4)	0.11
Complete blood cell count, $10^9/L$				
White blood cell, median (IQR)	7.99 (4.59–13.31)	6.28 (4.04–8.38)	13.05 (6.76–18.13)	**<0.0001***
Neutrophil, median (IQR)	6.6 (3.43–12.32)	4.29 (2.74–6.67)	11.88 (4.83–16.93)	**<0.0001***
Lymphocyte, median (IQR)	0.71 (0.38–1.09)	0.98 (0.62–1.47)	0.50 (0.27–0.78)	**<0.0001***
Liver and renal function				
Alanine transaminase, U/L, median (IQR)	30.0 (18.5–52.5)	27.0 (19.0–49.0)	32.0 (18.0–64.0)	0.5783
Aspartate transaminase, U/L, median (IQR)	37.0 (23.0–55.0)	30.0 (20.0–51.0)	41.5 (29.0–64.0)	**0.0299***
TBIL, μmol/L, median (IQR)	14.1 (9.5–21.7)	11.8 (9.1–17.8)	15.2 (10.1–24.4)	0.1216
Direct bilirubin, μmol/L, median (IQR)	5.3 (3.4–9.8)	4.1 (3.0–6.5)	6.6 (4.0–13.2)	**0.0047***
Lactate dehydrogenase, U/L, median (IQR)	315.0 (179.5–470.5)	185.0 (154.0–352.0)	407.0 (288.0–599.0)	**<0.0001***
eGFR, mL/(min*1.73 m^2), mean±SD	105.6 ± 47.0	121.1 ± 41.6	86.5 ± 44.5	**0.0003***
Blood urea nitrogen, mmol/L, median (IQR)	5.7 (3.9–11.1)	4.5 (3.2–5.8)	9.0 (5.2–15.9)	**<0.0001***
Uric acid, μmol/L, median (IQR)	234.0 (183.5–305.5)	235.0 (184.0–305.0)	230.5 (182.0–310.0)	0.9494
Cardiac biomarker				
Troponin-I, ng/mL, median (IQR)	0.01 (0.01–0.06)	0.01 (0.01–0.01)	0.05 (0.03–0.25)	**<0.0001***
Electrolytes				
Potassium, mmol/L, median(IQR)	4.04 (3.64–4.40)	3.87 (3.56–4.27)	4.19 (3.64–4.70)	**0.0354***
Sodium, mmol/L, median (IQR)	139.0 (136.0–142.0)	139.0 (135.0–141.0)	139.0 (136.0–145.0)	0.2992
Chloride, mmol/L, median (IQR)	102.0 (98.5–106.0)	103.0 (100.0–106.0)	101.5 (98.0–106.0)	0.6473
Calcium, mmol/L, mean ± SD	2.03 ± 0.18	2.09 ± 0.16	1.97 ± 0.18	**0.0018***
Coagulation profiles				
Prothrombin time, s, median (IQR)	13.4 (12.4–14.9)	13.0 (12.0–13.8)	13.9 (12.8–16.7)	**0.0030***
APTT, s, median (IQR)	35.5 (31.8–39.8)	35.1 (32.4–38.9)	35.5 (30.7–42.5)	0.6165
Fibrinogen, g/L, median (IQR)	3.39 (2.31–4.77)	3.39 (2.31–5.09)	3.40 (2.35–5.87)	0.8567
D-dimer, μg/mL, median (IQR)	2.03 (1.22–1.00)	1.37 (0.83–1.99)	6.96 (3.25–24.20)	**<0.0001***
Inflammatory biomarkers				
Procalcitonin, ng/mL, median(IQR)	0.45 (0.12–1.12)	0.23 (0.04–0.49)	1.01 (0.39–3.51)	**<0.0001***
hsCRP, mg/L, median (IQR)	13.80 (5.74–20.50)	6.09 (1.52–15.86)	18.00 (13.45–21.50)	**<0.0001***
Blood gas analysis				
PaO_2, mmHg, median (IQR)	71.0 (57.8–92.0)	78.5 (57.5–104.5)	68.5 (56.5–86.0)	0.4867
$PaCO_2$, mmHg, median (IQR)	41.0 (34.0–48.8)	39.5 (33.5–43.5)	42.5 (34.0–57.9)	0.1589
Lactic acid, mmol/L, median (IQR)	1.95 (1.40–2.40)	1.80(1.30–2.15)	2.00 (1.60–2.75)	0.1634
BNP, pg/mL, median (IQR)	92.0 (32.5–299.5)	34.0 (15.0–48.0)	299.5 (180.0–548.0)	**<0.0001***
Death, *n* (%)	32 (35.16)	5 (11.11)	27 (58.70)	**<0.0001***

*Continuous variables are presented as means ± SD if they conform to normal distribution, or median with interquartile range if not. Age is presented as median with range (minimum–maximum). Categorical variables are presented as percentage (%). *$p < 0.05$. TBIL, total bilirubin; eGFR, estimated glomerular filtration rate (calculated by MDRD formula); APTT, activated partial thromboplastin time; hsCRP, high-sensitive C-reactive protein; BNP, brain natriuretic peptide. Bold values means $p < 0.05$ which have statistics significance.*

on them (**Supplementary Figure 4**). Interestingly, pathways associated with infectious disease were positively enriched in NCMs, except for NK-T Cells/Monocytes. GO terms associated with mitochondrial matrix and ATP synthesis were positively enriched in smooth muscle cells, NK-T Cells/Monocytes and fibroblasts, which is consistent with the observation at CMs. GO term associated with muscle structure and function (**Supplementary Figures 4A,E**) and leukocyte mediated immunity were negatively enriched in ACE2+ cells of smooth muscle cells, fibroblasts, and endothelial cells (**Supplementary Figure 4B**). GO term associated with viral expression is positively enriched in ACE2+ granulocytes, while GO term associated with immunocyte mediated immunity is negatively enriched in ACE2+ granulocytes and ACE2+ NK-T Cells/Monocytes. These findings suggest an impaired cellular immunological response in HF patients, which may increase their vulnerability to various pathogens (**Supplementary Figures 4C,D**).

Clinical Characteristics of COVID-19 Patients

The median age of these 91 COVID-19 patients was 66 years [range, (27–89)]. Forty-six patients (50.5%) have elevated BNP (≥100 pg/mL). Both BNP level [56.0 (29.5, 259.0) vs. 105.5 (34.75, 307.3), $p = 0.309$) and proportion of higher BNP (57.41 vs. 40.54%, $p = 0.138$) were similar between male and female. HF patients have increased BNP plasma concentrations which are generally co-related with the degree of cardiac dysfunction. Thus,

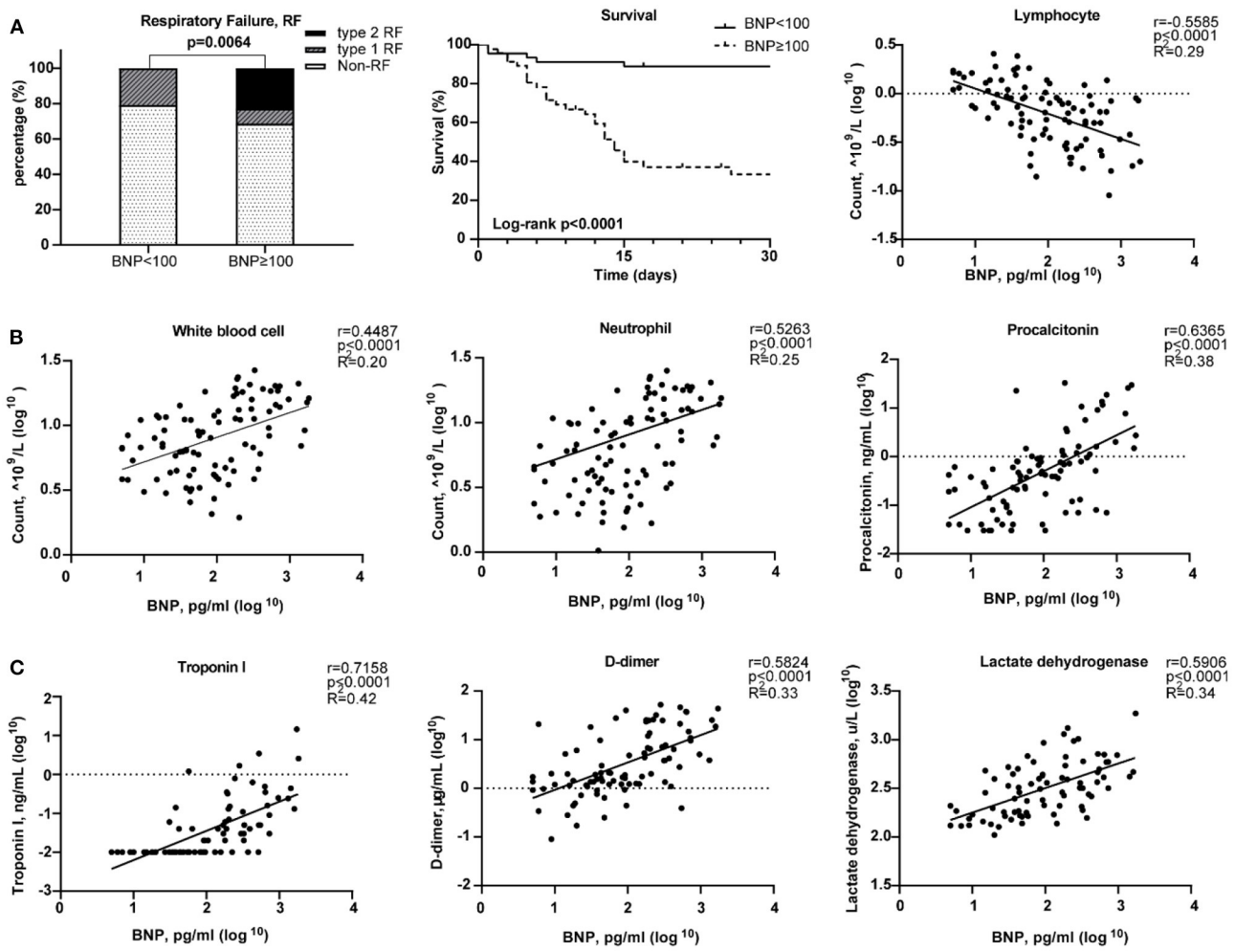

FIGURE 6 | Relationships between Brain natriuretic peptide (BNP) level and clinical assessments. **(A)** Measurement of disease severity. Left figure described the constitution of non-respiratory failure (RF), type 1 RF, and type 2 RF patients. Middle figure showed K–M estimation of the mortality in high BNP group. Right figure showed the significant negative correlation of lymphocyte count and BNP level. **(B)** The severity of infection. From left to right, white blood cell, neutrophil, and lymphocyte counts were positively correlated with BNP level. **(C)** The relationship between organ impairment and BNP. Left figure depicted the strong positive relationship of cardiac injury and blood BNP level. Middle figure showed the disturbance of coagulation as BNP level increased. Right figure showed the positive correlation between lactate dehydrogenase and BNP level.

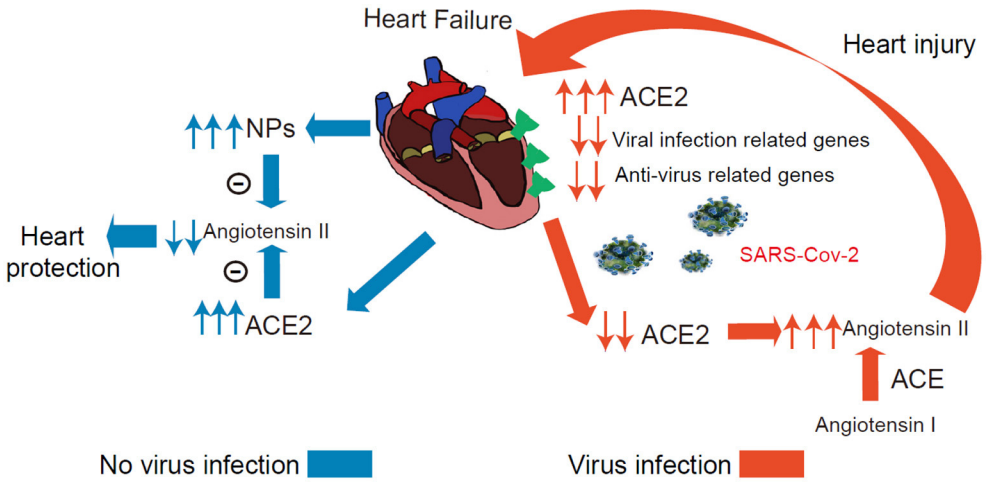

FIGURE 7 | Conceptual Schematic diagram highlighting the central role of SARS-CoV-2 and the NPs, RAAS in the potentially deleterious (red) and protective (blue) effects. **Left:** Schematic diagram showed that the ACE2 and NPs can protect heart from heart failure noted in blue. **Right:** The process under virus infection was noted in red to speculate the underlying relationship for the higher susceptibility and worse prognosis in HF.

BNP is often used as a biochemical marker for HF (34). Patients with a higher BNP were older [median age, 71 (IQR 44–89) vs. 62 (27–79), $p<0.0001$; **Table 1**]. Compared with the lower BNP group, patients in the higher BNP group have significantly higher levels of white blood cells ($p < 0.0001$) and neutrophils ($p < 0.0001$), although significantly lower number of lymphocytes ($p < 0.0001$; **Table 1**). The high BNP group has significant increased procalcitonin ($p < 0.0001$) and C-reactive protein ($p < 0.0001$) as compared with the low BNP group (**Table 1**). The high BNP group also showed imbalanced electrolyte levels and aberrant coagulation profiles as compared with the low BNP group. Furthermore, more severe organ dysfunction was observed in the high BNP group, including worse liver function indicated by higher aspartate transaminase ($p < 0.03$), direct bilirubin ($p < 0.005$), and lactate dehydrogenase ($p < 0.0001$; **Table 1**). The high BNP group also showed worse renal function as indicated by a reduced glomerular filtration rate ($p < 0.0003$) and increased blood urea nitrogen ($p < 0.0001$; **Table 1**). Cardiac TNI ($p < 0.0001$) was significantly increased in the higher BNP group, suggesting more cardiac injury in these patients (**Table 1**). Noteworthy, the high BNP group had a higher incidence of respiratory failure (RF, 31.43%, $p = 0.0064$; **Figure 6A**, left), and a significantly increased mortality rate (58.70%, $p < 0.0001$; **Figure 6A** middle, **Table 1**), and a negative correlation with the lymphocyte count (**Figure 6A**, right). Infective markers were positively correlated with the BNP level (**Figure 6B**). Markers of coagulative disturbance and organ impairment were positively correlated with the BNP level (**Figure 6C**, middle and right).

DISCUSSION

The present study has several major findings. First, the study systematically investigated the ACE2 expression dynamics in CMs, and Non-CMs in human normal and failing hearts at the single-cell level. We found that ACE2 was expressed in some CMs, vascular endothelial cells, and smooth muscle cells in both normal and failing hearts. Second, we demonstrated that ACE2 expression was selectively increased in the CM4 subset from 0 to 7.01% after heart failing, suggests that CM4 may be more vulnerable to SARS-CoV-2 infection than CM1. Third, we demonstrated for the first time that NPs transcripts are markedly enriched in ACE2+ CMs, which suggest that ACE2 and NPs may share similar signaling pathway and ACE2+NPPB+/ACE2+NPPA+ CMs may play important role in viral infection of HF patients. Fourth, we demonstrated that ACE2 expression was associated with the dynamic changes of a group of genes specific for the networks of viral infection and immunity in CMs (**Figure 7**).

We found that ACE2 was expressed in ∼5% normal ventricular or atrial CMs (2% in lung AT2 cells) and ACE2+ cells frequency was increased in the CM1, CM3, and CM4 subset in failing hearts. Our finding that ACE2 was expressed in normal hearts appears to contradict a previous report that pericytes, but not the CMs express ACE2 in normal hearts (35). The discrepancy may due to the fact that the previous study used the single nucleus RNA-seq approach, which generally captures fewer transcripts as compared with the more sensitive and comprehensive SMART-seq using whole-cell in our study. In the context that SARS-CoV-2 causes myocarditis and cardiac injury (36), it is reasonable to believe that the increased ACE2+ in CMs in the failing heart could make these CMs vulnerable to SARS-CoV-2 infection in COVID-19 patients.

Several studies have defined a critical role for ACE2 in protecting the heart against HF, systemic and pulmonary hypertension, myocardial infarction, and diabetic cardiomyopathy (18, 36, 37). Another very interesting finding is that both NPs transcripts were markedly enriched in ACE2+ CMs, and that NPs and ACE2 can form two negative feedback loops respectively associated with the RAAS/Ang II signaling pathway (38). Since ACE2 degrade Ang-II, the expression of

ACE2 in CMs is likely to protect these CMs through reducing local Ang-II content under conditions without SARS-CoV-2 infection. Circulating NPs can promote diuresis, natriuresis and vasodilation, which is critical for the maintenance of intravascular volume homeostasis (**Figure 7**) (19). In addition, GRN showed that ACE2 and NPs might be co-regulated during HF development.

SARS-CoV-2 binding to ACE2 could result in ACE2 degradation or dysfunction (37, 38). A recent study demonstrated that the plasma Ang-II level from SARS-CoV-2 infected patients was markedly elevated and the plasma Ang-II linearly correlated with the viral load and lung injury in COVID-19 patients (2, 39, 40). Our clinical data indicate that COVID-19 patients with a higher level of BNP had a more severe dysfunction of the heart and significantly higher mortality. Whether this increased BNP leads to higher mortality or constitutes an endogenous cardioprotective strategy in the settings of SARS-CoV-2-mediated inflammation remains to be confirmed. As patients are infected by SARS-CoV-2 in cardiac tissues, the overall virus defense capacity would be attenuated in CMs (**Figure 7**), SARS-CoV-2 infection in ACE2+ CMs could certainly cause or exacerbate cardiac injury and consequent cardiac dysfunction. Drug ACEI or ARBs can break this positive feedback loop by reducing Ang II (**Figure 7**). Importantly, another study found that plasma ACE2 was not related to ACEI or ARBs use in HF patients which alludes to the fact that using ACEI or ARBs will not increase the risk of virus infection (41). We speculate that patients may benefit from these types of drugs partly attributed to these explanations. Indeed, another study demonstrated that in hospitalized COVID-19 patients with hypertension, patient's use of ACEI/ARB was associated with lower risk of all-cause mortality compared with ACEI/ARB non-users (38, 42, 43).

In the failing heart, the upregulation of ACE2, virus infection and oxidative phosphorylation associated genes could facilitate SARS-CoV-2 virus entry and replication. These findings may advance our understanding of the underlying pathobiology of myocarditis associated with COVID-19 and new treatment strategy. The direct relationship between ACE2 and NPs and the role of ACE2+NPPB+/ACE2+NPPA+ CMs in viral infection would be the focus of our future studies. We will also further explore the new marker of failing CMs, especially CM4, and the mechanism of the virus susceptibility in failing hearts.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Approval No. L2020-Y-013 and No. SHSY-IEC-4.1/20-63/02. 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

XX and DX: conception and design. XX: scRNA-seq data collection and analysis. XH, MC, and MZ: provision of study materials or patients, collection, and assembly of clinical data. XX, MM, YX, DX, YS, YC, S-BO, and HL: results interpretation and manuscript writing. XX, DX, YC, HL, S-BO, YS, MM, YX, XH, MC, and MZ: final approval of manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.628885/full#supplementary-material

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Lower Plasma Elabela Levels in Hypertensive Patients With Heart Failure Predict the Occurrence of Major Adverse Cardiac Events: A Preliminary Study

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Background: Elabela, a novel cardiac developmental peptide, has been shown to improve heart dysfunction. However, the roles and correlation of Elabela in predicting adverse cardiac events in hypertensive patients with heart failure (HF) remain largely unclear.

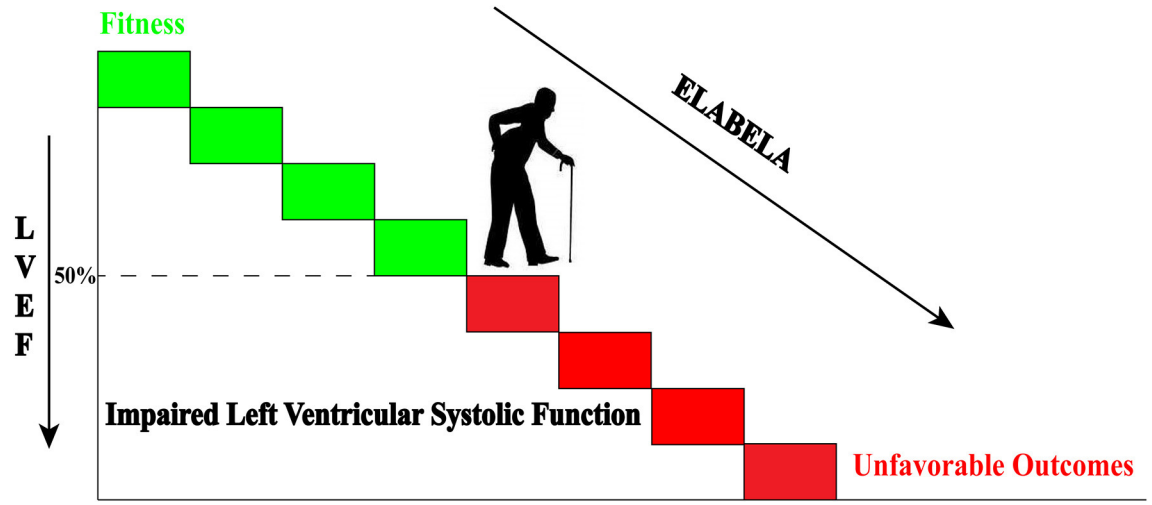
Objective: To measure plasma levels of Elabela in hypertensive patients with HF and evaluate its prognostic value.

Methods: A single-site, cohort, prospective, observational study was investigated with all subjects, including control subjects and hypertensive patients with or without HF, whom were recruited in Beijing Chaoyang Hospital Affiliated to Capital Medical University form October 2018 to July 2019. The subjects among different groups were matched based on age and sex. The clinical characteristics were collected, and plasma Elabela levels were detected in all subjects. The hypertensive patients with HF were followed up for 180 days, and the major adverse cardiac events (MACE) were recorded. The Cox regression was used to explore the correlation between Elabela level and MACE in hypertensive patients with or without HF. The receiver operating characteristic curves were used to access the predictive power of plasma Elabela level.

Results: A total of 308 subjects, including 40 control subjects, 134 hypertensive patients without HF, and 134 hypertensive patients with HF were enrolled in this study. Plasma levels of Elabela were lower in hypertensive patients compared with control subjects [4.9 (2.8, 6.7) vs. 11.8 (9.8, 14.0) ng/ml, $P < 0.001$]. Furthermore, HF patients with preserved ejection fraction had a higher plasma Elabela level than those with impaired left ventricular systolic function (heart failure with mid-range ejection fraction and heart failure with reduced ejection fraction). The hypertensive patients with HF and higher plasma Elabela levels had a better readmission-free and MACE-free survival than those with lower plasma Elabela levels in survival analysis. The Cox regression analysis revealed that plasma Elabela levels were negatively associated with MACE (HR 0.75, 95% CI 0.61–0.99, $P = 0.048$) in hypertensive patients with HF.

Conclusion: Plasma Elabela levels were decreased in hypertensive patients with left ventricular systolic dysfunction. Thus, Elabela may be potentially used as a novel predictor for MACE in hypertensive patients with HF.

Keywords: heart failure, hypertension, Elabela, prognosis factor, major adverse cardiac events



GRAPHICAL ABSTRACT | Declined plasma Elabela levels are associated with impaired left ventricular systolic function of hypertensive patients. The declined plasma Elabela levels predicated the unfavorable outcomes in hypertensive patients with heart failure during a 180-day follow-up.

INTRODUCTION

Congestive heart failure (HF), which is often accompanied by multiple comorbidities, is a leading cause of mortality and morbidity worldwide (1). Hypertension, as one of the possible causes of heart failure, has a rapidly increasing incidence with the aging population. The optimized comprehensive management has greatly improved the outcomes of hypertensive patients, except those with HF. Early identification and effective risk stratification are crucial for the management of these patients (2, 3). BNP has become a widely-used biomarker and valuable adverse events predictor for patients with HF (4). However, its low specificity limits its predictive power in a clinical application (5).

Elabela (also called Toddler or Apela) was identified as a novel endogenous ligand of the APJ receptor that had an important role in cardiac development (6). Further study found that Elabela also exerts the important biological effects (anti-hypertension, positive inotropic action, diuresis, anti-remodeling, antifibrotic action, as well as cardiorenal protection) in adult animals through Elabela/APJ signaling (7, 8). Clinical studies suggested that patients with hypertension had lower plasma Elabela levels than a healthy control group (9), and plasma Elabela levels were negatively associated with the extent of albuminuria in patients with type 2 diabetes (10).

Recent preclinical studies further confirmed that Elabela/APJ axis could prevent pressure overload HF and angiotensin II-induced cardiac damage through depressing ACE and FoxM1 expression and activating ERK1/2 pathway (11). The Elabela also improved hemodynamic parameters, including increased E-wave velocity and left ventricular end-diastolic volume (12). These results indicated that Elabela might take part in the prevention of HF. The correlation between Elabela and patients with hypertension or albuminuria (that are both independent risk factors for HF) suggested that Elabela may be an important biomarker for HF (13–15). So far, no studies have investigated plasma Elabela level and its prognostic value in patients with HF. Thus, in the present study, we measured the plasma Elabela levels and investigated the association between plasma Elabela and the outcomes in hypertensive patients with HF.

MATERIALS AND METHODS

Study Population

This was a single-site, cohort, prospective, and observational study. All subjects were recruited in the Heart Centre of Beijing Chaoyang Hospital, Capital Medical University, between October 2018 and July 2019. Hypertensive patients with or without HF were consecutively recruited into the HF group and non-HF group, respectively. The control subjects without cardiovascular diseases from our Health Examination Center were consecutively enrolled in the healthy control group during the same period. The subjects from different groups were matched 1:1 based on the same-sex with a maximum age difference of 5 years. The exclusion criteria were: (1)

congenital heart disease; (2) cardiomyopathy; (3) severe renal dysfunction with estimated glomerular filtration rate (eGFR) $\leq$30 ml/min/1.73 m^2 at baseline; (4) tumor; (5) severe infection, autoimmune disease, and mental disorder; (6) any other non-cardiovascular diseases which lead the life expectancy of fewer than 6 months; (7) acute exacerbation of chronic bronchitis and exacerbated asthma. Written informed consent and clinical characteristics were obtained from all subjects at the time of enrollment. All the laboratory assessments, except plasma Elabela levels, were conducted in the clinical laboratory center according to the standard protocols. The eGFR were estimated by using MDRD Study China equation [eGFR = $175\times$(serum creatinine mg/dl)$^{-1.234}\times$age$^{-0.179}\times$0.79 (if female)] (16).

All echocardiogram measurements were obtained by two experienced attending doctors. All of the HF patients received the optimized treatment as outlined in the 2016 ESC Guidelines (4). The flow diagram of the study (from enrollment to follow-up) was shown in **Supplementary Figure 1**.

Diagnostic Criteria

Criteria for hypertension diagnosis were: (1) systolic blood pressure (SBP) $\geq$140 mmHg and/or diastolic blood pressure (DBP) $\geq$90 mmHg in the office or clinic following repeated examination, (2) SBP $\geq$135 mmHg and/or DBP $\geq$85 mmHg at home, (3) 24-h average SBP $\geq$130 mmHg and/or DBP $\geq$80 mmHg, day time average SBP $\geq$135 mmHg and/or DBP $\geq$85 mmHg, or night time average SBP $\geq$120 mmHg and/or DBP $\geq$70 mmHg in ambulatory blood pressure monitoring (17). Criteria for diagnosis and classification of HF were based on the 2016 ESC Guidelines for the diagnosis and treatment of HF (4). Briefly, the typical signs (dyspnea), symptoms (crackles on lung auscultation), elevated brain natriuretic peptide (BNP) levels, X-ray examination (signs of pulmonary congestion and enlarged heart shadow), and ultrasound cardiogram report (impaired left ventricular diastolic and or systolic function) were all considered when the HF diagnosis was made. Heart failure with reduced ejection fraction (HFrEF) was defined as EF $\leq$40% (4); heart failure with preserved EF (HFpEF) as EF $\geq$50% (4); heart failure with mid-range EF (HFmrEF) as EF between 41 and 49% (4). The optimized treatment of HF was received but was not limited to the usage of diuretics, renin-angiotensin-aldosterone system antagonist, and beta-blockers (4).

Elabela Enzyme Immunoassay

All the blood samples were collected from a peripheral vein. Upon collection, venous blood samples were immediately processed with a centrifuge at 4°C and 3,000 rpm for 10 min. Plasma samples were then stored at −80°C until use. The commercialized human Elabela Elisa Kit (S-1508, Peninsula Laboratories International, Inc. USA) was used to measure plasma Elabela level with the test range: 0–100 ng/ml and average IC50: 2 ng/ml. The operation procedures followed the instructions of Elisa Kit. As the instructions suggested, the samples were appropriately extracted.

Follow-Up and Endpoints

The primary follow-up endpoint was the occurrence of major cardiac adverse events (MACE), including all-cause mortality and HF readmission. The length of hospital stay was used as the secondary endpoint. All 134 hypertensive patients with HF were divided into two groups (high-level group and low-level group) by the median of plasma Elabela level and then were followed up for 180 days (from November 2018). Telephone follow-up was conducted at a fixed time every month (**Supplementary Figure 2**).

Statistical Analysis

Continuous data were presented as mean ± standard deviation (SD) or median and interquartile range (IQR), and categorical data as number and percentage. Student's *t*-test was used for normally distributed variables when comparing continuous variables between two groups, and Mann–Whitney *U*-tests for non-normally distributed variables. In a comparison of the continuous variable among more than two groups, one-way analysis of variance was used for normally distributed variables, and the Kruskal-Wallis test was used for non-normally distributed variables. The Student Newman Keuls test was employed in pairwise comparison among three groups, and the adjusted *p*-value was provided. Fisher's exact test was used for categorical variables. BNP value was seriously skewed, and the logarithmic transformation was used for data conversion. Spearman correlation analyses were used to assess the relationship between plasma Elabela levels and study variables, including age, sex, BMI, Log_{10} BNP, blood lipid, renal function, and echocardiographic parameters. Kaplan–Meier survival curves with a log-rank test analyzed MACE, HF readmission, and survival. Cox regression was invited to explore the predictors of MACE in hypertensive patients with HF. Only variables with $P < 0.10$ in univariate analysis were included in the multivariable model. All continuous variables which entered the Cox regression met the linearity assumption. To analyze the predictive power of selected predictors, receiver operating characteristic curves (ROC) were calculated, and the area under the curve (AUC) was determined. The hazard ratio (HR) and 95% confidence intervals (CI) were reported. The MACE predictive cut-off point was selected according to the Youden index. All tests were 2-sided, and statistical significance was set at a value of $P < 0.05$. The statistical analysis was performed using the SPSS software version 23 (IBM Corporation, Armonk, NY).

RESULTS

The Baseline Characteristics of Hypertensive Patients

The baseline characteristics of 268 hypertensive patients (134 patients with HF and 134 age sex-matched patients without) were shown in **Table 1**. Data from laboratory examinations revealed that plasma BNP levels, serum creatinine levels, hemoglobin A1C levels, and high-sensitivity C-reactive protein (hs-CRP) levels were all higher in the HF group compared to the non-HF group ($P < 0.05$). In contrast, the high-density lipoprotein cholesterol (HDL-c) levels were significantly lower in the HF

TABLE 1 | Baseline characteristics and laboratory data of hypertensive patients.

	Total ($n = 268$)	Non-HF group ($n = 134$)	HF group ($n = 134$)	*P*
Age, years	67.8 ± 10.8	67.8 ± 10.5	68.8 ± 11.1	0.426
Male sex	173 (64.6%)	87/134 (64.9%)	86/134 (64.2%)	0.898
BMI, kg/m^2	25.5 ± 3.3	25.7 ± 3.2	25.3 ± 3.4	0.295
Comorbidities				
Coronary heart disease	185/268 (69%)	89/134(66.4%)	96/134 (71.6%)	0.355
Atrial fibrillation	118/268 (44.0%)	57/134 (42.5%)	61/134 (45.5%)	0.377
Diabetes Mellitus	120/268 (44.8%)	56/134 (41.8%)	64/134 (47.8%)	0.326
Chronic renal failure	35/268 (13.1%)	7/134 (5.2%)	28/134 (20.9%)	<0.001
Hyperlipidemia	173/268 (64.6%)	87/134 (64.9%)	86/134 (64.2%)	0.898
Laboratory data				
BNP level, pg/ml	151.0 (55.3, 560.5)	59.5 (26.7, 132.5)	506.0 (194.7, 1438.7)	<0.001
Creatine level, umol/l	72.4 (62.8, 82.1)	70.7 (61.0, 80.5)	76.4 (64.9, 114.5)	<0.001
eGFR, ml/(min·1.73 m^2)	84.4 ± 30.6	91.7 ± 32.3	77.2 ± 26.8	0.027
Hemoglobin A1C, %	6.4 ± 1.0	6.3 ± 1.0	6.6 ± 1.1	0.040
Triglyceride, mmol/l	1.4 ± 0.8	1.5 ± 0.9	1.3 ± 0.7	0.140
LDL-c, mmol/l	2.3 ± 0.9	2.3 ± 0.8	2.3 ± 0.9	0.606
HDL-c, mmol/l	1.0 ± 0.3	1.1 ± 0.3	0.9 ± 0.3	0.002
Total cholesterol, mmol/l	4.1 ± 1.1	4.2 ± 1.0	4.0 ± 1.2	0.107
Hs-CRP, mg/l	2.7 (1.1, 9.0)	1.6 (0.8, 3.2)	6.3 (2.4, 14.0)	<0.001
Troponin I, ng/ml	0.02 (0.00, 0.10)	0.01 (0.00, 0.02)	0.05 (0.02, 0.18)	<0.001
Elabela, ng/ml	4.4 (2.3, 6.2)	4.7 (3.0, 7.4)	3.9 (1.9, 5.4)	<0.001
Echcardiography				
LAD, mm	42.3 ± 7.1	39.9 ± 6.7	44.6 ± 7.4	<0.001
LVEDd, mm	51.4 ± 4.5	47.0 ± 4.2	55.1 ± 4.8	<0.001
LVEDs, mm	35.9 ± 5.6	29.7 ± 5.0	41.3 ± 6.2	<0.001
PASP, mmHg	27.1 (24.5, 29.4)	25.9 (24.2, 27.5)	28.9 (25.4, 48.3)	<0.001
LVEF, %	60.0 (42.0, 66.0)	65.0 (61.0,69.0)	44.0 (37.8, 58.0)	<0.001
Nyha function class				
Class II	44/268 (16.4%)	NA	44/134 (32.8%)	NA
Class III	44/268 (16.4%)	NA	44/134 (32.8%)	NA
Class IV	46/268 (17.2%)	NA	46/134 (34.3%)	NA

eGFR, estimated glomerular filtration rate; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LAD, left atrial diameter; LVEDd, left ventricular end-diastolic dimension; LVEDs, left ventricular end-systolic diameter; PASP, pulmonary arterial pressure; LVEF, left ventricular ejection fraction; NA, not available.

group compared to the non-HF group. Significant differences were also observed between the HF and non-HF group in echocardiographic parameters, including left atrial diameter, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, pulmonary arterial pressure (PASP), and left ventricular ejection fraction (LVEF).

Plasma Elabela Levels in Hypertensive Patients With HF and Without HF

Due to the differences in age among control subjects and hypertensive patients, the Elabela plasma levels in 40 control subjects (45.0% female, mean age 56.6 ± 6.0 years) and 40 age and sex-matched hypertensive patients with or without HF from the 268 hypertensive patients (45.0% female, mean age 57.5 ± 5.6 years) were compared. Plasma Elabela levels were significantly lower in hypertensive patients with or without HF compared to control subjects [4.9 (2.8, 6.7) vs. 11.8 (9.8, 14.0) ng/ml, $P < 0.001$]. Moreover, plasma Elabela levels were significantly lower in hypertensive patients with HF when compared with control subjects [3.0 (1.9, 4.9) vs. 11.8 (9.8, 14.0) ng/ml, $P < 0.001$] (**Supplementary Figure 3**).

In 268 hypertensive patients, plasma Elabela levels were significantly lower in the HF group compared to the non-HF group [3.9 (1.9, 5.4) vs. 4.7 (3.0, 7.4) ng/ml, $P < 0.001$]. We further divided the 134 patients with HF into HFrEF (50/134), HFmrEF (42/134), and HFpEF (42/134) groups according to LVEF. The mean plasma Elabela level of the Non-HF group and HFpEF group were similar [4.7 (3.0, 7.4) vs. 4.8 (2.4, 6.8) ng/ml, $P = 0.999$]. HFpEF group, like non-HF group, had higher plasma levels of Elabela than HFrEF and HFmrEF group [4.8 (2.4, 6.8) vs. 2.6 (1.9, 4.9) ng/ml, $P = 0.010$ and 4.8 (2.4, 6.8) vs. 2.7 (1.8, 5.4) ng/ml, $P = 0.037$ separately], while no significant differences

were observed between HFrEF and HFmrEF groups. HF patients were further divided into another three subgroups (class II, III, and IV groups) according to the classification of NYHA. Intriguingly, plasma Elabela levels were significantly higher in the class II group of HF patients than in class III and class IV groups [4.9 (2.1, 6.8) vs. 2.2 (1.8, 4.8), $P = 0.007$ and 4.9 (2.1, 6.8) vs. 3.0 (1.8, 4.9) ng/ml, $P = 0.011$ separately] with no significant differences observed between class III and class IV groups (**Figure 1**).

Correlation Between Elabela and Study Variables

We further analyzed the correlation between Elabela and study variables in all subjects (**Supplementary Table 1**). Edema($r = -0.23$, $P < 0.001$), Third heart sound ($r = -0.22$, $P < 0.001$), Rales ($r = -0.21$, $P < 0.001$), Jugular venous distention($r = -0.20$, $P = 0.001$), Log10 BNP ($r = -0.20$, $P = 0.001$), creatinine levels (r =-0.13, $P = 0.029$), troponin I levels ($r = -0.19$, $P = 0.002$), left atrial diameter ($r = -0.14$, $P = 0.027$), left ventricular end diastolic diameter ($r = -0.34$, $P < 0.001$), left ventricular end systolic diameter (r =-0.29, $P < 0.001$) and PASP ($r = -0.27$, $P < 0.001$) were negatively related to plasma Elabela levels, whereas eGFR ($r = 0.13$, $P = 0.034$) and LVEF ($r = 0.23$, $P < 0.001$) were positively correlated to plasma Elabela levels.

Baseline Clinical Characteristics and Outcome of Patients With Different Levels of Elabela

According to the median of plasma Elabela level, all the hypertensive patients with HF were divided into two groups, the high-level group and the low-level group (**Supplementary Table 2**). Low-level group had more male patients (77.6 vs. 50.7%, $P = 0.001$), higher BNP levels [594.0 (342.0, 1917.0) vs. 367.0 (133.0, 1044.0) pg/ml, $P = 0.032$], lower total cholesterol levels (3.7 ± 1.2 vs. 4.2 ± 1.2 mmol/l, $P = 0.049$) and lower plasma Elabela levels [1.9 (1.6, 2.3) vs. 5.4 (4.8, 6.7) ng/ml, P <0.001] than those in high-level group. Echocardiographic data indicated that the low-level group had larger atrial and ventricular chambers and worse left ventricular systolic function than the high-level group ($P < 0.05$). After the 180-day follow-up, 15 out of 67 patients (22.4%) from the low-level group were admitted for HF recurrence, while only 5 out of 67 patients (7.5%) from the high-level group were readmitted ($P = 0.015$). Although the all-cause mortality had no statistical difference between the two groups (6.0 vs. 4.5%, $P = 0.698$), the MACE rate in the low-level group was higher than those in the high-level group (28.4 vs. 11.9%, $P = 0.018$). The high-level group had better readmission-free and MACE-free survival (**Figure 2**). No significant difference was found in the median lengths of hospital stay between the two groups.

Predictors of Baseline Characteristics for the Unfavorable Outcome of HF

To analyze the prognostic value of Elabela, we divided hypertensive patients with HF into a favorable outcome group (107 patients without MACE) and an unfavorable outcome group (27 patients with MACE). The baseline characteristics were shown in **Supplementary Table 3**. In univariate Cox proportional hazards analysis, $\log_{10}$ BNP levels [HR 5.05, 95% CI (2.28–11.17), $P < 0.001$], eGFR [HR 0.98, 95% CI (0.97–0.99), $P = 0.006$], plasma Elabela levels [HR 0.73, 95% CI (0.58–0.91), $P = 0.006$], classification of NYHA [HR 3.16, 95% CI (1.74–5.74), $P < 0.001$] and PASP [HR 1.03, 95% CI (1.00–1.05), $P = 0.025$] were closely associated with the occurrence of MACE. These factors were then incorporated into the multivariate analysis. Finally, plasma Elabela levels [HR 0.75, 95% CI (0.61–0.99), $P = 0.048$] and $\log_{10}$ BNP [HR 4.04, 95% CI (1.82–9.00), $P = 0.001$] were associated with the occurrence of MACE (**Supplementary Table 4**). ROC curve was used to assess the predictive value of plasma Elabela levels and BNP levels for the occurrence of MACE (**Supplementary Figure 4**). The AUC area of Elabela was 0.70 (95% CI 0.59–0.82), and the predictive cut-off point was 2.60 ng/ml (sensitivity 0.74, specificity 0.79). In contrast, the AUC area of $\log_{10}$ BNP was 0.76 (95% CI 0.67–0.85), and the predictive cut-off point was 2.58 ng/ml (sensitivity 0.93, specificity 0.50). Furthermore, the AUC area of the combination of Elabela and $\log_{10}$ BNP was 0.78 (95% CI 0.70–0.88). The predictive cut-off point of Elebala was 2.86 ng/ml, and $\log_{10}$ BNP was 2.58 (sensitivity 0.89, specificity 0.58).

DISCUSSION

To the best of our knowledge, this is the first study that investigated the predictive value of plasma Elabela levels in hypertensive patients with HF. Our study revealed that the declined plasma Elabela level was a promising predictor of HF readmission for HF patients. Moreover, we found that plasma Elabela levels were positively correlated with LVEF and negatively associated with the size of the left ventricle. These findings highlighted the need for conducting research on the biological action and mechanism of Elabela in the context of HF.

This study showed that plasma Elabela levels were significantly lower in hypertensive patients, especially in those with HF when compared with those in control subjects. A previous study reported lower plasma Elabela levels in patients with essential hypertension (9). The primary causes were recognized as the loss of hypotensive effect and endothelial protection from Elabela. So far, no study investigated plasma Elabela levels in other cardiovascular diseases such as coronary heart disease and atrial fibrillation. The differences in plasma Elabela levels among control subjects and hypertensive patients in this study revealed an underlying relationship between Elabela deficiency and cardiovascular diseases. In addition, due to the similar bioeffects with Apelin, it was indirectly implied that Elabela might be a protective factor preventing cardiovascular disease (18, 19). Apelin shared the same APJ receptor as Elabela and had been found to be significantly decreased in the plasma of HF patients (20–23). However, until now, plasma Elabela levels in HF patients had rarely been investigated. Our data showed that plasma Elabela levels in patients with HF were significantly depressed compared with those without HF. Elabela was essential for diverse biological processes and has important

roles in regulating fluid homeostasis, myocardial contractility, vasodilation, angiogenesis, myocardial fibrosis, apoptosis and proliferation, thus, contributing to the prevention of HF (11, 12, 24, 25). Our findings also indirectly supported previous studies that showed lower concentrations of plasma Elabela in patients with hypertension and renal impairment (9, 10), which were independent risk factors for HF development.

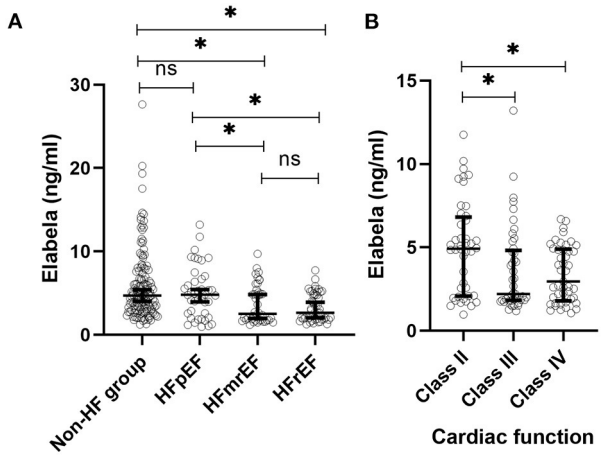


FIGURE 1 | Plasma Elabela levels in hypertensive patients with different types of HF. **(A)** The mean plasma Elabela level of the non-HF group were similar with that of HFpEF group. Plasma Elabela levels of patients with HFrEF were similar with that of HFmrEF. Furthmore, patients without HF had a higher mean plasma level of Elabela compared with patients with HFrEF and HFmrEF separately [4.7 (3.0, 7.4) vs. 2.6 (1.9, 4.9) ng/ml, $P = 0.01$ and 4.7 (3.0, 7.4) vs. 2.7 (1.8, 5.4) ng/ml, $P < 0.001$ separately]. Importantly, patients with HFpEF had higher plasma levels of Elabela than patients with HFrEF and HFmrEF separately [4.8 (2.4, 6.8) vs. 2.6 (1.9, 4.9) ng/ml, $P = 0.010$ and 4.8 (2.4, 6.8) vs. 2.7 (1.8, 5.4) ng/ml, $P = 0.037$ separately]. **(B)** There were no difference of plasma Elabela levels between HF patients with NYHA class III and IV; Plasma Elabela levels were significantly higher in HF patients with NYHA class II than those with NYHA class III and class IV separately [4.9 (2.1, 6.8) vs. 2.2 (1.8, 4.8), $P = 0.007$ and 4.9 (2.1, 6.8) vs. 3.0 (1.8, 4.9) ng/ml, $P = 0.011$ separately]. ELA, Elabela; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with middle-range fraction; HFpEF, heart failure with preserved ejection fraction; NYHA, New York Heart Association.

We further analyzed plasma Elabela levels in different types of HF. The HF patients with HFrEF or HFmrEF had lower plasma Elabela levels than HF patients with HFpEF and patients without HF. Interestingly, neither plasma Elabela levels between the non-HF and HFpEF group, nor plasma Elabela levels between HFrEF and HFmrEF group showed notable differences. These results showed a close relationship between Elabela and impaired left ventricular systolic function. This may be attributed to the positive inotropic effect of Elabela that was previously demonstrated in animal research (12, 26). It has also been reported that Elabela limited the area of cardiac fibrosis and downregulated the expression of profibrotic genes (11). Therefore, HF development might be ascribed to the adverse left ventricular remodeling and the systolic dysfunction due to lower plasma Elabela levels. Consistent with the above results, plasma Elabela levels were lower in patients with worse NYHA classification. Pulmonary hypertension is an independent predictive factor for adverse events in patients with HF (27). Previous studies showed that Elabela expression in human pulmonary hypertension (PHT) lung was significantly reduced comparing with healthy lung (7). Consistent with the previous results, plasma Elabela levels was inversely associated with PASP in our study. The similar trends in both tissues and circulation indicated a strong relationship between Elabela and pulmonary arterial pressure. Based on this, the correlation between Elabela and PASP might become stronger in patients with HF and PHT who often has a worse prognosis.

The multiple bioeffects of Elabela have a vital role in the progression of HF. The signs of HF are important clues for HF diagnosis. The characteristic signs of HF include the Edema, third heart sound, rales, and jugular venous distention. Recently, it was reported that these signs had independent prognostic value even beyond symptoms and natriuretic peptides (28). We found that plasma Elabela levels were negatively correlated with these signs. These results indirectly indicated that plasma Elabela levels were associated with prognosis of HF. Chronic kidney disease and HF are closely related. They interact with each other and

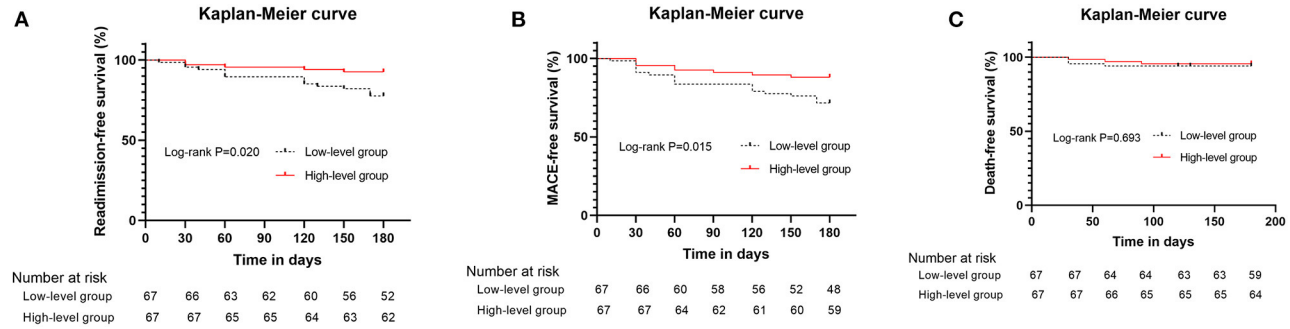


FIGURE 2 | Kaplan–Meier curves for hypertensive patients with heart failure above and below the median values for plasma Elabela level. **(A)** Kaplan–Meier curves (heart failure readmission) for patients above and below the median values for plasma Elabela levels; **(B)** Kaplan–Meier curves (composite outcomes, MACE) for patients above and below the median values for plasma Elabela levels; **(C)** Kaplan–Meier curves (all-cause mortality) for patients above and below the median values for plasma Elabela levels.

deteriorate patient's condition. Accordingly, kidney function is a well-established risk predictor in HF patients (29). In our study, the correlations between Elabela and eGFR and creatinine levels suggested that declined plasma Elabela levels might be associated with renal impairment. Evidence from the previous basic research and clinical study was in line with our findings. It was also reported that Elabela protected against podocyte injury in diabetic mice (30). In addition, declined plasma Elabela levels were associated with albuminuria in patients with type 2 diabetes (10). Given this evidence, declined plasma Elabela levels might increase the incidence of HF development via renal impairment and its dysfunction. Plasma Elabela levels had a positive correlation with HDL-c levels, which is a protective factor in cardiovascular diseases. This result revealed that Elabela might work as adipocytokines like Apelin taking part in metabolic regulation (31). Plasma levels of Elabela were much higher in patients with good cardiac function (NYHA class II) than those with poor cardiac function (NYHA class III and IV). These results showed a trend that patients with lower plasma Elabela level had an exacerbated cardiac dysfunction than those with higher Elabela plasma level. It is well-established that the worsen heart function is an independent risk factor for adverse events in patients with HF (32). The relationship between plasma Elabela level and heart function might be connected the declined plasma Elabela level to the adverse events in patients with HF. Notably, Elabela was also negatively related to both BNP and troponin I in our study. The relation between Elabela and BNP revealed that the anti-HF effects of Elabela might include the positive inotropic effect and the inhibition of cardiac remodeling. The negative relationship between Elabela and troponin I demonstrated the effect of Elabela on combating myocardial injury. The anti-inflammatory and antioxidant effects of Elabela might effectively prevent and limit myocardial injury. Hence, plasma levels of Elabela might be used as a new tool for the severity stratification of patients with HF in the future. Notably, further studies should also be conducted to investigate the interactions among Elabela, BNP, and troponin I.

It has been proved that BNP levels are associated with HF severity and mortality (33). Unfortunately, BNP has a poor predictive power on specificity (34). Our results suggest that Elabela might be a novel promising biomarker for HF severity. In our study, multivariate analysis revealed lower plasma Elabela levels as a useful predictor of a worse prognosis. Plasma Elabela levels were predictive for HF readmission and MACE. The predictive ability of Elabela might be attributed to its multiple protective effects, including antihypertensive effect, protection of renal function, inhibition of cardiac remodeling, suppression of inflammatory response, and impairment of myocardial injury (11, 12, 35). These results were in line with the previous conclusions advocating that BNP was an important predictor for adverse events in HF patients (36). Although the BNP had a significantly greater predictive sensitivity compared to Elabela, Elabela was superior to BNP in predictive specificity. Taking Elabela and BNP into consideration greatly improved physician's predictive ability for adverse events in HF patients. Importantly, our study revealed that the MACE was clearly driven by HF hospitalization, not by mortality. These results were due to the positive inotropic action of Elabela. Cardiac remodeling, an important impact factor on mortality, is a slow process (37). Thus, the short follow-up period might explain the lack of difference in mortality and a longer follow-up period is necessary for the certification of the long-term protective effect of Elabela for HF patients.

This study still had a few limitations. Firstly, the sample size was small and follow-up time was short. Secondly, control subjects in this study were younger than overall hypertensive patients with or without HF. So, we had to compare age and gender-matched patients. It still remained unknown whether there are differences between healthy volunteers and patients of advantage age. Thirdly, although the patients with and without HF had similar incidences of cardiovascular diseases in this study, confounders and interactions were inevitable.

CONCLUSIONS

The present study demonstrated for the first time that plasma Elabela levels were declined in hypertensive patients with HF, especially in those with left ventricular systolic dysfunction. Plasma Elabela levels were associated with multiple risk factors for HF. Lower plasma Elabela level might be used as a promising predictor for MACE in hypertensive patients with 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 Chaoyang Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

X-CY and J-CZ contributed to conception and design. ZM and LZ conducted the study and drafted the manuscript. YZ contributed to acquisition, analysis, and interpretation. YD contributed to analysis. SM critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.2021.638468/full#supplementary-material

Supplementary Figure 1 | Flow diagram for study from enrollment to follow-up.

Supplementary Figure 2 | Flow diagram for follow-up. Firstly, whether the subject is alive or not were confirmed. If the subject has died, the death details will be further investigated and death certificate will provide important information. Secondly, whether the subject is readmission or not were confirmed. If yes, we will further get the details information including the symptom, the diagnosis and medication records. Our team will clarify the reason of readmission. If no, we will ask the subject whether he/she had any symptoms associated with the deterioration of heart failure. If yes, we will assess the necessity of further outpatient visits and readmission.

Supplementary Figure 3 | Plasma Elabela levels in control subjects and age-sex-matched hypertensive patients. **(A)** The comparison between control subjects and hypertensive patients; **(B)** The comparison among control subjects, hypertensive patients with and without HF.

Supplementary Figure 4 | ROC curve of the prognostic value of plasma Elabela levels in predicting major adverse cardiac events in patients with HF.

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Alteration of m6A RNA Methylation in Heart Failure With Preserved Ejection Fraction

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Background: Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous disease, in which its pathogenesis is very complex and far from defined. Here, we explored the N^6-methyladenosine (m6A) RNA methylation alteration in patients with HFpEF and mouse model of HFpEF.

Methods: In this case–control study, peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood samples obtained from 16 HFpEF patients and 24 healthy controls. The change of m6A regulators was detected by quantitative real-time PCR (RT-PCR). A "two-hit" mouse model of HFpEF was induced by a high-fat diet and drinking water with 0.5 g/L of N^{ω}-nitro-l-arginine methyl ester (L-NAME). MeRIP-seq was used to map transcriptome-wide m6A in control mice and HFpEF mice, and the gene expression was high-throughput detected by RNA-seq.

Results: The expression of m6A writers *METTL3*, *METTL4*, and *KIAA1429*; m6A eraser *FTO*; and reader *YTHDF2* was up-regulated in HFpEF patients, compared with health controls. Furthermore, the expression of *FTO* was also elevated in HFpEF mice. A total of 661 m6A peaks were significantly changed by MeRIP-seq. Gene Ontology (GO) analysis revealed that protein folding, ubiquitin-dependent ERAD pathway, and positive regulation of RNA polymerase II were the three most significantly altered biological processes in HFpEF. The pathways including proteasome, protein processing in the endoplasmic reticulum, and PI3K-Akt signaling pathway were significantly changed in HFpEF by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Conclusions: The expression pattern of m6A regulators and m6A landscape is changed in HFpEF. This uncovers a new transcription-independent mechanism of translation regulation. Therefore, our data suggest that the modulation of epitranscriptomic processes, such as m6A methylation, might be an interesting target for therapeutic interventions.

Keywords: heart failure with preserved ejection fraction, N6-methyladenosine, epitranscriptomics, METTL3, FTO

INTRODUCTION

Heart failure is the main cause of mortality worldwide. Furthermore, heart failure with preserved ejection fraction (HFpEF) accounts for 50% or higher of heart failure. With the development of aging and the increasing prevalence of obesity, hypertension, and diabetes, this ratio will be higher (1). Because of the complex pathophysiological mechanism and heterogeneity of this syndrome, there is no evidence-based therapy for HFpEF, and treatment proven effective in heart failure with reduced ejection fraction (HFrEF) cannot improve survival in HFpEF (2). In this setting, exploration of various molecular and cellular mechanisms contributing to the morbidity of HFpEF is very crucial.

Recently, emerging evidences have demonstrated that epigenetics plays critical roles in the pathophysiological responses of HFpEF, such as DNA methylation, chromatin remodeling, histone modifications, and microRNA-depending gene expression (3). DNA methylation has been shown a causality role in diabetes-induced HFpEF (4). Aging affects the progress of HFpEF through the regulation of DNA methylation and histone modifications (5). Moreover, the alteration of microRNAs, such as down-regulation of miRNA-1 and up-regulation of miRNA-195, controls cardiac hypertrophy, oxidative stress, ischemic susceptibility, and fibrosis in HFpEF through histone modification (3, 6). Recently, Wallner et al. (7) reported that inhibition of histone deacetylases (HDAC) activity with suberoylanilide hydroxamic acid improves cardiopulmonary function, i.e., preserved lung structure, compliance, blood oxygenation, and reduced perivascular fluid cuffs around extra-alveolar vessels in HFpEF. Furthermore, Jeong et al. (8) found that HDAC inhibition with ITF2357 (givinostat) ameliorates the impairment of cardiac myofibril relaxation, cardiac fibrosis, and cardiac hypertrophy and changes in cardiac titin and myosin isoform expression in Dahl salt-sensitive rats with HFpEF, indicating that epigenetic regulation also significantly contributes to HFpEF.

N^6-methyladenosine (m6A) is the most common post-transcriptional modification of mRNA in mammals (9, 10). Recent studies have demonstrated that it is important for the regulation of various biological processes, such as embryonic development, cell differentiation, regeneration, and tumorigenesis (11–15). However, a study related to m6A in the cardiovascular field is still rare. It is reported that the global level of m6A is increased in myocardial infarction, ischemia–reperfusion injury, and HFrEF, and decreased m6A may enhance autophagic flux and improve cardiac function (16–19). Consistent with these roles, m6A modification is emerging as a key pathway influencing the pathological progress of HFrEF. However, how m6A modification affects heart function and which underlying mechanisms mediate these changes remain unknown. Given the critical role of m6A in regulating mRNA modification related to various biological processes by influencing mRNA stability, splicing, translation, and localization (20–26), it is reasonable to speculate that m6A may be involved in HFpEF. However, its role in HFpEF has not been studied.

Lacking relevant experimental models to accurately recapitulate the heterogeneity of this complex disease leads to the lack of effective treatments for HFpEF, as it is increasingly recognized as a complex interaction of multiple impairments throughout the body rather than cardiomyocyte disorder (27). Multiple comorbidities, such as diabetes, obesity, and hypertension, have been demonstrated to increase the risk of HFpEF (28). Recently, Hill et al. (29) proposed a "two-hit" mouse model of HFpEF, which mimicked concomitant metabolic and hypertensive stress in mice. In this model, a high-fat diet (HFD) induces the metabolic stress (obesity, glucose intolerant, and metabolic syndrome), and hypertension is caused by a drug called N^{ω}-nitro-L-arginine methyl ester (L-NAME), which inhibits nitric oxide (NO) synthase. This model recapitulates the numerous systemic and cardiovascular characteristics of HFpEF, including impaired cardiac filling, cardiac hypertrophy, cardiac fibrosis, reduced myocardial capillary density, pulmonary hyperemia, reduced exercise tolerance, and increased levels of inflammatory markers (29). Thus, this *bona fide* model of HFpEF was used in this study.

In order to explore the epigenetic modifications of RNA in HFpEF and their diagnostic value, we analyzed the m6A regulators in patients with HFpEF and healthy controls and the m6A methylation profiles in the setting of a "two-hit" mouse model of HFpEF (29). By analyzing of RNA and m6A methylation levels, we have identified potential novel targets that can provide a basis for further intervention in HFpEF.

METHODS

Patients and Control Subjects

In the part of case–control study, 16 HFpEF patients in our hospital from November 2020 to December 2020 were enrolled, and 24 cases who took health examination at the same period were recruited as healthy controls. The study complied with the Declaration of Helsinki and was registered (ChiCTR2000040038). The research program was approved by the ethics committee (No. B2020-356R) at Zhongshan Hospital, Fudan University, China. All patients provided written informed consent. Patients with HFpEF were eligible for the study (30). Exclusion criteria included (1) age <18 years, (2) participate in other clinical trials in the previous 3 months, (3) cancers, (4) chronic kidney disease at stage 2 or above, (5) severe hepatic insufficiency, (6) blood systemic diseases, such as leukemia, and (7) unlikely cooperation in the study. Baseline characteristics of study subjects were obtained, including age, gender, body mass index, hypertension, diabetes, atrial fibrillation, coronary heart disease, laboratory parameters, and echocardiography parameters.

Blood Sampling and Peripheral Blood Mononuclear Cells Extraction

Peripheral blood samples (8–10 ml) were collected into ETDA anticoagulant vacutainer (Becton Dickinson, San Jose, CA, USA) from HFpEF patients and healthy controls. Peripheral blood mononuclear cells (PBMCs) were extracted by Ficoll-isopaque centrifugation as mentioned previously (31). Briefly, peripheral

blood samples were centrifuged at 3,000 rpm for 10 min to obtain complete blood cell. After dilution with phosphate-buffered saline (PBS) at a ratio of 1:1, the diluted complete blood cell was transferred to lymphocyte separation medium (TBDsciences, Tianjin, China) and then centrifuged at 3,000 rpm for 10 min again to obtain PBMCs.

Animals and a "Two-Hit" HFpEF Model

Eight-week-old, male C57/BL6 mice were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). A "two-hit" mouse model of HFpEF was induced as described previously (29). Briefly, HFpEF mice were fed with a HFD [60% kilocalories from fat (lard)] and drinking water with 0.5 g/L of L-NAME (Sigma, N5751) for 10 weeks; control mice were fed with a standard diet. Mice were maintained in a 12/12-h light/dark cycle environment with a 22°C constant temperature. All animal experimental processes followed the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996), and were reviewed and approved by the animal ethics committee at Zhongshan Hospital, Fudan University, China.

RT-PCR

The total RNA of PBMCs and heart tissue was extracted with TriZol reagent (Invitrogen, USA), and the quality and quantity of RNA were assessed by NanoDrop 2000 (Thermo Fisher Scientific, USA). The reverse transcription was performed by using PrimeScript RT reagent kit (Takara, Japan). Then, real-time PCR (RT-PCR) was performed by using SYBR Premix Ex Taq II (Takara, Japan) in CFX96 Real-Time System (Bio-Rad, USA). Relative gene expression was normalized by 18S. The primers are listed in **Supplementary Table 1**.

MeRIP-seq

Total RNA was extracted from heart tissue with TriZol reagent (Invitrogen, USA), and polyA$^+$ RNA was enriched from total RNA with oligo-dT magnetic beads. Then, the polyA$^+$ RNA was fragmented to ∼100 nt long fragments by using RNA fragmentation buffer (Millipore Sigma, USA). The fragment RNA was divided into two parts; one was enriched with m6A antibody that could capture m6A for m6A-IP, and the other was used as input to construct normal transcriptome sequencing library. After the RNA fragment with m6A was enriched, the conventional sequencing library was constructed. The constructed sequencing libraries were sequenced by using the sequencing platform Illumina Hiseq X Ten (OE Biotech, China).

GO Analysis and KEGG Pathway Analysis

In order to annotate the altered m6A peaks, Gene Ontology (GO) enrichment analysis was used to describe the function of genes related to differential peaks. GO analysis of differentially expressed peaks was performed by using R based on the hypergeometric distribution. The number of genes related to the altered peaks in each GO term was counted, and the significance of enrichment of genes in each GO term was calculated by hypergeometric distribution test. GO categories from "biological process," "cellular component," and "molecular function" were extracted and plotted with their $-\log_{10}$ *P*-value. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed by using R, and hypergeometric distribution test was used to calculate the significance of genes related to altered peaks in each pathway term.

Statistical Analysis

All statistical analysis was performed by GraphPad Prism 7.0. Data were expressed as mean ± standard deviation (SD). Normal distribution was evaluated by Shapiro–Wilk test. Differences between two groups were determined by using unpaired Student's *t*-test. Furthermore, the association between m6A regulators and blood fasting glucose and blood lipids was determined by Pearson correlation test. Statistical significance was considered when $P < 0.05$.

RESULTS

Expression of m6A Regulators Was Changed in HFpEF Patients and HFpEF Mice

A total of 16 HFpEF patients and 24 healthy controls were analyzed in this study. The average age was 53 ± 15 years, and 65% of them were male. In order to investigate whether m6A methylation status was changed in HFpEF patients, we evaluated the m6A regulators in peripheral blood in HFpEF patients and healthy controls by RT-PCR: writers: *METTL3*, *METTL14*, *WTAP*, *METTL4*, and *KIAA1429*; erasers: *FTO* and *ALKBH5*; and readers: *YTHDF1-3* and *YTHDC1-2*. The expression of *METTL3*, *METTL4*, *KIAA1429*, *FTO*, and *YTHDF2* was significantly up-regulated in HFpEF patients (**Figures 1A,C,E,F,I**), compared with healthy controls. Furthermore, the expression of *WTAP* has a decreased trend (**Figure 1D**), and *ALKBH5* has an increased trend (**Figure 1G**), but the significance was near the border ($P = 0.07$). The expression of *METTL14*, *YTHDF1*, *YTHDF3*, *YTHDC1*, and *YTHDC2* remained unchanged between these two groups (**Figures 1B,H,J–L**). Then, we detected the m6A regulators in the hearts of HFpEF mice. In addition, we found that *FTO* was also up-regulated in HFpEF mice compared with control mice (**Figure 1M**), consistent with the finding in peripheral blood of HFpEF patients. However, *METTL3* was down-regulated, and the expression of *METTL4*, *KIAA1429*, and *YTHDF2* was not significantly changed (**Figure 1M**). Interesting, the expression of *YTHDC1* was up-regulated in HFpEF mouse (**Figure 1M**), which remained unchanged in HFpEF patients. The expression pattern changes of these m6A regulators could lead to a dynamic change in the m6A methylation in HFpEF patients and HFpEF mice.

Association of m6A Regulators With Risk of HFpEF

It is known that blood lipids and fasting glucose are risk factors for HFpEF (32, 33); then, we explored the association of the m6A regulators with total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and fasting glucose of HFpEF patients

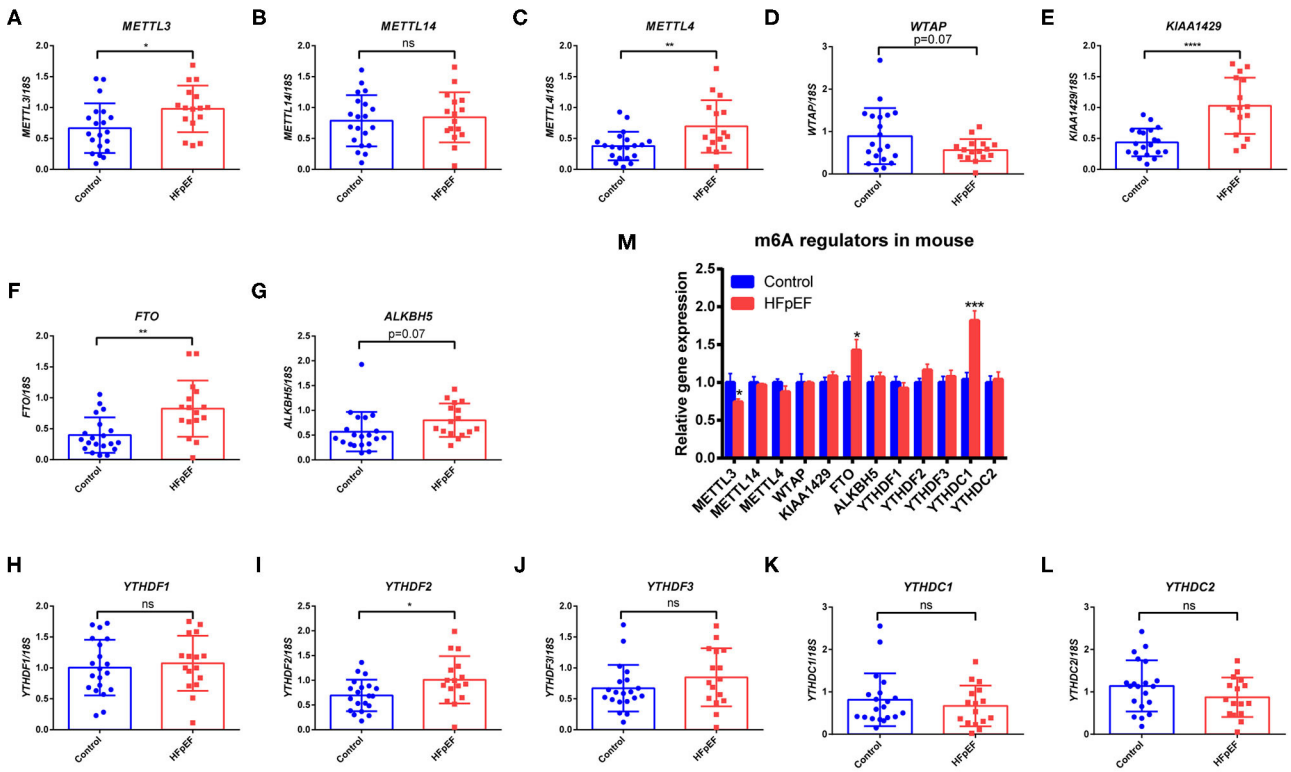

FIGURE 1 | The expression pattern of m6A regulators in HFpEF patients and mice. **(A–L)** RT-PCR of m6A writers (*METTL3*, *METTL14*, *METTL4*, *WTAP*, and *KIAA1429*), erasers (*FTO* and *ALKBH5*), and readers (*YTHDF1-3* and *YTHDC1-2*) in peripheral blood of HFpEF patients ($n = 16$) and healthy controls ($n = 24$). **(M)** RT-PCR of m6A regulators in the hearts in a "two-hit" mouse model of HFpEF ($n = 6$ per group). Data were shown as mean ± SD, and the differences were determined by Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$, compared with the control group.

by Pearson correlation test. Furthermore, we found that the expression of *METTL4* was negatively correlated with TC (r = −0.3632, $P = 0.0295$) and HDL-C ($r = -0.4186$, $P = 0.0111$) (**Figure 2A**). *KIAA1429* was negatively correlated with TC ($r = -0.4137$, $P = 0.0121$) and LDL-C ($r = -0.3457$, $P = 0.0389$) (**Figure 2B**). In addition, the association of *METTL3*, *KIAA1429*, and *FTO* with TC or HDL-C was near the border of significance (*P*-value range from 0.05 to 0.1) (**Figures 2C–E**). However, there was no correlation of m6A regulators with fasting glucose (data not shown).

Topological Distribution of m6A Peaks in HFpEF Mice

In order to determine the m6A modification levels of the HFpEF mice and control mice, we performed a transcriptome-wide m6A-seq analysis by MeRIP-seq. Compared with the high-throughput data between IP samples and their corresponding inputs, m6A methylation peaks were distinguished, including 1,852 distinct m6A peaks for 1,182 genes in the HFpEF group and 1,326 m6A peaks for 899 genes in the control group (**Supplementary Table 2**). The chromosomes with the most m6A modification sites in control mice were chromosomes 2, 4, and 7 with 104, 94, and 87 m6A modification sites in 69, 61, and 66 genes, respectively (**Figure 3A**, **Supplementary Table 2**). Furthermore, in HFpEF mice, they were chromosomes 4, 11, and 2 with 130, 125, and 122 m6A methylation sites in 79, 84, and 77 genes, respectively (**Figure 3A**, **Supplementary Table 2**). Moreover, the number of m6A methylation sites on the genes ranged from 1 to 13 in both groups, with 86.80% of genes having one or two m6A modification sites and 13.20% of genes having three or more m6A modification sites in the HFpEF group and 89.77% of genes having one or two m6A modification sites and 10.23% of genes having three or more m6A modification sites in the control group (**Supplementary Table 2**). For example, *Dync1h1* located on chromosome 12 was identified with the maximum number of m6A modification sites (13 sites) in the HFpEF group, and *Cmya5* located on chromosome 13 was also identified with the maximum number of m6A modification sites (13 sites) in control mice (**Supplementary Table 2**).

Then, the distribution patterns of m6A peaks across mRNA transcripts were analyzed, and we found that the frequency of m6A peaks across all transcripts was mostly distributed on the coding sequence (CDS) region and there was also distinct enrichment at the 5′UTR and 3′UTR regions (**Figure 3B**). Three representative genes (*Sumo1*, *Mapkapk2*, and *Zbed6*) were chosen to present the m6A modification pattern (**Figure 3C**). The peak of *Sumo1* was located at the 3′UTR region, the two peaks of *Mapkapk2* were both located at the 3′UTR regions, and the

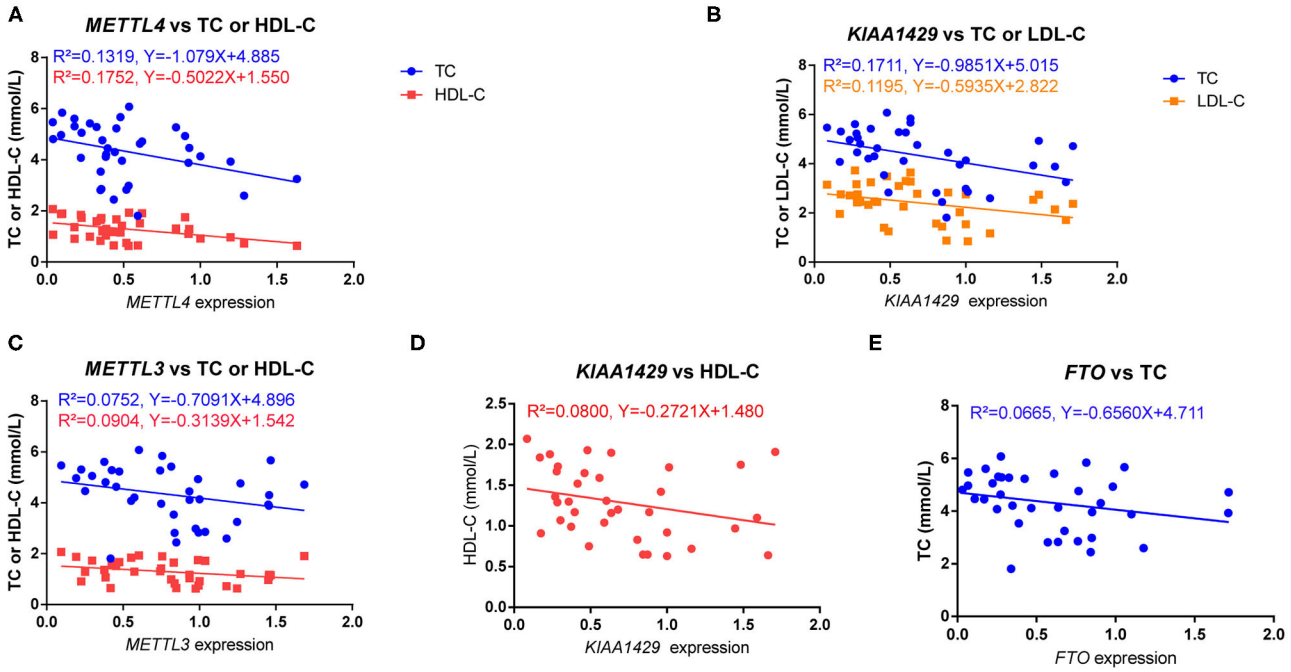

FIGURE 2 | The relationship of m6A regulators with serum lipids. **(A)** The expression of *METTL4* was negatively correlated with TC ($r = -0.3632$, $P = 0.0295$) and HDL-C ($r = -0.4186$, $P = 0.0111$). **(B)** The expression of *KIAA1429* was negatively correlated with TC ($r = -0.4137$, $P = 0.0121$) and LDL-C ($r = -0.3457$, $P = 0.0389$). **(C–E)** The association of *METTL3*, *KIAA1429*, and *FTO* with TC or HDL-C was near the border of significance (*P*-value range from 0.05 to 0.1). The relation was determined by Pearson correlation test.

peaks of *Zbed6* were located at the CDS and the 5′UTR regions. A total of 1,325 and 1,844 peaks were identified in control mice and HFpEF mice (**Figure 3D**), respectively. The average logarithmic fold-enrichment in HFpEF mice and control mice was 3.72 (**Figure 3E**). Furthermore, the average peak length of HFpEF mice was 1,799.86 bp and 1,943.19 bp in control mice (**Figure 3F**).

Significant m6A Methylation Alteration in HFpEF

To clarify the function of m6A modification in HFpEF, we compared the m6A modification levels of mouse hearts between the HFpEF mice and control mice. A total of 661 m6A peaks were significantly altered between two groups, and 443 of them were up-regulated, and 228 peaks were down-regulated in the HFpEF group (**Figure 4A**), compared with the control group. The top 20 differently expressed m6A marked mRNAs were presented in **Table 1**. Compared with the input sample, the average logarithmic fold-enrichment of differently expressed peaks was 2.98 (**Figure 4B**), and the average peak length was 1,140.81 bp (**Figure 4C**). The distribution of *P*-value of the altered peaks was presented in **Figure 4D**. The exact distribution pattern of altered peaks in HFpEF mice was shown in **Figure 4E**. There were 270 peaks distributed in the 3′UTR and exon regions, 167 peaks in the exon region, and 131 peaks in the intron and exon regions. Three representative mRNAs with significantly altered peaks were shown in **Figure 4F**. The m6A methylation levels of *Alb*, *Ehd1*, and *Hmgcs2* were significantly up-regulated in HFpEF by 29, 8.34, and 3.27 times, respectively.

Functional Annotation of the m6A Methylation by GO and KEGG Analyses

reveal the role of m6A modification in HFpEF, the mRNAs with significantly altered m6A methylation level were subjected to gene functional annotation by the GO and KEGG pathway analyses. GO analysis was divided into three parts: biological process, cellular component, and molecular function (**Figure 5A**). Protein folding, ubiquitin-dependent ERAD pathway, and positive regulation of RNA polymerase II were the three most significantly enriched in biological process. Mitochondrion, proteasome complex, and myelin sheath were the three most significantly enriched in cellular component. Furthermore, protein binding, proteasome-activating ATPase activity, and TBP-class protein binding were the three most significantly enriched in molecular function. Through KEGG pathway analysis, we annotated the mRNAs with significantly altered m6A modification levels. These mRNAs were mostly enriched in the pathways including proteasome, protein processing in the endoplasmic reticulum, and PI3K-Akt signaling pathway (**Figure 5B**).

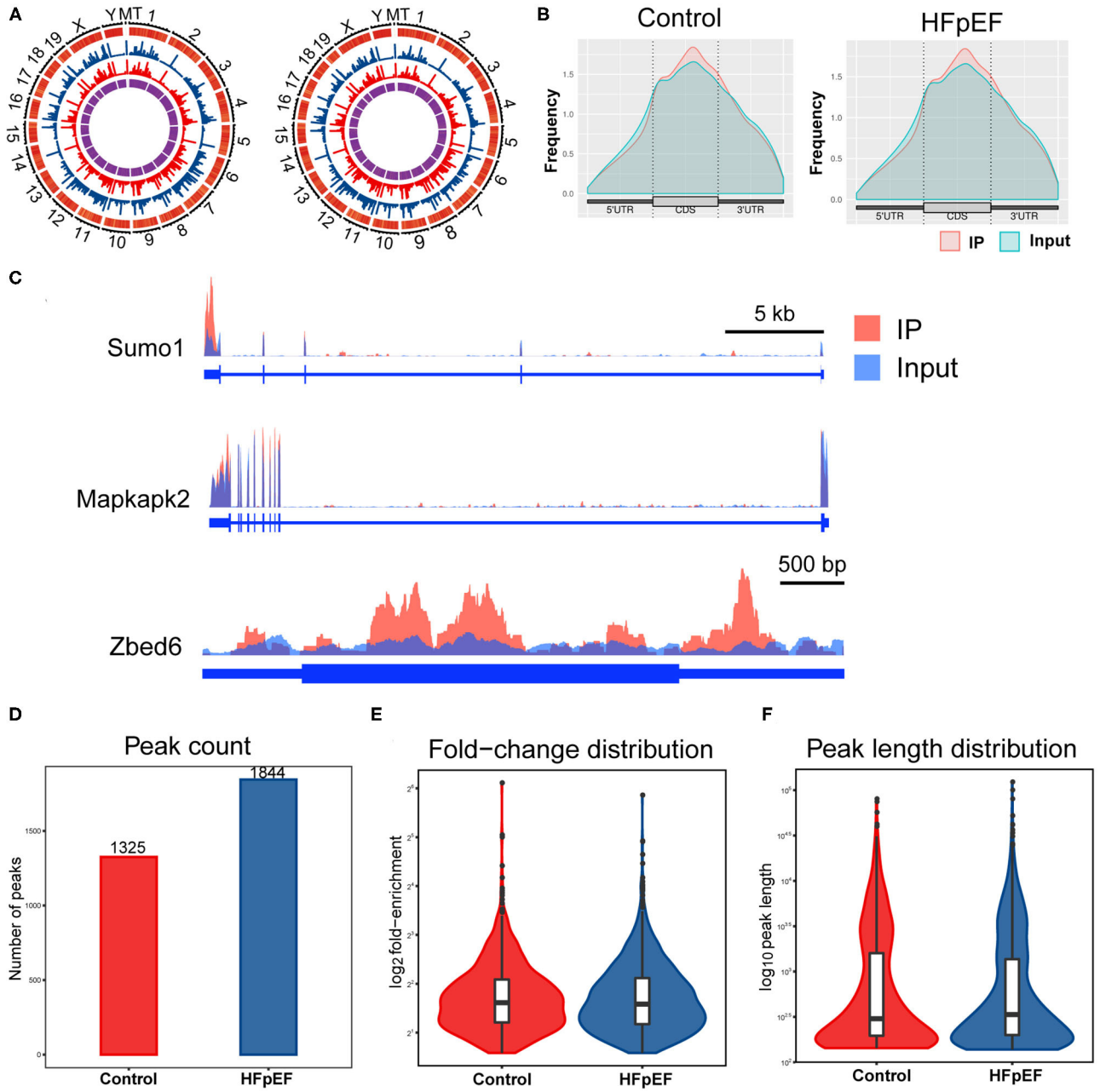

FIGURE 3 | The topological distribution of m6A peaks in HFpEF mice. **(A)** The distribution patterns of m6A peaks in different chromosomes of control mice and HFpEF mice. **(B)** The distribution patterns of m6A methylation peaks in gene structures of mRNA. **(C)** Visualization of representative m6A peaks in the genes of *Sumo1*, *Mapkapk2*, and *Zbed6*, using Integrative Genomics Viewer. The peak of *Sumo1* was located at the 3′UTR region, the two peaks of *Mapkapk2* were both located at the 3′UTR regions, and the peaks of *Zbed6* were located at the CDS and the 5′UTR regions. **(D)** The number of m6A peaks in the indicated groups. **(E)** The fold-enrichment of indicated groups. **(F)** The peak length distribution of indicated groups.

Combined Analysis of m6A Modification and Gene Expression in HFpEF

To further demonstrate the association between m6A modification and gene expression, the level of mRNA alteration was high-throughput detected in HFpEF mice and control mice by RNA sequencing data of input experiments. A total of 4,255 differently expressed genes were identified ($P < 0.05$, $\log_2$ FC >1), in which 2,155 genes were significantly up-regulated and 2,100 genes were significantly down-regulated in HFpEF (**Figure 6A**), compared with control. The top 20 significantly altered mRNAs are presented in **Table 2**. The volcano plot (**Figure 6B**) shows the

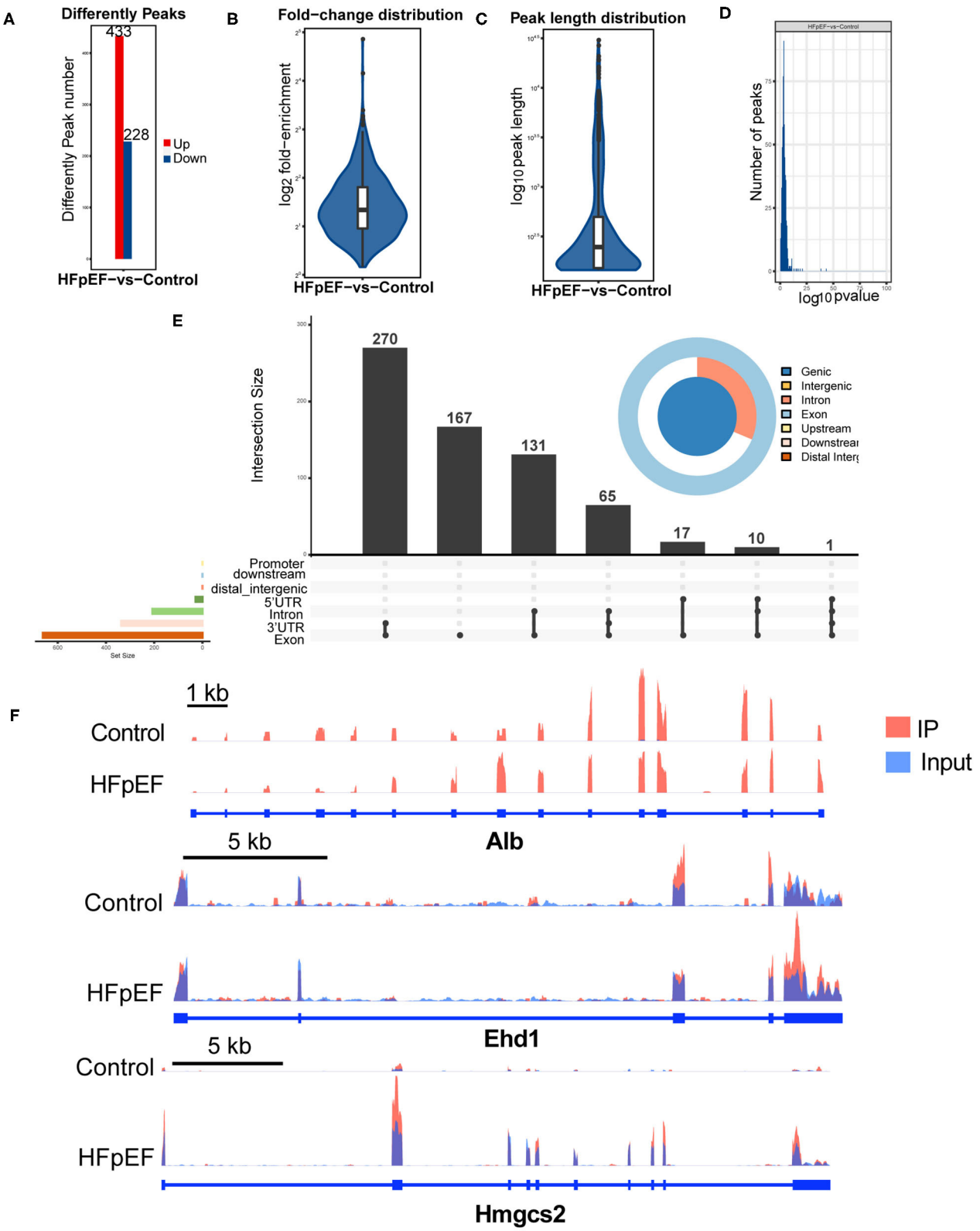

FIGURE 4 | The difference analysis of identified m6A peaks in HFpEF mice and control mice. **(A)** The up-regulated and down-regulated peak numbers in HFpEF mice, compared with control mice. **(B–D)** The fold-enrichment **(B)**, length **(C)**, and distribution of *P*-value **(D)** of altered m6A peaks. **(E)** The exact distribution pattern of

(Continued)

FIGURE 4 | significantly altered peaks in HFpEF mice. There were 270 peaks distributed in the 3′UTR and exon regions, 167 peaks in the exon region, and 131 peaks in the intron and exon regions. **(F)** Three representative genes with significantly changed peaks. The m6A levels of *Alb*, *Ehd1*, and *Hmgcs2* were significantly up-regulated in HFpEF by 29, 8.34, and 3.27 times, respectively.

TABLE 1 | The top 20 differently expressed m6A methylation peaks based on *P*-value.

mRNA	Chromosome	Peak region	Lg (*P*-value)	$\log_2$ (fold-change)	Up/down
Hspa1a	Chr17	3′UTR	−9.8	−3.95	Down
Ehd1	Chr19	3′UTR	−8.04	3.06	Up
Hmgcs2	Chr3	5′UTR	−7.66	1.71	Up
Fzd4	Chr7	Exon	−6.78	1.71	Up
Fbxl22	Chr9	Exon	−6.67	−1.82	Down
1810013L24Rik	Chr16	3′UTR	−5.71	1.99	Up
Adamts1	Chr16	3′UTR	−5.69	1.86	Up
Kdm3b	Chr18	Exon	−5.57	1.86	Up
Snapin	Chr3	Exon	−5.45	2.48	Up
Kank2	Chr9	3′UTR	−5.44	2.44	Up
Acot1	Chr12	Exon	−5.4	3.04	Up
Sdha	Chr13	3′UTR	−5.39	−0.611	Down
Lhfp	Chr3	5′UTR	−5.27	2.02	Up
Cfh	Chr1	Exon	−5.19	0.946	Up
Ywhae	Chr11	5′UTR	−5.01	1.4	Up
Fem1a	Chr17	3′UTR	−5.01	1.13	Up
Jun	Chr4	Exon	−4.91	−1.82	Down
Lbh	Chr17	3′UTR	−4.86	1.7	Up
Lmcd1	Chr6	3′UTR	−4.84	1.94	Up
Tmed7	Chr18	3′UTR	−4.77	1.51	Up

significantly up-regulated and down-regulated mRNAs between two groups.

Combined analysis of mRNA m6A modification and gene expression levels used peaks with $\log_2$ FC >0.58, $P < 0.01$ and the mRNA with $\log_2$ FC >1, $P < 0.05$. The association of m6A methylation and gene expression is presented in the quadrant graph (**Figure 6C**) and Venn diagram (**Figure 6D**). As shown, there were 58 mRNAs where both their m6A peaks and mRNA levels were altered significantly, among which the levels of 19 mRNAs were both down-regulated and the levels of 20 mRNAs were both up-regulated. Besides, there were five genes with down-regulated m6A peaks and up-regulated mRNA expression and 14 genes with up-regulated m6A peaks and down-regulated mRNA expression (**Figures 6C,D**). Furthermore, the protein–protein interaction network was performed to exhibit the junction between the proteins encoded by these identified genes (**Figure 6E**).

DISCUSSION

To the best of our knowledge, this is the first study to detect the m6A regulators in peripheral blood in cardiovascular diseases and the first study to explore the role of m6A methylation in HFpEF. Combined with the clinical case–control study and animal experiment, we showed the different expression patterns of m6A regulators in HFpEF patients and healthy controls and their association with the risk of HFpEF. Through MeRIP-seq, we obtained an m6A methylation panorama in a "two-hit" mouse model of HFpEF, which extended our knowledge of the critical role of m6A modification in HFpEF epigenetics.

Because it is difficult to obtain heart tissue samples from HFpEF patients, we detected the m6A regulators in PBMCs, although m6A RNA methylation in PBMCs may not reflect the post-transcriptional situation in the gene expression related to the function of the myocardium. The expression pattern of m6A regulators in the hearts of HFpEF mice is different from that in PBMCs of HFpEF patients, in which *FTO* and *YTHDC1* are up-regulated and *METTL3* is down-regulated. This may be explained by different sources of tissues. The altered expression pattern of m6A regulators in PBMCs of HFpEF patients represents the diagnostic potential of m6A regulators; however, the changed m6A regulators in heart tissue give inspiration to the pathological mechanisms and treatments.

Compared with the healthy controls, HFpEF showed higher expression of *METTL3*, *METTL4*, *KIAA1429*, *FTO*, and *YTHDF2* in peripheral blood (**Figure 1**). Similarly, it is reported that *METTL3*, *FTO*, *METTL14*, and *WTAP* are up-regulated in diabetes patients (34). Previous study reveals that the mRNA expression of *FTO* is positively correlated with glucose in diabetes patients (34); however, there is no correlation of m6A regulators with fasting glucose in our study. However, the correlation of m6A regulators with blood lipids is revealed. *METTL4* was

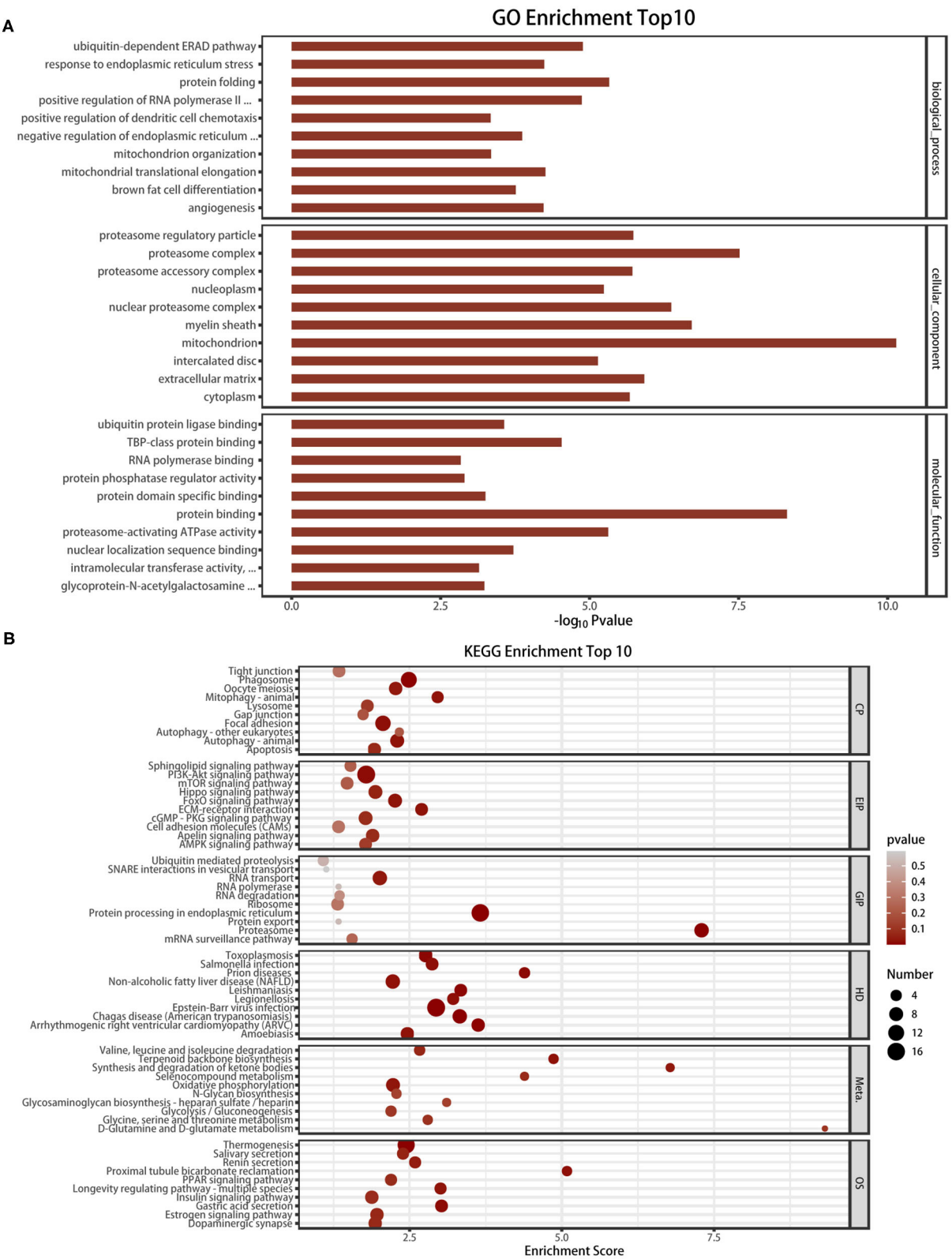

FIGURE 5 | GO analysis and KEGG pathway analysis of the altered mRNA m6A modification. **(A)** The top 10 enriched GO terms of the m6A peaks. **(B)** The top 10 enriched pathways of m6A peaks.

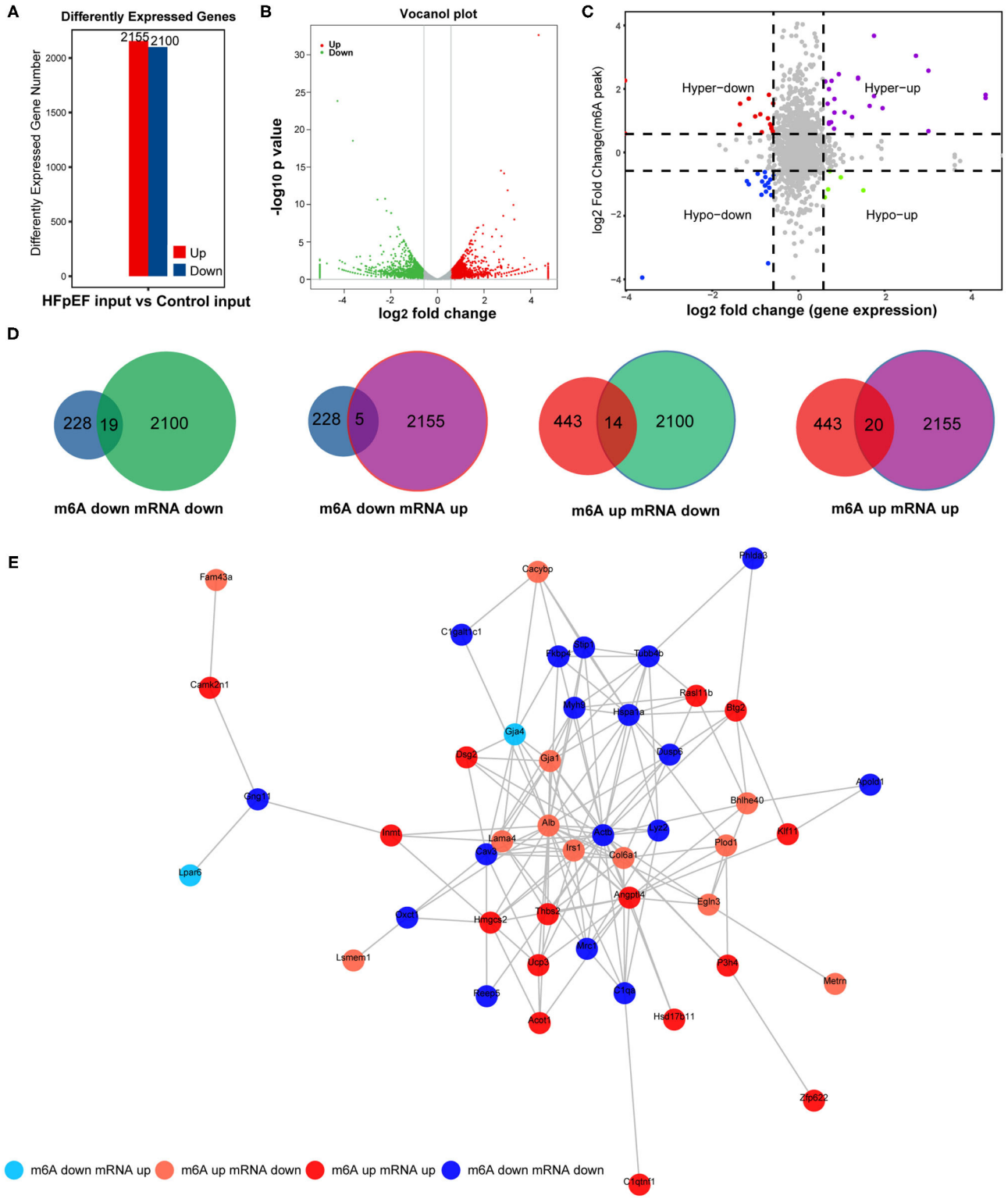

FIGURE 6 | The combined analysis with MeRIP-seq and RNA-seq. **(A)** The up-regulated and down-regulated mRNAs by RNA-seq in HFpEF mice and control mice. **(B)** The volcano plot of differently expressed genes. **(C,D)** Four quadrant graph and Venn diagram show the correlation between mRNA m6A methylation and its mRNA expression. **(E)** The protein–protein interaction network shows the connection between the proteins of the genes used in the combined analysis.

TABLE 2 | The top 20 differently expressed mRNAs based on *P*-value.

mRNA	*P*-value	log_2 (fold-change)	Up/down
Hmgcs2	2.25E−33	4.337564	Up
Hspa1b	1.45E−24	−4.29178	Down
Hspa1a	3.04E−19	−3.63182	Down
Acot1	2.95E−15	2.72059	Up
Spock2	6.94E−15	2.858137	Up
Angptl4	1.26E−12	3.011051	Up
Hsph1	1.70E−11	−2.2474	Down
Itgb6	2.26E−11	−2.57659	Down
Tcf23	1.16E−10	3.257808	Up
Cpxm2	6.96E−10	−2.18919	Down
Bdh1	1.39E−09	−1.98944	Down
Pth1r	3.28E−09	2.82245	Up
Myh7	1.00E−08	3.295993	Up
Scd4	5.83E−08	1.962302	Up
Col3a1	1.06E−07	−1.69663	Down
Hsd17b11	1.22E−07	1.747722	Up
Fmo2	1.35E−07	1.698478	Up
Serpinh1	1.98E−07	−1.6577	Down
Dusp4	2.06E−07	2.513462	Up
Thbs2	4.58E−07	1.646042	Up

negatively correlated with TC and HDL-C, and *KIAA1429* was negatively correlated with TC and LDL-C. Due to the cardioprotective function of HDL and the opposite roles of TC and LDL-C, KIAA1429 may play a cardioprotective role in HFpEF, but the role of METTL4 may be more complex. Interesting, *FTO* is up-regulated in both peripheral blood of HFpEF patients and hearts of HFpEF mice. As the core of the m6A methyltransferases, METTL3 can form a complex with METTL14 and WTAP to catalyze m6A modification on RNA (35). In contrast, a demethylase FTO could mediate the reversion of m6A methylation of RNA (36). Due to the alteration of m6A regulators in HFpEF, m6A modification resulted in both up-regulated peaks (433) and down-regulated peaks (228) in HFpEF. Recent studies have revealed that the expression pattern of m6A regulators and global level of m6A were changed in myocardial infarction, ischemia–reperfusion injury, myocardial hypertrophy, and HFrEF (16–19). METTL3 is increased in ischemia–reperfusion injury, but METTL14 was not significantly changed (18). Furthermore, FTO is decreased in HFrEF (16). The difference expression of m6A methyltransferases and demethylases in different cardiovascular diseases might be caused by physiopathologic differences or different organ sources.

By high-throughput measure of the m6A modification by MeRIP-seq, we found that the m6A modification levels of several mRNAs (i.e., *Alb*, *Ehd1*, *Hmgcs2*) related to the pathophysiological processes of HFpEF were significantly altered. The level of albumin (Alb) is an important hallmark of nutritional state, and a low serum Alb has been demonstrated to be a marker of myocardial fibrosis and exacerbates the prognosis of HFpEF (37, 38). Furthermore, we found that the m6A methylation of Alb was up-regulated in HFpEF and the mRNA of Alb was down-regulated. Ehd1 (Eps15 homology domain-containing protein 1) is recently identified as a novel interactor of Cx43 in the heart and plays a critical role in the pathological remodeling of gap junctions (39). Hmgcs2 is a ketone metabolic enzyme, and the level of Hmgcs2 in patients with arrhythmogenic cardiomyopathy is elevated, which leads to elevated plasma beta-hydroxybutyrate (β-OHB) and predicts major adverse cardiovascular events (MACE) (40). The altered m6A peaks in HFpEF have been associated with several protein processing by GO enrichment and KEGG pathway analyses, such as protein folding, ubiquitin, and protein binding, which means that the dysfunctional m6A methylation of the protein process plays a vital role in the development of HFpEF. Zhang et al. proved that activating the proteolytic function of the ubiquitin–proteasome system improves mouse survival in HFpEF (41). However, the results of the GO analysis and KEGG pathway analysis were not confirmed by phenotypical study, and we suggested using the METTL3 conditional knockout mouse model or FTO inhibitor to further verify the role of m6A in HFpEF. In addition, further research studies could be performed to confirm the exact protein level of these m6A methylated mRNAs.

Recent studies have revealed that m6A methylation induces the dysfunction of mRNA half-life and leads to mRNA instability (42). To better understand the mechanisms of m6A modification in HFpEF, we screened all the altered m6A peaks combined with the differentially expressed mRNAs. Consequently, 58 mRNAs were identified, whose m6A peak and gene level were both altered significantly, which could be divided into four parts: mRNA and m6A peaks both down-regulated (19), mRNA and m6A peaks both up-regulated (20), the m6A peak down-regulated and mRNA up-regulated (5), and the m6A peak up-regulated and mRNA down-regulated (14). The differently expressed level of m6A methylation will be recognized by "reader" protein and then induces different outcomes, for example, mRNA decay, mRNA stability, and mRNA translation (43). The m6A "reader" protein YTHDF2 is identified to control the half-life of target transcripts by mediating mRNA degradation, whereas YTHDF1 promotes translational effect.

In conclusion, to the best of our knowledge, this is the first study to explore the role of m6A methylation in HFpEF. Our study shows that the expression pattern of m6A regulators is changed in HFpEF. By MeRIP-seq, 661 m6A peaks were identified to be significantly altered in HFpEF mice, compared with control mice. The further combined analysis of m6A peaks and genes expression disclosed that there were 58 mRNAs significantly altered in HFpEF. These identified genes may be the critical regulators to interfere in the epigenetic regulation of HFpEF, and further exploring the fine regulation mechanism of m6A could open up a way to effective treatment for HFpEF.

Limitations

Firstly, the sample size is small in this study, and we will further expand the sample size and explore the association of m6A regulators with the prognosis of HFpEF in the

future. Secondly, the precise mechanism of m6A regulators in HFpEF needs to be studied in the future, for example, by using conditional knockout mouse model. Thirdly, m6A RNA methylation in PBMCs may not reflect the post-transcriptional situation in the gene expression related to the function of the myocardium.

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 at: https://www.ncbi.nlm.nih.gov/, PRJNA691715; https://www.ncbi.nlm.nih.gov/, PRJNA691685.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee at Zhongshan Hospital, Fudan University, China. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Animal Ethics Committee at Zhongshan Hospital, Fudan University, China.

AUTHOR CONTRIBUTIONS

BZ, AS, and JG were responsible for the design of the study and the writing the manuscript. YX and XC were responsible for data analysis work. HJ, WL, XW, YW, and YZ were responsible for the edit of the manuscript. All authors read and approved the final manuscript.

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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.2021.647806/full#supplementary-material

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MINI REVIEW
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Treatment of Heart Failure With Mid-Range Ejection Fraction: A Summary of Current Evidence

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Heart failure (HF) is a complex syndrome causing heavy burden in public health, and the modern objective assessment of it is based on the left ventricular ejection fraction (LVEF). In 2016, the European Society of Cardiology classified the "gray area" in HF with LVEF of 40–49% as a new HF phenotype (HFmrEF) in an attempt to uncover the specific characteristics and treatment of these patients, which might recover or worsen to HFpEF or HFrEF, respectively, or conversely from these two subtypes. Up to now, many studies have demonstrated that patients with HFmrEF would possibly gain more benefits from some targeted therapies with HFrEF than those with HFpEF. This review summarizes what is known about the findings in the treatment of HFmrEF and discusses what should be done to better define the peculiar HF phenotype in the future.

Keywords: heart failure, HFmrEF, HFPEF, treatment, review

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INTRODUCTION

Heart failure (HF) is a complex clinical syndrome characterized by reduced cardiac output and/or elevated filling pressures at rest or with exertion. Recognizing different heart failure (HF) subtypes is so important, not only because it broadly frames differences in the underlying pathophysiology, but also because each of the HF subtypes presents different outcomes in therapeutic approaches (1, 2). The modern management of heart failure (HF) is primarily guided by clinical objective assessments of left ventricular ejection fraction (LVEF), which has been proven to be an efficacious predictive method of adverse outcomes even in patients without symptomatic HF.

In 2016, the European Society of Cardiology classified HF with mid-range ejection fraction (HFmrEF) as LVEF of 40–49% (3), which has often been considered a "gray" area in HF, as HFmrEF remains insufficiently characterized compared with the HFrEF and HFpEF subtypes in the past years. This new classification, as acknowledged in the guidelines, is an attempt to stimulate research and resolve critical clinical questions, rather than a true admittance of an independent phenotype different from the other groups. And as expected, there has been research on the clinical entity of HFmrEF in recent years, which presented us with expanding insights into epidemiology, pathophysiology, clinical characteristics, morbidity and mortality, and treatment for patients with HFmrEF. Clinical trial data suggest a HFmrEF prevalence of 14–24% among the overall HF population (4–8).

The EF may change with treatment and over time, and the heterogeneity is deduced by the different etiology of HF. A considerable number of patients transition to either HFrEF or HFpEF while on treatment. Coronary artery disease seems to be common, and it seems to play a critical

role for worsening from HFpEF to HFmrEF or from HFmrEF to HFrEF. As there are a flurry of findings that HFmrEF specifies the aspects resembling the other two HF categories, which provide us with a feasible explanation of the controversies about why some researchers thought HFmrEF was a "transition phase" of HFrEF and HFpEF. This raises a question of which potential therapies thus far reserved for patients with HFrEF may be beneficial in those with intermediate LVEF.

As there are difficulties in the enrollment of patients with HFmrEF, there have been no randomized controlled trials (RCTs) dedicated to evaluate the effect of therapy. Therefore, we could only find some information on the overlap between HFmrEF and other groups, as we did from the CHARM, TOPCAT, and PARAGON clinical trials, which all showed an effect of different drugs in the lower end of the LVEF spectrum included in these studies, such as 40–50% or 45–50%. We have made some progress in understanding the treatment efficacy of neurohormonal antagonists, including angiotensin-converting enzyme inhibitors/ACEI, angiotensin receptor blockers/ARB, beta-blockers, and mineralocorticoid receptor antagonists/MRA, in patients with HFmrEF. In this review, we will present an overview about the updated therapies for patients with HFmrEF.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (ACEI) AND ANGIOTENSIN RECEPTOR BLOCKERS (ARB)

In a *post-hoc* analysis of a randomized clinical trial, named the CHARM program, in which the 7,598 patients with available integer digit EF were divided into three parts: HFrEF, HFmrEF, and HFpEF (9), the authors evaluated the treatment effect of candesartan in patients with HFmrEF, and found there was a smaller risk of primary outcome [HR: 0.76; 95% CI (0.61–0.96); $p = 0.02$] and recurrent HF hospitalization [HR: 0.48, 95% CI (0.33–0.70), $p < 0.001$] in the treatment group during the mean follow-up of 2.9 years. It is notable that the treatment efficacy of candesartan was constant at a lower EF and generally began to decline at EF > 50%. However, in the randomized controlled I-PRESERVE trial with LVEF >45% (10), there was no difference between the irbesartan treatment group compared with the placebo group, though the average LVEF was higher in this trial (mean LVEF, 59%) compared with the CHARM-preserved trial (mean LVEF, 54%). In an observational study (11), the OPTIMIZE-HF trial, HF patients with LVEF >40% also did not benefit from ACEI/ARB therapy in the first 60 to 90 days of follow-up.

BETA-BLOCKERS

Cleland et al. (12) used a meta-analysis of randomized controlled trials to demonstrate that beta-blockers may reduce CV death in HFmrEF patients in sinus rhythm compared with placebo [HR 0.48; 95% CI (0.24–0.97); $p = 0.04$] and improve left ventricular systolic function with a higher LVEF using data from double-blind, randomized, placebo-controlled trials. Similar to the outcomes above, several observational studies suggested that beta-blockers treatment may have benefits in cardiovascular outcomes in the HFmrEF population. In the multicenter prospective registry CHART-2 cohort (13), beta-blocker use was associated with reduced mortality among those with HFmrEF. Similarly, in the Swedish Heart Failure Registry (6), beta-blockers were associated with reduced mortality only in the presence of CVD (HR up to 1 year 0.74, 95% CI 0.59–0.92), nevertheless, ACEI/ARBs and statins were associated with lower 1-year all-cause mortality with or without CVD. However, in the OPTIMIZE-HF trial (14), initiation of beta-blockers did not show improved outcomes in the HF patients with LVEF >40%, and another study also revealed that there were no improvements in all-cause mortality in those with EF >40% (15).

MINERALOCORTICOID RECEPTOR ANTAGONISTS (MRAs)

A study used data from a randomized controlled trial called TOPCAT (16) to assess the relationship between efficacy and outcome of spironolactone and LVEF, found that LVEF modified the treatment efficacy of spironolactone, those with LVEF between 45 and 50% had a lower primary outcome [HR 0.72, 95% CI (0.50, 1.05)] and HF hospitalization [HR 0.76, 95% CI (0.46, 1.27)]. Along with this, in a prospective study (17), during a mean follow-up of 2.2 years, Enzan et al. found that patients with spironolactone had a lower incidence rate of primary outcome (all-cause death and or HF rehospitalization) than those without it [RR 0.61, 95 CI (0.44–0.86), $P = 0.004$].

SACUBITRIL/VALSARTAN

There were 4,822 patients with LVEF >45% who were randomly assigned to sacubitril/valsartan or valsartan groups in the PARAGON-HF trial (18). The primary composite outcome of total hospitalizations for heart failure and death from cardiovascular causes had no statistical significance between the two groups. Although statistically non-significant, it is noticeable that sacubitril/valsartan had a lower rate of hospitalization for heart failure than valsartan alone (rate ratio 0.87, 95%CI 0.75–1.01, $p = 0.06$). And of the 12 pre-specified subgroups, two showed a benefit in patients with an ejection fraction in the lower part (45–57%) of the study and in women. Along with this, Solomon and colleagues (19) combined data from the PARADIGM-HF (LVEF eligibility ≤40%; $n = 8,399$) and PARAGON-HF trials, as the two studies had similarities in many aspects such as eligibility criteria, similar control groups (enalapril or valsartan, respectively), and outcome assessment. The pooled analysis containing a total cohort of 13,195 patients suggested that patients with LVEF lower than normal, including HFmrEF or borderline ejection fraction, would possibly benefit, particularly in the combined end-point of cardiovascular mortality and first hospitalization for HF, from sacubitril/valsartan compared with RAS inhibition. And these therapeutic benefits appeared to extend to a higher LVEF range in women compared with men. A study suggested that combination use of sacubitril/valsartan rather than valsartan

TABLE 1 | Overview of the main studies investigating patients with HFmrEF.

References	Study type	Inclusion criteria	LVEF	Patient number	Outcome for HFmrEF
Lund et al. (9)	*Post-hoc* analysis of randomized trial	Patients enrolled in CHARM program	Full spectrum	7,599	Primary outcome for candesartan vs. placebo: [HR: 0.76, 95% CI (0.61, 0.96), $p = 0.02$]; recurrent HF hospitalization: [HR: 0.48, 95% CI (0.33, 0.70), $p < 0.001$]
Solomon et al. (16)	*Post-hoc* analysis of randomized trial	Patients with HF and LVEF ≥45% enrolled in TOPCAT	>45%	3,444	Primary outcome for spironolactone vs. placebo: [LVEF < 50%,HR: 0.72, 95% CI (0.50, 1.05), $p = 0.046$]; heart failure hospitalization [LVEF < 50%, HR: 0.76, 95% CI (0.46, 1.27), $p = 0.039$]
Cleland et al. (12)	Meta-analysis of randomized controlled trials	Included all patients with baseline LVEF and an electrocardiogram (ECG) that showed either sinus rhythm or AF/atrial flutter	Full spectrum	14,262	Beta-blockers may reduce CV death in HFmrEF patients in sinus rhythm compared with placebo [HR: 0.48, 95% CI (0.24, 0.97), $p = 0.04$]
Solomon et al. (18)	*Post-hoc* analysis of randomized trial	Patients with HF and LVEF ≥45% enrolled in PARAGON-HF	>45%	4,822	Primary events for sacubitril–valsartan vs. valsartan: [RR: 0.87, 95% CI (0.75, 1.01), $p = 0.06$]
Abdul-Rahim et al. (20)	*Post-hoc* analysis of randomized trial	Patients enrolled in DIG. HF patients with LVEF ≤45% and were in normal sinus rhythm (6,800 patients). HF patients with LVEF >45% were enrolled in an ancillary trial (988 patients)	Full spectrum	7,788	Digoxin had an intermediate effect in HFmrEF [HR: 0.80, 95% CI (0.63, 1.03)] compared with HFrEF and HFpEF; the composite of HF death or HF hospitalization [HR: 0.83, 95% CI (0.66, 1.05)]
Massie et al. (10)	Randomized controlled trial	Patients with HF and LVEF ≥45% in I-PRESERVE	≥45%	4,128	The primary outcome in the irbesartan group vs. the placebo group: [HR: 0.95, 95% CI (0.86, 1.05), $p = 0.35$); the secondary outcome: rates of death from any cause in the irbesartan group and the placebo group: [HR: 1.00, 95% CI (0.88, 1.14), $p = 0.98$]; rates for protocol-specified hospitalization: [HR: 0.95, 95% CI (0.85, 1.08), $p = 0.44$]
Enzan et al. (17)	Multicenter prospective registry	Patients with HF and with LVEF of ≥40 and <50% from JCARE-CARD	40–50%	457	Primary outcome for spironolactone vs. placebo: [IRR: 0.61, 95% CI (0.44, 0.86); $p = 0.004$]; composite of all-cause death or HF rehospitalization [adjusted HR: 0.63, 95% CI (0.44, 0.90), $P = 0.010$]
Tsuji et al. (13)	Multicenter prospective registry	Patients with HF and LVEF ≥45% enrolled in CHART-2	Full spectrum	3,480	Beta-blockers were positively associated with HFmrEF [HR: 0.57, 95% CI (0.37, 0.87), $p = 0.010$]; diuretics were negatively associated with improved mortality in HFmrEF [HR: 2.01, 95% CI (1.24, 3.28), $p = 0.004$]
Fonarow et al. (11)	Prospective registry	Patients with HF and LVEF ≥40% and left ventricular systolic dysfunction (LVSD) with reduced EF enrolled in OPTIMIZE-HF	≥40%	41,267	60- to 90-day mortality: [HR: 1.141, 95% CI (0.812, 1.603), $p = 0.447$] and rehospitalization rates [HR: 0.909, 95% CI (0.692, 1.196), $p = 0.497$] for ACEI/ARB; 60- to 90-day mortality: [HR: 1.209, 95% CI (0.872, 1.875), $p = 0.255$] and rehospitalization rates [HR: 0.923, 95% CI (0.723, 1.179), $p = 0.523$] for beta-blockers
Lund et al. (9)	Nationwide prospective registry	Patients with HF enrolled in SwedeHF	Full spectrum	51,060	Beta-blockers use and 1-year mortality in HFmrEF: mortality was reduced in HFmrEF with CAD [HR up to 1 year 0.74, 95% CI (0.59, 0.92)] but not in HFmrEF without CAD [HR 0.99, 95% CI (0.78, 1.26)]; angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARBs)/statins were associated with reduced risk in all HFmrEF groups with or without CAD (all $p \leq 0.004$)

HF, heart failure; LVEF, left ventricular ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; CI, confidence interval; CAD, coronary artery disease; IRR, incidence rate ratio; AF, atrial fibrillation; ACEI, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers.

TABLE 2 | Medical therapy in heart failure.

	ACEI	ARB	Beta-blocker	MRA	ARNI	SGLT2I	Diuretic
HFrEF	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	**?**
HFmrEF	**?**	↑	↑	↑	↑	**?**	**?**
HFpEF	×	↑	×	↑	↑	**?**	**?**

↑↑: Proven cardiovascular benefit.
↑: Potential cardiovascular benefit.
×: No cardiovascular benefit.
***?**: Uncertain cardiovascular benefit.*
ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist; ARNI, angiotensin receptor—neprilysin inhibitor; SGLT2I, sodium glucose cotransporter 2 inhibitors.

alone with MRA appeared to be associated with a lesser decline of renal function and no increase in severe hyperkalemia in patients with LVEF >45% in the PARAGON-HF trial, which would provide us with a new insight of benefit of combined therapy (21). In the PARALLAX trial (22), randomizing 2,572 patients with a LVEF of >40%, NT-proBNP was significantly reduced in the sacubitril/valsartan group after 12 weeks vs. individualized medical therapy, and was associated with a reduction of left atrial size. But it is noticeable that there was a significantly difference in terms of NTproBNP cut-off diastolic dysfunction analysis and comorbidities compared with other trials. There was a lower risk of worsening renal function with sacubitril/valsartan in HF patients with LVEF >40% than those with LVEF ≤40% (23), according to a systematic review and meta-analysis of randomized controlled trials. Excitingly, the FDA panel has supported the expanded indication for sacubitril/valsartan, which would allow it to be a treatment for certain patients with HFpEF, and it is possible that sacubitril/valsartan would be efficacious in those with HFmrEF.

OTHER THERAPEUTICS

Digoxin had an intermediate effect on HFmrEF [HR: 0.80, 95% CI (0.63–1.03)] compared with HFrEF and HFpEF, and did not significantly reduce HF hospitalization in the HFmrEF population (20). Diuretics seem to be negatively associated with improved mortality in HFmrEF (13). Sodium glucose cotransporter 2 inhibitors (SGLT-2I), an inhibitor of a new pathway of HF treatment different from the neurohormonal one, are associated with reduced HF hospitalizations and CV death in patients with type 2 diabetes mellitus regardless of history of HF (24, 25), and the ongoing EMPEROR-Preserved, DELIVER, and SOLOIST-WHF trials may confirm the effect of these drugs on HF outcomes in patients with LVEF >45%. The summary of the effect of the main HF therapies on outcomes specifically in the HFmrEF population is reported in **Table 1**.

HFmrEF is not a stable phenotype, but a heterogenous condition with variable evolutions, which is proven by the fact that without any change in underlying pathophysiology, a number of HF patients move in and out of the HFmrEF range on serial echocardiograms (6, 26). The treatment and management of coronary artery disease and atrial fibrillation seems to be important in the process of heart failure phenotype transition. As indicated by the HF Long-Term Registry of the European Society of Cardiology (27), the prevalence of AF was higher with increasing LVEF (27% in HFrEF, 29% in HFmrEF, and 39% in HFpEF) and AF was associated with worse outcomes (combined HF hospitalization and all-cause mortality) in HFpEF [HR = 1.36, 95% CI (1.15–1.62), $p < 0.001$] and HFmrEF [HR = 1.30, 95% CI (1.06–1.61), $p = 0.014$], but not in HFrEF [HR = 0.96, 95% CI (0.84–1.09), $p = 0.502$]. In the SwedeHF trial (6), HFmrEF resembled HFrEF most notably for CAD (HFrEF 54%, HFmrEF 53%, HFpEF 42%, $p < 0.001$), and notable adjusted odds ratios (ORs) were similar for CAD [HFmrEF vs. HFpEF: OR 1.52, 95% CI (1.41–1.63); HFmrEF vs. HFrEF: OR 0.94, 95% CI (0.88–1.00)]. Although targeting patients of HFmrEF specifically, like we did in other HF groups, is efficacious for resolving questions that disturbed us, many have failed due to the difficulties in patient enrollment. In addition, the variability of LVEF measurements based on echocardiography influences the accuracy of EF evaluation. Potential solutions to these issues might include the following: (1) expanding the EF range of HFrEF and/or HFpEF to include HFmrEF or the entire EF spectrum, as we did in some research evaluating ARB, MRA, and ARNI therapy in HFmrEF, and (2) evaluating EF in a dynamic and serial way, as beyond evaluating baseline LVEF, the implications of longitudinal LVEF are becoming more important.

In the era of precision medicine, the future management of HFmrEF or HF patients may involve accurately evaluating cardiac function and identifying features of each patient with HF, which might provide us with more information about how to scientifically stratify risk factors and choose appropriate therapies beyond what is predicted by LVEF alone, help doctors discern true myocardial recovery from myocardial remission which includes reverse cardiac remolding, but the absence of signs of complete reversal of damage, and multiparametric approaches, such as biomarkers and image parameters, should be taken into account for the discovery of new more effective treatments.

CONCLUSION

The expanding insights of HFmrEF indicate to us that it is an intermediate phenotype between HFrEF and HFpEF in terms of baseline characteristics, outcomes, and prognosis, but mildly resembles more that of the HFrEF subtype than HFpEF. As summarized in **Table 2**, ARB, beta-blockers, MRA, and ARNI may have potential cardiovascular benefits for patients with HFmrEF, but it is uncertain whether ACEI or SGLT-2I has

cardiovascular benefits. Future research, especially RCTs, is needed to explore the expanding insights into this peculiar phenotype and to identify strategies that will best achieve improvements in cardiovascular outcomes.

AUTHOR CONTRIBUTIONS

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

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Prognostic Implication of Liver Function Tests in Heart Failure With Preserved Ejection Fraction Without Chronic Hepatic Diseases: Insight From TOPCAT Trial

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Background: Liver dysfunction is prevalent in patients with heart failure (HF), but the prognostic significance of liver function tests (LFTs) remains controversial. Heart failure with preserved ejection fraction (HFpEF) had been introduced for some time, but no previous study had focused on LFTs in HFpEF. Thus, we aim to evaluate the prognostic significance of LFTs in well-defined HFpEF patients.

Methods and Results: We conveyed a *post-hoc* analysis of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial (TOPCAT). The primary outcome was the composite of cardiovascular mortality, HF hospitalization, and aborted cardiac arrest, and the secondary outcomes were cardiovascular mortality and HF hospitalization. In Cox proportional hazards models, aspartate transaminase (AST) and alanine transaminase (ALT) were not associated with any of the outcomes. On the contrary, increases in total bilirubin (TBIL) and alkaline phosphatase (ALP) were associated with increased risks of the primary outcome [TBIL: adjusted hazard ratio (HR), 1.17; 95% confidence interval (CI) 1.08–1.26; ALP: adjusted HR, 1.12; 95% CI 1.04–1.21], cardiovascular mortality (TBIL: adjusted HR, 1.16; 95% CI 1.02–1.31; ALP: adjusted HR, 1.16; 95% CI 1.05–1.28), and HF hospitalization (TBIL: adjusted HR, 1.22; 95% CI 1.12–1.33; ALP: adjusted HR, 1.12; 95% CI 1.03–1.23).

Conclusion: Elevated serum cholestasis markers TBIL and ALP were significantly associated with a poor outcome in HFpEF patients without chronic hepatic diseases, while elevated ALT and AST were not.

Keywords: heart failure with preserved ejection fraction, liver function tests, prognosis, cholestasis, congestive hepatopathy

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INTRODUCTION

Liver dysfunction is prevalent in patients with chronic heart failure (CHF) (1). Both hypoperfusion due to reduced cardiac output and congestion secondary to volume and pressure overload could lead to hepatic injury (2). Although, it is known that CHF patients with severe hepatic dysfunction had a poor outcome (3), the prognostic value of abnormal liver function tests (LFTs) has not been established. Several studies focusing on this issue reported conflicting results. Some studies demonstrated strong prognostic values of increased serum aminotransferase (aspartate transaminase and alanine transaminase) in CHF patients (4, 5), while others found an association of worse clinical outcomes with the increase in cholestatic measurements, such as total bilirubin, alkaline phosphatase, and γ-glutamyltransferase, instead of aminotransferase (1, 6, 7).

It has been reported that patterns of abnormal LFTs were associated with congestion and hypoperfusion of the liver in the setting of heart failure CHF (8), suggesting that changes in LFTs might be indicators of hemodynamic disturbance in CHF. Recently, HF with preserved ejection fraction (HFpEF) has been recognized to be a distinct disease entity from HF with reduced ejection fraction (HFrEF) (9); however, no previous study had focused on LFTs in HFpEF patients.

Therefore, this study aimed at evaluating the prognostic implication of LFTs, including aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP), in well-defined HFpEF patients. To avoid the influence of hepatic dysfunction, we further excluded patients with known hepatic diseases in the present study.

MATERIALS AND METHODS

Study Population

This was a *post-hoc* analysis of data from the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial (TOPCAT), which was a phase 3, multicenter, international, randomized, double-blinded, placebo-controlled trial. Totally, 3,445 HFpEF patients were included and randomized to receive spironolactone or placebo treatment. Specifically, patients with known chronic hepatic diseases with AST or ALT >3.0 times the upper limit of normal were excluded from the study. The design and results of TOPCAT were published elsewhere (10, 11). Patients or the public was not involved in the design, or conduct, or reporting, or dissemination of our research.

Data analyzed in this study were obtained from the National Institutes of Heart, Lung, and Blood Institute's Biologic Specimen and Data Repository Information Coordinating Center. Data from Russia and Georgia were excluded because of concerns about the representativeness of HFpEF patients in these two countries (12), leaving 1,767 patients from the Americas for analysis. Among these patients, those with missing data on LFTs or any of the potential confounders mentioned below were excluded. No exclusion criteria for drugs that might affect liver function was applied. Finally, there were 1,657 patients included in the analyses. The present study was approved by the Medical Ethics Commission of the First Affiliated Hospital of Sun Yat-sen University, China.

Liver Function Tests

Serum AST, ALT, TBIL, and ALP were measured at baseline. Based on routine laboratory standards, the upper limits of normal were 35 U/L for AST and ALT, 1.0 mg/dl for TBIL, and 120 U/L for ALP (13).

Outcome of Interest

The primary outcome was a composite of cardiovascular mortality, HF hospitalization, and aborted cardiac arrest. Secondary outcomes were cardiovascular mortality and HF hospitalization.

Statistical Analysis

As patients with known chronic hepatic diseases were excluded from TOPCAT, most of the elevated LFT results did not exceed two times the upper limit of normal. Continuous variables were presented as mean ± SD and compared by Student's *T*-test. Categorical variables were presented as percentages and compared by chi-squared test. Kaplan–Meier curves with log-rank tests were performed to observe differences in primary and secondary outcomes between elevated vs. normal LFTs groups. Multivariate Cox proportional hazards models were used to evaluate the association of LFTs and clinical outcomes. To adjust for potential confounders, age, gender, race, New York Heart Association (NYHA) classification (III and IV vs. I and II), previous HF hospitalization, history of myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus, smoking, alcohol use, heart rate, systolic blood pressure, body mass index, ejection fraction, hemoglobin, estimated glomerular filtration rate, and randomized treatment were also included in the models as covariates. Five proportional hazards models were established to comprehensively evaluate the prognostic significance of each liver function measurement. In model 1, liver function measurement was included as a categorical variable (elevated vs. normal). In model 2, the measurements were included as continuous variables. Variables in models 3 and 4 were the same as models 1 and 2, but to rule out the influence of extreme values, patients with liver function measurement >2 times the upper limit of normal were excluded. To explore potential non-linear relation, in model 5, liver function measurements were included as continuous variables with restricted cubic remodeling. Three knots were located to the 10th, 50th, and 90th percentiles following Harrell's suggestion (14). Liver function measurements were also limited to two times the upper limit of normal because restricted cubic remodeling could be affected by extreme values. Baseline brain natriuretic peptide (BNP) or N-terminal-pro-BNP (NT-proBNP) levels were available in only 992 patients; thus, we calculated standardized *z*-scores of BNP and NT-proBNP as previously reported (15) and included them in multivariate models as a sensitivity analysis. Statistical analyses were performed using STATA (version 13) and IBM SPSS (version 25). Hazard ratios (HRs), confidence intervals (CIs), and *P*-values were reported. $P < 0.05$ was regarded as statistical significance.

TABLE 1 | Baseline characteristics of patients with and without abnormal liver function tests.

	AST		ALT		TBIL		ALP	
	Normal *N* = 1,454	Elevated *N* = 203	Normal *N* = 1,378	Elevated *N* = 279	Normal *N* = 1,468	Elevated *N* = 189	Normal *N* = 1,340	Elevated *N* = 317
Age, y	71.9 ± 9.6	70.1 ± 10.2*	72.1 ± 9.7	69.4 ± 9.5*	71.5 ± 9.7	73.1 ± 9.9*	71.9 ± 9.7	70.6 ± 9.8*
Male, *n* (%)	721 (49.6)	106 (52.2)	674 (48.9)	153 (54.8)	702 (47.8)	125 (66.1)*	687 (51.3)	140 (44.2)*
Caucasian, *n* (%)	1,144 (78.7)	159 (78.3)	1,076 (78.1)	227 (81.4)	1,147 (78.1)	156 (82.5)	1,056 (78.8)	247 (77.9)
Previous HF hospitalization, *n* (%)	853 (58.7)	118 (58.1)	807 (58.6)	164 (58.8)	868 (59.1)	103 (54.5)	768 (57.3)	203 (64.0)*
NYHA III and IV, *n* (%)	501 (34.5)	73 (36.0)	493 (35.8)	81 (29.0)*	505 (34.4)	69 (36.5)	456 (34.0)	118 (37.2)
EF, %	58.3 ± 7.7	57.9 ± 7.8	58.2 ± 7.7	58.4 ± 7.8	58.3 ± 7.8	57.9 ± 7.4	58.1 ± 7.7	58.8 ± 8.1
Spironolactone arm, *n* (%)	734 (50.5)	95 (46.8)	696 (50.5)	133 (47.7)	741 (50.5)	88 (46.6)	660 (49.3)	169 (53.3)
Myocardial infraction, *n* (%)	301 (20.7)	37 (18.2)	292 (21.2)	46 (16.5)	302 (20.6)	36 (19.0)	278 (20.7)	60 (18.9)
Diabetes, *n* (%)	658 (45.3)	77 (37.9)	617 (44.8)	118 (42.3)	677 (46.1)	58 (30.7)*	595 (44.4)	140 (44.2)
COPD, *n* (%)	243 (16.7)	32 (15.8)	227 (16.5)	48 (17.2)	245 (16.7)	30 (15.9)	243 (18.1)	32 (10.1)*
Current smoking, *n* (%)	88 (6.1)	16 (7.9)	87 (6.3)	17 (6.1)	93 (6.3)	11 (5.8)	84 (6.3)	20 (6.3)
Alcohol use, *n* (%)	372 (25.6)	64 (31.5)	344 (25.0)	92 (33.0)*	380 (25.9)	56 (29.6)	368 (27.5)	68 (21.5)*
Heart rate, bpm	69.0 ± 11.0	69.2 ± 12.5	69.0 ± 11.1	69.0 ± 11.4	68.9 ± 11.1	70.2 ± 11.4	68.3 ± 11.0	71.9 ± 11.4*
SBP, mmHg	128.2 ± 15.8	122.7 ± 15.8*	127.9 ± 15.8	125.8 ± 16.2*	127.9 ± 15.9	124.9 ± 15.5*	127.3 ± 16.0	128.6 ± 15.3
BMI, kg/m^2	33.9 ± 8.0	32.8 ± 8.6	33.6 ± 8.0	34.2 ± 8.4	33.9 ± 8.1	32.7 ± 8.2	33.7 ± 8.1	33.7 ± 8.1
Hemoglobin, g/dL	12.8 ± 1.6	13.1 ± 1.7*	12.7 ± 1.6	13.5 ± 1.6*	12.8 ± 1.6	13.2 ± 2.0*	12.9 ± 1.6	12.9 ± 1.7
eGFR, ml/min	64.2 ± 21.4	66.9 ± 22.8	64.2 ± 21.8	66.3 ± 20.3	64.8 ± 21.9	62.3 ± 18.9	64.4 ± 20.7	64.9 ± 25.1
Use of statins	946 (65.1)	128 (63.1)	886 (64.3)	188 (67.4)	957 (65.2)	117 (61.9)	894 (66.7)	180 (56.8)*

HF, heart failure; NYHA, New York Heart Association Classification; EF, ejection fraction; COPD, chronic obstructive pulmonary disease; SBP, systolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.
**P < 0.05 when compared with the normal group.*

RESULTS

Baseline Characteristics

Among patients included in analyses, proportions of patients with elevated AST, ALT, TBIL, and ALP were 12.3, 16.8, 11.4, and 19.1%, respectively. Baseline characteristics are summarized in **Table 1**. Compared with the normal groups, patients with elevated AST and ALT were younger, had lower systolic blood pressure, and higher hemoglobin levels. Besides, patients with elevated ALT also had higher proportions of NYHA I or II and alcohol use. Patients with elevated TBIL were older, more likely to be male and non-diabetic, with a lower systolic blood pressure but a higher hemoglobin level. Those with elevated ALP were younger, more likely to be female with previous HF hospitalization, and had a faster heart rate. However, alcohol use, a history of chronic obstructive pulmonary disease, and use of statins were less common than the normal group.

Liver Function Tests and Clinical Outcomes

Crude rates of outcome events are shown in **Table 2**. Kaplan–Meier curves (**Figure 1**) illustrated that elevated AST or ALT had a comparable risk of the primary outcome, cardiovascular mortality, and HF hospitalization compared with the normal groups. Elevated ALP had a higher risk of the primary outcome but comparable risks of cardiovascular mortality and HF hospitalization compared with the normal group, while patients with elevated TBIL had higher risks of the primary outcome, cardiovascular mortality, as well as HF hospitalization.

TABLE 2 | Numbers and percentages of outcome events.

	Normal	Elevated	*P*
The primary outcome			
AST, *n/N* (%)	429/1,454 (29.5)	60/203 (29.6)	0.988
ALT, *n/N* (%)	417/1,378 (30.3)	72/279 (25.8)	0.137
TBIL, *n/N* (%)	415/1,468 (28.3)	74/189 (39.2)	0.002
ALP, *n/N* (%)	382/1,340 (28.5)	107/317 (33.8)	0.066
Cardiovascular mortality			
AST, *n/N* (%)	184/1,454 (12.7)	27/203 (13.3)	0.796
ALT, *n/N* (%)	180/1,378 (13.1)	31/279 (11.1)	0.373
TBIL, *n/N* (%)	175/1,468 (11.9)	36/189 (19.0)	0.006
ALP, *n/N* (%)	165/1,340 (12.3)	46/317 (14.5)	0.291
HF hospitalization			
AST, *n/N* (%)	331/1,454 (22.8)	44/203 (21.7)	0.728
ALT, *n/N* (%)	322/1,378 (23.4)	53/279 (19.0)	0.112
TBIL, *n/N* (%)	318/1,468 (21.7)	57/189 (30.2)	0.009
ALP, *n/N* (%)	294/1,340 (21.9)	81/317 (25.6)	0.167

AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.

The results of model 1 are shown in **Table 3**, and those of model 2 are summarized in **Table 4**. In model 1, ALT and AST were not associated with any of the outcomes as categorical variables, while elevated TBIL was associated with

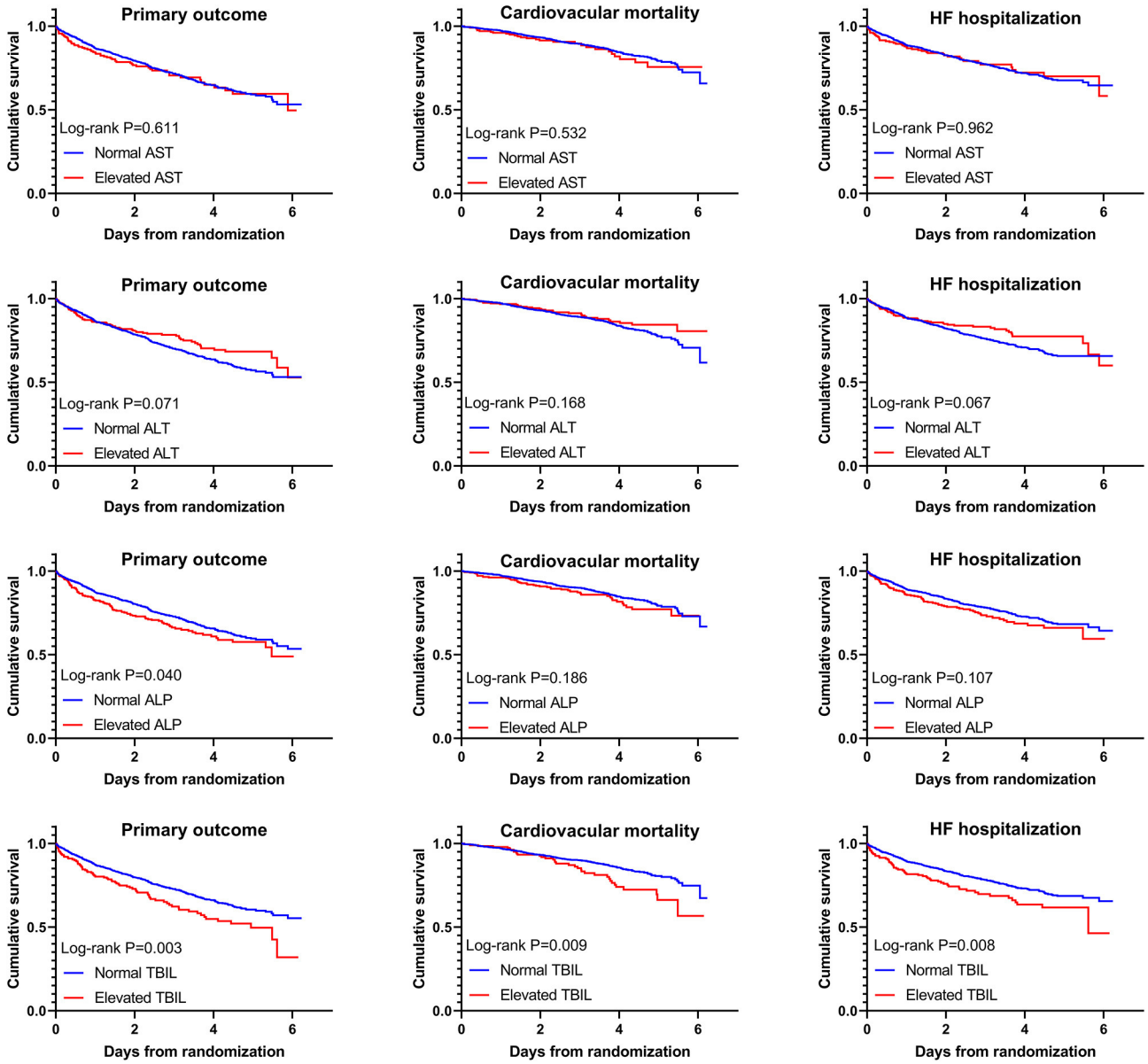

FIGURE 1 | Kaplan–Meier curves with log-rank tests for comparison of elevated vs. normal aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP) to primary and secondary outcomes.

increased risks of the primary outcome (HR, 1.51; 95% CI 1.17–1.94; $P = 0.002$), cardiovascular mortality (HR, 1.45; 95% CI 1.01–2.10; $P = 0.047$), and HF hospitalization (HR, 1.58; 95% CI 1.18–2.10; $P = 0.002$). Elevated ALP was associated with increased risk of the primary outcome (HR, 1.25; 95% CI 1.00–1.56; $P = 0.046$) but not cardiovascular mortality or HF hospitalization. When these markers were included as continuous variables in model 2, ALT and AST were still not associated with any of the outcomes, but increase in ALP and TBIL were associated with increased risks of the primary outcome (TBIL: HR, 1.17; 95% CI 1.08–1.26; $P < 0.001$; ALP: HR, 1.12; 95% CI, 1.04–1.21; $P = 0.003$), cardiovascular mortality (TBIL: HR, 1.16; 95% CI 1.02–1.31; $P = 0.022$; ALP: HR, 1.16; 95% CI, 1.05–1.28; $P = 0.004$), and HF hospitalization (TBIL: HR, 1.22; 95% CI, 1.12–1.33; $P < 0.001$; ALP: HR, 1.12; 95% CI, 1.03–1.23; $P = 0.012$). Models 3 and 4 excluded patients with LFTs >2 times the upper limit of normal, which yielded similar results except that associations of cardiovascular mortality and TBIL and ALP were no longer significant (**Tables 3, 5**). Although ALT and AST did not have significant results in the above Cox proportional hazards models, a non-linear association could not be excluded. Thus, we conducted the restricted cubic remodeling analysis; however, the result did not indicate non-linear relations of all four LFTs and outcomes

TABLE 3 | Associations of liver function tests as binary variable (Normal vs. Elevated) and clinical outcomes.

	The primary outcome		Cardiovascular mortality		HF hospitalization	
	Adjusted HR* (95% CI)	***P***	**Adjusted HR* (95% CI)**	***P***	**Adjusted HR* (95% CI)**	***P***
Model 1						
Elevated AST	1.17 (0.89–1.54)	0.254	1.19 (0.79–1.80)	0.399	1.13 (0.82–1.56)	0.448
Elevated ALT	0.92 (0.71–1.18)	0.501	0.85 (0.58–1.26)	0.424	0.91 (0.68–1.23)	0.543
Elevated TBIL	1.51 (1.17–1.94)	0.002	1.45 (1.01–2.10)	0.047	1.58 (1.18–2.10)	0.002
Elevated ALP	1.25 (1.00–1.56)	0.046	1.28 (0.92–1.80)	0.147	1.24 (0.96–1.60)	0.096
Model 3						
Elevated AST	1.13 (0.83–1.53)	0.440	1.22 (0.77–1.92)	0.404	1.09 (0.77–1.55)	0.619
Elevated ALT	0.94 (0.72–1.23)	0.656	0.89 (0.59–1.33)	0.567	0.96 (0.70–1.30)	0.786
Elevated TBIL	1.41 (1.07–1.85)	0.015	1.40 (0.94–2.08)	0.100	1.40 (1.02–1.93)	0.038
Elevated ALP	1.15 (0.90–1.46)	0.259	1.14 (0.79–1.66)	0.483	1.14 (0.86–1.49)	0.360
Sensitivity analysis (n = 992)						
Elevated AST	1.22 (0.84–1.77)	0.291	1.51 (0.86–2.67)	0.152	1.01 (0.64–1.58)	0.976
Elevated ALT	0.98 (0.70–1.37)	0.892	1.05 (0.63–1.75)	0.846	0.91 (0.61–1.35)	0.639
Elevated TBIL	1.33 (0.96–1.84)	0.085	1.34 (0.82–2.18)	0.241	1.37 (0.95–1.98)	0.094
Elevated ALP	1.35 (1.00–1.82)	0.054	1.47 (0.92–2.35)	0.108	1.28 (0.91–1.80)	0.160

**Covariates for adjustment included age, gender, race, NYHA classification (III and IV vs. I and II), previous HF hospitalization, history of myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus, smoking, alcohol use, heart rate, systolic blood pressure, body mass index, ejection fraction, hemoglobin, estimated glomerular filtration rate, and randomized treatment.*

TABLE 4 | Associations of liver function tests and clinical outcomes.

	The primary outcome		Cardiovascular mortality		HF hospitalization	
	Adjusted HR* (95% CI)	***P***	**Adjusted HR* (95% CI)**	***P***	**Adjusted HR* (95% CI)**	***P***
AST	1.05 (0.96–1.15)	0.322	0.97 (0.84–1.12)	0.662	1.06 (0.96–1.18)	0.246
ALT	0.95 (0.86–1.04)	0.248	0.88 (0.75–1.02)	0.095	0.94 (0.84–1.05)	0.288
TBIL	1.17 (1.08–1.26)	<0.001	1.16 (1.02–1.31)	0.022	1.22 (1.12–1.33)	<0.001
ALP	1.12 (1.04–1.21)	0.003	1.16 (1.05–1.28)	0.004	1.12 (1.03–1.23)	0.012

HR, hazard ratio; CI, confidence interval; HF, heart failure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.
**Covariates for adjustment included age, gender, race, NYHA classification (III and IV vs. I and II), previous HF hospitalization, history of myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus, smoking, alcohol use, heart rate, systolic blood pressure, body mass index, ejection fraction, hemoglobin, estimated glomerular filtration rate, and randomized treatment. HRs were calculated as per standard deviation increase.*

TABLE 5 | Associations of liver function tests and clinical outcomes after excluding patients with liver function test > 2 times upper limit of normal.

	The primary outcome		Cardiovascular mortality		HF hospitalization	
	Adjusted HR* (95% CI)	***P***	**Adjusted HR* (95% CI)**	***P***	**Adjusted HR* (95% CI)**	***P***
AST	1.04 (0.93–1.17)	0.465	0.98 (0.82–1.17)	0.803	1.07 (0.94–1.22)	0.300
ALT	0.96 (0.85–1.07)	0.421	0.89 (0.75–1.06)	0.193	0.98 (0.86–1.11)	0.721
TBIL	1.24 (1.09–1.41)	0.002	1.19 (0.97–1.45)	0.088	1.31 (1.13–1.52)	<0.001
ALP	1.15 (1.03–1.30)	0.018	1.19 (1.00–1.42)	0.051	1.16 (1.02–1.33)	0.029

HR, hazard ratio; CI, confidence interval; HF, heart failure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.
**Covariates for adjustment included age, gender, race, NYHA classification (III and IV vs. I and II), previous HF hospitalization, history of myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus, smoking, alcohol use, heart rate, systolic blood pressure, body mass index, ejection fraction, hemoglobin, estimated glomerular filtration rate, and randomized treatment. HRs were calculated as per standard deviation increase.*

as well (**Figure 2**), further, confirming that ALT and AST were not associated with the risks of outcomes. Interestingly, according to **Figure 2**, the positive association of ALP, TBIL, and outcome risk was not limited to abnormal results. Instead, this positive association began below the upper limit of the normal range.

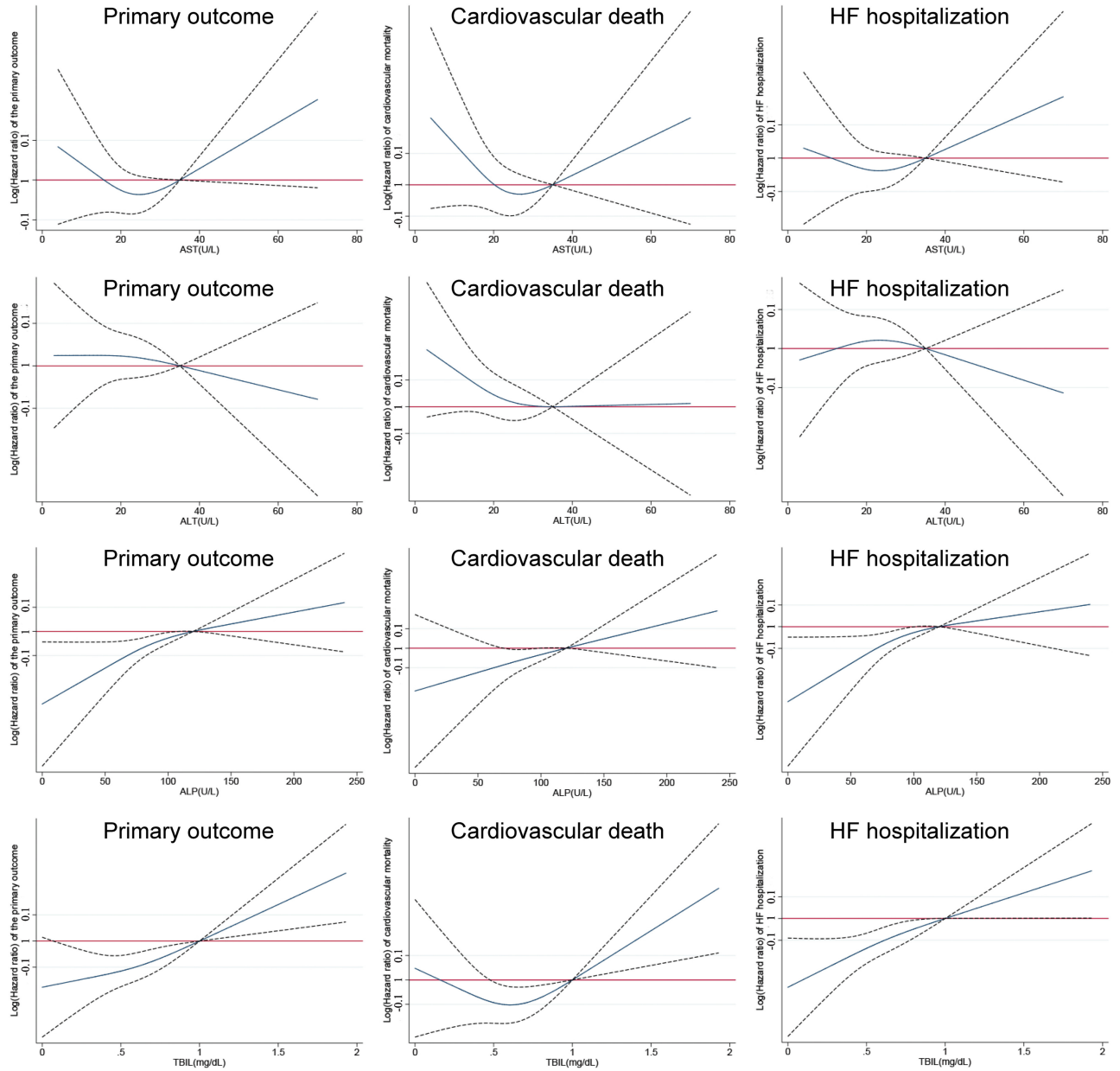

FIGURE 2 | Restricted cubic remodeling of Cox proportional hazards models of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP) to primary and secondary outcomes (solid lines represent relative hazard ratios; dot lines represent upper and lower limits of 95% confidence intervals).

Sensitivity Analysis

In the sensitivity analysis, we further adjusted BNP/NT-proBNP *z*-scores in multivariate analysis in 992 patients with available baseline BNP or NT-proBNP levels. When included as a categorical variable, none of the four LFTs was associated with the risk of the primary outcome, cardiovascular mortality, or HF hospitalization (**Table 3**). However, when included as a continuous variable, the results showed that the increase in TBIL was significantly associated with higher risks of the primary outcome (HR, 1.13; 95% CI 1.01–1.26; $P = 0.031$), cardiovascular mortality (HR, 1.20; 95% CI 1.02–1.42; $P = 0.029$), and HF hospitalization (HR, 1.17; 95% CI 1.05–1.32; $P = 0.007$). Similarly, the increase in ALP was significantly associated with higher risks of the primary outcome (HR, 1.19; 95% CI 1.05–1.35; $P = 0.006$), cardiovascular mortality (HR, 1.32; 95% CI 1.10–1.58; $P = 0.002$), and HF hospitalization (HR, 1.16; 95% CI 1.00–1.34; $P = 0.044$). AST and ALT were still not associated with any of the outcomes (**Table 6**).

TABLE 6 | Associations of liver function tests and clinical outcomes in enrolled patients with BNP/NT-proBNP available ($n = 992$).

	The primary outcome		Cardiovascular mortality		HF hospitalization	
	Adjusted HR* (95% CI)	*P*	Adjusted HR* (95% CI)	*P*	Adjusted HR* (95% CI)	*P*
AST	1.07 (0.95–1.21)	0.276	1.15 (0.96–1.38)	0.143	1.04 (0.90–1.20)	0.603
ALT	0.98 (0.86–1.11)	0.713	0.90 (0.72–1.12)	0.339	0.99 (0.86–1.15)	0.910
TBIL	1.13 (1.01–1.26)	0.031	1.20 (1.02–1.42)	0.029	1.17 (1.05–1.32)	0.007
ALP	1.19 (1.05–1.35)	0.006	1.32 (1.10–1.58)	0.002	1.16 (1.00–1.34)	0.044

HR, hazard ratio; CI, confidence interval; HF, heart failure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.
**Covariates for adjustment included age, gender, race, NYHA classification (III and IV vs. I and II), previous HF hospitalization, history of myocardial infarction, chronic obstructive pulmonary disease, diabetes mellitus, smoking, alcohol use, heart rate, systolic blood pressure, body mass index, ejection fraction, hemoglobin, estimated glomerular filtration rate, randomized treatment, and BNP/NT–proBNP z-scores. HRs were calculated as per standard deviation increase.*

DISCUSSION

In the present study, we demonstrated the independent prognostic significance of cholestatic LFTs—TBIL and ALP—instead of AST and ALT within a cohort of well-defined HFpEF patients without known hepatic diseases.

Abnormalities of LFTs were frequently seen in both chronic and acute HF (AHF) patients and closely related to hepatic perfusion and congestion (8). A recent review divided the abnormality of LFTs in HF into two subtypes according to different primary pathophysiology (16). Passive venous congestion that resulted in "congestive hepatopathy (CH)," which was supposed to be associated with increased bilirubin levels and high ALP levels from an increased central venous pressure (CVP) (17), was a common sign of congestive heart failure (18). Low cardiac output and arterial hypoperfusion resulted in "acute cardiogenic liver injury (ACLI)," which was associated with increased levels of AST and ALT in heart failure that was attributed to hepatocellular damage from decreased perfusion (17). As the liver's complex dual blood supply makes it relatively resistant to hepatocellular damage from hemodynamic perturbations, ACLI was expected only in cases of marked hypotension or hypoperfusion (16). Low cardiac output and arterial hypoperfusion were more common in HFrEF patients and/or AHF patients, which could lead to the elevation of ALT and/or AST (19–21). But in the TOPCAT trial, participants were chronic HFpEF patients, suggesting that they were unlikely to suffer from low cardiac output or arterial hypoperfusion. In terms of CH, elevated CVP could be transmitted directly to the hepatic veins, leading to hepatic congestion and impairment of the biliary system (22). Recently, Cogger et al. showed that hepatic congestion increased pressure within the hepatic sinusoid, leading to disruption of the liver sinusoidal endothelial cells and subsequent pressure increase in zonula occludens, which were the tight junctions between hepatocytes that separate the extravascular space from the bile canaliculus. Thus, disruption of the zonula occludens would expose the bile canaliculus directly to the sinusoidal blood causing the elevation of cholestasis markers (23). Additionally, Allen et al. (6) found that total bilirubin was significantly higher in patients who had evidence of volume overload on physical examination. CHF patients, unlike patients with AHF, did not frequently suffer from hypotension (18); therefore, changes in AST and ALT might be caused by other conditions or severe congestion, which leads to hepatocellular damage in CHF. By contrast, moderate congestion and elevated CVP were common in CHF (18), which could lead to CH and be reflected by the increases in TBIL and ALP. Some previous studies about LFTs in HF patients presented the same hypothesis, which found that TBIL and ALP were more likely to be associated with outcomes in CHF patients (1). Thus, the prognostic value of TBIL and ALP might represent the association of increased CVP and poor outcome (24). However, further study is needed to validate the hypothesis.

As discussed above, the changes in LFTs were associated with the alteration of hemodynamics in HF. Several studies have pointed out that the hemodynamic changes in HFpEF were different from HFrEF (25–27). Previous studies about LFTs in CHF patients showed inconsistent results. The average LVEF of these studies ranged from 28 to 51% (1, 5–7), implying that there was a large difference in the proportions of HFpEF and HFrEF in these studies. Additionally, Vyskocilova et al. (28) found that ALT and AST pattern predominated in the left-sided forward AHF (more likely presented by reduced EF), while cholestatic profile occurred mainly in the bilateral and right-sided AHF. The heterogeneity of CHF patients resulting from pooling HFpEF and HFrEF could be a reason for these inconsistent results. A recent *post-hoc* analysis of the PARADIGM-HF trial found that ALT was associated with worse prognosis in chronic HFrEF patients, as well as TBIL, but not AST (29). Of note, as they included chronic HFrEF patients, some of them could be with bilateral HF. Our study only focused on the HFpEF patients who were less likely to have left-sided HF to eliminate the heterogeneity caused by HF categories, and thus, the results were more convincing. Another reason for the conflicting results of previous studies could be the influence of coexisted hepatic diseases. None of the studies mentioned above (1, 4–7) set any exclusion criterion about the hepatic diseases. Indeed, proportions of abnormal LFTs at baseline differ significantly among studies mentioned above (1, 4–7). As discussed above, the elevated TBIL and ALP might reflect hemodynamic changes in our study. However, it would be a different story if abnormal LFTs were caused by hepatic diseases. As hepatic diseases could cause much larger changes in LFTs than hemodynamics of heart failure, the prognostic value of LFTs would be very hard to interpret. The present study had excluded patients with known chronic hepatic diseases, and further, in models 3 and 4, patients with potential unknown

hepatic diseases at admission had also been excluded (those whose liver function measurement >2 times the upper limit of normal). Thus, the results were not confounded by coexisted hepatic diseases and revealed that TBIL and ALP, instead of ALT and AST, had significant prognostic value. As far as we know, this is the first study to evaluate the prognostic value of LFTs in sole HFpEF patients without chronic hepatic diseases. Sensitivity analysis further confirmed the independent prognostic value of TBIL and ALP from BNP and NT-proBNP.

However, there are some limitations to our study. We had no data on hemodynamic parameters (e.g., CVP) of enrolled patients and could not further investigate the relationship between LFTs and hemodynamic parameters. In addition, it was reported that TBIL was strongly correlated with GGT and its prognostic value lost in a multivariable model including GGT (1), but we had no data on GGT, and thus, this potential confounder could not be adjusted. Besides, all patients enrolled in the TOPCAT trial are with chronic HFpEF, so we could not compare the prognostic value of LFTs with patients with AHF or HFrEF.

CONCLUSIONS

Among HFpEF patients without chronic hepatic diseases, elevated TBIL and ALP, two serum cholestasis markers, were significantly associated with poor outcome. On the contrary, AST and ALT had no prognostic significance. The results suggested a potential role of TBIL and ALP measurement in HFpEF. More studies are needed to validate the correlation of TBIL, ALP, and hemodynamic parameters in HFpEF.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://biolincc.nhlbi.nih.gov/studies/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Commission of First Affiliated Hospital of Sun Yat-sen University. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

WL, XH, YD, and CL: conceptualization. WL, XH, and DW: methodology. WL: software. RX, BD, and MO-A: validation. WL and XH: formal analysis. DW: investigation and original draft preparation. LC: resources. ZY: data curation. MO-A, YD, and CL: manuscript review and editing. JZ, YD, and CL: supervision. DW, RX, BD, JZ, YD, and CL: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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MINI REVIEW
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Are HFpEF and HFmrEF So Different? The Need to Understand Distinct Phenotypes

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Traditionally, patients with heart failure (HF) are divided according to ejection fraction (EF) threshold more or <50%. In 2016, the ESC guidelines introduced a new subgroup of HF patients including those subjects with EF ranging between 40 and 49% called heart failure with midrange EF (HFmrEF). This group is poorly represented in clinical trials, and it includes both patients with previous HFrEF having a good response to therapy and subjects with initial preserved EF appearance in which systolic function has been impaired. The categorization according to EF has recently been questioned because this variable is not really a representative of the myocardial contractile function and it could vary in relation to different hemodynamic conditions. Therefore, EF could significantly change over a short-term period and its measurement depends on the scan time course. Finally, although EF is widely recognized and measured worldwide, it has significant interobserver variability even in the most accredited echo laboratories. These assumptions imply that the same patient evaluated in different periods or by different physicians could be classified as HFmrEF or HFpEF. Thus, the two HF subtypes probably subtend different responses to the underlying pathophysiological mechanisms. Similarly, the adaptation to hemodynamic stimuli and to metabolic alterations could be different for different HF stages and periods. In this review, we analyze similarities and dissimilarities and we hypothesize that clinical and morphological characteristics of the two syndromes are not so discordant.

Keywords: ejection fraction, heart failure with mid-range ejection fraction, phenotype, biomarkers, systolic function

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INTRODUCTION

Despite the last ESC guidelines introducing a new category for heart failure (HF) classification including those patients with mild ejection fraction (EF) reduction ranging from 40 to 49%, this subtype is still underdetermined and poorly represented in most clinical trials (1). Current gaps arise from the recent introduction of this HF class and the indeterminate profile between heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) that probably account for different phenotypes. Indeed, the ESC classification is an attempt to identify specific biological and pathophysiological mechanisms in subjects with clinical manifestations typical for HF, increased natriuretic peptides, and moderate structural cardiac dysfunction (2). Perhaps, HFmrEF is a mixed model related to the intermediate clinical profile between HFpEF and HFrEF, encompassing patients with phenotypic and clinical characteristics

typical for both reduced and preserved EF (3). Indeed, few studies analyzing HFmrEF subtypes demonstrated some discrepancies in terms of comorbidity and etiology (4). However, the simple categorization based only on EF keeps some weaknesses related to the intrinsic limitation of EF, its change over a time period, and the natural history of HF. Therefore, EF measurement depends on several intrinsic variables such as preload and afterload, heart rate, stable or unstable condition, myocardial contractile forces, and presence of valve disease (5). Of note, both American and recent Australian HF guidelines preferred to maintain the traditional classification of HFpEF and HFrEF based on EF cutoff of 50%, so as not to create a misunderstanding and overlap in HF nomenclature (6, 7). Thus, the attempt to classify HF population based on simple EF categorization is probably inappropriate, and the identification of a unique profile for HFmrEF is a pending issue (8). Classification of HF patients into the whole spectrum of different phenotypes within EF assessment remains a challenge for future research.

CLINICAL CHARACTERISTICS OF HFmrEF

HFmrEF is an heterogeneous group poorly characterized in terms of baseline characteristics, clinical presentation, and outcome (4). Most of the data came from subanalysis investigating the features of patients with HFpEF and borderline EF or from HFrEF trials analyzing patients with recovered systolic function (9, 10). In order to bypass this gap, recent studies have focused on the HFmrEF distribution and risk profile investigation; unfortunately, most of them are single center with unrepresentative sample size and with incomplete standardized diagnostic criteria based only on EF cutoff. Clinical characteristics, cardiovascular (CV) risk profile, extracardiac comorbidities, and echocardiographic features are often neglected, leading to a further confusion in HFmrEF recognition and some discrepancies between studies. Of note, most of the data can be extrapolated by larger clinical trials with a relevant follow-up period including this category. The CHARM preserved study that included patients with EF >40% showed that most patients with mildly reduced EF were females with intermediate mean age values and hypertension prevalence between HFrEF and HFpEF (11). Therefore, HFmrEF has a similar prevalence to coronary artery disease (CAD) and atrial fibrillation (AF) compared with HFrEF, whereas creatinine values and NYHA class distribution were intermediate between HFrEF and HFpEF. Despite different clinical characteristics, the study revealed a reduced trend of HF-related hospitalization and death for CV causes with respect to HFrEF.

The restrospective analysis of the DIG trial demonstrated that HFmrEF resembled patients with HFrEF in terms of similar mean age, sex, and ischemic etiology (12). In the TOPCAT trial involving patients with mean EF above 45%, mean age and female prevalence were higher in those with mildly reduced EF, hypertension was higher in HFmrEF, whereas other comorbidities such as chronic kidney disease (CKD), CAD, AF, and diabetes were similar between groups (13). Interestingly, a Korean registry revealed different prevalence rates of AF that tend to increase according to EF values with different occurrences in reduced (29%), midrange (40%), and preserved (45%). Additionally, AF has a negative prognostic impact only in HFpEF (14).

The ESC observational registry confirmed that patients affected by HFmrEF resembled the HFpEF group in some features including age, female prevalence, and hypertension. However, CAD prevalence was more similar to the HFrEF group. Mortality rate at 1 year significantly differed between HFpEF and HFmrEF (6.3 vs. 7.6%, respectively) (15). A validated analysis using MAGGIC score including a wide range of cardiac and extracardiac and demographic characteristics demonstrated that an increased burden of extracardiac diseases in those with higher EF with a significant prevalence of lung diseases increased body mass index and diabetes (16). Accordingly, a Japanese registry confirmed an intermediate profile of HFmrEF patients supposing that the current condition may be a transitional status between HFpEF and HFrEF (17). In a recent Swedish registry analysis comparing three common comorbidities such as AF diabetes and CKD, HFmrEF revealed an intermediate prevalence of CKD and AF, whereas diabetes was similarly expressed in all HF groups (18). Finally, the combined analysis of PARADIGM and PARAGON confirmed an intermediate range regarding age, female sex, body mass, natriuretic peptides, and hypertension, whereas history of myocardial infarction resembled HFrEF (19).

Current findings are related to chronic HF conditions, but acute patients presenting with HFmrEF are less extensively investigated: in the ALARM HF trial that stratified patients for EF tertiles, majority of the patients were male with consistent prevalence of older age more than 75 years, obesity, hypertension, and dyslipidemia; with intermediate prevalence of CAD; and lower prevalence of CKD with respect to HFrEF. No differences were observed in terms of anemia, lung diseases, vascular diseases, and liver disease (20). The main causes of hospitalization were acute coronary syndrome (ACS) in 38.6%, arrhythmias in 25.8%, and valvular disease in 15.4%. Clinical presentation differed between HFmrEF and HFrEF in terms of less peripheral edema, jugular vein distention, and prevalence of cold extremities. Current findings considerably differ from those observed in the DIG in which HFmrEF had less prevalence of orthopnea and additional cardiac sound compared with HFrEF (12). Conversely, exertional dyspnea, dyspnea at rest, and peripheral edema were similar in both HFrEF and HFmrEF (**Table 1**).

Aside from clinical characteristics and presentation, a few discrepancies are related to the outcome and mode of death of this group: although some studies reported a similar mortality rate independently of EF, some authors revealed an intermediate clinical profile and risk between HFpEF and HFrEF, and there is a general agreement in considering the outcome of HFmrEF much more similar to HFpEF (21, 22). Despite that CV events are considerably more in HFrEF, prognosis in those with HFmrEF is more strictly related to non-CV events and this tends to balance the overall mortality rate (23).

TABLE 1 | Clinical trials describing prevalent risk factors, comorbidities, and causes of HFmrEF.

Clinical Trial	Type of study	Population enrolled	NYHA Class	Outcome
CHARM preserved 2018	*Post-hoc* analysis including 1,322 pt	Mean age 65 year, mean EF 44%, 30% females, BMI 27.8, 67% CAD, 56% hypertension, 25% AF	57% II 41% III	HF hospitalization reduction (HR 0.48) Mortality reduction per year (HR 0.76)
DIG trial 2018	Retrospective analysis including 1,195 pt	Mean age 64.5 year, mean EF 43%, females 29%, BMI 27.7, previous MI 63%, hypertension 53%, AF not reported	3 % I 62% II 20 % III	Composite endpoint HF-hospitalization /mortality HR 0.83
TOPCAT trial 2016	Retrospective analysis including 520 pt	Mean age 66 years, mean EF<50%, females 36.5%,BMI 31.5, previous MI 44%, hypertension 86%, AF not reported, diabetes 29%	3% I 61% II 35%III	CV death per 100 patient-years HR 4.1 HF hospitalization per 100 patient-years HR 7.2
Korean HF registry 2020	Prospective observational study including 875 acute pt	Mean age 69 years, Mean EF 49%, females 45%, BMI not reported, CAD 29%, hypertension 59%, AF 27% AF, diabetes 36%	18% II 41%III 41%IV	Composite end point for all cause mortality and readmission HR 1.14
ESC -HF registry 2017	Observational research program of 2,212 pt	Mean age 64 years; females 31%, BMI 28.6, previous CAD 42%, hypertension 10%, AF 22%, Diabetes 30.5%, CKD 16,5%	82% I/II 18% III/ IV	Mortality at one year 7.6% in HFmEF vs. 6.3% in HFpEF and 8.8% in HFrEF
chart-2 investigators 2017	Japanese registry including 596 pt	Mean age 69 years, mean EF 45%, females 28%, BMI 23, previous MI 53%, hypertension 90%, AF 43.5%, diabetes 36%, CKD not reported	18.5% I 70% II 11% III	HFmrEF patients had intermediate incidences of all-cause death, and CV admission between HFpEF and HFrEF; 44% transitioned from HFmEF to HFpEF
Swedish HF registry 2019	Categorial analysis including 8,942 pt	Mean age 74 years, mean EF 44%, Females 38%, BMI 28, previous CAD 62%, hypertension 71%, AF 27%, diabetes 24%, CKD 46%	16% I 48% II 37%III 4% IV	HFmrEF had lowest crude risk of all CV and HF events, but it was intermediate between HFpEF and HFrEF for the crude risk of non-CV events
PARAGON and PARADIGM combined data matched for EF categories	*Post-hoc* analysis including 1,427 pt	Mean age 71 years, mean EF 48%, females 40%, previous MI 32%, hypertension 94%, AF 34%, diabetes 44%	3%I 76% II 21% III	Total heart failure hospitalization and CV death 0.81 in HFmEF vs. 1.06 in HFpEF
ALARM-HF prospective trial 2017	Multicenter survey including 811 acute pt	Mean age not reported, Mean EF 44%, females 35%, history of CAD 29%, hypertension 76%,AF 42 %, diabetes 46%	9.8% I 8.3 %II 47% III 35 %IV	Mortality in HFmEF was similar in HFmEF and HFpEF (HR 1.02 vs. 0.97)

AF, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; EF, ejection fraction; MI, myocardial infarction.

LABORATORY PROFILE OF HFmrEF

The division of HF across the EF spectrum comprises different biochemical and neurohormonal profiles that help to explain the neutral effects of interventional trials testing neurohormonal antagonism in HFpEF. N-terminal pro-B-type natriuretic peptide (NT-proBNP), plasma renin activity (PRA), aldosterone, and norepinephrine are increased in a substantial proportion of patients with HFpEF and HFmrEF with the same levels between the above groups and with lower levels when compared with HFrEF. Vergaro et al. demonstrate that 10% of HFpEF patients had elevated PRA, aldosterone, and norepinephrine vs. 8% in HFmrEF and 21% in HFrEF. The prognosis of HF patients seems to correlate with the number of neurohormones elevated, and different degrees of neurohormonal activation are evident across the whole EF spectrum, suggesting a positive effect of renin–angiotensin–aldosterone system inhibitors (RAASi) and adrenergic antagonists in patients with a significant increase

of the aforementioned biomarkers (24). A specific biomarker analysis from the Swedish Heart Failure Registry revealed similar NT-proBNP levels in HFmrEF and HFpEF, but significantly lower to HFrEF. However, body mass index (BMI), CKD, diabetes, hypertension, and heart rate significantly influence NT-proBNP levels. Nevertheless, NT-proBNP shows a greater prognostic in HFmrEF and may be a useful tool for diagnosis and stratification of CV risk (25).

The PROTECT trial analyzes several biomarkers of cardiac stretch and inflammation in acute HF setting. The network analysis demonstrates that inflammation is the main reason of interactions between biomarkers in HFpEF [e.g., galectin-3 (Gal-3) or C-reactive protein (CRP)], whereas in HFrEF, biomarker interactions are mostly related to cardiac stretch [e.g., NT-proBNP or high-sensitivity troponin (hs-TnT)]. Patients with acute HFmrEF show an intermediate profile between those of HFrEF and HFpEF. A small proportion of patients enrolled in the HFmrEF group are considered with "recovered LVEF," and interestingly, NT-proBNP, Gal-3, and hs-TnT are lower than in patients with persistent EF reduction, suggesting a different biomarker profile in this phenotype. However, in both HFpEF and HFmrEF, inflammatory markers at admission are both predictive for all-cause mortality and rehospitalization (26). Similarly, the Singapore Heart Failure Outcomes and Phenotypes (SHOP) study show intermediate values of hs-TnT with significant increased values compared with HFpEF (27).

The study with better laboratory and biological profile investigation is currently the HOMAGE trial; unfortunately, the laboratory analysis is limited to patients with a high risk of HF occurrence, history of CAD, and evidence of borderline EF dysfunction above 45%, but without specific signs and symptoms suggestive of HF (28). Patients with EF below the normal range experienced raised plasma B-type natriuretic peptides (BNP) and fibrosis biomarkers, whereas an increased level of inflammatory and collagen markers has been recruited in those with significant cardiac hypertrophy. Spironolactone significantly reduced natriuretic peptides, biomarkers of collagen, and inflammation (29).

Another study reported the bioprofile and the bioprognostication of several biomarkers of neurohormonal activation, extracellular matrix, inflammation, oxidative stress, and myocardial injury in patients with HFmrEF. Cystatin-C levels were significantly lower in patients with HFmrEF when compared with patients with HFpEF. The results of soluble suppression of tumorigenicity (sST2) levels, a member of the interleukin family, in HFmrEF patients are controversial which may be due to confounding factors such as race, HF congestion status, population enrolled, and disease time course. However, sST2 levels correlate with advanced NYHA class, pulmonary arterial systolic pressure, hs-CRP, cTnT, NT-proBNP, and the high frequency of diuretics use. Conversely, Gal-3 seems to be lower in HFmrEF than in HFpEF, showing the highest prognostic capability in the latter group (30).

In a selective group of patients with type 2 diabetes mellitus and HFpEF or HFmrEF, C-terminal propeptide of procollagen type I (PICP) and N-terminal propeptide of procollagen type III (PIIINP) are significantly increased in patients with HFmrEF compared with those with HFpEF. Glucometabolic impairment stimulated fibroblast proliferation and activated transcription and secretion of extracellular matrix proteins. The changes found in both markers of fibrosis may suggest a shift in balance toward type I collagen synthesis in HFmrEF compared with HFpEF in diabetic patients (31). Finally, we could assume that analyzing the various biomarker profiles in all HF population does not take into account the several mechanisms that are shared across the entire EF range. Some processes are more relevant at the extremities (HFrEF myocyte death vs. HFpEF inflammation or fibrosis), and in this spectrum, HFmrEF represents a continuum without a predominant underlying pathophysiology (32, 33). In this era in which a new precision phenotype is emerging in patients with HF, knowledge of different pathophysiologic pathways and of the laboratory profile of each patient may contribute to therapeutic decision and prognostic stratification (**Table 2**).

TABLE 2 | Biomarker characteristics and differences existing between HFmrEF and HFpEF.

Biomarkers profile in HF patients according to EF spectrum		
Diagnosis	**HFpEF**	**HFmEF**
	NT-proBNP↑	NT-proBNP↑↑
	hs-TnT ↑	hs-TnT ↑↑↑
	Plasma renin activity ↑	Plasma renin activity ↑
	Aldosterone ↑↑	Aldosterone ↑
	Norepinephrine ↑	Norepinephrine ↑
	hs-CRP ↑↑	hs-CRP ↑
	Cystatin-C ↑↑	Cystatin-C ↑
	Galectin-3 ↑↑	Galectin-3 ↑
	Neprilysin ↑	Neprilysin ↑↑
	ST2 ↑ ↑	ST2 ↑
	PICP ↑	PICP ↑↑
	PIIINP ↑	PIIINP ↑↑
Prognosis	NT-proBNP+	NT-proBNP+ + +
	hs-TnT +	hs-TnT ++
	hs-CRP ++	hs-CRP +
	Cystatin-C +	Cystatin-C +
	Galectin-3 +	Galectin-3 +
	Neprilysin +	Neprilysin +
	ST2 ++	ST2 +

EF, Ejection Fraction; NTproBNP, N-terminal pro-brain natriuretic peptide; hs-TnT, high-sensitivity troponin T; hs-CRP, high-sensitivity C-reactive protein; ST2, soluble suppression of tumorigenicity 2; PICP, C-terminal propeptide of procollagen type I; PIIINP, N-terminal propeptide of procollagen type III; ↑, diagnostic accuracy to detect heart failure subtypes; +, prognostic significance for each biomarker.

LIMITATIONS RELATED TO EF ASSESSMENT

The EF threshold constitutes the hallmark variable for HF subtype identification and categorization. Notably, EF offers some advantages related to immediate comprehension, short scan time, and feasibility—not requiring specific expertise (34, 35). Therefore, EF can be calculated easily by using

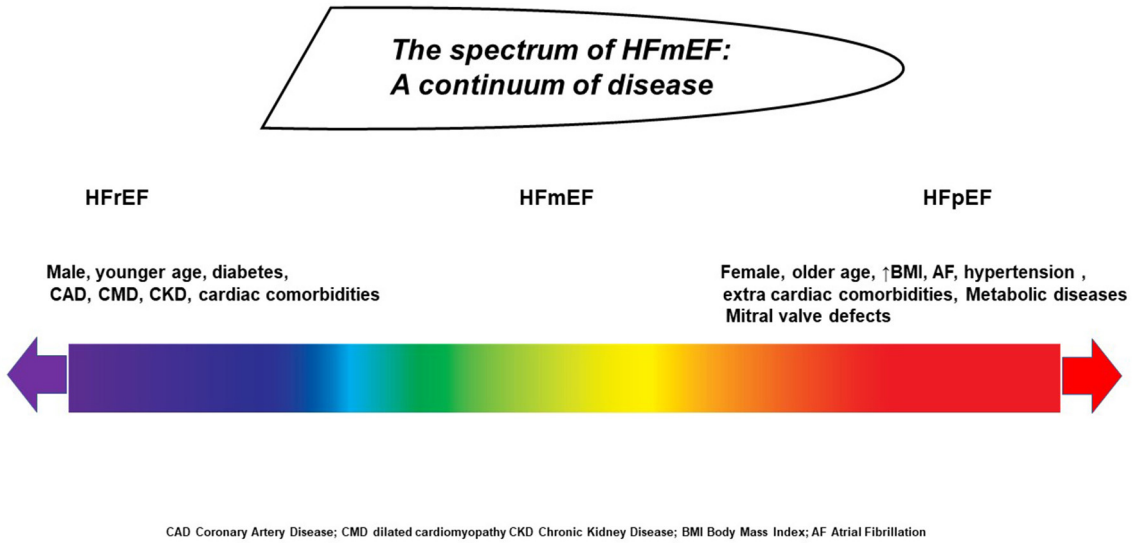

FIGURE 1 | The spectrum of patients with HF ranging from severe ejection fraction reduction to preserved function, according to baseline phenotype, disease time course, response to therapy, and loading conditions.

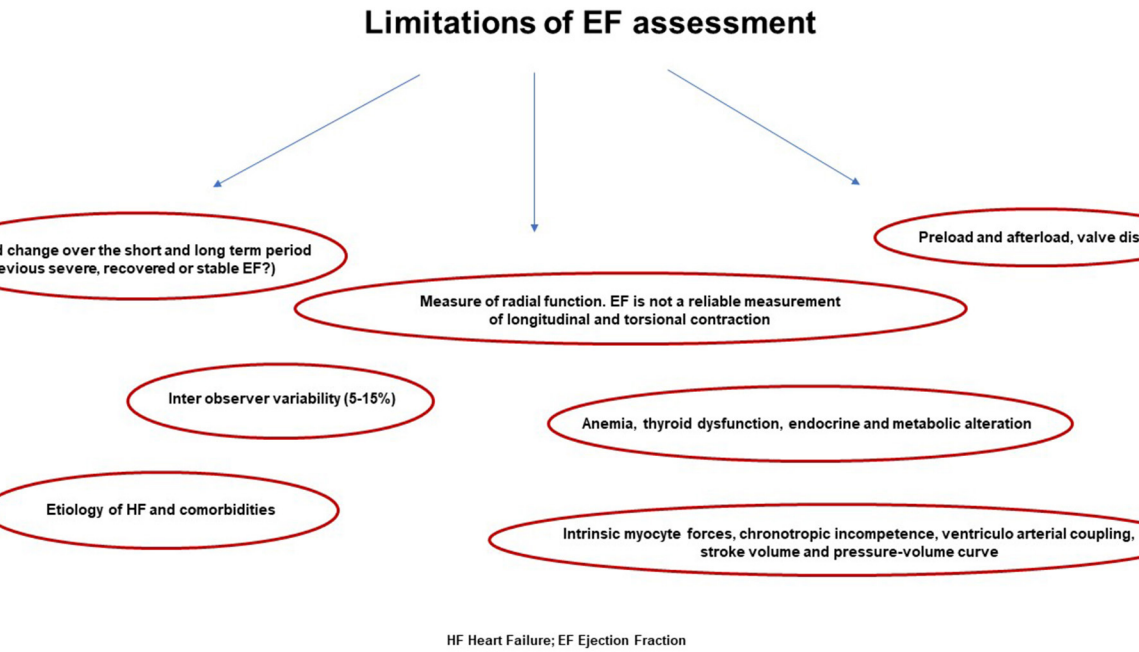

FIGURE 2 | Main weakness in ejection fraction calculation that does not comprise several features revealing the real systolic function of the left ventricle.

echocardiographic application, and it can be assessed visually even without a specific background. Moreover, EF provides the basis for structural and functional phenotype classification, and it is universally accepted in clinical practice and in study research (36). Beyond these features, EF assessment and related HF classification has demonstrated several gaps due to mechanistic, methodological, and hemodynamic pitfalls that do not really describe the true contractile ventricular function and pressure–volume relationship status (37).

EF is sensitive to sudden changes in preload and afterload forces, and sudden elevation in systemic blood pressure or vascular stiffness could impair the measurement. Conversely, a reduction in preload, causing a decrease in the atrioventricular blood afflux, makes the LV emptying more efficacious by a reduction of parietal strain forces (5, 38). In the presence of a valve defect, EF may be over- or underestimated: in case of significant mitral regurgitation, EF will be augmented because of the reduced workload during cardiac contraction.

Otherwise, during aortic stenosis, an increase of afterload occurs along with a delay in outflow time peak and consequent EF reduction (39). Other factors such as intrinsic myocyte forces, distension capacity, cronotropic incompetence, ventriculo-arterial coupling, and pressure–volume curve adaptation during exercise are all potential confounders for EF estimation (40). Chronic heart rate increase or decrease could underestimate or overestimate the values, respectively. Similarly, sympathetic activity or vagal stimulation and other systemic conditions such as anemia, thyroid dysfunction, and endocrine and metabolic alterations are all features that could potentially influence EF assessment. Behind these features, the HFmrEF subtype can be derived from patients with a previous and more severe EF reduction having a good response to therapy as well as from subjects with initial preserved EF experiencing initial systolic dysfunction (41). All these concerns highlight the need for a more comprehensive approach including environmental, social, genetic, and metabolomic factors in order to better characterize this syndrome. Therefore, patients' history, associated risk factors, comorbidities, body size conformation, and response to therapy should be taken into account beyond the simple EF calculation (42). The real challenge is to concretize and combine several epidemiological, biohumoral, mechanistic, and cardiac functional data across a spectrum of different phenotypes in which each subject has a specific HF onset, development, and pathophysiological pathways (43). Indeed, the population included in the HFmrEF category is extremely variable, encompassing patients with different disease triggers, demographic characteristics, associated diseases, and mortality risks (**Figure 1**).

EF is usually measured by echocardiography; unfortunately, the interobserver variability even in accredited echo laboratories ranges from 5 to 18% with broader limits for less experienced physicians (44). Thus, the current ESC cutoff distinguishing HFpEF (for patients with EF >50%) from midrange EF (for patients with EF between 40 and 49%) makes this classification hard to distinguish, and it could reveal significant misclassification depending on the laboratory site and the physician's experience and skills. Finally, EF is erroneously considered a measurement of systolic function, but it is just an estimation of radial function. EF is not a reliable measurement of longitudinal and torsional contraction although the whole systolic function results from all three variables. This reflects the different course and geometrical alignment of myocardial fibers that are not homogeneous inside the myocardial wall and in the different cardiac sites varying from basal to apical segments (45, 46). Accordingly, several studies that included patients with preserved EF showed significant longitudinal global function impairment, despite an apparently normal systolic function (47, 48). These difficulties represent a challenge for future investigation and could be overcome with the extensional use of cardiac magnetic resonance and 3D echo by the construction of a specific software algorithm.

Although it is not strictly related to the real forward flow, EF is erroneously considered as an indicator for LV remodeling. Indeed, an enlargement of diastolic dimension works as a compensatory factor in order to maintain an adequate stroke volume even during the occurrence of dilated systolic volume (46, 49). Conversely, in case of concentric remodeling, the stroke volume may be maintained although end diastolic volume is within the normal range and the ratio to systolic volume has altered. Notably, EF is inversely related to systolic volume but poorly related to stroke volume; thus, it is a mirror of systolic dysfunction in eccentric remodeling, whereas in concentric remodeling, it does not reflect effective contractile decline (50, 51) (**Figure 2**). For all these reasons, EF cannot be considered the only one reference of systolic function and may be contextualized into different cardiac remodeling, loading conditions, filling pressure, and hemodynamic status.

CONCLUSIONS

HFmrEF represents a mixed model between HFpEF and HFrEF. Demographic, structural, and laboratory data resembled HFpEF, whereas the CAD prevalence and the response to management are likely associated with HFrEF. Because of distinct phenotype, HFmrEF might be differentiated from other HF subgroups, but it deserves further research investigating cardiac and extracardiac diseases influencing its appearance. Therefore, the simple HFmrEF categorization based only on EF cutoff appears misleading, and it should be contextualized with other variables comprising both CV risk factors and detailed cardiac morphological assessment.

AUTHOR CONTRIBUTIONS

All authors participated in the manuscript draft review and design. The author warrants that his/her contribution is original and that he/she has full power to make this grant. The author signs for and accepts responsibility for releasing this material on behalf of any and all co-authors. The copyright transfer covers the exclusive right and license to reproduce, publish, distribute, and archive the article in all forms and media of expression now known or developed in the future, including reprints, translations, photographic reproductions, microform, electronic form (offline, online), or any other reproductions of similar nature. On Behalf of all authors AP.

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REVIEW
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Heart Failure With Mid-range Ejection Fraction: A Distinctive Subtype or a Transitional Stage?

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Heart failure with mid-range ejection fraction (HFmrEF) was first proposed by Lam and Solomon in 2014, and was listed as a new subtype of heart failure (HF) in 2016 European Society of Cardiology guidelines. Since then, HFmrEF has attracted an increasing amount of attention, and the number of related studies on this topic has grown rapidly. The diagnostic criteria on the basis of left ventricular ejection fraction (LVEF) are straightforward; however, LVEF is not a static parameter, and it changes dynamically during the course of HF. Thus, HFmrEF may not be an independent disease with a uniform pathophysiological process, but rather a collection of patients with different characteristics. HFmrEF is often associated with various cardiovascular and non-cardiovascular diseases. Thus, the pathophysiological mechanisms of HFmrEF are particularly complex, and its clinical phenotypes are diverse. The complexity and heterogeneity of HFmrEF may be one reason for inconsistent results between clinical studies. In fact, whether HFmrEF is a distinctive subtype or a transitional stage between HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF) is controversial. In this review, we discuss the clinical characteristics, treatment and prognosis of patients with HFmrEF, as well as the differences among HFmrEF, HFrEF, and HFpEF.

Keywords: heart failure, mid-range ejection fraction, preserved ejection fraction, angiotensin receptor-neprilysin inhibitors, sodium-glucose co-transporter 2 inhibitors

INTRODUCTION

Heart failure (HF) is a serious complication or an end-stage manifestation of various cardiovascular (CV) diseases. It is a complex clinical syndrome with a poor prognosis. Over the last three decades, despite continuous in-depth understanding and considerable progress in HF management, the morbidity and mortality of patients with HF have remained very high, causing a heavy social and economic burden (1, 2).

Historically, the classification of HF is complicated and often confused in different guidelines.

Previously descriptive terms of HF include systolic HF, diastolic HF, HF with preserved systolic function, and HF with normal ejection fraction, amongst others (3–7). Since left ventricular ejection fraction (LVEF) is a commonly used parameter to evaluate cardiac function and a significant prognostic predictor of HF, patients with HF are classified into two categories on the basis of LVEF, namely HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF) (8, 9). However, the majority of clinical trials on HFrEF or HFpEF exclude patients with a LVEF of between 40 and 50%; this group were once considered as an intermediate group or a "gray-zone" group (8, 9). Interestingly, some characteristics differ between these patients and patients with HFrEF or HFpEF. Therefore, in 2014, Lam and Solomon proposed a new term to describe such patients, namely HF with mid-range ejection fraction (HFmrEF). They pointed out that HFmrEF deserves more attention due to its special clinical, echocardiographic, hemodynamic, and prognostic characteristics (10). Subsequently, HFmrEF was classified formally as a new phenotype of HF in 2016 European Society of Cardiology (ESC) guidelines (11). From then on, clinical studies devoted to HFmrEF have rapidly emerged. However, the results of studies on HFmrEF are not consistent, and are sometimes contradictory, suggesting that HFmrEF may have complex characteristics. Thus, our current understanding of HFmrEF is still insufficient. This leads to a debate about whether HFmrEF is a unique subtype of HF or a transitional stage between HFrEF and HFpEF.

DEFINITION AND DIAGNOSIS

HFmrEF, which previously fell into the category of HFpEF, was once known as "borderline" HFpEF in 2013 American Heart Association/American College of Cardiology Foundation (AHA/ACCF) guidelines (9). HFmrEF was defined as HF with a LVEF of between 40 and 49%, and was listed as a new subtype of HF for the first time in 2016 ESC guidelines (11). According to these guidelines, the diagnosis of HFmrEF includes four elements: HF symptoms with or without signs, LVEF in the range of 40–49%, elevated brain natriuretic peptide (BNP) concentration (>35 pg/ml) or N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration (>125 pg/ml), and relevant structural heart disease or diastolic dysfunction (11).

Although this definition gives a clear diagnostic cut-off value for LVEF, HFmrEF is not as simple as it seems, because LVEF changes dynamically with an improvement or deterioration in the patient's condition and is not the only parameter used to measure cardiac function (12). Moreover, as the most commonly used technique, echocardiographic measurement of LVEF is not entirely accurate due to possible interobserver and intraobserver variability (13). From this point of view, HFmrEF resembles a container for a crowd of patients with HF with a LVEF of between 40 and 49%. Nevertheless, these patients may have different trajectories and prognoses. Therefore, for further recognition and understanding, HFmrEF can be classified as "HFmrEF improved" or "HFmrEF recovered" (previously a LVEF of <40%), "HFmrEF unchanged" (previously a LVEF of 40–49%), and "HFmrEF deteriorated" (previously a LVEF of ≥50%) based on changes in LVEF over time (14–16). This detailed classification may contribute to a deeper understanding of the pathophysiological process of HFmrEF and partly explain the inconsistent results between clinical studies.

EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS

Prevalence

Based on recent clinical trials and registries, HFmrEF accounts for ∼13–24% of HF cases (10, 17–20). For example, in the SwedeHF Registry, which enrolled 42,061 patients with HF, 21% had HFmrEF, whereas 56% had HFrEF and 23% had HFpEF (21). A similar proportion of HFmrEF was observed in the ESC-HF-LT Registry (22). However, the proportion of patients with HFmrEF was inconsistent between studies. In the PINNACLE Registry for first-visit patients with HF, only 7.5% of patients (82,292 of 1,103,386) were classified into HFmrEF category (23).

In addition, data from the GWTG-HF Registry showed that the proportion of patients with HFmrEF was relatively stable over time (between 13 and 15%), whereas the proportion of patients with HFpEF increased from 33 to 39%, and that of patients with HFrEF declined from 52 to 47% (24). In another study examining age-dependent differences in patients with HF, the prevalence of HFmrEF increased slightly with age, whereas the prevalence of HFpEF markedly increased and that of HFrEF significantly decreased (25).

Demographic Characteristics

Previous cohort and registry studies showed that patients with HFmrEF have intermediate features between those of HFrEF and HFpEF, but closer to those of HFpEF (**Table 1**) (26–28). However, patients with HFmrEF tend to be younger, and HFmrEF is more common in males compared with HFpEF (18, 19, 21, 22, 26–28).

Etiology

Despite once being considered as a borderline classification similar to HFpEF, HFmrEF shows different etiological features compared with HFpEF. The ESC-HF-LT Registry suggested that the main causes of HFmrEF are similar to those of HFrEF, including ischemic heart disease (IHD) in 41.8% of HFmrEF and 48.6% of HFrEF patients, and idiopathic dilated cardiomyopathy in 27.6% of HFmrEF and 35.1% of HFrEF patients. In contrast, IHD and idiopathic dilated cardiomyopathy account for only 23.7 and 11.6% of patients with HFpEF, respectively (22). Similarly, in the TIME-CHF study, the primary cause of HFmrEF or HFrEF was coronary artery disease (CAD), whereas the primary cause of HFpEF was hypertensive heart disease (18). In the ALARM-HF study, patients with HFmrEF or HFrEF were more likely to be hospitalized for acute coronary syndrome compared with those with HFpEF (20). In addition, previous myocardial infarction was more common in patients with HFmrEF or HFrEF compared with those with HFpEF (29, 30).

In short, IHD is the primary cause of HFmrEF and HFrEF, whereas the underlying diseases of patients with HFpEF often consist of hypertensive heart disease and valvular heart

TABLE 1 | Clinical characteristics of patients with HFmrEF compared with patients with HFrEF and HFpEF.

	GWTG-HF (n = 39,982)			SwedeHF (n = 42,061)			ESC-HF-LT (n = 9,134)			CHART-2 (n = 3,480)			ALARM-HF (n = 3,257)			OPTIMIZE-HF (n = 37,511)			TIME-CHF (n = 622)		
	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF	HFrEF	HFmrEF	HFpEF
Patients	18,398 (46%)	3,285 (8.2%)	18,299 (45.8%)	23,402 (56%)	9,019 (21%)	9,640 (23%)	5,460 (59.8%)	2,212 (24.2%)	1,462 (16%)	730 (21%)	596 (17.1%)	2,298 (66%)	1,698 (52%)	811 (25%)	748 (23%)	20,118 (53.6%)	7,321 (19.5%)	10,072 (26.9%)	402 (65%)	108 (17%)	112 (18%)
Age, yrs.	79.0	81.0	82.0	72.0	74.0	77.0	64.0	64.2	68.6	66.9	69.0	71.7	–	–	–	70.4	74.3	75.6	75.5	79.0	80.2
Female, %	41.0	51.5	67.6	29.0	39.0	55.0	21.6	31.5	47.9	23.3	28.2	39.2	29.9	35.1	51.6	38.0	52.0	68.0	32.6	46.3	64.3
BMI, kg/m^2	25.6	26.8	27.3	26.0	27.0	28.0	27.8	28.6	28.4	22.7	22.8	23.2	–	–	–	–	–	–	25.3	25.5	27.0
SBP, mmHg	132.0	141.0	143.0	124.0	131.0	133.0	121.6	126.5	131.0	117.9	124.7	127.9	123.4	139.8	144.9	–	–	–	117.0	127.0	136.0
DBP, mmHg	73.0	74.0	72.0	73.0	74.0	73.0	–	–	–	69.8	71.8	71.9	–	–	–	–	–	–	71.0	73.0	74.0
Heart rate, beats/min	82.0	80.0	79.0	74.0	73.0	74.0	72.9	73.2	72.5	74.0	73.4	71.7	108.5	106.6	108.7	–	–	–	76.0	76.0	74.0
Smoking, %	10.9	8.0	7.4	60.0	55.0	50.0	12.7	10.7	8.1	–	–	–	64.7	58.9	46.1	–	–	–	63.5	60.2	41.1
Hypertension, %	69.9	75.3	77.9	56.0	64.0	72.0	55.6	60.1	67.0	84.7	89.8	91.2	65.5	76.5	71.6	66.0	74.0	77.0	68.9	82.4	85.7
Diabetes mellitus, %	38.3	41.6	38.8	27.0	27.0	28.0	32.3	30.5	29.3	38.1	36.1	33.8	44.0	45.7	41.8	39.0	44.0	41.0	33.6	39.8	39.3
Hyperlipidemia, %	43.5	44.0	40.2	–	–	–	–	–	–	82.2	80.2	78.8	44.7	47.8	39.5	34.0	35.0	31.0	52.2	48.1	36.6
CAD, %	56.8	55.1	43.5	54.0	53.0	42.0	48.6	41.8	23.7	–	–	–	37.8	28.7	20.3	–	–	–	73.9	79.6	63.4
Atrial fibrillation, %	34.5	37.4	38.9	51.0	58.0	63.0	18.3	22.3	32.2	38.1	43.5	51.8	24.2	24.6	26.2	28.0	33.0	32.0	30.0	39.6	42.9
CKD, %	19.4	18.8	17.6	45.0	48.0	56.0	19.5	16.5	19.9	–	–	–	23.1	17.9	18.2	–	–	–	54.0	63.9	61.6
Stroke or TIA, %	14.91	15.98	16.33	–	–	–	9.4	8.3	9.8	18.9	22.1	21.9	–	–	–	–	–	–	14.9	15.7	18.8
Anemia, %	14.73	19.40	20.03	31	35	41	–	–	–	–	–	–	13.2	13.6	14.9	–	–	–	23.6	38.0	34.8
Lung disease, %	25.91	26.87	29.44	28	30	35	15.2	11.6	14.0	–	–	–	22.9	22.4	23.3	–	–	–	20.6	21.3	16.1

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CAD, coronary artery disease; CKD, chronic kidney disease; TIA, transient ischemic attack; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction.

disease. Therefore, from an etiological point of view, patients with HFmrEF are more similar to those with HFrEF rather than HFpEF.

Comorbidities

In the GWTG-HF Registry, patients with HFmrEF had a similar prevalence of anemia, atrial fibrillation, chronic obstructive pulmonary disease (COPD) or asthma, depression, hypertension, and chronic kidney disease (CKD) compared with those with HFpEF. However, a significantly higher prevalence of IHD was observed in patients with HFmrEF or HFrEF, compared with HFpEF (17). In the ESC-HF-LT Registry, patients with HFmrEF showed a lower incidence of COPD and CKD, compared with the other two groups. An intermediate prevalence of atrial fibrillation in the HFmrEF group was observed. Notably, the incidence of IHD in HFmrEF group was similar to that of HFrEF group, but significantly higher than that of HFpEF group (22). Similar trends in the incidence of IHD among three groups were observed in the MACARF program, TIME-CHF study, and SwedeHF Registry (18, 30, 31). Moreover, patients with HFmrEF or HFrEF carried a higher risk of new IHD events compared with those with HFpEF (30).

In brief, although the characteristics of diseases concomitant with HFmrEF are not consistent in clinical studies, a consistent finding is that patients with HFmrEF have a significantly greater incidence of IHD compared with those with HFpEF (32) (**Table 1**).

Prognosis

LVEF is widely considered as an important predictor of CV events in patients with HF. In the CHARM study, when LVEF was <45%, all-cause mortality increased by 39% with every 10% decline in LVEF. With an improvement in LVEF, all-cause mortality and CV death declined. However, once elevated to >45%, an increase in LVEF did not contribute to a further decline in either all-cause mortality or CV death (33). In a meta-analysis, along with an improvement in LVEF, all-cause mortality and CV death declined progressively in patients with HFrEF; however, a similar trend was not observed in patients with a LVEF of ≥40% (34). These findings indicate that LVEF is not an adequate prognostic predictor in patients with HFmrEF or HFpEF.

In a study analyzing the precipitating clinical factors in patients with HF, in-hospital death was significantly lower in patients with HFmrEF compared with those with HFrEF or HFpEF (17). However, in the GWTG-HF Registry, the HFmrEF group showed no difference compared with the other two groups in terms of 5-year mortality. Nevertheless, CV and HF readmission rates were higher in both the HFmrEF group and the HFrEF group compared with the HFpEF group (35).

In the ESC-HF-LT Registry, the 1-year mortality rate of patients with HFrEF, HFmrEF, and HFpEF was 8.8, 7.6, and 6.4%, respectively. By pairwise comparison, there was no significant difference in all-cause mortality of patients with HFmrEF compared with patients with HFrEF or HFpEF. Non-CV mortality in patients with HFmrEF was similar to that of patients with HFpEF, but higher than that of patients with HFrEF. In terms of HF hospitalization rate, the HFmrEF group was similar to the HFpEF group, but significantly lower than HFrEF group (22).

In the SwedeHF Registry, adjusted all-cause mortality in patients with HFmrEF or HFpEF was lower compared with those with HFrEF (21). In the CHART-2 study, patients with HFmrEF showed an intermediate risk of all-cause death, CV death, and hospitalization for HF compared with the other two groups (19).

In terms of patients with acute HF, short-term mortality was lower in patients with HFmrEF or HFpEF, compared with patients with HFrEF in the ALARM-HF study (20). However, in another study of patients suffering from acute decompensatory HF, patients with HFmrEF had similar short-term outcomes compared with those of other categories (36).

In a recent meta-analysis including >600,000 adult patients, patients with HFmrEF demonstrated similar all-cause mortality compared with those with HFpEF, but significantly lower than that of HFrEF patients. Cardiac death was more common in patients with HFpEF, whereas non-cardiac death was significantly more common in the HFrEF group. In addition, no significant differences in all-cause and HF-related hospitalization were observed among the three groups (37).

PATHOPHYSIOLOGY

HF is a complex clinical syndrome with a series of abnormalities in cardiac structure and function. Due to obvious differences in epidemiology, pathophysiology, comorbidity, response to treatment, and prognosis, HFrEF and HFpEF are considered as two distinct pathophysiological entities (38). HFrEF, previously called systolic HF, is generally characterized by impaired left ventricular contractility accompanied by a marked decline in LVEF. The major structural abnormality of HFrEF is eccentric remodeling, followed by progressive ventricular dilatation and volume overload. In contrast, HFpEF, previously called diastolic HF, is predominantly characterized by concentric remodeling accompanied by impaired myocardial relaxation and increased stiffness, resulting in pressure overload (39). In fact, systolic dysfunction and diastolic dysfunction often coexist whether in HFrEF or HFpEF.

Once a component of HFpEF, the exactly pathophysiological mechanisms of HFmrEF remain unclear. According to 2016 ESC guidelines, patients with HFmrEF may have both mild systolic dysfunction and diastolic dysfunction (11). However, this seemingly simple statement may not adequately explain its complex characteristics.

In a recent study of biomarkers in acute HF with different LVEF values, patients with HFmrEF demonstrated an intermediate biomarker feature with interactions between cardiac stretch and inflammation, whereas the biomarker profile of HFrEF was predominantly associated with cardiac stretch and HFpEF with inflammation (38, 40). In another study, epicardial adipose tissue volume was significantly higher in patients with HFmrEF and HFpEF compared to healthy individuals (41). These findings suggested that metabolic and inflammatory mechanisms were involved in the development of HFmrEF.

In the TIME-CHF study, NT-proBNP levels were elevated similarly in the HFrEF group and the HFmrEF group, but were significantly higher than that in the HFpEF group. In addition, NT-proBNP-guided therapy showed similar benefit in HFrEF and HFmrEF, but not in HFpEF, compared with standard therapy (18). In another study, sympathetic activation was greatest associated with adverse outcomes in HFmrEF patients compared with that in HFrEF or HFpEF patients (42). These findings suggested that neurohormonal system activation may play an important role in the pathogenesis of HFmrEF. However, in another study, elevated levels of neuroendocrine hormones including plasma renin activity, aldosterone and norepinephrine were detected in 10% of HFpEF patients, 8% of HFmrEF patients and 21% of HFrEF patients, suggesting neurohormonal activation may only be involved in pathogenesis of a small subset of patients with HFmrEF (43).

In a study evaluating the prognostic value of soluble suppression of tumorigenicity 2 (sST2) in patients with HF, sST2 was an independent predictor of all-cause death and HF rehospitalization for all three groups, indicating that myocardial fibrosis may be a potential pathogenesis of HFmrEF (44). Besides, myocardial dysfunction was also associated with the pathophysiology of HFmrEF (45).

Overall, HFmrEF demonstrates mixed pathophysiological characteristics between HFrEF and HFpEF in existing studies. Although a variety of pathophysiological mechanisms may attribute to the occurrence and development of HFmrEF, extensive data are still lacking, and further studies are required.

THERAPY

Thus far, no prospective studies have specially assessed the effect of pharmacological therapy in patients with HFmrEF. Existing evidences on pharmacological therapy for patients with HFmrEF are based on *post-hoc* analyses of studies that partially or wholly include HF patients with a LVEF of between 40 and 49%, as discussed below.

Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers

In the OPTIMIZE-HF Registry, ACEI/ARB treatment showed no significant beneficial effects in patients with HF with a LVEF ≥40% (26). In the CHARM-PRESERVED trial, which aimed to assess the effect of candesartan in patients with HF with a LVEF >40%, moderate benefit was observed in preventing HF hospitalization when compared with placebo (46). However, candesartan did not significantly reduce CV death compared with placebo, which may be due to the fact that patients were not classified specially into HFmrEF or HFpEF group (46).

Notably, in a recent analysis using CHARM data to evaluate the effect of candesartan in patients with HF across the entire LVEF spectrum, the HFmrEF group accounted for 17% of all enrolled patients. Candesartan significantly reduced the incidence of CV death or hospitalization in both the HFrEF group and the HFmrEF group, but not in the HFpEF group. Also, candesartan substantially reduced the incidence of recurrent HF hospitalization in patients with HFmrEF (29).

In several studies using data from the SwedeHF Registry, ACEIs/ARBs reduced all-cause mortality in patients with HFmrEF and HFpEF (47–49). Similarly, in a further analysis of the same registry, of 42,061 patients, 21% were classified into the HFmrEF group. ACEIs/ARBs significantly reduced mortality, whether CAD was present or not (21). Similar findings were observed in other studies (18, 19).

In early studies on HFpEF (LVEF ≥40%), ACEIs/ARBs did not demonstrate significant benefit in improving primary outcomes, such as all-cause mortality and CV death. However, subsequent evidence suggested that patients with a LVEF of 40–49% respond differently to treatment compared with those with a LVEF ≥50%. In recent studies specially on patients with HFmrEF, an increasing amount of evidence suggested that ACEIs/ARBs improve clinical outcomes in this group.

In summary, ACEIs/ARBs may be an effective treatment option for patients with HFmrEF. In recent Brazilian Society of Cardiology guidelines, ACEIs or ARBs (if ACEIs are not tolerated) are recommended for patients with HFmrEF (50). Further prospective studies that are focused on this population are required.

Angiotensin Receptor-Neprilysin Inhibitors

Since the PARADIGM-HF trial was published, ARNIs has been proven to significantly reduce incidence and mortality in patients with HFrEF. Based on this powerful evidence, ARNIs are recommended as a cornerstone pharmacological therapy for HFrEF (11, 51–53). However, the effect of ARNIs in patients with HFmrEF and HFpEF remains unclear.

In the PARAMOUNT trial, ARNIs reduced NT-proBNP levels to a greater extent compared with ARBs. In addition, ARNIs reduced left atrial volume, indicating an improvement in left atrial remodeling (54).

In the subsequent PARAGON-HF trial, which enrolled 4,822 symptomatic HF patients with a LVEF ≥45% and an elevated BNP level, sacubitril/valsartan did not further reduce the risk of total HF hospitalization and CV death compared with valsartan (55). However, in subgroup analyses, a potential benefit was observed in patients with a relatively lower LVEF (45–57%), suggesting that patients with HFmrEF characterized by a mildly reduced LVEF may benefit from sacubitril/valsartan (55, 56). In subsequent analyses based on PARAGON-HF data, pulse pressure and serum uric acid were considered as independent predictors of adverse outcomes in patients with HFpEF, and ARNI reduced pulse pressure and serum uric acid compared with valsartan (57, 58).

In a recent meta-analysis on >5,500 patients, compared with ACEIs and ARBs, ARNIs did not significantly reduce CV death and all-cause mortality. However, ANRIs significantly reduced HF hospitalization and improved physical capacity in patients with HFmrEF or HFpEF. This suggested that ARNIs may reduce HF hospitalization and improve clinical symptoms in patients with HFmrEF or HFpEF (59).

PARALLAX, which is a prospective, randomized, controlled, and double-blind multi-center clinical trial, enrolled patients

with HFmrEF and HFpEF to assess the effect of ARNIs on functional capacity (60). In the 2020 ESC Congress-Clinical Trials Hotline Session, the results of the PARALLAX trial were first reported. Compared with individualized medical therapy, ARNIs further reduced NT-proBNP level by 16% at 12 weeks after treatment, and they also significantly reduced the risk of first hospitalization for HF by 51% and of composite events (HF hospitalization, mortality) by 36%.

Given the above evidence, ANRIs may be a useful pharmacological treatment for patients with HFmrEF, as well as patients with HFpEF with a relatively lower LVEF.

Mineralocorticoid Receptor Antagonists

Mineralocorticoid receptor antagonists have been proven to improve the prognosis of patients with HFrEF. To date, the most important study to assess the effects of spironolactone in patients with HFpEF (LVEF ≥45%) is the TOPCAT study (61). In this study, spironolactone did not significantly improve primary composite outcomes (CV death, aborted cardiac arrest, and HF hospitalization) compared with placebo (61). Interestingly, in a *post-hoc* analysis, a greater potential benefit of spironolactone was observed in patients with a relatively lower LVEF (45–49%) in terms of the primary composite outcome (62), suggesting that patients with HFmrEF may benefit from spironolactone treatment.

Consistent findings were observed in other studies. In a Chinese study examining the role of spironolactone in patients with HFmrEF, spironolactone significantly reduced primary composite outcomes (all-cause death, HF re-hospitalization) compared with placebo (63). In another study, the use of spironolactone at discharge significantly reduced composite outcomes (all-cause death, HF re-hospitalization) in patients with HFmrEF during a mean follow-up period of 2.2 years (64). In a recent meta-analysis of 11 randomized controlled trials (RCTs) with over 4,500 patients, spironolactone treatment reduced HF hospitalization and BNP levels, and improved functional class in patients with HFmrEF or HFpEF (65). These benefits may be partly attributed to alleviation of myocardial fibrosis using spironolactone (65, 66).

Based on these favorable outcomes, mineralocorticoid receptor antagonists are recommended (class IIb) in patients with HFmrEF in recent update to AHA/ACCF guidelines (52, 67).

Beta-Blockers

Since a large number of RCTs have consistently demonstrated that beta-blockers can significantly improve both short- and long-term outcomes, such as all-cause mortality, CV death, HF hospitalization, and cardiac arrest, these agents are widely recognized as a standard therapy in patients with HFrEF (8, 9, 11, 52). However, whether patients with HFmrEF or HFpEF also benefit from beta-blockers remains unclear.

In the OPTIMIZE-HF Registry, beta-blockers showed no benefit in patients with HFpEF (LVEF ≥40%) (26). Even when the subsequent analysis was refined to patients with a LVEF in the range of 40–49%, beta-blockers did not significantly reduce the risk of mortality and re-admission (68).

Conversely, beta-blockers improved clinical outcomes and reduced mortality in both HFmrEF and HFrEF patients in the CHART-2 study (19). Interestingly, in the SwedeHF Registry, beta-blockers reduced 1-year mortality in patients with HFrEF whether CAD was present or not, but in patients with HFpEF, beta-blockers were only effective in the absence of CAD. In contrast, beta-blockers reduced 1-year mortality in patients with HFmrEF only in the presence of CAD (21). In a meta-analysis of 11 RCTs, beta-blockers were associated with an increased LVEF and improved the prognosis of patients with HFmrEF and HFrEF in sinus rhythm, whereas for patients with atrial fibrillation at baseline, beta-blockers only increased LVEF in the HFmrEF and HFrEF groups, but did not improve prognosis. No significant benefit of beta-blockers was observed in patients with HFpEF whether in sinus rhythm or atrial fibrillation (69). In a nationwide retrospective study, beta-blockers treatment reduced in-hospital mortality in post-acute coronary syndrome patients with HFmrEF (70). However, a recent observational study indicated that beta-blockers did not improve the long-term prognosis in patients with HFmrEF with IHD. Conversely, significant benefits were observed in patients with HFrEF with IHD in terms of long-term outcomes after beta-blockers therapy (71).

In terms of acute HF, in the ALARM-HF study, patients with HFmrEF were intermediate frequently treated with beta-blockers compared with patients with HFrEF or HFpEF (20). In an analysis of data from the KorAHF Registry, beta-blockers improved LVEF in patients with HFmrEF (72).

In brief, according to 2016 ESC guidelines, which recommended that therapy for patients with HFmrEF should be based on the evidence in patients with HFpEF, beta-blockers are not recommended for patients with HFmrEF or HFpEF (11). Similar recommendations were also released in 2017 update to American Heart Association/American College of Cardiology Foundation guidelines (52). However, some studies suggested that beta-blockers may be beneficial for patients with HFmrEF, especially those who have recovered from prior HFrEF after treatment (73, 74). In 2018 Brazilian Society of Cardiology guidelines, beta-blockers are recommended for patients with HFmrEF (50).

Diuretics

In the SwedeHF Registry, diuretics showed an adverse impact on 1-year all-cause mortality in patients with HFrEF and HFmrEF, but not in patients with HFpEF (21). A similar unfavorable impact on prognosis was observed in the CHART-2 study (19).

Therefore, diuretics are recommended to alleviate symptoms or signs in patients with HFmrEF only in the presence of congestion (11, 52).

Digoxin

Digoxin is often used as an adjunctive therapy in patients with HFrEF (11). In an analysis of the DIG trial, digoxin reduced HF hospitalization in patients with HFrEF (75). In another study including >11,000 hospitalized patients with HFrEF in the Medicare-linked OPTIMIZE-HF Registry, digoxin reduced HF re-hospitalization, but not all-cause mortality, in older patients

with HFmrEF receiving guideline-directed medical therapy (76). Also, in this study, discontinuation of pre-admission digoxin increased the risk of all-cause mortality and the combined endpoint (77).

However, the benefit of digoxin in patients with HFpEF or HFmrEF remains controversial. In a study on 7,374 hospitalized patients with HFpEF in the Medicare-linked OPTIMIZE-HF Registry, the impact of digoxin on short-term (30-day) and long-term (6-year) outcomes was neutral in older hospitalized patients with HFpEF (78). In an observational and multi-center study, digoxin increased the risk of all-cause death and/or re-hospitalization in older patients with HFpEF discharged after acute HF (79).

A retrospective study on the DIG trial included 7,788 patients, 1,195 of whom were diagnosed with HFmrEF. In this group, digoxin reduced primary composite outcomes (CV death or HF hospitalization), mainly reduced HF hospitalization. Interestingly, the effect was greatest in patients with HFrEF, intermediate in patients with HFmrEF, and smallest in patients with HFpEF (80).

Statins

In early randomized trials, statins did not improve clinical outcomes in patients with HFrEF. In contrast, statins showed a beneficial effect on clinical outcomes, such as mortality, in patients with HFpEF, in the presence or absence of CAD (81–83). The effect of statins in patients with HFmrEF remains unclear.

In the CHART-2 study, statins reduced all-cause mortality in patients with HFpEF, but not in patients with HFrEF or HFmrEF (19). This is consistent with prior studies. However, of note, in the SwedeHF Registry, statin use was associated with a reduction in 1-year mortality in all three groups, irrespective of the presence of CAD (21).

Sodium-Glucose Co-transporter 2 Inhibitors

Although originally classified as anti-hyperglycemic drugs, SGLT2 inhibitors reduced the risk of HF hospitalization, CV death, and all-cause mortality in patients with HFrEF (84–86). In the 2021 update to the 2017 ACC Expert Consensus Decision Pathway for Optimization of Heart Failure Treatment, addition of SGLT2 inhibitors to standard treatment was recommended to improve clinical outcomes in patients with HFrEF (53). In the newly proposed therapeutic algorithm for patients with HFrEF, simultaneous administration with a beta-blocker and a SGLT2 inhibitor was recommended as the initial treatment (87).

Thus far, whether patients with HFmrEF or HFpEF will benefit from SGLT2 inhibitors remains unclear. Ongoing studies, such as EMPEROR-Preserved, DELIVER, and PRESERVED-HF, will assess the effects of SGLT2 inhibitors in these populations. If the expectations are achieved, SGLT2 inhibitors may be an optional treatment for patients with HFmrEF or HFpEF.

Other Therapies

Ivabradine is the first selective inhibitor of I_f-channel. Due to its benefit in reducing the composite outcomes of mortality or HF hospitalization in patients with HFrEF, it is recommended as an additional therapy to alleviate clinical symptoms and improve outcomes for these patients (11, 52). Heart rate is an essential predictor of clinical outcomes in patients with HF (74). Regarding the importance of heart rate control, ivabradine may also be effective in patients with HFmrEF or HFpEF, but this required further validation.

Tolvaptan is a vasopressin V_2 receptor antagonist. Its efficacy and safety in patients with HFrEF have been proven in previous studies (88). In a prospective, multi-center, post-marketing surveillance study on 1,741 patients, 286 (16.4%), 795 (45.7%), and 660 (37.9%) patients were categorized as HFmrEF, HFpEF, and HFrEF, respectively. Tolvaptan showed similar benefit in all three groups, suggesting that it may be an effective and safe pharmacological therapy for patients with HFmrEF or HFpEF (88).

Levosimendan is a calcium-sensitizing cardiotonic agent that promotes calcium sensitization of the contractile apparatus without increasing intracellular calcium concentration compared with other inotropes (89). In the LION-HEART multi-center randomized trial, levosimendan reduced plasma NT-proBNP concentration and HF hospitalization, and improved health-related quality of life in outpatients with advanced chronic HF (90). In a recent meta-analysis, intravenous levosimendan was associated with a reduced BNP concentration, an increased LVEF, and reduced short-term mortality in patients with advanced HF (91). Therefore, levosimendan is mainly used in patients with acute HF or chronic decompensated HF. However, no studies have yet investigated the effect of levosimendan in patients with HFmrEF or HFpEF.

Vericiguat is a novel oral soluble guanylate cyclase agonist. It improves myocardial and vascular function by stimulating the activity of guanylate cyclase and increasing the production of cyclic guanosine monophosphate. In the VICTORIA study, which enrolled >5,000 patients with chronic HF and an LVEF of ≤45%, vericiguat was associated with a reduced risk of CV death or HF hospitalization (92). However, in the VITALITY-HFpEF randomized trial, 24-week treatment with vericiguat did not demonstrate a beneficial effect on quality of life in patients with HFpEF and recent decompensation (93). Since patients with HFmrEF were partly included in these two studies, whether these patients can benefit from vericiguat remains uncertain; thus, further studies are required in this population.

CDR132L, the first microRNA-132 inhibitor, is a synthetic special antisense oligonucleotide. In preclinical models, CDR132L demonstrated beneficial effects on improving and even reversing HF. In the first-in-human study of CDR132L, which enrolled patients with a LVEF in the range of 30–50% or an NT-proBNP concentration of >125 ng/L, CDR132L improved cardiac function and ameliorated cardiac fibrosis (94). CDR132L may be a promising drug for patients with HFmrEF or HFrEF; however, this requires further validation.

Iron deficiency is prevalent in patients with HFrEF, HFpEF, and HFmrEF. Progression of iron deficiency accelerates HF deterioration (95). Intravenous iron treatment improved exercise capacity, relieved HF symptoms, and improved quality of life in patients with HFrEF and iron deficiency (96). However, whether

TABLE 2 | Treatment response of patients with HFrEF, HFmrEF, and HFpEF.

	ACEI	ARB	ARNI	MRA	Beta-blocker	SGLT2 inhibitor	Statins
HFrEF	++	++	++	++	++	++	?
HFmrEF	+	+	++	+	+	?	?
HFpEF	/	+	++	+	/	?	++

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor-neprilysin inhibitor; MRA, mineralocorticoid receptor antagonist; SGLT2 inhibitor, sodium-glucose co-transporter 2 inhibitor; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction. The symbol +, ++, /, ? represent moderately effective, significantly effective, noneffective, and probably effective, respectively.

patients with HFmrEF or HFpEF patients can benefit from intravenous iron remains uncertain (97).

In general, despite HFmrEF have intermediate features between HFrEF and HFpEF, patients with HFmrEF demonstrate a comparable response to guideline-directed medical therapies as patients with HFrEF (**Table 2**).

SIMILARITIES AND DIFFERENCES BETWEEN ACUTE HFmrEF AND CHRONIC HFmrEF

Since the majority of studies on HFmrEF enrolled patients with chronic HF (CHF), studies specially for HFmrEF patients with acute HF (AHF) were relatively few.

In current studies on HFmrEF patients with AHF, the proportions of HFmrEF patients were ∼14–25% (20, 36, 98). These patients demonstrated intermediate features between HFrEF patients and HFpEF patients. HFmrEF patients were older and more commonly male compared with HFrEF patients, whereas they were younger and more likely to be female compared with HFpEF patients. Similar characteristics were observed in patients with CHF (18, 20, 22).

In terms of biomarkers of AHF patients, the HFmrEF group also showed intermediate characteristics between the other two groups (40). However, in CHF patients, HFmrEF resembled more closely HFrEF except lower BNP level (99). In addition, some biomarkers played an important role in prognostic prediction. For example, elevated BNP level predicted an increased risk of mortality in all three groups (100). The difference was that in AHF patients, the prognostic significance of BNP was higher in HFrEF compared with that in HFmrEF and HFpEF (100), while in CHF patients, BNP was most closely associated with the prognosis of the HFmrEF group compared with other two groups (99).

Considering the etiological aspect, IHD was the leading cause of HFmrEF patients whether with AHF or CHF. From this viewpoint, HFmrEF was closer to HFrEF but not HFpEF. However, regarding short-term mortality, HFmrEF patients showed a lower risk compared with HFrEF patients, but a similar risk to HFpEF patients (20, 98). However, in discharge AHF patients, the long-term all-cause mortality of all three groups was comparable high (98).

Regarding pharmacological treatment, neurohormonal activation was associated with an increased risk of all-cause mortality and CV death in HF patients. Previous studies showed that this association was greatest in HFmrEF patients, while it was weakest in HFpEF (42). These findings suggested that neurohormonal therapies may be effective for HFmrEF patients, which was consistent with observations in clinical trials, such as SwedeHF registry (21). However, in acute HFmrEF patients receiving guideline-directed medical therapy, only beta-blockers showed favorable effect on in-hospital mortality, whereas ACEIs/ARBs and MRAs did not improve outcomes (98). Therefore, further studies are required to evaluate the effect of ACEIs/ARBs or MRAs in acute HFmrEF patients.

TRANSITIONS AMONG THE THREE HF GROUPS

According to LVEF, recent clinical guidelines classify HF into three groups: HFrEF, HFpEF, and HFmrEF (11). As a gray zone between HFrEF and HFpEF, this new definition has encouraged research into the potential characteristics, pathophysiology, and treatment of HFmrEF (101). Of note, despite LVEF is widely used as the basis for classifying HF in recent guidelines (9, 11), it is not a precise indicator of cardiac function, which may be influenced by many factors. For example, LVEF may provide imprecise implications in the presence of mitral regurgitation, aortic stenosis, or ventricular hypertrophy (102). In addition, there is substantial variability among different imaging techniques for LVEF measurement (103). Even when using the same imaging method, interobserver variability may exist. Especially noteworthy is the fact that LVEF is a dynamic index and may increase or decrease during the course of HF. In several studies, transitions in LVEF were observed (12, 104–106), suggesting that the cut-off value of LVEF is artificial, and LVEF may change dynamically over time. In other words, transitions among these three groups require more attention rather than a static LVEF value.

In a cohort study examining the natural history of LVEF over time in patients with HF, patients who suffered from previous myocardial infarction were more likely to transition from HFpEF to HFrEF, whereas females and those using beta-blockers tended to transition from HFrEF to HFpEF (105). Similarly, in a community-based cohort study, average LVEF decreased by 5.8% over 5 years in patients with HFpEF, and a greater decline was observed in older individuals and individuals with CAD. In contrast, average LVEF increased by 6.9% over 5 years in patients with HFrEF, and a greater increase was observed in females, younger patients, individuals without CAD, and those receiving guideline-directed medical therapy (12). In a recent study evaluating the prognostic implications of longitudinal LVEF change in HF, transitions among the three groups were observed during follow-up. Increases in LVEF occurred in 25% of HFmrEF patients and 26% of HFrEF patients, whereas decreases in LVEF occurred in 39% of HFpEF patients and 37% of HFmrEF patients (107). Predictors of increased LVEF included younger, female, lower severity of HF, fewer

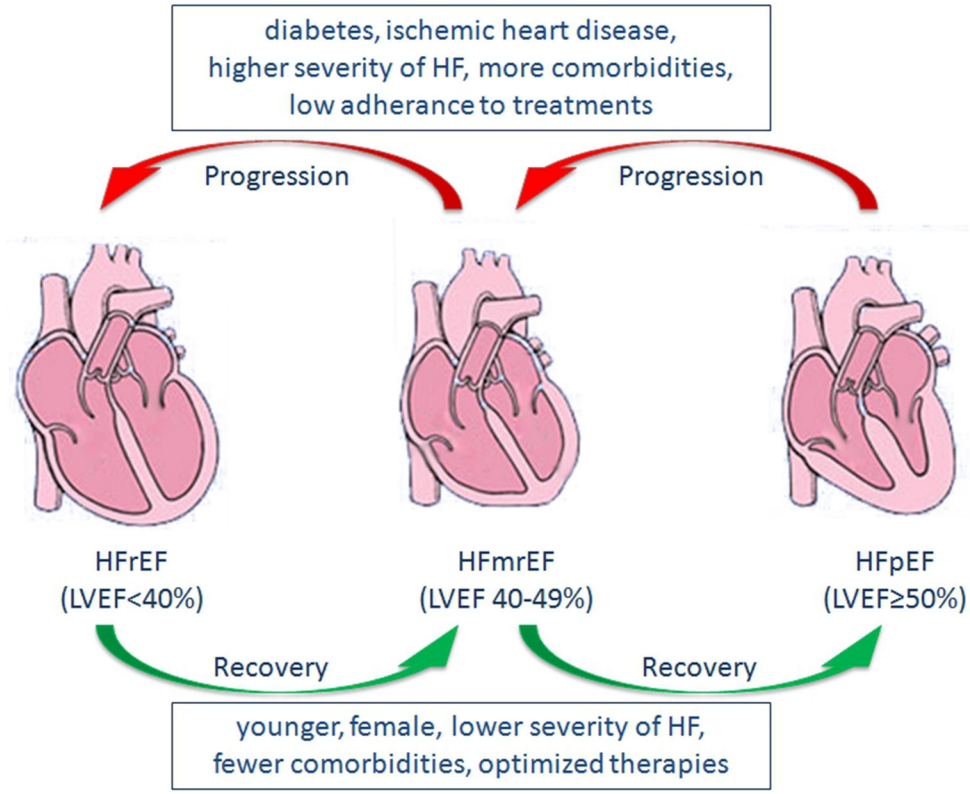

FIGURE 1 | Predictors of changes in LVEF, and Transitions among HFrEF, HFmrEF, and HFpEF. LVEF, left ventricular ejection fraction; HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HF, heart failure.

comorbidities, optimized therapies, and predictors of decreased LVEF included diabetes, IHD, higher severity of HF (107, 108) (**Figure 1**). Moreover, a decrease in LVEF over time is associated with increased mortality and/or HF hospitalization, whereas an increase in LVEF is associated with reduced mortality and/or hospitalization (12, 107, 108).

Considering the trajectory of LVEF over time, HFmrEF may occur either as a recovery from HFrEF, or a deterioration from HFpEF. Also, it may be the initial presentation of patients with HF (109). Thus, HFmrEF represents a large group of patients with heterogenous features and consists of at least three subgroups, including HFmrEF improved, HFmrEF unchanged, and HFmrEF deteriorated (15, 16). Although both are categorized as HFmrEF, HFmrEF improved (an increase in LVEF after treatment for prior HFrEF) may have a distinct pathophysiological process, treatment response, and prognosis compared with HFmrEF deteriorated (declined LVEF from prior HFpEF) (**Figure 1**). In a recent study examining the epidemiology, pathophysiology and clinical outcomes of HFmrEF, HFmrEF improved patients showed significantly better clinical outcomes compared with HFmrEF deteriorated individuals, whereas no significant differences were observed in clinical outcomes between the HFmrEF deteriorated group and matched patients with HFpEF (110). Similar findings were observed in the CHART-2 study (19).

In summary, despite a universal diagnosis of HFmrEF, patients may have different characteristics, pathophysiological features, clinical courses and prognoses according to diverse changes in LVEF (107, 111). By recognizing the continuous spectrum of HF and the limitations of LVEF, we should pay attention to the trajectory of LVEF over time, refine the classification of HF based on pathophysiological homogeneity rather than LVEF value alone (112–114), and design an individualized, evidence-based therapeutic strategy (50, 114).

CONCLUSION

As a new HF classification, HFmrEF demonstrates intermediate characteristics between those of HFrEF and HFpEF. Whether HFmrEF represents a distinct subtype of HF or is a transitional stage between HFrEF and HFpEF remains controversial. In terms of the longitudinal trajectory of LVEF and transitions among the three HF groups, HFmrEF resembles a transitional stage between HFrEF and HFpEF rather than a unique subtype, including patients who have recovered from previous HFrEF, patients who have deteriorated from previous HFpEF, and patients with a relatively stable LVEF in the range of 40–50%. More importantly, different LVEF trajectories of

patients with HFmrEF often indicate different prognoses. A refined classification may be helpful to further understand the clinical characteristics and pathophysiology of HFmrEF, and to make optimized and individualized treatment decisions.

AUTHOR CONTRIBUTIONS

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

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MELD-XI Score Is Associated With Short-Term Adverse Events in Patients With Heart Failure With Preserved Ejection

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Aim: Accumulating evidence suggests that MELD-XI score holds the ability to predict the prognosis of congestive heart failure. However, most of the evidence is based on the end-stage heart failure population; thus, we aim to explore the association between the MELD-XI score and the prognosis in heart failure with preserved ejection fraction (HFpEF).

Methods: A total of 30,096 patients hospitalized for HFpEF in Fujian Provincial Hospital between January 1, 2014 and July 17, 2020 with available measures of creatinine and liver function were enrolled. The primary endpoint was 60-day in-hospital all-cause mortality. Secondary endpoints were 60-day in-hospital cardiovascular mortality and 30-day rehospitalization for heart failure.

Results: A total of 222 patients died within 60 days after admission, among which 75 deaths were considered cardiogenic. And 73 patients were readmitted for heart failure within 30 days after discharge. Generally, patients with an elevated MELD-XI score tended to have more comorbidities, higher NYHA class, and higher inflammatory biomarkers levels. Meanwhile, the MELD-XI score was positively correlated with NT-pro BNP, left atrial diameter, E/e' and negatively correlated with LVEF. After adjusting for conventional risk factors, the MELD-XI score was independently associated with 60-day in-hospital all-cause mortality [hazard ratio(HR) = 1.052, 95% confidential interval (CI) 1.022–1.083, $P = 0.001$], 60-day in-hospital cardiovascular mortality (HR = 1.064, 95% CI 1.013–1.118, $P = 0.014$), and 30-day readmission for heart failure (HR = 1.061, 95% CI 1.015–1.108, $P = 0.009$). Furthermore, the MELD-XI score added an incremental discriminatory capacity to risk stratification models developed based on this cohort.

Conclusion: The MELD-XI score was associated with short-term adverse events and provided additional discriminatory capacity to risk stratification models in patients hospitalized for HFpEF.

Keywords: heart failure with preserved ejection fraction, MELD-XI score, prognosis, short-term, risk stratification

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INTRODUCTION

Patients with heart failure with preserved ejection fraction(HFpEF) account for 22–73% of the total population of heart failure (1). Compared with patients with heart failure with reduced ejection fraction(HFrEF), the prognosis of patients with HFpEF appears to be better, but there have not been many improvements throughout the years (2). Previous studies underscored that HFpEF is often accompanied by liver dysfunction and renal dysfunction, which are independent predictors for a poor prognosis (3–7). The mutual pathophysiological mechanism shared by HFpEF, impaired liver, and renal function is speculated to be the combination of insufficient perfusion, hormonal imbalance, and inflammation (3, 6, 8).

As an indicator of liver dysfunction and renal dysfunction, model of end-stage liver dysfunction (MELD-XI), has been shown to be negatively correlated with the survival rate in end-stage heart failure patients undergoing cardiac transplantation. It is used as a simple tool to assess appropriate candidates (9, 10). Furthermore, recent studies also suggested that the MELD-XI score is associated with poor prognosis in congestive and acute heart failure (11, 12).

To our knowledge, there is sparse evidence addressing the predictive value of the MELD-XI score in HfpEF, and this study may represent the largest real-world cohort to evaluate the utility of the MELD-XI score calculated at admission as a predictor for short-term prognoses in patients hospitalized for HFpEF.

METHODS

Data Source and Study Definition

Study Population

A total of 30,096 patients hospitalized for HFpEF in Fujian Provincial Hospital between January 1, 2014 and July 17, 2020 with available measures of liver function were retrospectively enrolled. HFpEF was defined based on the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, which is characterized by a left ventricular ejection fraction (LVEF) $\geq$50% (1). Patients with acute coronary syndrome, acute phase of stroke, advanced cancer, pregnancy, active rheumatic disease, advanced liver cirrhosis, and undergoing dialysis were excluded.

Endpoints

The primary endpoint was 60-day in-hospital all-cause mortality. Secondary endpoints were 60-day in-hospital cardiovascular mortality and 30-day rehospitalization for heart failure. The 30-day readmission for heart failure was defined as hospital admission for decompensated heart failure from 24 h to 30 days after discharge. In-hospital cardiovascular mortality was death, consistent with a ventricular tachyarrhythmia, and occurred in the absence of a known non-cardiac condition as the proximate cause of the death (13).

TABLE 1 | Baseline characteristics between patients with elevated and normal MELD-XI score.

	Elevated MELD-XI score (n = 3,429)	Normal MELD-XI score (n = 26,652)	*P*-value
Demographics			
Age(years)	67.5 ± 14.0	70.9 ± 12.6	<0.001
Gender(Male)%	2,330(67.9%)	16,741(62.8%)	<0.001
BMI(kg/m^2)	23.5(21.0–26.0)	24.2(21.9–26.6)	<0.001
Medical history			
Hypertension(%)	3189(92.9%)	20803(78.1%)	<0.001
Diabetes(%)	1780(51.9%)	10325(38.7%)	<0.001
Atrial fibrillation(%)	345(10.1%)	3948(14.8%)	<0.001
Ischemic heart disease(%)	1212(35.3%)	16398(61.5%)	<0.001
Valvular heart disease(%)	399(11.6%)	3,477(13.0%)	0.02
Myocardial infarction(%)	296(8.6%)	2,507(9.4%)	<0.001
Stroke(%)	174(5.1%)	1,132(4.2%)	0.025
Chronic kidney disease(%)	464(13.5%)	209(0.8%)	<0.001
Liver disease(%)	297(8.7%)	1,329(5.0%)	<0.001
NYHA class 2(%)	1,138(38.0%)	17,520(70.7%)	<0.001
NYHA class 3(%)	1,156(38.6%)	5,707(23.0%)	
NYHA class 4(%)	704(23.5%)	1,562(6.3%)	
Laboratory Measures			
Troponin I(ng/ml)	0.060(0.027–0.142)	0.013(0.006–0.034)	<0.001
NT-pro BNP(pg/ml)	9,167(2,861–28,464)	369(101–1,446)	<0.001
HDL(mmol/L)	0.94 ± 0.40	1.10 ± 0.37	<0.001
LDL(mmol/L)	2.46 ± 1.13	2.57 ± 1.02	<0.001
Triglyceride(mmol/L)	1.73 ± 1.47	1.51 ± 1.15	<0.001
Total cholesterol(mmol/L)	4.13 ± 1.54	4.08 ± 1.19	0.049
WBC (10^9/L)	6.48 ± 4.21	4.93 ± 2.89	<0.001
Hemoglobin(g/L)	89.72 ± 24.85	127.49 ± 22.41	<0.001
Platelet(10^9/L)	200.64 ± 90.12	212.22 ± 80.18	<0.001
Na+(mmol/L)	137.11 ± 5.52	139.53 ± 4.64	<0.001
K+(mmol/L)	4.35 ± 0.82	4.05 ± 0.49	<0.001
CRP(mg/L)	23.1(5.3–78.1)	12.2(2.81–43.8)	<0.001
HbA1c (%)	6.37 ± 1.38	6.64 ± 1.39	<0.001
D-dimer(mg/L)	1.58(0.81–3.11)	0.58(0.30–1.28)	<0.001
Echocardiography			
LVEF	0.58 ± 0.04	0.59 ± 0.05	<0.001
Left Atrial Diameter(cm)	4.12(3.60–4.57)	3.72(3.36–4.21)	<0.001
E/e'	13.00 (10.00–17.4)	11.00 (8.83–14.00)	<0.001
Medication			
ACEIs/ARBs/ARNIs(%)	1,410(41.2%)	16,760(63.1%)	<0.001
MRAs(%)	574(16.8%)	6,732(25.3%)	<0.001
Diuretics(%)	2,536(74.0%)	10,279(38.7%)	<0.001
Digoxin(%)	448(13.1%)	3,590(13.5%)	0.481
I.v. inotropes(%)	568(16.6%)	4,120(15.5%)	0.104
Beta-blockers(%)	1,677(48.0%)	12,725(47.9%)	0.246
Lipid-Lowering Agents(%)	2,283(66.6%)	17,396(65.5%)	0.179
Nitrates(%)	1,128(32.9%)	8,430(31.7%)	0.158
Anticoagulants(%)	1,851(54%)	14,088(53%)	0.27

BMI, body mass index; LVEF, left ventricular ejection fraction; NYHA class, New York Heart Association class; NT-pro BNP, N-terminal pro brain natriuretic peptide; HDL, high density lipoprotein; LDL, low density lipoprotein; WBC, white blood cell count; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; ARNI, angiotensin II type 1 receptor blockade and neprilysin inhibitor; MRA, mineralcorticoid receptor antagonist.

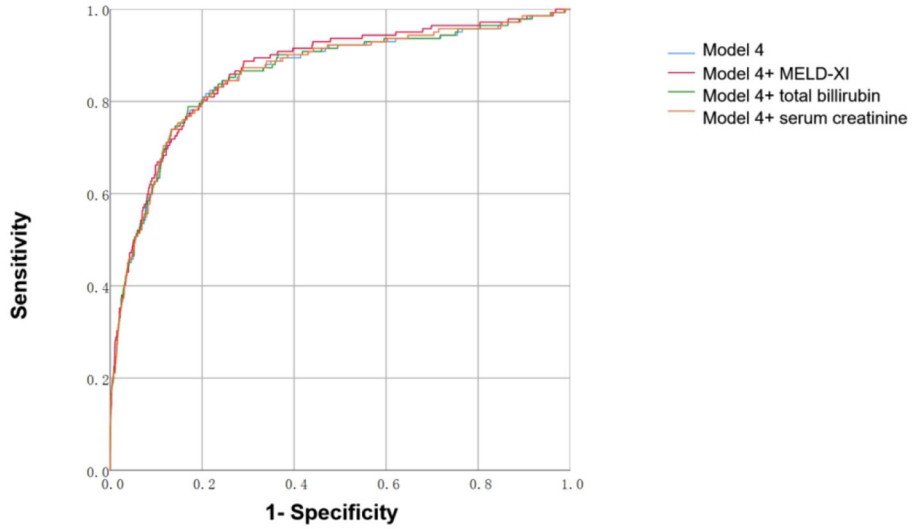

FIGURE 1 | Kaplan–Meier estimates of short-term clinical outcomes of according to baseline MELD-XI score.

TABLE 2 | Risk of short-term events for each 1-point increase in MELD-XI score.

	HR(95% CI)	*P*-value
60-Day In-Hospital All-Cause Mortality		
Model 1	1.126 (1.090–1.164)	<0.001
Model 2	1.115 (1.093–1.093)	0.001
Model 3	1.052 (1.022–1.083)	0.001
60-Day In-Hospital Cardiovascular Mortality		
Model 1	1.125 (1.087–1.163)	<0.001
Model 2	1.063 (1.012–1.117)	0.015
Model 3	1.064 (1.013–1.118)	0.014
30-Day Readmission		
Model 1	1.108 (1.069–1.148)	<0.001
Model 2	1.064 (1.018–1.111)	0.006
Model 3	1.061 (1.015–1.108)	0.009

Model 1: *Adjusted for age and gender.*
Model 2: *Adjusted for age, gender, BP at admission >140/90 mmHg, history of diabetes, history of atrial fibrillation, history of stroke, history of myocardial infarction, New York Heart Association (NYHA) class, left ventricular ejection fraction (LVEF), white blood cell count, triglyceride.*
Model 3: *Adjusted for age, gender, BP at admission >140/90 mmHg, history of diabetes, history of atrial fibrillation, history of stroke, history of myocardial infarction, NYHA class, LVEF, white blood cell count, triglyceride, use of digoxin, use of intravenous inotropes, use of beta-blocker, use of lipid-lowering agents.*

Biochemical Measurements

Liver function tests and renal function tests were quantified in a core laboratory (Roche, Modular-P chemical analyzer). Based on routine laboratory standards in Fujian Provincial Hospital, the upper limit was 23 μmol/L (1.35 mg/dl) for total bilirubin, similar to the standard published by previously studies (7).

The MELD-XI score was calculated as follows: 5.11 × Ln (total bilirubin as mg/dl)+11.76 × Ln (creatinine as mg/dl) + 9.44 (14). A MELD-XI score > 9.44 was considered elevated (12). eGFR was calculated using the simplified modification of diet in renal disease (MDRD) formula described by the National Kidney Foundation as follows: eGFR (ml/min/1.73 m^2) $= 175 \times (\text{Scr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female})$ (15).

Statistical Analysis

Continuous variables were presented as mean ± SD when normally distributed, as median and interquartile range (IQR) when skewed. Categorical variables were presented as frequencies and percentages. Differences between groups were evaluated by Student t, Mann-Whitney *U* test, or chi-square test as appropriate. The Spearman correlation test was applied to explore the association between the MELD-XI score and NT-pro BNP. A total of nine cox regression models were developed to estimate the association between the baseline MELD-XI score and the three study endpoints. Covariates in model 1 included age and gender. Covariates in model 2 included age, gender, elevated blood pressure (BP) at admission (>140/90mmHg), history of diabetes, history of atrial fibrillation, history of stroke, history of myocardial infarction, New York Heart Association (NYHA) class, LVEF, white blood cell count (WBC), triglyceride. Covariates in model 3 included age, gender, elevated BP at admission, history of diabetes, history of atrial fibrillation, history of stroke, history of myocardial infarction, NYHA class, NT-pro BNP, LVEF, WBC, triglyceride, use of digoxin, use of intravenous inotropes, use of beta-blocker, use of lipid-lowering agents. Models 1, 2, and 3 were used to estimate the risk of the three endpoints separately. Logistic regression and area under the receiver operating characteristic (ROC) curve were applied to evaluate the discriminatory capacity of the models for predicting 60-day all-cause in-hospital mortality. After adjusting for covariates stated above, age, LVEF, NYHA class, WBC, triglyceride remained independent risk factors, and thus were included in model 4.

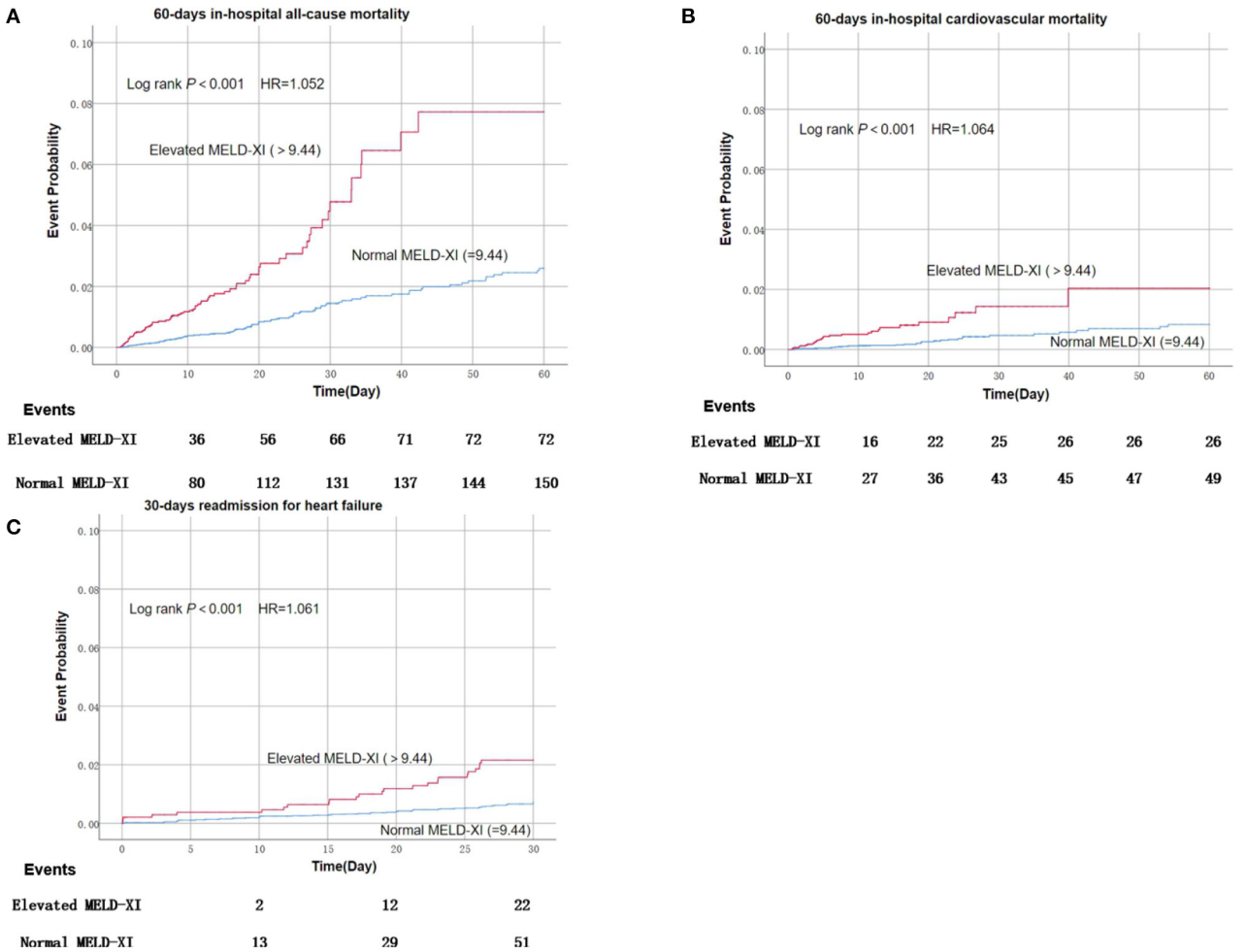

FIGURE 2 | (A-C) Area under the receiver operating characteristic curve of models predicting 60-day in-hospital all-cause mortality and cumulative events. Model 4: Adjusted for age, LVEF, NYHA class, WBC, triglyceride. Calculated as: $-0.015 + 0.019 \times$ age $-14.604 \times$ LVEF $+ 1.293 \times$ NYHA class III (1) [OR $2.871 \times$ NYHA Class IV (1)] $+ 0.046 \times$ WBC(10^9/L) $+ 0.118 \times$ triglyceride(mmol/L). Model 4+MELD-XI: $-0.420 + 0.024 \times$ age $-14.488 \times$ LVEF $+ 1.135 \times$ NYHA class III (1) [OR $2.567 \times$ NYHA Class IV (1)] $+ 0.043 \times$ WBC(10^9/L) $+ 0.109 \times$ triglyceride(mmol/L) $+ 0.053 \times$ MELD-XI. Model 4+serum creatinine: $-0.163 + 0.021 \times$ age $-14.593 \times$ LVEF $+ 1.267 \times$ NYHA class III (1) [OR $2.830 \times$ NYHA Class IV (1)] $+ 0.046 \times$ WBC(10^9/L) $+ 0.116 \times$ triglyceride(mmol/L) $+ 0.031 \times$ serum creatinine(mg/dl). Model 4+total bilirubin: $-0.128 + 0.020 \times$ age $-14.639 \times$ LVEF $+ 1.282 \times$ NYHA class III (1) [OR $2.842 \times$ NYHA Class IV (1)] $+ 0.044 \times$ WBC(10^9/L) $+ 0.118 \times$ triglyceride(mmol/L) $+ 0.083 \times$ total bilirubin (mg/dl).

These analyses were conducted using a statistical software package (SPSS version 25.0, IBM, Armonk, NY, USA). A *P*-value < 0.05 was considered statistically significant.

RESULTS

Study Population

Participants had a mean age of 70.7 ± 12.8, 64.1% were male, 19,048(57.6%) had an ischemic etiology of heart failure, 1,627(5.4%) had history of liver disease, 2,299(7.6%) had elevated total bilirubin(>1.35 mg/dl), 673(2.2%) had a previous diagnosis of chronic kidney disease(CKD), and 9,715(32.3%)had an eGFR < 60 ml/min/1.73 m^2.

Generally, patients with elevated MELD-XI score tended to be male, had worse cardiac function, and higher inflammatory biomarker levels. Furthermore, patients with elevated MELD-XI score were more likely to have previous diagnosis of hypertension and diabetes mellitus. However, patients with an elevated MELD-XI score seemed to receive less prescription of angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blocker (ARB), angiotensin II type 1 receptor blockade and neprilysin inhibitor (ARNI), and mineralcorticoid receptor antagonist (MRA) (**Table 1**).

Association Between Cardiac Function and MELD-XI Score

Patients with elevated MELD-XI scores had significantly higher NT-pro BNP levels (Median = 9,167 pg/ml vs. 369 pg/ml, $P <$ 0.001), higher left atrial diameter (Median = 4.12 cm vs. 3.72 cm,

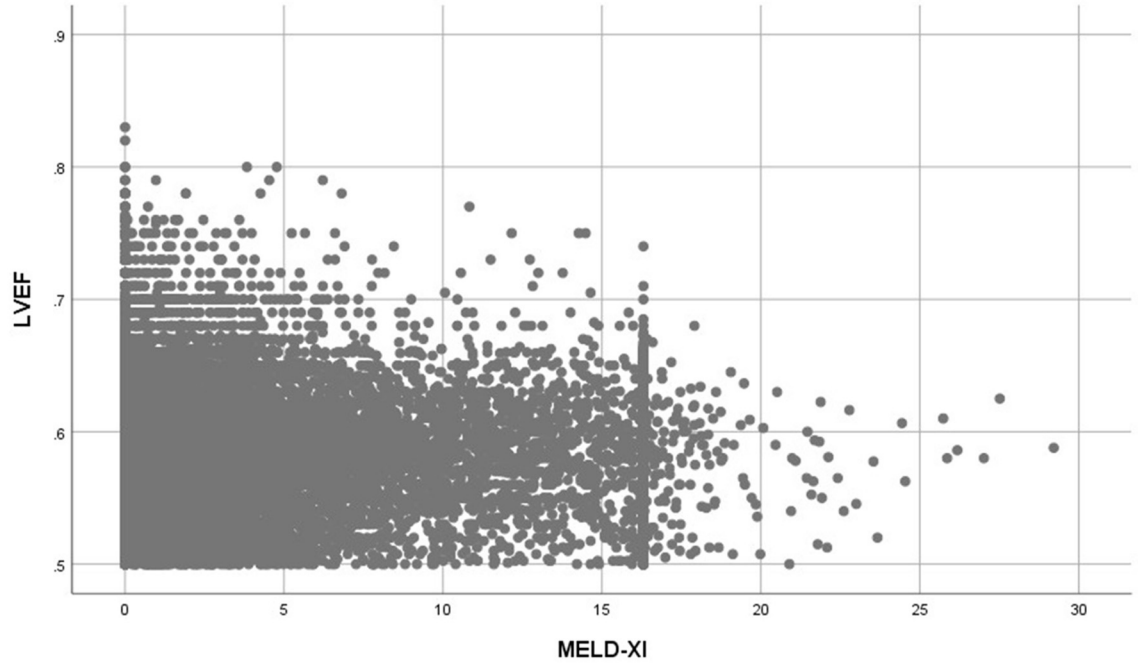

FIGURE 3 | Scatter plot between LVEF and MELD-XI score.

TABLE 3 | AUC of models predicting 60-day in-hospital all-cause mortality.

	AUC (95% CI)	*P*-value
Model 4	0.858 (0.821–0.894)	<0.001
Model 4+ MELD-XI	0.868 (0.835–0.901)	<0.001
Model 4+ total bilirubin	0.859 (0.823–0.895)	<0.001
Model 4+ serum creatinine	0.86 (0.824–0.895)	<0.001

$P < 0.001$), higher E/e' (Median = 13.00 vs. 11.00, $P < 0.001$) and lower LVEF (0.58 ± 0.04 vs. 0.59 ± 0.05, $P < 0.001$) (**Table 1**). The MELD-XI score had a moderate correlation with NT-pro BNP (Spearman rho = 0.497, $P < 0.001$) and a weak correlation with left atrial diameter (Spearman rho = 0.209, $P < 0.001$) and E/e' (Spearman rho = 0.090, $P < 0.001$). A weak and inverse correlation was also determined between the MELD-XI score and LVEF (Spearman rho = −0.084, $P < 0.001$) (**Figure 3**).

MELD-XI Score and Short-Term Adverse Events

A total of 222 patients died within 60 days after admission, among which 75 deaths were considered cardiogenic. And among 29,844 living patients within 30 days after discharge, 73 were readmitted for heart failure within 30 days after discharge. Patients with elevated MELD-XI scores suffered a significantly higher risk of mortality, cardiovascular mortality, and readmission for heart failure compared with those with normal MELD-XI (**Figure 1**). After adjusting for conventional risk factors, the MELD-XI score remained a predominant predictor for 60-day in-hospital all-cause mortality (HR = 1.052, 95%CI 1.022–1.083, $P = 0.001$), 60-day in-hospital cardiovascular mortality (HR = 1.064, 95% CI 1.013–1.118, $P = 0.014$), and 30-day readmission (HR = 1.061, 95% CI 11.015–1.108, $P = 0.009$) (**Table 2**).

Predictive Value of Models Includes MELD-XI

We constructed risk stratification models for prediction of in-hospital all-cause mortality. As shown in **Figure 2** and **Table 3**, adding the MELD-XI score slightly increased the discriminatory capacity of model 4 (AUC = 0.868 vs. 0.858, $P = 0.0162$), which was better than models including total bilirubin or serum creatinine (AUC = 0.868 vs. 0.859, $P = 0.0296$; AUC = 0.868 vs. 0.860, $P = 0.0161$).

DISCUSSION

It has been noticed that both liver dysfunction and renal dysfunction are common comorbidities during congestive heart failure as well as associated with ominous prognosis (3–7). As a combined index of liver function and renal function, it has been shown that the MELD-XI score holds the ability to predict adverse events in patients with acute heart failure and end-stage heart failure (11, 12). To our knowledge, this study is the first to find the MELD-XI score to be a risk factor for short-term adverse events, improving risk stratification models for patients hospitalized with HFpEF.

The predictive ability of liver dysfunction and renal dysfunction have been confirmed in heart failure. A study

by Allen et al. (16) demonstrated that abnormal liver function tests are prevalent in patients with heart failure, and total bilirubin could be one of the most powerful predictors for poor prognosis among all liver function tests. Furthermore, Prenner et al. (5) conducted a *post-hoc* study of TOP-CAT trial and indicated that albumin was also a strong predictor of adverse events in patients with HFpEF. Data from a most recent *post-hoc* study of TOP-CAT suggested that not only kidney function tests but also their variability between visits are independently correlated with clinical outcomes (17).

As a modification of the MELD score, the MELD-XI score excludes international normalized ratio and consists of only total bilirubin and serum creatinine, thus could be applied to heart failure patients receiving anticoagulants. Previous investigators confirmed that higher MELD-XI value is associated with mortality in patients with advanced heart failure undergoing heart transplantation, thus could serve as a simple quantitative tool for screening appropriate candidates receiving heart transplantation (11, 18). Afterward, the MELD-XI score was also validated in patients with acute heart failure as an innovative prognosticator (12, 19). Recently Abe et al. (20) retrospectively analyzed 562 patients with decompensated heart failure and concluded that MELD-XI was an independent predictor of mortality in general heart failure patients. In our cohort, 53% patients received anticoagulants; hence, MELD-XI seems an appropriate approach to estimate liver and renal function compared to original MELD score and MELD-Na score. And to extend this conclusion from previous study, we analyzed short-term events separately and confirmed the prognostic role of MELD-XI in the setting of HFpEF.

Analyzing each possible risk factor is the foundation of accurate risk stratification, which ultimately contributes to the management strategy of patients with HFpEF (1). A Seattle Heart Failure Model was derived from the population of general heart failure then validated in 9,942 patients, which provided an overall AUC of 0.729 (21). The MUSIK risk score, which contains 10 variables (eGFR), was developed in a cohort of 992 ambulatory heart failure patients and demonstrated good performance of predicting mortality (22). Risk stratification models developed in our study contained only six variables and yielded good discriminatory capacity for short-term adverse events, which was improved slightly by adding the MELD-XI score.

The pathophysiological connection between MELD-XI and HFpEF might consist of several aspects. First, decompensated cardiac function provides insufficient perfusion to both the kidneys and liver, thus leading to decreased glomerular filtration rate as well as tissue damage of renal and liver (6, 8, 23). Second, elevated right atrial pressure due to heart failure, especially HFpEF, usually results in passive hepatic venous congestion, lipid metabolism disorder, and even cirrhosis (4, 23–25). Third, neurohumoral disorder results in vascular contractility disorder in HfpEF, which further reduces renal blood flow and liver perfusion (25, 26). And in turn, impaired liver and renal function contribute to hemodynamic changes and inflammatory activity, eventually resulting in cardiac stiffness (3, 6, 24). Data from our study have confirmed the association between MELD-XI and NT-pro BNP, which reinforced that volume overload is the reflexion of the vicious cycle formed by HFpEF and impaired liver and renal function. And we also found that MELD-XI was correlated with cardiac function indexes derived from echocardiography, which supports that impaired liver and renal function is responsible for the deteriorated cardiac stiffness during HFpEF.

Our study demonstrated that the MELD-XI score not only was associated with short-term adverse events but also offered increased discriminatory ability for risk stratification models in patients hospitalized for HFpEF.

LIMITATION

First, as a retrospective study based on the data of a single center, the conclusion should be further validated in different regions and different populations. Second, we failed to analyze the association between long-term prognosis and the MELD-XI score since we have not been able to complete the follow-up procedure due to the large scale of this study, although it is still crucial to focus on the risk stratification of long-term events. Third, considering the extendibility of the conclusion, we only excluded patients with advanced liver cirrhosis and those undergoing dialysis, not all patients with previously diagnosed liver disease and renal disease.

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 Fujian Provincial Hospital Ethics Committee (福建省立医院伦理委员会). 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

SW and YW built this large scale database containing the clinical information of heart failure patients in Fujian Provincial Hospital. SW and XiaoX conducted the statistical analysis. YG is the overseer of this study. Rest of the team contributed to the idea gathering. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: YW is a software engineer employed by Fujian Yirong Information Technology Corporation and she provided her information technological support for free.

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.

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CORRECTION
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Corrigendum: MELD-XI Score Is Associated With Short-Term Adverse Events in Patients With Heart Failure With Preserved Ejection

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Keywords: heart failure with preserved ejection fraction, MELD-XI score, prognosis, short-term, risk stratification

A Corrigendum on

MELD-XI Score Is Associated With Short-Term Adverse Events in Patients With Heart Failure With Preserved Ejection

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There is an error in the title. The correct title for "MMMELD-XI Score Is Associated With Short-Term Adverse Events in Patients With Heart Failure With Preserved Ejection Fraction" is "MELD-XI Score Is Associated With Short-Term Adverse Events in Patients With Heart Failure With Preserved Ejection."

In the published article, there was an error regarding the affiliation(s) for Xiaoxu Xie. Instead of "Department of Cardiology, Shengli Clinical Medical College, Fujian Medical University, Fuzhou, China," it should be "Department of Epidemiology and Health Statistics, School of Public Health, Fujian Medical University, Fuzhou, China." In the published article, there was also an error regarding the affiliation(s) for Yuwei Wang. Instead of "Department of Cardiology, Shengli Clinical Medical College, Fujian Medical University, Fuzhou, China," it should be "Fujian Yirong Information Technology Corporation, Fuzhou, China." In the published article, there was an error regarding the affiliation(s) for Yansong Guo, Sunying Wang, Manqing Luo, Kaiyang Lin, Na Lin, Qingyong Yang, Tian Zou, Xinan Chen, and Xianwei Xie. Instead of affiliation(s) "Department of Cardiology, Shengli Clinical Medical College, Fujian Medical University, Fuzhou, China," it should be "Department of Cardiology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou, China."

In the published article, there was an error regarding the affiliation(s) for Yansong Guo. As well as having affiliation 1 "Department of Cardiology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou, China," they should also have affiliation 4 "Fujian Provincial Key Laboratory of Cardiovascular Disease, Fujian Provincial Center for

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The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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ORIGINAL RESEARCH
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Weight Change and Mortality Risk in Heart Failure With Preserved Ejection Fraction

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Aims: The aim of the study was to determine the associations of weight loss or gain with all-cause mortality risk in heart failure with preserved ejection fraction (HFpEF).

Methods and Results: Non-lean patients from the Americas from the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist study were analyzed (n = 1,515). Weight loss and weight gain were defined as a decrease or increase in weight ≥5% between baseline and 1 year. To determine the associations of weight change and mortality risk, we used adjusted Cox proportional hazards models and restricted cubic spline models. The mean age was 71.5 (9.6) years. Weight loss and gain were witnessed in 19.3 and 15.9% patients, respectively. After multivariable adjustment, weight loss was associated with higher risk of mortality (HR 1.42, 95% CI 1.06–1.89, P = 0.002); weight gain had similar risk of mortality (HR 0.98, 95% CI 0.68–1.42, P = 0.932) compared with weight stability. There was linear relationship between weight change and mortality risk. The association of weight loss and mortality was different for patients with and without diabetes mellitus (interaction p = 0.009).

Conclusion: Among patients with HFpEF, weight loss was independently associated with higher risk of all-cause mortality, and weight gain was not associated with better survival.

Clinical Trial Registration: https://clinicaltrials.gov, Identifier: NCT00094302.

Keywords: HFpEF, weight gain, weight loss, mortality, heart failure

INTRODUCTION

Prior studies (1–3) of patients with established heart failure (HF) demonstrated more favorable prognosis in patients with obesity vs. normal weight. The "obesity paradox" led to further investigations on weight change and mortality in patients with chronic HF. Both the American College of Cardiology/American Heart Association guideline (4), and the European Society of

Cardiology guideline (5) in HF have not provide conclusive recommendations about weight control. Several informative studies (6–10) that mainly focus on HF with reduced ejection fraction (HFrEF) have shown that both weight loss and weight gain were associated with poor prognosis. However, robust evidence regarding the relation of weight change and long-term prognosis in heart failure with preserved ejection fraction (HFpEF) is missing, despite HFpEF accounts for over half of the overall HF burden all over the world (11–13). Moreover, prior reviews (14–16) raised the differences in baseline characteristics of patients, including gender and prevalence of comorbidities that may account for the "obesity paradox." Whether patients' characteristic-related differences existed on weight change and HF prognosis remains unknown.

The TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) was a large international trial among patients with HFpEF, where the effect of the spironolactone was compared with placebo for mortality and morbidity. The main aim of this analysis was to assess the effect of weight loss or gain over a 1-year follow-up period on subsequent mortality in patients with HFpEF enrolled in the Americas in TOPCAT, with further exploration of the interaction between weight change and patients' characteristics and spironolactone treatment.

METHODS

TOPCAT Study Design and Objectives

The design of the TOPCAT trial has been described in detail previously (17). Briefly, TOPCAT was a multicenter, international, randomized, double blind, placebo-controlled trial of spironolactone in adults with HFpEF recruited from over 270 clinical sites. The trial was funded by the National Heart, Lung, and Blood Institute as a contract with the Brigham and Women's Hospital (Clinical Coordinating Center) and the New England Research Institute (Data Coordinating Center). Enrollment began in August 2006 and ended in January 2012, and the primary results of the trial were published in April 2014 (18). The primary aim was to determine whether treatment with spironolactone, compared with placebo, can produce a clinically meaningful reduction in the composite outcome of cardiovascular mortality, aborted cardiac arrest, or HF hospitalization in adults with symptomatic HF and documented LVEF ≥45%. All study participants provided written informed consent.

Data on vital signs, including body weight and height, were collected at baseline. Patients were followed at 1, 2, 4, 8, 12, and 18 months, and every year thereafter, at which times data on vital signs, including body weight, were collected. Patients were followed for a median of 3.5 years (18).

For the present study, we excluded (i) patients from Russia and Georgia ($n = 1{,}678$), given the significant regional differences previously described (19), (ii) missing body weight or body mass index (BMI) < 18.5 kg/m^2 at baseline ($n = 16$), (iii) missing body weight at both 1-year follow-up and the follow-up close to it (8 and 18 months) ($n = 183$), and (iv) died before 1-year follow-up ($n = 12$). Death from all causes was the main outcome.

Definition of Weight Change and Obesity

Weight change was defined as the change in body weight from the baseline measurement to the end of the first year of follow-up. For 118 participants (7.8%) missing body weight at 1-year follow-up, we impute with measures at 8 or at 18 months if it missing at 8 months. A positive value means increased weight, and a negative value means decreased weight. Patients were classified according to weight change into three strata as follows: weight loss (weight witnessed a decrease of ≥5%), weight stability (weight change <5%), and weight gain (weight witnessed an increase of ≥5%). BMI was analyzed according to weight and height at baseline using the formula weight (kg)/(height in m)^2. Obesity was defined as BMI ≥ 30 kg/m^2 based on the criteria defined by the World Health Organization (WHO Technical Report Series, no 854, Geneva, 1999). In present analysis, non-obesity was defined as BMI 18.5 to <30 kg/m^2.

Statistical Analysis

Categorical variables were described by frequencies with percentages, and continuous variables were described by median with interquartile ranges (IQR) or mean with standard deviation (SD). Demographic and clinical characteristics were compared among weight change groups using the Kruskal–Wallis test for continuous variables and chi-squared tests for categorical variables.

Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals for mortality, starting from the first year follow-up, associated with weight loss and weight gain using weight stability as reference. Multivariable models adjusted for age, gender, race, smoking status, New York Heart Association (NYHA) class, estimated glomerular filtration rate (eGFR), heart rate, systolic blood pressure (SBP), ejection fraction, diabetes status, atrial fibrillation, peripheral arterial disease, previous hospitalization for HF, prior myocardial infarction, stroke, chronic obstructive pulmonary disease (COPD), baseline BMI, presence of edema, and assignment to spironolactone vs. placebo, using stepwise selection. Covariates were chosen based upon a combination of clinical relevance and previous prognostic implication in the TOPCAT. In addition, we did the Cox regression multivariable analyses using standardized weight change as continuous variable (with 1 SD decrease). To assess for possible non-linearity, we fitted restricted cubic spline models with five knots at the 5, 25, 50, 75, and 95th percentiles of standardized weight change.

Subgroups analyses were conducted to explore interactions on weight change and mortality. Cox regression multivariable analyses using weight change as both categorical and continuous variable were repeated after stratifying patients into different subgroups as follows: obesity or non-obesity, with or without diabetes mellitus, women or men, and allocated to spironolactone or placebo.

All statistical analyses were conducted using SAS statistical software version 9.4 (SAS Institute Inc.), and the forest plot was made using Excel version 15.23 (Microsoft Institute Inc.).

TABLE 1 | Baseline demographic and clinical characteristics by weight change groups.

	All (1,515)	Weight loss (*n* = 293)	Weight stability (*n* = 981)	Weight gain (*n* = 241)	*p*
Weight change, mean (SD), (kg)	−0.5 (6.43)	−9.1 (5.8)	−0.2 (2.6)	8.6 (4.4)	<0.001
Demographic					
Age, median (IQR), year	72 (64–79)	73 (63–79)	73 (65–80)	68 (62–76)	<0.001
Women, *n* (%)	749 (49.4)	162 (55.3)	471 (48.0)	116 (48.1)	0.083
Race, *n* (%)					0.186
White	1,199 (79.1)	223 (76.1)	790 (80.5)	186 (77.2)	
Black	247 (16.3)	56 (19.1)	144 (14.7)	47 (19.5)	
Clinical					
Randomization to spironolactone, *n* (%)	771 (50.9)	151 (51.5)	502 (51.2)	118 (49.0)	0.803
Current smoker, *n* (%)	89 (5.9)	25 (8.5)	48 (4.9)	16 (6.6)	0.040
Medical history, *n* (%)					
Previous hospitalization for CHF	873(57.6)	166 (56.7)	538 (54.8)	169 (70.1)	<0.001
Previous myocardial infarction	323 (21.3)	59 (20.1)	217 (22.1)	47 (19.5)	0.587
Stroke	141 (9.3)	36 (12.3)	91 (9.3)	14 (5.8)	0.036
COPD	239 (15.8)	58 (19.8)	139 (14.2)	42 (17.4)	0.048
Hypertension	1,360 (89.8)	250 (85.3)	891 (90.8)	219 (90.9)	0.030
Peripheral arterial disease	182 (12.0)	36 (12.3)	123 (12.5)	23 (9.5)	0.433
Atrial fibrillation	657 (43.4)	121 (41.3)	437 (44.5)	99 (41.1)	0.470
Diabetes mellitus	667 (44.0)	120 (41.0)	420 (42.8)	127 (52.7)	0.011
Physical examination					
NYHA class III/IV, *n* (%)	515 (34.0)	117(39.9)	316 (32.2)	82 (34.0)	0.051
Presence of edema, *n* (%)	1,077 (71.1)	213 (72.7)	707 (72.1)	157 (65.1)	0.232
Heart rate, median (IQR), (bpm)	68 (60–76)	68 (62–76)	68 (60–75)	68 (61–76)	0.532
SBP, median (IQR), (mmHg)	129 (118–138)	126 (116–138)	130 (118–138)	128 (118–138)	0.122
Body mass index, median (IQR), (kg/m^2)	32.8 (28.1–38.4)	33.1 (27.9–39.4)	32.7 (28.2–37.9)	32.9 (28.0–39.1)	0.525
Laboratory and imaging testing, median (IQR), %					
Ejection fraction	59 (53–65)	57 (53–60)	60 (53–65)	59 (51–65)	0.094
eGFR	61.6 (49.6–76.5)	63.0 (51.1–78.1)	60.8 (48.9–75.1)	63.5 (49.8–79.4)	0.119
Medication					
Diuretics	1,343 (88.6)	255 (87.0)	876 (89.3)	212 (88.0)	0.550

COPD, chronic obstructive pulmonary disease; CHF, chronic heart failure; NYHA, New York Heart Association; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate.

All comparisons were two sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

Among all study populations, 1,515 participants met the inclusion criteria for the present analysis. The mean (SD) age was 71.5 (9.6) years; 49.4% were women, and 79.1% were White. The median weight change was −0.45 kg (IQR −3.63 to 2.76, range −50.3 to 27.2) during the first year of follow-up. Among all patients, 19.3% experienced weight loss, and 15.9% experienced weight gain. **Table 1** lists the baseline characteristics of the study population, stratified by weight change groups. Patients in the weight loss group were more often current smoker, more often had history of stroke and COPD, and less often had history of hypertension, and patients in the weight gain group were younger age, more commonly had diabetes mellitus and previous hospitalization for HF, and less commonly had history of stroke.

Association of Weight Change and All-Cause Mortality

During a mean subsequent follow-up of 2.5 years after the first year, all-cause mortality occurred in 65 (22.2%), 175 (17.8%), and 36 (14.9%) patients with weight loss, weight stability, and weight gain, respectively. In the multivariable model adjusted for age, gender, race, smoking status, NYHA class, eGFR, heart rate, SBP, ejection fraction, diabetes status, atrial fibrillation, peripheral arterial disease, previous hospitalization for HF, prior myocardial infarction, stroke, COPD, baseline BMI, presence of edema and assignment to spironolactone, weight loss was associated with a higher risk of mortality (HR 1.42, 95% CI 1.06–1.89, $P = 0.002$), and weight gain had similar risk of mortality (HR 0.98, 95% CI 0.68–1.42, $P = 0.932$), compared with weight stability (**Table 2**). Findings from restricted cubic spline analysis demonstrate that there was a linear relationship between weight change as a continuous variable and all-cause mortality ($P = 0.194$ for overall relationship) (**Figure 1**). Similar linear relationship was found between relative changes in body weight

TABLE 2 | Multivariable Cox regression analysis for all-cause mortality.

Covariates	HR	95% CI	*p*
Weight loss*	1.42	1.06–1.89	0.018
Weight gain*	0.98	0.68–1.42	0.932
SBP	0.99	0.98–1.00	0.008
Age	1.04	1.03–1.06	<0.001
Women	0.63	0.49–0.81	<0.001
Black race#	1.90	1.15–3.12	0.012
Other race#	0.90	0.60–1.35	0.624
Previous hospitalization for CHF	1.37	1.06–1.76	0.015
Diabetes mellitus	1.43	1.11–1.85	0.006
eGFR	0.99	0.98–1.00	0.007

*Using weight stability as reference.
#Using white race as reference.
SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate.

and mortality (**Supplementary Figure 1**). One SD decrease in weight was associated with 21% higher risk of mortality (HR 1.21, 95% CI 1.08–1.36, $P = 0.001$) (**Supplementary Table 1**).

Subgroup Analysis

Figure 2 shows the association between weight change groups and all-cause mortality for several patient subgroups. We explored whether the link between weight change and mortality risk was different for patients with and without obesity: no such interaction was found. However, among HF patients with obesity, weight loss was associated with higher risk of mortality than that observed in patients without obesity. The impact of weight loss on mortality was related to diabetes mellitus (interaction $p = 0.009$). Weight loss was significantly associated with remarkable higher mortality among patients with diabetes mellitus (adjusted HR 2.29, 95% CI, 1.52–3.44, $P < 0.001$), but was non-significant among patients without diabetes mellitus (adjusted HR 0.95, 95% CI, 0.62–1.43, $P = 0.793$). A similar interaction was found using weight change continuous variable (interaction $p = 0.02$) (**Supplementary Figure 2**). The impact of weight loss on mortality appeared more pronounced in women (interaction $p = 0.008$), but no such interaction was found when using weight change as continuous variable. The link between weight loss and mortality risk was similar in patients on spironolactone and on placebo.

DISCUSSION

We have found that both weight loss and weight gain were common in patients with HFpEF. Weight loss was associated with increased mortality risk from all causes, and weight gain was not associated with lower mortality risk. In addition, the impact of weight loss on mortality may be interacted by diabetes status and gender. Findings from the current study extended previous evidence to a less known population, HFpEF, and raised novel interaction in a broad spectrum of subgroup analysis.

Unintentional weight loss was witnessed in 14–21% of patients with HF in prior studies (6–8, 20), with results quite similar to ours. These studies have provided important information on association of weight loss with outcomes in HF. Anker et al. (21) first demonstrated that weight loss of at least 7.5% during at least 6 months in HF was an independent risk factor for poor prognosis in a small-sample and single-center study. *Post-hoc* analysis of the SOLVD and V-HeFT II trials (10) identified which level of weight loss gave the strongest discrimination and proposed 6% of weight loss to define cachexia in HFrEF. In an analysis of the CHARM study (9), those patients with 5% or greater weight loss in 6 months had over 50% increase in hazard both for mortality compared with those with stable weight. Analysis of patients with HFrEF from the Val-HeFT study (7) found that 5% or greater weight loss in 1 year was independently associated with mortality and adverse cardiovascular outcomes. Zamora et al. (6) also reported that 5% or greater weight loss in 1 year was associated with an increased 89% risk of mortality in patients with ambulatory HFrEF. The present study is the first one focusing on patients with HFpEF, which accounts for over half of the HF population. We demonstrated that weight loss was also an independent prognostic factor in patients with HFpEF, that 5% or greater weight loss in 1 year was associated with an increased 42% risk of subsequent long-term mortality compared with patients with stable weight. More precise estimation achievable with restricted cubic spline demonstrated that 1 SD decrease in weight was associated with 21% higher risk of mortality. Thus, in addition to routine monitoring of body weight that was recommended by HF guidelines, calling for vigilance on apparent weight loss is also suggested throughout long-term HF management.

Although lacking in robust evidence, the potential benefit of intentional weight loss was suggested in several pilot studies in established HF patients with obesity. Weight loss through bariatric surgery and non-surgical approaches has been found to improve LVEF (22, 23), exercise capacity (24), NYHA class (25, 26), and quality of life (27, 28). The controversy effect between intentional and unintentional weight loss suggested different mechanisms during this course. The onset of unintentional weight loss may be a signal of HF progress to imbalance between catabolic and anabolic states, and the subsequent wasting outlook of the patients. A few studies (10, 29, 30) have found hormonal and immune activations such as interleukin-6 and tumor necrosis factor-α in patients with cardiac cachexia. Nevertheless, further research on the underlying mechanisms are still needed. In a previous study (24) on intentional weight loss by caloric restriction or aerobic exercise training, the change in peak Vo2 was positively correlated with the change in percent lean body mass and the change in thigh muscle:intermuscular fat ratio. Another study (31) assessing mortality based on body fat and lean mass, rather than BMI or weight alone, reported that subjects losing body fat, rather than lean mass, have a lower mortality. Thus, improvement in body composition, instead of indiscriminate weight loss, is a promising target in future HF programs.

Unintentional weight gain in established HF was less investigated in previous studies. This study showed that weight gain was almost as frequent as weight loss in patients with HFpEF. The *post-hoc* analysis of the CHARM study (9) found

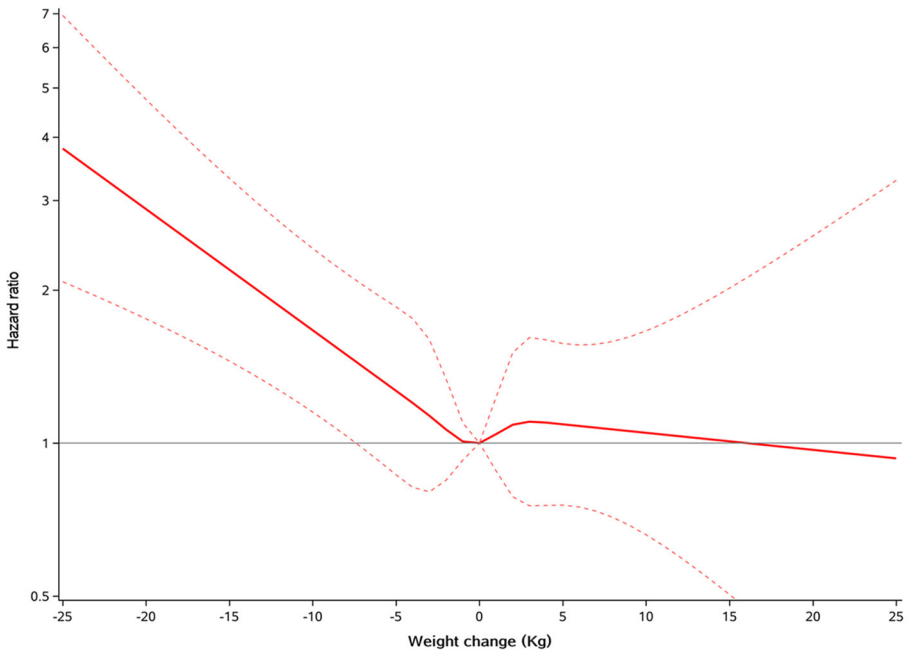

FIGURE 1 | Restricted cubic spline plots for all-cause mortality by weight change. A positive value means increased weight, and a negative value means decreased weight.

Subgroup	No. of patients	Weight loss	HR (95% CI)	Weight gain	HR (95% CI)	p for interaction
Overall	1515		1.42 (1.06-1.89)		0.98 (0.68-1.42)	
BMI(kg/m^2)						0.290
≥30	983		1.70 (1.17-2.47)		0.96 (0.58-1.58)	
<30	532		1.05 (0.66-1.67)		0.97 (0.67-1.66)	
Diabetes mellitus						0.009
Yes	667		2.29 (1.52-3.44)		1.16 (0.71-1.88)	
No	848		0.95 (0.62-1.43)		0.86 (0.49-1.51)	
Gender						0.008
Women	749		2.21 (1.44-3.37)		1.85 (1.08-3.17)	
Men	766		1.02 (0.68-1.54)		0.67 (0.40-1.10)	
Allocated treatment						0.497
Spironolactone	771		1.55 (1.04-2.32)		0.81 (0.46-1.45)	
Placebo	744		1.31 (0.86-1.99)		1.07 (0.67-1.73)	
Reference, weight stable		0 1 2 3 4		0 1 2 3 4		

FIGURE 2 | Multivariable Cox regression analysis for various subgroups. BMI, body mass index.

that weight gain was associated with modestly increased short-term mortality risk. Similar results were also reported in the sub-analysis of patients from the GISSI-HF and Val-HeFT studies (7). On the contrary, we demonstrated the neutral role of weight gain on mortality risk compared with weight stability in patients with HFpEF, and results from the restricted cubic spline analysis confirmed this association. Difference in HF population may account for this discrepancy that the majority of patients enrolled in prior studies was HFrEF. Hitherto, there has been no evidence that patients with established HF can benefit from weight gain. We demonstrated that weight gain was not associated with better prognosis even in HF patients without obesity.

Notably, the effect of weight loss on all-cause mortality was remarkable among patients with diabetes mellitus, but

was non-significant among patients without diabetes mellitus. One possible explanation is that unintentional weight loss may result from insufficient antidiabetic treatment, and the body subsequently starts burning fat and muscle for energy in patients with diabetes mellitus. Such unintentional weight loss related to progression of disease would be expected to increase mortality (32). We also show that the link of weight loss to mortality may be different between women and men in established HF, whereas this gender difference need to be tested in a larger study. The CHARM study (9) showed that the impact of weight loss on mortality appeared more pronounced in patients not receiving angiotensin-converting-enzyme inhibitors (ACEI) (interaction $P = 0.01$) compared with those receiving ACEI. However, no such interaction was observed for spironolactone in the present analysis.

There are several limitations to our study because participants were from a clinical trial database that had several exclusion criteria that might affect the generalizability. The cutoff of weight change equal to or <5% can be considered arbitrary as were all the definitions used in previous studies (6, 7); however, no definite cutoff exists. We have no further anthropometric measures (muscle or fat mass wasting assessments), and we cannot fully ascertain whether weight change was in part intentional or non-intentional. Although we have adjusted multiple patient characteristics including presence of edema at baseline, a higher prevalence of relevant risk factors, such as COPD in the weight-loss group, and the average younger age in the weight-gain group may have played a role in the incidence of death, and bias due to unmeasured confounders are possible. Due to the limitation of the sample size, we did not analyze the specific cause of death.

In conclusion, this study shows that weight loss is an independent factor of poor prognosis in HFpEF with normal to overweight, especially in patients with diabetes, though this interaction needs further investigation. Weight gain was not associated with better prognosis, either. Indiscriminate advice to lose or gain weight in HFpEF might not be indicated, and the underlying mechanism of weight change on mortality merits further research.

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**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the First Affiliated Hospital of Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study. Ethical review and approval was received for the original clinical trial.

AUTHOR CONTRIBUTIONS

BD, PH, and ZG design the research. CL, WL, YW, XH, and WZ analyse the data. PH, JZ, YD, and YY write the article. All authors contributed to the article and approved the submitted version.

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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.2021.681726/full#supplementary-material

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Comparative Effectiveness of Exercise Training for Patients With Chronic Thromboembolic Pulmonary Hypertension After Pulmonary Endarterectomy: A Systematic Review and Meta-Analysis

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Background: Patients with chronic thromboembolic pulmonary hypertension (CTEPH) still experience reduced exercise capacity despite pulmonary endarterectomy (PEA). Exercise training improves the exercise capacity and quality of life (QoL) in patients with PH, but data on the effects of exercise training on these patients are scarce. The aim of this meta-analysis and systematic review was to evaluate the effectiveness and safety of exercise training in CTEPH after PEA.

Methods: We searched the relevant literature published before January 2020 for the systematic review and meta-analysis using the PubMed, EMBASE, and Cochrane Library databases. The primary outcome was a change in the 6-min walking distance (6 MWD). We also assessed the effect of exercise on the peak oxygen uptake (VO_2) or peak VO_2/kg, oxygen uptake anaerobic threshold, workload, oxygen pulse, hemodynamics, arterial blood gases, oxygen saturation, N-terminal pro-brain-type natriuretic peptide (NT-proBNP), quality of life (QoL) and pulmonary function tests.

Results: We included 4 studies with 208 exercise-training participants. In the pooled analysis, short-term exercise training can improve the 6 MWD of 58.89 m (95% CI: 46.26–71.52 m, $P < 0.0001$). There was a significant increase in the peak VO_2/kg or peak VO_2 after exercise training (3.15 ml/min/kg, 95% CI: 0.82–5.48, $P = 0.008$; 292.69 ml/min, 95% CI: 24.62–560.75, $P = 0.032$, respectively). After exercise training, the maximal workload and O_2 pulse significantly improved. Three months of exercise training increased the right ventricular ejection fraction by 3.53% (95% CI: 6.31–11.94, $P < 0.00001$, $I^2 = 0$) independently of PEA surgery. In addition, NT-proBNP plasma levels significantly improved with exercise training after PEA [weighted mean difference (WMD): −524.79 ng/L, 95% CI: 705.16 to −344.42, $P < 0.0001$, $I^2 = 0$]. The partial pressure of oxygen and pH improved progressively over 12 weeks of exercise training (WMD: 4 mmHg, 95% CI: 1.01–8.33, $P = 0.01$; WMD: 0.03, 95% CI: 0.02–0.04, $P < 0.0001$,

respectively). Subscales of the QoL measured by the SF-36 questionnaire had also improved. In addition, exercise training was well-tolerated with a low dropout rate, and no major adverse events occurred during exercise training.

Conclusion: Exercise training may be associated with a significant improvement in the exercise capacity and QoL among CTEPH patients after PEA and was proven to be safe. However, more large-scale multicentre studies are needed to confirm the effectiveness and safety of exercise training in CTEPH patients after PEA.

PROSPERO registration number: CRD42021235275.

Keywords: pulmonary endarterectomy, pulmonary hypertension, exercise intolerance, cardiorespiratory fitness, exercise training

INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is characterized by stenosis and/or occlusion of pulmonary arteries caused by organized thrombus (1). Early diagnosis and suitable treatment are critical because CTEPH has a high rate of mortality and right-side heart failure (2). Pulmonary endarterectomy (PEA) is a proven curative treatment for operable CTEPH (3, 4). PEA surgery is recommended as the first-line therapy for operable CTEPH patients (ClassI, evidence C) (5, 6). However, some patients still suffer limited exercise capacity despite PEA (7), especially when their pulmonary hemodynamics do not normalize (8, 9). An improvement in exercise tolerance, physical function, and the ability to cope with daily living should be targets during the comprehensive management of these patients (10).

Rehabilitation programmes, including aerobic exercise training, have strong evidence of effectiveness in improving the exercise capacity, dyspnoea, and health-related quality of life (QoL) in different etiologies of pulmonary hypertension (PH) (11–17). Exercise training has also improved muscular and right ventricular function, QoL and pulmonary hemodynamics in idiopathic pulmonary arterial hypertension (PAH), PAH associated with connective tissue diseases and inoperable CTEPH (11–16, 18–20).

Exercise training has improved the exercise capacity for up to 3 months in patients with CTEPH after PEA, independent of the post-surgery hemodynamic response (17, 21–23). However, the evidence of the effect of exercise training on patients with CTEPH after PEA is limited.

Therefore, we performed the meta-analysis and systematic review to assess the efficacy and safety of an exercise training program in CTEPH patients after PEA.

METHODS AND ANALYSIS

Data Sources and Search Strategy

We designed the study according to the PRISMA statement (**Supplementary Table 1**) (24). We searched the PubMed, EMBASE, and the Cochrane Collaboration databases using the key words "exercise training," "chronic thromboembolic pulmonary hypertension," "pulmonary endarterectomy," and "rehabilitation" to identify studies that evaluated the efficacy and safety of exercise training for CTEPH patients after PEA surgery. Our search included articles published from the database beginning up to January 2020. The search was limited to human studies and English language articles.

Study Selection

We included five observational studies comparing the effectiveness of exercise training in CTEPH patients after PEA. The inclusion criteria were (1) patients were diagnosed with CTEPH and (2) the patients underwent exercise training after PEA. We excluded studies if they were conference abstracts, case reports, reviews, letters, or editorials.

Data Extraction

Two independent reviewers (Y-L. Z, R.J) performed the literature search, data extraction, and methodological grading. Disagreements were resolved by consensus. We extracted the information of each study, such as the author, year of publication, demographic characteristics, nature of the study, hemodynamics, and pre- and post-exercise intervention results.

Outcomes

The primary outcome was a change in the 6-min walking distance (6 MWD). The secondary outcomes were as follows:

1) Changes in exercise tolerance by cardiopulmonary exercise testing (CPET): oxygen consumption at peak exercise (peak VO_2 and peak VO_2/kg), oxygen uptake anaerobic threshold (VO_2 at AT), and workload;
2) Changes in cardiac function by CPET: oxygen pulse, resting and peak heart rate (HR), and resting and peak oxygen saturation;
3) Changes in pulmonary hemodynamics by right heart catheterization: right atrial pressure (RAP), mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), pulmonary capillary wedge pressure (PCWP), transpulmonary gradient (TPG), systemic vascular resistance (SVR), total pulmonary resistance (TPR), cardiac output (CO), cardiac index (CI) and right ventricular ejection fraction (RVEF).
4) Changes in the resting and peak exercise systolic pulmonary arterial pressure (sPAP) by echocardiography;

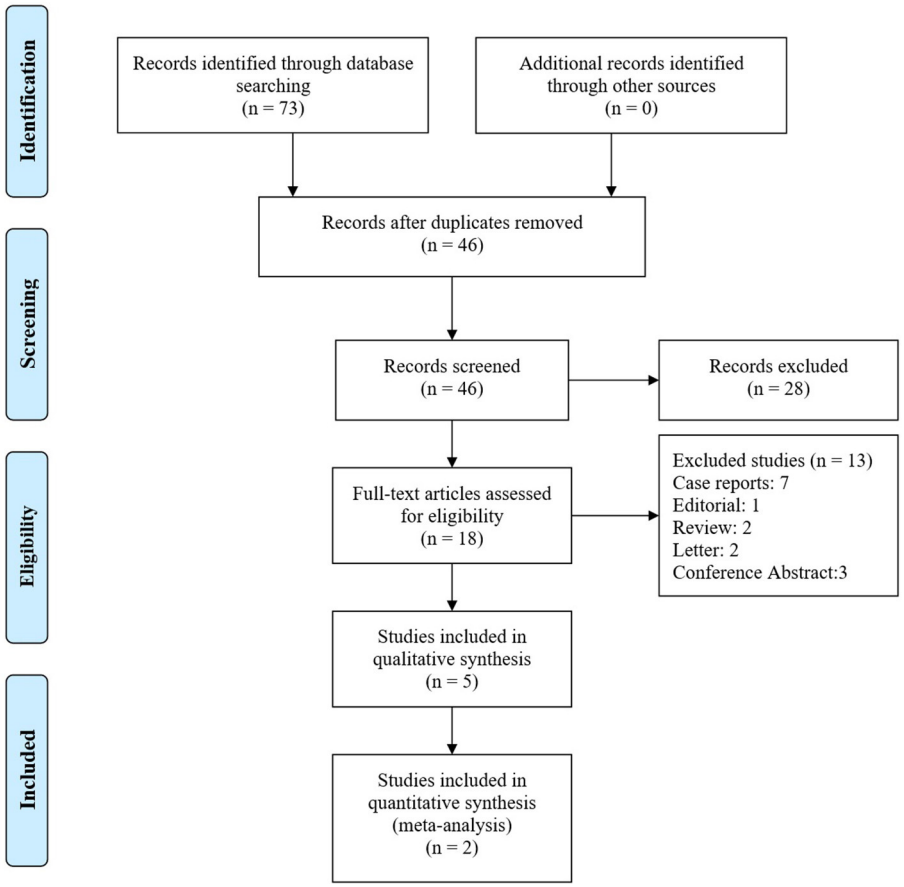

FIGURE 1 | Flow diagram.

5) Changes in N-terminal pro-brain-type natriuretic peptide (NT-proBNP);
6) Changes in arterial blood gases: pH, oxygen partial pressure (PaO_2), and partial pressure of carbon dioxide ($PaCO_2$);
7) Changes in QoL scales assessed by the SF-36 questionnaire;
8) Changes in echocardiography, including the resting and peak systolic pulmonary pressure (sPAP);
9) Changes in pulmonary function tests: forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV1).

Methodological Quality

We used the NIH quality assessment tool to assess the quality of pre-post interventional studies (25). Due to the insufficient number of research articles, publication bias could not be assessed.

Data Synthesis and Statistical Analysis

We conducted meta-analyses for comparisons when two or more studies reported the same outcome. Continuous values are presented as the mean ± SD and analyzed using weighted mean differences (WMDs). We used random-effect models to quantitatively synthesize the evidence and to calculate the summary estimates. Pooled analysis was calculated using fixed-effect models, and random-effect models were applied in cases of significant heterogeneity.

Certain studies reported continuous variables in the form of interquartile ranges or 95% confidence intervals (CI) other than the SD and needed to be converted into SDs. Cases in which converted SDs could not be obtained were excluded. We considered $P < 0.05$ as significant.

Statistical analysis was performed using Stata version 15 software (Stata Corp., College Station, Texas) and RevMan 5.4 (The Cochrane Collaboration, Copenhagen, Oxford, UK).

RESULTS

Characteristics of the Studies

We retrieved 73 articles for more detailed analysis after 68 initial articles were identified by the search and included 4 studies in our systematic review (**Figure 1**). There were four pre-post interventional studies, encompassing 208 exercise-training participants between 2012 and 2020 (17, 21–23). All studies used a supervised exercise training programme combined with aerobic exercise (treadmill or bicycle Ergometer) and resistance training. One study was performed at an outpatient rehabilitation

TABLE 1 | Characteristics of all included studies.

References	No. of patients	Exercise training intervention	Duration and time interval between PEA and the exercise programme	Primary endpoint	Results
Nagel et al. (17)	$n = 45$; Female:16; Age: 61 ± 15 years	• In the Rehabilitation Clinic (first 3 weeks): Interval bicycle ergometer, walking, respiratory training (low workloads, 5 days/week, a minimum of 1.5 h/day), single muscle groups (low weights). • Training at home (for 12 weeks): Bicycle ergometer (≥ 30 min/day at 5 days a week). • Psychological support and mental training.	3 weeks; 15 weeks. Time interval: not described	6 MWD; CPET variables; stress Doppler echocardiography; HR; blood pressure; Borg dyspnoea index; WHO-FC; SF-36; NT-proBNP; gas exchange	6 MWD↑; SF-36 scores for physical functioning and vitality↑; peak VO_2↑; peak VO_2/kg↑; workload↑; an increase of maximal HR↑; NT-proBNP↓; WHO-FC(-).
Inagaki et al. (23)	$n = 8$; Age: 64 ± 12 years	In-hospital each week (40–60 min) and home-based programme, including: • Lower-limb endurance training (walking exercises, a cycle ergometer at 60% of the target HR) • Lower and upper limb strength training • Respiratory exercises • Education	12 weeks Time interval: not described	Echocardiography; BNP; exercise capacity; dyspnoea severity and the functional status; pulmonary function; peripheral muscle force; physical activity.	6 MWD↑; TDI scores↑; QF↑; Ex↑; SGRQ scores↑; No change in MRC scores, BDI scores, HRR1, and WHO-FC.
La Rovere et al. (21)	Group 1 = 84, Age: 60.4 ± 13.8 years, Female: 6; Group 2 = 26, Age: 57.9 ± 13.1 years, Female: 31.	Daily sessions of: • Incremental exercise training (30 min, at 50–70% of the maximal load); • Abdominal, upper, and lower limb muscle activities including lifting progressively increasing light weights (0.30–0.50 kg), and shoulder and full arm circling; • Education; • Nutritional programmes and psychosocial counseling.	3 months Time interval: not described	6 MWD; pulse oximetric oxygen saturation, lung function tests; arterial blood gases; hemodynamics.	PAP↓; TPG↓; RVEF↑; PVR↓; PaO2↑; pH↓; No changes in RAP, PCWP, CO, CI, SVR, PVR,TPR, PaCO2, FEV1,% predicted, FVC, % predicted, and FEV1/FVC, % predicted*.
Nagel et al. (22)	$n = 45$; Female: 22 Age: 57.6 ± 12.4 years.	• In the rehabilitation clinic (first 3 weeks): Interval bicycle ergometer, walking, respiratory training (low workloads, 5 days/week, a minimum of 1.5 h/day), single muscle groups (low weights). • Training at home (for 19 weeks): Bicycle ergometer (≥ 15 min/day at 5 days a week). • Psychological support and mental training	3 weeks; 19 weeks. Time interval: 3.3 ± 0.9 (median 3.1) weeks	WHO-FC; 6 MWD; BDI scores; echocardiography; lung function; blood gas; SF-36; CPET with stress echocardiography.	6 MWD↑; SF-36 scores for physical functioning and vitality↑; peak VO_2↑; peak VO_2/kg↑; workload↑; NT-proBNP↓; RA area↓; RV area↓; sPAP↓; TAPSE↑; left ventricular eccentricity index↓; tissue Doppler imaging s RV free wall↑; oxygen pulse↑; $EqCO_2$↓; BDI↓; HR↓; peak HR↓; O_2 at AT↑.

VE, ventilation; VO_2, oxygen consumption; VCO2, carbon dioxide output; WHO-FC, WHO functional class; NT-proBNP, N-terminal pro-brain natriuretic peptide; 6 MWD, 6-minute walking distance; TRPG, tricuspid regurgitation pressure gradient; sPAP, systolic pulmonary arterial pressure; BNP, brain natriuretic peptide; MRC, Medical Research Council dyspnoea grade; BDI, dyspnoea index; TDI, Transition dyspnoea index. QF, quadriceps force; HF, handgrip force; HHR1, heart rate recovery during the first minute; HR, heart rate; NRADL, Nagasaki University Respiratory ADL Questionnaire; SGRQ, St. George's Respiratory Questionnaire; Ex, amount of exercise; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; CO, cardiac output; RVEF, right ventricular ejection fraction; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; TPR, transpulmonary resistance; FVC, forced vital capacity; FEV1, forced expiratory volume at 1 second; RA, right atrial; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion; SaO_2, oxygen saturation; $EqCO_2$:respiratory equivalent for CO_2.
**COMPARED integrated two groups with 3-month vs. after surgery (before rehabilitation).*

center, while three studies were performed in-hospital for the first few weeks followed by home-based exercise training. All training participants underwent low workload aerobic exercise training with some form of resistance and respiratory training. The exercise intensity was titrated at 50–70% of the peak exercise capacity. The characteristics of all included studies are presented in **Table 1**.

Quality Assessment

The quality assessment of the pre-post interventional studies had a detailed description. All included studies had a clearly stated study question, prespecified eligibility/selection criteria, CTEPH patient's representative in the real world, clearly defined intervention and outcome variables, and low rate of loss to follow-up (**Supplemental Table 2**).

Six-Minute Walking Distances

In Christian Nagel's study (22), the 6 MWD significantly improved by 55.31 ± 53.67 m (95% CI: 39.19–71.43, $P < 0.0001$) and 65.11 ± 63.96 m (95% CI: 43.14–87.09, $P < 0.0001$) with 3- or 19-week exercise training after PEA in patients with CTEPH, respectively. By pooling analysis, short-term exercise training can improve the 6 MWD of 58.89 m (95% CI: 46.26–71.52 m, $P < 0.0001$) (**Figure 2**). Ekkehard Grünig evaluated the effects of exercise training in patients with inoperable or residual CTEPH in a prospective study (17). The 6 MWD significantly improved by 71 ± 70 m after 15 weeks among 35 inoperable or residual CTEPH patients ($P = 0.001$). Jiro Terada also evaluated the changes in the 6 MWD after a 12-week exercise training programme in 8 inoperable or residual CTEPH patients (23). After completion of the pulmonary rehabilitation programme, the 6 MWD significantly improved by 33.3 ± 25.1 m compared with the baseline ($P < 0.01$). However, we could not extract information on residual CTEPH from the studies of Ekkehard Grünig and Jiro Terada.

Cardiopulmonary Exercise Testing

Patients performed a gradually increasing work rate CPET to maximal tolerance on an electromagnetically braked cycle ergometer in the upright position. The peak VO_2, peak VO_2/kg, VO_2 at AT, peak workload, oxygen pulse, HR and oxygen saturation were evaluated to assess the CPET capacity.

Regarding exercise tolerance, Christian Nagel's study (22) was included to analyse the peak VO_2, peak VO_2/kg, VO_2 at AT and peak workload by pooling analysis.

There was a significant increase in the peak VO_2/kg or peak VO_2 after exercise training (3.15 ml/min/kg, 95% CI: 0.82–5.48, $P = 0.008$; 292.69 ml/min, 95% CI: 24.62–560.75, $P = 0.032$, respectively). However, there were no significant increases in VO_2 at AT (136.32 ml/min, 95% CI: −66.78–339.41, $P = 0.19$). After exercise training, the max workload during exercise had significantly improved (26.69 Watt, 95% CI: 9.41–43.98, $P = 0.002$) (**Table 2**).

With regard to cardiac function, we pooled the study of Christian Nagel to analyse the oxygen pulse, resting and peak HR, and resting and peak oxygen saturation (22). By exercise training, the O_2 pulse and oxygen saturation had significantly improved (1.55, 95% CI: 0.40–2.70, $P = 0.008$; 0.55%, 95% CI: 0.02–1.08, $P = 0.043$), with a tread of an improved of maximal HR of 10.41 bpm (0.66, 95% CI: −0.66 to 21.48, $P = 0.065$) and maximal saturation of 1.18% (95% CI: −0.18 to 2.54, $P = 0.088$) during exercise (**Table 2**). In Ekkehard Grünig's study (17), among patients with inoperable or residual CTEPH after PEA,

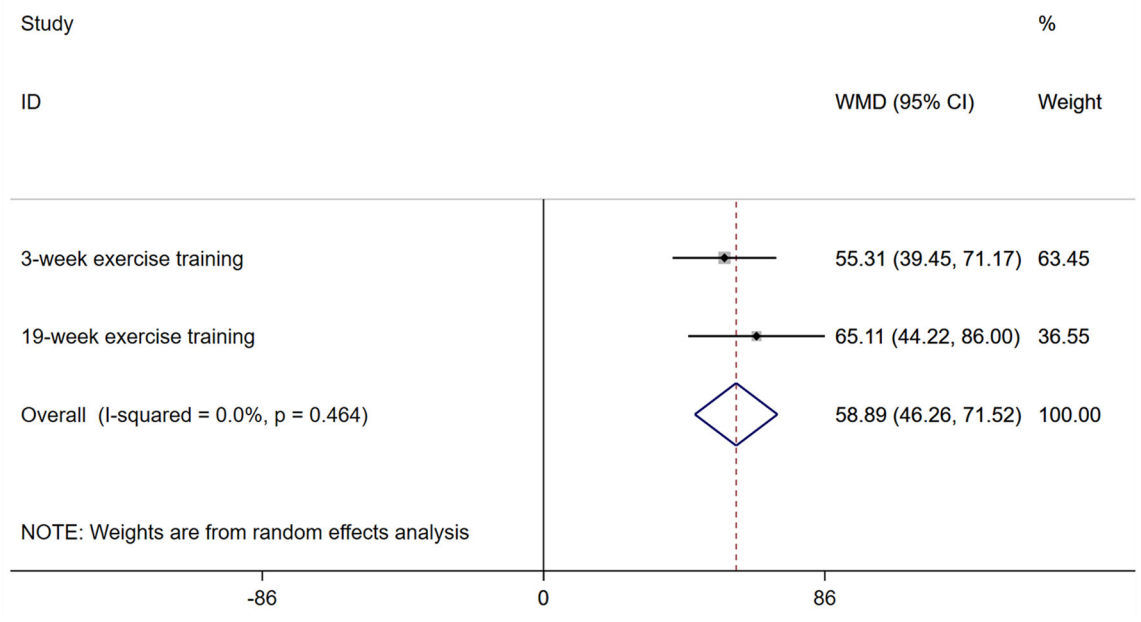

FIGURE 2 | Forest plots for the 6-min walking distance. 6MWD, six-minute walking distance.

TABLE 2 | Changes in cardiopulmonary exercise testing after exercise training in CTEPH patients with PEA.

Variable (22)	WMD (95% CI)	% Weight	*P*-value
Exercise tolerance			
Peak VO_2/kg	3.15 (0.82, 5.48)	53.78	0.008
after 3 weeks	1.99 (1.48, 2.50)	16.26	
after 12/15 weeks	4.37 (3.48, 5.26)	16.17	
Peak VO_2	292.69 (24.62, 560.75)	3.45	0.032
after 3 weeks	158.50 (122.50,194.50)	1.08	
after 12/15 weeks	432.09 (349.76, 514.42)	0.22	
VO_2 at AT	136.32 (−66.78, 339.41)	1.11	0.188
after 3 weeks	37.07 (−34.72, 108.86)	0.29	
after 12/15 weeks	244.50 (133.71, 355.29)	0.12	
Workload max	26.69 (9.41, 43.98)	41.65	0.002
after 3 weeks	18.18 (13.05, 23.31)	12.69	
after 12/15 weeks	35.83 (27.53, 44.13)	9.33	
Cardiac function			
O_2 pulse	1.55 (0.40, 2.70)	33.09	0.008
after 3 weeks	0.98 (0.48, 1.48)	11.41	
after 12/15 weeks	2.15 (1.50, 2.80)	10.97	
HR rest	−2.70 (−7.75, 2.35)	10.8	0.295
after 3 weeks	−0.36 (−3.16, 2.44)	4.09	
after 12/15 weeks	−5.54 (−9.76, −1.32)	2.21	
HR max	10.41 (−0.66, 21.48)	4.66	0.065
after 3 weeks	4.95 (−0.20, 10.10)	1.58	
after12/15 weeks	16.25 (9.69, 22.81)	1.03	
SaO_2 rest	0.55 (0.02, 1.08)	31.10	0.043
after 3 weeks	0.39 (−0.24, 1.02)	11.02	
after 12/15 weeks	0.94 (−0.05, 1.93)	9.72	
SaO_2 max	1.18 (−0.18, 2.54)	20.35	0.088
after 3 weeks	0.57 (−1.31, 2.45)	6.41	
after 12/15weeks	1.85 (−0.11, 3.81)	6.16	

SaO_2, oxygen saturation; PEA, pulmonary endarterectomy; HR, heart rate; Oxygen consumption, O_2; WMD, weighted mean difference.

the peak oxygen consumption, maximal workload and maximal HR had improved (**Supplementary Table 3**).

Effect of PEA on Hemodynamic Measurements

In Nicolino Ambrosino's study (21), CTEPH patients were divided into Group 1 ($n = 84$) and Group 2 ($n = 26$) according to the post-surgery hemodynamic response. Group 1 patients met at least one of the following criteria: (1) mPAP ≤25 mm Hg; (2) ≥50% reduction in mPAP; and (3) ≥70% reduction in PVR. Group 2 included patients who did not meet any of these criteria. In this study, we combined the mean and SD of the two groups before PEA, after PEA and after exercise training.

CTEPH patients had a decreased mPAP of 18.10 mmHg, PCWP of 1.46 mmHg, TPG of 20.01 mmHg, SVR of 548.87 dynes/cm^{-5}, PVR of 528.61 dynes/cm^{-5}, and TPR of 532 dynes/cm^{-5} after PEA surgery. PEA surgery increased the CO, CI and RVEF. However, the RAP remained unchanged (**Table 3**).

Effect of Exercise Training After PEA on Hemodynamic Measurements

Table 3 shows the changes in hemodynamics from immediately after the surgery (before rehabilitation) to 3 months after the exercise training. Three months of exercise training increased the RVEF by 3.53% (95% CI: 6.31–11.94, $P < 0.00001$, $I^2 = 0$). However, 3 months of exercise training did not influence the RAP, mPAP, PCWP, TPG, CO, CI, SVR, PVR or TPR (**Table 3**).

NT-proBNP, Arterial Blood Gases, Echocardiography, and Pulmonary Function Test

By pooling analysis in Christian Nagel's study (22), we found that the NT-proBNP plasma levels had continuously decreased by 524.79 ng/L (95% CI: −705.16 to −344.42, $P < 0.0001$, $I^2 = 0$) when CTEPH patients underwent 3–19 weeks of exercise training after PEA surgery (**Table 4**).

By combining the mean and SD of the two groups in Nicolino Ambrosino's study (21), PEA surgery significantly improved the PaO_2 by 9.30 mmHg, $PaCO_2$ by 1.72 mmHg and pH by 0.01 (**Table 4**). Three months of exercise training after PEA surgery further improved the PaO_2 and the pH (WMD: 4 mmHg, 95% CI: 1.01–8.33, $P = 0.01$; WMD: 0.03, 95% CI: 0.02–0.04, $P < 0.0001$, respectively), but not the $PaCO_2$ (**Table 4**).

In Christian Nagel's study (22), the resting sPAP or maximal sPAP during exercise training improved with the extension of rehabilitation (**Table 4**). Ekkehard Grünig's study (17) and Jiro Terada's study (23) also assessed the sPAP by echocardiography. In the two interventional studies (17, 23), there were no significant improvements in resting sPAP or maximal sPAP. We cannot conduct quantitative meta-analysis of the sPAP in the above three studies because Ekkehard Grünig's and Jiro Terada's studies included some inoperable CTEPH patients.

In addition, the right atrial and right ventricular areas, tricuspid annular plane systolic excursion (TAPSE), left ventricular eccentricity index, pulmonary artery diameter, and tissue Doppler imaging of the RV free wall, as assessed by echocardiography, improved by varying degrees with exercise training (22).

In Christian Nagel's study (22), neither the FVC% predicted, FEV1% predicted nor FEV1/FVC% predicted improved after 12 weeks of exercise training after PEA.

Quality of Life

Christian Nagel's study described the QoL change after exercise training in CTEPH patients after PEA (22). The subscales for physical function (WMD: 29.78 points, 95% CI: 15.26–44.30, $P < 0.0001$), physical role functioning (WMD: 29.76 points, 95% CI: 13.68–45.84, $P < 0.0001$), bodily pain (WMD: 11.05 points, 95% CI: 2.02–20.08, $P = 0.017$), and social role functioning (WMD: 13.13 points, 95% CI: 2.25–24.01, $P = 0.018$) improved during the 19-week exercise training after PEA (**Figure 3**). However, physical function, general health perception, physical role functioning, vitality, emotional role and mental health had not significantly improved (**Figure 3**).

TABLE 3 | Forest plots of pulmonary hemodynamic measurements.

Variables (21)	Pulmonary hemodynamic measurements after PEA before rehabilitation		Pulmonary hemodynamic measurements with 3 months of exercise training after PEA	
	MD (95% CI)	***P*-value**	**MD (95% CI)**	***P*-value**
RAP, mmHg	−0.04 (−2.26, 2.18)	0.97	−0.14 (−0.94, 0.66)	0.74
mPAP, mmHg	−18.10 (−29.35, −6.84)	0.002	−0.18 (−5.60, 5.25)	0.95
PCWP, mmHg	1.46 (0.04, 2.87)	0.04	−0.24 (−1.10, 0.63)	0.59
TPG, mmHg	−20.01 (−29.98, −10.03)	<0.0001	0.34 (−4.36, 5.05)	0.89
CO, L/min	0.92 (0.59, 1.25)	<0.00001	0.20 (−0.10, 0.50)	0.19
CI, L/min/m^2	0.49 (0.20, 0.77)	0.0009	0.10 (−0.04, 0.24)	0.17
RVEF, %	9.12 (6.31, 11.94)	<0.00001	3.53 (1.11, 5.95)	0.004
SVR, dyn·s·cm^{-5}	−548.87 (−687.29, −410.44)	<0.00001	92.20 (−15.24, 199.63)	0.09
PVR, dyn·s·cm^{-5}	−538.61 (−744.64, −332.59)	<0.00001	1.19 (−78.37, 80.75)	0.98
TPR, dyn·s·cm^{-5}	−532.00 (−773.86, −290.13)	<0.0001	−10.72 (−103.32, 81.87)	0.82

RAP, right atrial pressure; mPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; TPG, transpulmonary gradient; CO, cardiac output; CI, cardiac index; RVEF, right ventricular ejection fraction; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; TPR, transpulmonary resistance; PEA, pulmonary endarterectomy; MD, mean difference.

TABLE 4 | The effect of exercise training on NT-proBNP, arterial blood gases, echocardiography, and pulmonary function test in patients with CTEPH after PEA.

Variables	WMD (95% CI)	I^2 (%)	*P*-value
NT-proBNP, ng/L (22)	−524.79 (−705.16, −344.42)	0	<0.0001
Changes after 3 weeks	−482.30 (−698.90, −269.70)		
Changes after 19 weeks	−622.21 (−955.09, −289.33)		
Arterial blood gases (21)			
From before surgery to after surgery (before rehabilitation)			
PaO_2, mmHg	9.30 (6.04, 12.55)	0	<0.00001
$PaCO_2$, mmHg	1.72 (0.54, 2.89)	48	0.004
pH	0.01 (0, 0.02)	0	0.04
From after surgery (before rehabilitation) to 3 months rehabilitation			
PaO_2, mmHg	4.67 (1.01, 8.33)	0	0.01
$PaCO_2$, mmHg	0.90 (−0.28, 2.08)	0	0.13
pH	−0.03 (−0.04, −0.02)	0	<0.00001
Echocardiography (22)			
After 3 weeks	−1.20 (−3.14, 0.74)	0	0.225
sPAP rest	−1.29 (−3.55, 0.97)		
sPAP max	−0.95 (−4.74, 2.84)		
After 19 weeks	7.42 (3.87, 10.97)	0	<0.0001
sPAP rest	6.37 (2.23, 10.51)		
sPAP max	10.35 (3.44, 17.26)		
Pulmonary function test (21)			
FVC % predicted	4.10 (−0.63, 8.83)	0	0.09
FEV1 % predicted	3.30 (−2.72, 9.32)	0	0.28
FEV1/FVC % predicted	2.70 (−1.13, 6.53)	0	0.17

NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PEA, pulmonary endarterectomy; PaO_2, oxygen partial pressure; $PaCO_2$, partial pressure of carbon dioxide; sPAP, systolic pulmonary arterial pressure; FVC, forced vital capacity; FEV1, forced expiratory volume at 1 second; WMD, weighted mean difference.

Safety of Exercise Training

In most of the included studies, exercise training was well-tolerated with an overall dropout rate of 5%. Approximately 0.9% of the training patients experienced syncope or palpitations, half of which were related to exercise training (2.2%). Furthermore, during exercise training, no major adverse events, such as symptom progression, right heart failure, or death, occurred among participants (**Table 5**).

DISCUSSION

The principal finding of our study is that exercise training significantly improves exercise capacity and cardiorespiratory fitness among CTEPH patients after PEA. There was also a significant reduction in the NT-proBNP and an improvement in the arterial blood gases. Furthermore, exercise training was well-tolerated with significant improvements in the QoL.

Our study has important clinical implications. Exercise rehabilitation has been actively discouraged in PAH patients because of the fear that it would worsen symptoms and negatively impact cardiac function. However, recently, with evidence of exercise rehabilitation in PH, supervised rehabilitation, including exercise training, has been recommended for patients with PH (Class I,A) (26). The 2019 ERS statement on exercise training and rehabilitation acknowledges the strong evidence of benefits from exercise training in PH (27). Exercise training has shown beneficial effects as an add-on to PAH-specific drug therapies among CTEPH patients (17). Current guidelines recommend PEA as a potentially curative first-choice treatment that is superior to medical therapies in inoperable CTEPH patients (28, 29). Our study findings provide comprehensive evidence to support the efficacy and safety of exercise training in patients with CTEPH after PEA.

In Angelo G. Corsico's study, most of the CTEPH patients recovered good exercise tolerance (30). However, ∼40% continue to suffer from the limitation of moderate intensity exercise (30). Exercise limitation 12 months after PEA is characterized by multifactorial etiologies involving lower RVEF, CI and

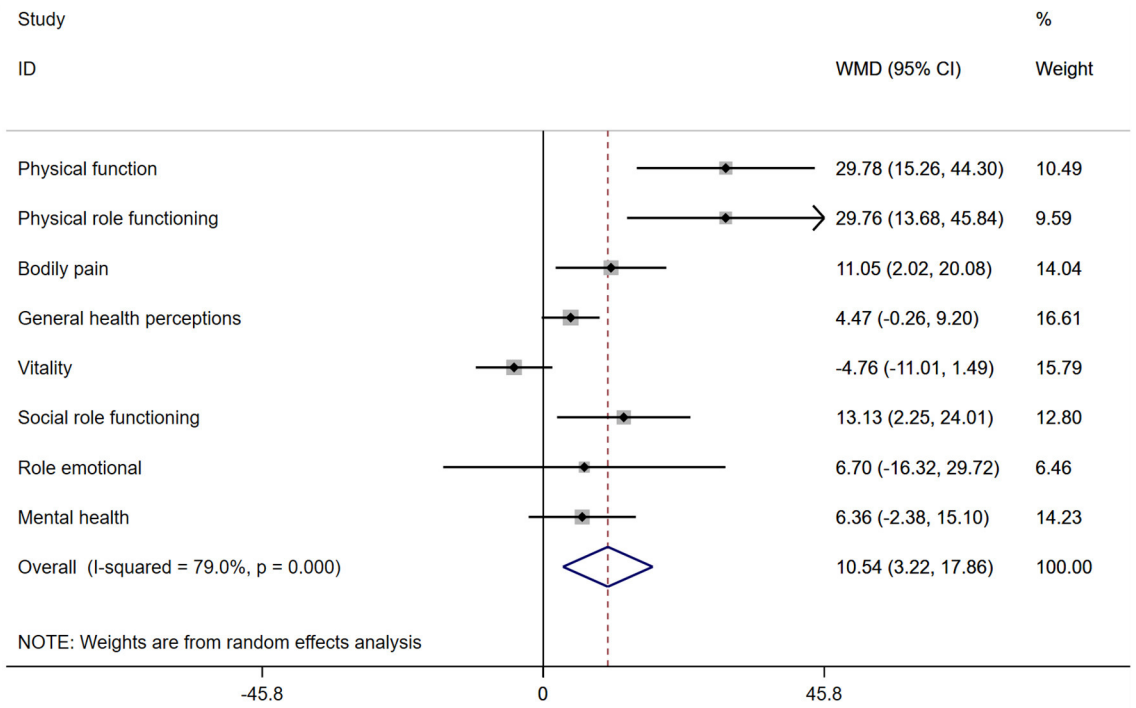

FIGURE 3 | Forest plots for the quality of life. IV, inverse variance.

TABLE 5 | Adverse effects reported in the included studies.

References	Total no. of exercise training participants	Exercise training related adverse events
Nagel et al. (17)	45	Syncope in one patient; Herpes zoster infection in one patient.
Shamseer et al. (24)	8	Low blood pressure and tachycardia in one patient
La Rovere et al. (21)	110	None
Nagel et al. (22)	45	None

pulmonary function test abnormalities (FVC, FEV1/FVC, and single breath carbon monoxide diffusing capacity).

Exercise Training on the Exercise Capacity and QoL

As the primary endpoint, the 6 MWD, peakVO_2 or peakVO_2/kg demonstrated significant increases in many studies (11, 19, 28, 29, 31). In our study, we observed significant improvements in exercise tolerance, shown as the 6 MWD, peak VO_2 or peak VO_2/kg, after exercise training.

Exercise training performed in different etiologies of PH improved not only the exercise capacity but also different aspects of the QoL, as shown in several studies (16–20, 27, 29).

From the ERS statement of summarized outcomes of the QoL in many studies (27), bodily pain and general health perception always have no significant differences. Bodily pain, general health perception and social role functioning were significantly improved in our study. Our study may indicate that exercise rehabilitation after PEA may be more effective in improving bodily pain, general health perception and social role functioning.

In addition, our study found that exercise training can improve the exercise capacity independent of the effect of the PEA surgery response.

In this systematic review, only Christian Nagel's study described the time interval between the PEA and the initiation of the exercise programme (22). Patients underwent exercise training 3.3 ± 0.9 (median 3.1) weeks after PEA. Other studies did not describe the time interval. There is little information about the acute and chronic effects of PEA on the exercise capacity and ventilatory efficiency in patients with CTEPH. N Nagaya's study examined the changes in exercise training and ventilatory efficiency as indicated by the peak VO_2 and the VE/VCO_2 slope after PEA (32). After PEA, the VE/VCO_2 slope decreased greatly from baseline (before surgery) to the early phase (1 month) and reached a steady level thereafter. In contrast, they noted a continued increase in the peakVO_2 from the early to the late phase (4 months) after surgery as well as from the baseline to the early phase (32). Surprisingly, the increase in the peak VO_2 after surgery did not correlate with the decrease in the PVR. The peak VO_2 is influenced not only by CO during exercise but also by oxygen extraction in skeletal muscles and vasodilatation of the nutrient arterioles within working skeletal muscles (33). From

another point of view, exercise training can improve skeletal muscle oxygen extraction and can continue to improve exercise tolerance in CTEPH patients independent of decreased PVR after PEA.

Hemodynamics, Echocardiography, and Cardiac Function

Thus far, most exercise training trials published in PH have focused on changes in the exercise capacity. Only one prospective, randomized, controlled trial was designed to systematically evaluate changes during rest and exercise by the invasive measurement of hemodynamics as secondary endpoints (31). Altogether, the study revealed significant increases in CI at rest or during peak exercise and decreases in mPAP and PVR at rest in the training group among 73 PAH or inoperable CTEPH patients. In Nicolino Ambrosino's study (21), CTEPH patients were divided into two groups, with and without "good" surgery hemodynamic response. We integrated the data of the two groups into one group before PEA, after PEA and after exercise training. Among the 110 CTEPH patients, they had decreased mPAP, PCWP, TPG, SVR, PVR, and TPR after PEA surgery. PEA surgery increased the CO, CI, and RVEF, respectively. After PEA, CTEPH patients continued exercise training and underwent evaluation at the 3-month follow-up. Compared with before training but after PEA, exercise training can increase the RVEF (Δ3.53%) but not other hemodynamics, such as the RAP, mPAP, PCWP, TPG, CO, CI, SVR, PVR, and TPR.

Most exercise training studies involved performing echocardiography to estimate sPAP and right ventricular functional variables (20). Although not all individual studies revealed significant improvements in echocardiographic parameters (31), the pooled analysis showed that exercise training had a significantly decreased resting sPAP of 3.7 mmHg. Our pooled analysis showed sPAP was assessed by echocardiography at 3 weeks or 12/15 weeks regardless of the resting sPAP or peak sPAP. In our study, we did not pool the analysis of right heart areas because only Christian Nagel's study assessed this outcome (22).

With regard to the cardiac function assessed by CPET (17, 22), the oxygen pulse had a trend toward an increase at 3 weeks. However, regardless of whether at rest or max, neither the HR nor oxygen saturation changed. Meanwhile, NT-proBNP plasma levels continued to decrease with 12 or 15 weeks of exercise training after PEA.

Interestingly, our pooled analysis showed the arterial blood gases at the end of the post-PEA 3-month exercise training significantly improved, similar to the improvement observed at the end of the PEA.

Limitations

Our study had several limitations. First, we found only a few studies that had assessed the safety and efficacy of exercise training in CTEPH patients after PEA. Studies of early rehabilitation after PEA are scarce, and our study may provide some insights into the safety, tolerability and clinical effects of CTEPH undertaking early exercise training programme after PEA. Second, most studies have not evaluated clinical endpoints, such as right heart failure and mortality. Therefore, we also cannot analyse the impact of exercise training on clinical endpoints. Third, it is difficult to assess the sustainability of the effects of exercise training interventions among CTEPH patients after PEA. Fourth, all included studies were single center and had a shorter duration of follow-up. In the future, multicentre randomized controlled trials with longer follow-up durations are needed to further verify the benefits of exercise training on CTEPH patients after PEA in the real world. Finally, as with all meta-analyses, selection bias cannot be completely excluded. Publication bias could not be assessed because of the insufficient number of research articles.

CONCLUSIONS

The findings of this systematic review and meta-analysis suggest that exercise training may be associated with a significant improvement in the exercise capacity and QoL among CTEPH patients after PEA and proved to be safe. Exercise training also improves arterial blood gases and NT-proBNP. However, additional large-scale and multicentre studies are needed to better evaluate the long-term effectiveness and safety of exercise training in CTEPH after PEA.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

LW, PY, and RJ contributed to conception and design of the study. Y-LZ, Q-HZ, and RZ organized the database. JH, S-GG, and C-JL performed the statistical analysis. H-LQ wrote the first draft of the manuscript. J-ML, Y-LZ, and PY wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.2021.664984/full#supplementary-material

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High Neutrophil to Lymphocyte Ratio and Its Gene Signatures Correlate With Diastolic Dysfunction in Heart Failure With Preserved Ejection Fraction

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Aims: To evaluate the interrelation between neutrophil to lymphocyte ratio (NLR) coupled with gene signatures, inflammation, and diastolic dysfunction in patients with heart failure (HF) with preserved ejection fraction (HFpEF).

Methods: The clinical profile of 172 patients with HFpEF (EF $\geq$ 50%) and 173 non-HF control individuals was analyzed retrospectively. The association between NLR and HFpEF and the predictive performance of NLR for HFpEF were assessed by the binary logistic regression analysis and the receiver operating characteristic curve (ROC). Multivariate linear regression models further examined the associations between NLR and high-sensitivity C-reactive protein (hs-CRP), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), and average septal-lateral E/e', respectively. The freshly isolated neutrophils from 30 HFpEF patients and 42 non-HF controls were subjected to transcriptomic profiling. The biomarkers related to neutrophil activation and inflammation were detected in serum samples.

Results: The HFpEF patients in Southeast China were lean and had comorbidity burden and worse cardiac structure/function. Compared with non-HF control individuals, HFpEF patients had a rise in NLR. NLR displayed an independent association with HFpEF [adjusted odds ratio, 2.351; 95% CI, 1.464–3.776; $p < 0.001$] and it predicted HFpEF with the area under the ROC 0.796 (95% CI, 0.748–0.845, $p < 0.001$). The positive associations between NLR and hs-CRP, NT-proBNP, and mitral E/e' were found in HFpEF patients. Moreover, patients had significantly elevated serum levels of neutrophil elastase and inflammatory biomarkers, both of which correlated with the mitral E/e' ratio. Finally, multiple molecules that drive neutrophil degranulation and inflammation, such as *S100A8/A9/A12* and *PADI4*, were transcriptionally up-regulated in neutrophils of HFpEF patients.

Conclusions: The high NLR coupled with transcriptional activation of neutrophils correlates with systemic inflammation and functional impairment in HFpEF patients, which may suggest a causative role of neutrophils in the pathogenesis of the disease.

Keywords: heart failure with preserved ejection fraction, neutrophil to lymphocyte ratio, inflammation, diastolic dysfunction, gene signature

INTRODUCTION

Heart failure (HF) with preserved ejection fraction (HFpEF) has conferred substantial morbidity and mortality on clinical patients. Its prevalence is increasing at an alarming rate, currently representing 50% of all HF worldwide (1). In contrast to positive outcomes in heart failure with reduced ejection fraction (HFrEF) treated by neurohumoral inhibition, most of the large-scale clinical trials to assess the efficiency of medical therapies for HFpEF have not shown positive results yet (2, 3). HFpEF represents a broad cohort of patients with a combination of multiple risk factors and comorbidities. As such, the failure of effective treatment for HFpEF is likely attributable to the heterogeneity in this clinical scenario (4). Despite the phenotypic diversity, an increasingly popular theory about HFpEF is that this syndrome reflects a pro-inflammatory state (5). By utilizing comprehensive proteomic approaches to analyze blood biomarkers of HFpEF patients, recent studies demonstrate that systemic inflammation is closely related to HFpEF symptomatology. Moreover, the inflammation appears to mediate the association between comorbidity burden, worse cardiac hemodynamic stress, and adverse outcomes (6, 7). The systemic inflammation is associated with increased cardiomyocyte passive tension and aberrant myocardial collagen deposition, both of which would result in impaired left ventricular (LV) compliance in HFpEF (5, 8). Intriguingly, the tissue or cellular source of these inflammatory biomarkers remains uncertain. Therefore, characterizing specific sources of inflammatory molecules involved in the pathogenesis of HFpEF is an essential issue to be clarified.

Neutrophils are the dominant type of leukocytes during acute inflammatory reactions. The emerging evidence that neutrophils contribute to the clinical manifestations of cardiovascular diseases has been well-discussed (9). In the context of congestive HF, the increased neutrophil lifespan positively correlates to the New York Heart Association (NYHA) class, plasma levels of C-reactive protein (CRP), and alkaline phosphatase (10). The I-PRESERVE trial (Irbesartan in Heart Failure with Preserved Ejection Fraction Study) demonstrates that high neutrophil counts serve as an independent risk factor associated with poor outcomes of HFpEF patients (11). The neutrophil to lymphocyte ratio (NLR) has been proposed as a valuable marker to stratify the risk of patients hospitalized with HFpEF (12). The high level of plasma myeloperoxidase secreted by neutrophils is thought to be suggestive footprints of microvascular endothelial inflammation in HFpEF patients (13). In the endomyocardial biopsy samples from HFpEF patients, a subset of inflammatory cells marked by CD11a and CD45 (pan-leukocyte markers) is increased, associated with the collagen accumulation and high tissue levels of transforming growth factor (TGF)-β (14). Our previous study reported a lean diabetic HFpEF mouse model. The HFpEF mice have diastolic dysfunction and LV stiffness, concurrent with apparent cardiac inflammation and interstitial fibrosis. Of note, these pathological alternations in mouse hearts are associated with massive neutrophil infiltration and neutrophil extracellular traps (NETs) formation (15).

It appears that neutrophils play significant roles in the pathogenic process of HFpEF. However, the pathological involvement of neutrophils in exacerbating the inflammation or functional impairment of HFpEF patients remains poorly understood. To this end, our study aimed to assess the interrelation between neutrophils coupled with transcriptomic profile, inflammatory biomarkers, and abnormal cardiac structure/function of clinical HFpEF patients.

METHODS

Study Population

First, in retrospective analysis, the clinical data were obtained from 172 in-patients diagnosed with HFpEF (EF $\geq$50%) between January 2016 and December 2019 (16). Meanwhile, 173 gender and age-matched in-patients with mild to moderate hypertension but no HF symptoms were recruited as non-HF controls (27 patients had Grade1 hypertension, 144 patients had Grade 2 hypertension, and two patients had Grade 3 hypertension) (**Table 1**). Patients' clinical profile, including demographic variables, medical history, laboratory values, and echocardiographic variables, was well-documented after admission. Patients who had pulmonary infection, hematopoietic disease, and autoimmune disease or were undergoing antibiotic or immunosuppressive therapy were excluded from this study. Second, a total of 30 in-patients newly diagnosed with HFpEF and 42 non-HF control individuals in our hospital (from January to December 2020) were enrolled in this study (**Supplementary Table 1**). The circulating neutrophils were freshly isolated from blood samples of patients for transcriptomic analysis. The serum samples of patients were subjected to a biomarker assay.

Assessment of Hematological Parameters

Laboratory variables, including complete blood cell counts, serum lipids, glucose, hemoglobin A1c (HbA1c), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), were examined and documented. The NLR ratio was constructed as follows: NLR = neutrophil count to lymphocyte count.

TABLE 1 | The clinical characteristics of non-HF control individuals and HFpEF patients.

	Non-HF ($n = 173$)	HFpEF ($n = 172$)	*p*-Value
Demographic characteristics			
Age	70.8 ± 5.8	71.1 ± 12.5	0.24
Female	82 (47.6)	83 (48.3)	0.91
BMI, kg/m^2	24.7 ± 3.2	23.7 ± 4.5	<0.05
Heart rate, beats/min	73.9 ± 13.1	84.3 ± 19.8	<0.05
Systolic BP, mm Hg	138.6 ± 16.5	133.1 ± 21.8	<0.05
Diastolic BP, mm Hg	80.9 ± 10.9	77.5 ± 15.5	<0.05
Medical history			
NYHA functional class			<0.05
I	5 (2.9)	2 (1.2)	
II	2 (1.2)	14 (8.1)	
III	0 (0)	41 (23.8)	
IV	0 (0)	115 (66.9)	
Hypertension	173 (100)	123 (71.5)	<0.05
Diabetes mellitus	31 (17.9)	58 (33.7)	<0.05
Hyperlipidemia	48 (27.7)	20 (11.6)	<0.05
Arrhythmia	33 (19.1)	111 (64.5)	<0.05
Coronary vascular disease	14 (8.1)	89 (51.7)	<0.05
Medication use			
Antiplatelet therapy	120 (69.3)	160 (93.0)	<0.05
Beta-blockers	71 (41.0)	150 (87.2)	<0.05
Calcium-channel blockers	117 (67.6)	39 (22.7)	<0.05
Diuretics	11 (6.4)	170 (98.8)	<0.05
ACE inhibitors or ARBs	95 (54.9)	124 (72.1)	<0.05
Statins	157 (90.8)	138 (80.2)	<0.05
Echocardiography			
LV mass, g	165 (139, 186)	253 (211, 315)	<0.05
LVEF, %	67 (65, 70)	61 (55, 65)	<0.05
E/e'	11.4 (8.9, 13.4)	18.5 (14.5, 26.2)	<0.05
LA diameter, cm	3.2 (3.0, 3.4)	4.2 (3.9, 4.7)	<0.05
Laboratory			
NT-proBNP, pg/ml	88 (53, 156)	3,320 (1,657, 7,991)	<0.05
hs-CTnI, ng/ml	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.27
Creatinine, μmol/L	65.5 (53.8, 76.6)	95.0 (71.6, 144.7)	<0.05
Total triglyceride, mmol/L	1.24 (0.95, 1.74)	0.94 (0.77, 1.55)	<0.05
Total cholesterol, mmol/L	4.27 (3.67, 4.98)	3.67 (3.16, 4.47)	<0.05
LDL-C, mmol/L	2.64 (2.05, 3.29)	2.08 (1.70, 2.79)	<0.05
HDL-C, mmol/L	1.22 (1.05, 1.39)	0.99 (0.82, 1.26)	<0.05
Fasting Glucose, mmol/L	5.15 (4.79, 5.73)	5.38 (4.72, 6.84)	<0.05
HbA1c, %	5.8 (5.5, 6.1)	6.1 (5.7, 6.7)	<0.05
hs-CRP, mg/L	1.50 (0.48, 2.73)	9.62 (3.03, 27.38)	<0.05
Hematological parameters			
WBC count, 10^9/L	6.19 (5.23, 7.29)	6.79 (5.54, 8.96)	<0.05
RBC count, 10^{12}/L	4.38 (4.04, 4.71)	4.10 (3.60, 4.62)	<0.05
Platelet count, 10^9/L	209 (178, 244)	198 (158, 248)	0.19
Hemoglobin, g/L	132 (124, 143)	119 (101, 136)	<0.05
Neutrophil, 10^9/L	3.79 (3.05, 4.48)	4.71 (3.58, 6.74)	<0.05
Lymphocyte, 10^9/L	1.79 (1.40, 2.16)	1.29 (0.92, 1.74)	<0.05
Monocyte, 10^9/L	0.49 (0.37, 0.61)	0.50 (0.33, 0.67)	0.24
NLR	2.21 (1.60, 2.74)	3.77 (2.43, 5.76)	<0.05

Data are given as mean (SD), median (IQR), or number (percent), as appropriate. Depending on the types of data, the Mann-Whitney test or Fisher exact test for unpaired observations was applied, and $p < 0.05$ was considered to indicate statistical significance. ACEI, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; BP, blood pressure; E/e', average septal-lateral E/e' ratio; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CTnI, high-sensitivity cardiac troponin I; hs-CRP, high-sensitivity C-reactive protein; LA, left atrial; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LV mass, left ventricular mass; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; NYHA, New York Heart Association; RBC, red blood cell; WBC, white blood cell; NLR, neutrophil count to lymphocyte count.

Neutrophil Isolation

The circulating neutrophils were freshly isolated from eight HFpEF patients and 12 non-HF control individuals. The blood sample (2 mL) was carefully layered over Polymorphprep™ reagent (Axis-Shield, Scotland). After centrifuging at 500 g for 35 min at room temperature, the plasma and mononuclear cells (upper band of cells) were removed, and neutrophils were harvested. After washing with Hepes-buffered saline [0.85% (w/v) NaCl], cell pellet was resuspended in ammonium chloride lysis buffer [0.83% (w/v) NH4Cl, 10 mM Hepes-NaOH, pH 7.4] to remove any residual erythrocyte contamination. Then cells were harvested by centrifugation and stored in TRIzol™ reagent (Thermo Fisher Scientific, USA) for subsequent RNA extraction.

Transcriptome Sequencing of Neutrophils

RNA isolation and purification were performed using TRIzol-chloroform and RNeasy Mini Kit (Qiagen, Germany). The quality of RNA was checked with Agilent 2100 Bioanalyzer (Agilent, USA). The RNA-seq library was prepared by the Beijing Genomics Institute (Shenzhen, China). Sequence reads were obtained using BGIseq500 (Illumina) and successfully mapped to the human genome (Genome Reference Consortium Human Build 38 patch release 13, GRCh38.p13). Read counts were normalized based on reads per kilobase million (RPKM). The DEseq2 method was used to filter differential genes (17). The adjusted *p*-value (Q value) <= 0.05 was acceptable to indicate the gene expression with a significant difference. According to the results of differential gene detection, the R package heatmap was used to perform hierarchical clustering analysis on the union set differential genes. The Reactome enrichment was subsequently performed to investigate the molecular function and biological pathways that genes participate.

Determination of Biomarkers in Serum

The serum samples were collected from 30 HFpEF patients and 42 non-HF control individuals. Biomarkers related to systemic inflammation [interleukin (IL)-1β, IL-6, IL-10, tumor necrosis

factor (TNFα), and soluble intercellular adhesion molecule-1 (sICAM-1)], tissue remodeling [matrix metalloproteinase 9 (MMP9)], as well as neutrophil activation [neutrophil elastase (NE)] were examined by the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (NeoBioscience, China, and Abcam, UK).

Statistical Analysis

In the cohort study, data are given as means and standard deviations (SD), medians and interquartile 25th and 75th percentiles (IQRs), or numbers and percentages, as appropriate. The statistical calculations were performed using IBM SPSS statistics software. Depending on the types of data, the non-parametric Mann-Whitney test or Fisher exact test for unpaired observations was applied for statistical comparison. Binary logistic regression analysis was carried out using HFpEF as the dependent variable to analyze the association between NLR and HFpEF after adjusting for potential confounders, including age, gender, body mass index (BMI), high-sensitivity CRP (hs-CRP), and diabetes. The crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated. Receiver operating characteristic (ROC) curves with area under the curve (AUC) were calculated to determine discriminative ability. The partial Pearson or the Spearman correlations were computed to describe the relationship between variables of interest after values were logarithmically transformed. Subsequently, the multivariate linear regression analysis was conducted to identify factors associated with hs-CRP, NT-proBNP, and average septal-lateral E/e', respectively. In the transcriptomic sequencing study, the fold changes of FPKM of interested genes were calculated and compared between the two groups. Independent samples were compared by a two-tailed unpaired *t*-test with Welch's correction. For all statistical analyses, $p < 0.05$ was considered to indicate statistical significance.

RESULTS

The Clinical Characteristics of HFpEF Patients

Baseline demographic characteristics and laboratory variables of the entire study population were shown in **Table 1**. The age and gender distribution of HFpEF patients were comparable to that of the non-HF control population. Of note, both groups of cohorts were lean and with an average BMI below 30 kg/m^2. The majority of HFpEF patients had severe cardiac function impairment. Approximately 90% of patients were classified in NYHA III to IV, together with a significant elevation of NT-proBNP in patients' blood. Besides hypertension, comorbid arrhythmia and coronary vascular disease were frequently present in HFpEF patients. Of note, we found diabetes was more common in the HFpEF group (30.8 *vs.*12.7% of cohorts who had HbA1c>6.5% for HFpEF *vs.* non-HF individuals). Compared with non-HF individuals, a greater proportion of HFpEF patients were on an antiplatelet, beta-blocker, diuretic, and angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARBs) therapy. Echocardiography examination demonstrated that HFpEF patients had an overall prevalence of LV hypertrophy. Although HFpEF patients exhibited a preserved LV ejection fraction (LVEF, 55–65%), the significantly increased average septal-lateral E/e' ratio, one of the echocardiographic markers of LV filling pressure (18), was present among patients. Moreover, HFpEF patients exhibited an apparent left atrial (LA) dilatation compared to non-HF controls. In terms of laboratory variables, HFpEF patients had significantly elevated hs-CRP levels in circulation, underpinning a systemic inflammatory state in patients.

TABLE 2 | Binary logistic regression analysis of factors associated with HFpEF.

Model	Variables	OR	95% CI	*p*
Crude model	NLR	2.626	(2.036 to 3.386)	<0.001
Adjusted model	NLR	2.351	(1.464 to 3.776)	<0.001
	Age	0.961	(0.910 to 1.015)	0.152
	Female	1.133	(0.453 to 2.835)	0.789
	BMI	0.899	(0.791 to 1.021)	0.102
	hs-CRP	1.719	(1.334 to 2.215)	<0.001
	Diabetes	1.319	(0.447 to 3.088)	0.616

Binary logistic regression analysis was applied to examine factors associated with the HFpEF (dependent variable) in the entire study population (n = 345). The p < 0.05 was considered to indicate statistical significance. OR, odds ratio; CI, confidence interval. Other abbreviations as in ***Table 1****.*

Correlations Between NLR, Inflammation, and Echo Characteristic

The total count of leukocyte, red blood cells, platelet, and monocytes of HFpEF patients was within normal range and was not distinctly different from that of non-HF controls. However, HFpEF patients had a higher neutrophil count but lower lymphocyte count than that of non-HF controls, which resulted in a significant rise in NLR of HFpEF patients (**Table 1**). The binary logistic regression analysis showed that NLR was significantly associated with HFpEF, independent of effects of age, gender, BMI, hs-CRP, and diabetes (adjusted OR, 2.351; 95% CI, 1.464–3.776; $p < 0.001$) (**Table 2**). Then, we calculated the AUC in the ROC curve to assess the predictive performance of NLR for HFpEF, which was 0.796 [95% CI (0.748–0.845), $p < 0.001$] (**Figure 1**).

The significant correlations between NLR and hs-CRP, NT-proBNP, and mitral E/e' ratio were found in HFpEF patients, which were not changed after adjusting age, gender, BMI, NYHA class, and diabetes (**Supplementary Table 1**). Furthermore, results of multivariate linear regression models suggested NLR was likely to be an independent predictor of hs-CRP ($p < 0.001$), NT-proBNP ($p < 0.01$), and mitral E/e' ratio ($p < 0.05$), respectively (**Table 3**). In contrast, NLR did not display any significant correlation with cardiac structural parameters in HFpEF patients (**Supplementary Table 1**).

Correlations Between Neutrophil Activation and Systemic Inflammation

The circulating levels of pro-inflammatory biomarkers were further examined in HFpEF patients and non-HF control

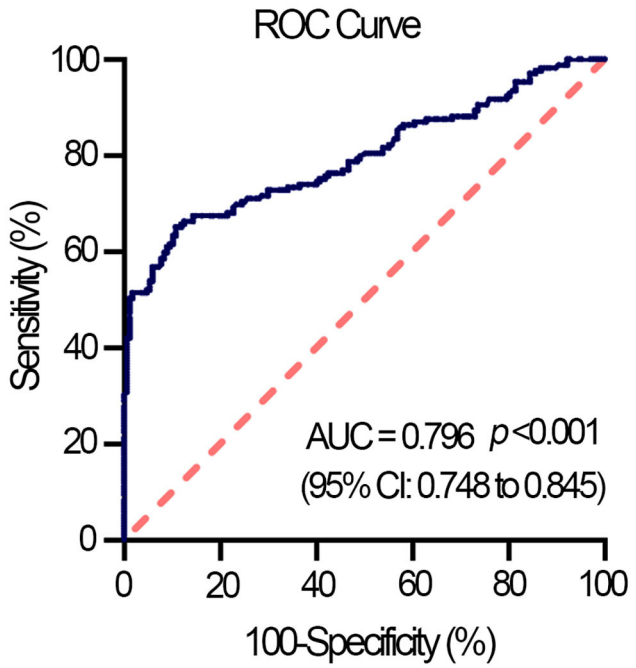

FIGURE 1 | Receiver operating characteristic (ROC) curve displays NLR as a predictor of HFpEF in the entire study population ($n = 345$). AUC, the area under the curve; CI, confidence interval; HFpEF, heart failure with preserved ejection fraction.

individuals (**Table 4**, **Supplementary Table 2**). Multiple pro-inflammatory biomarkers involved in systemic inflammation, such as TNFα, IL-1β, IL-6, IL-10, and sICAM-1 (6, 7), were substantially increased in HFpEF patients. Meanwhile, compared to non-HF controls, HFpEF patients had a higher level of MMP9, a serological marker of collagen turnover that predicts diastolic dysfunction and incidence of HFpEF (19). We also found serum level of NE, one of the neutrophil-derived serine proteases released upon neutrophil activation and degranulation (20), was significantly elevated in the HFpEF group (**Table 4**). The circulating level of NE correlated well with multiple inflammatory biomarkers, including TNFα, IL-1β, IL-6, and sICAM-1. Meanwhile, these inflammatory biomarkers had a significant correlation with the mitral E/e' ratio. Finally, a correlation between NE and the E/e' ratio ($r = 0.562$, $p < 0.01$) was observed in HFpEF patients. By contrast, although MMP9 showed a correlation trend with both NE and E/e' ratio, it did not reach a statistical significance (**Table 5**).

The Transcriptomic Characteristics of Neutrophils of HFpEF Patients

We further characterized the transcriptional plasticity of neutrophils collected from non-HF controls and HFpEF patients. A total of 19,813 genes were successfully identified by RNA-sequencing. Among them, 134 genes were filtered with a significantly differential expression between the two groups (**Supplementary Table 3**). Concretely, compared with neutrophils of the non-HF control group, there were 89 transcripts significantly increased, whereas 45 transcripts decreased in neutrophils of HFpEF patients. The representative gene expression profile in the form of a heatmap was generated (**Figure 2A**). The Reactome annotation classification was subsequently performed to enrich signaling pathways that genes participate. The most significant enrichments were found in signaling pathways relating to neutrophil degranulation (17 genes), immune system (33 genes), and innate immune system (20 genes) (**Figure 2B**). Importantly, we found all 17 genes involved in neutrophil degranulation were significantly up-regulated in the HFpEF group. In particular, the gene expression of *S100A8* and *S100A9*, both of them encoding small calcium-binding protein S100A8/A9 complex, were significantly increased in neutrophils of HFpEF patients (**Figure 2C**). S100A8/A9 complex triggers leukocyte degranulation by promoting protein synthesis of leukotriene B4 (21) or by mechanisms dependent on p38 and JNK (22). In addition, HFpEF patients showed transcriptional up-regulation of *S100A12* in circulating neutrophils. The S100A12 protein has been proved to mobilize neutrophils from bone marrow and activate the adhesion and migration of neutrophils toward inflammatory sites (23). Compared with the non-HF controls, neutrophils of the HFpEF patients also had much higher *PADI4* gene expression that encodes the peptidyl arginine deiminase 4 (PAD4), a protein that critically regulates chromatin de-condensation and NETs formation (**Figure 2C**) (24). The gene-level of *CD55*, encoding a glycoprotein involved in the complement cascade regulation, was elevated in the HFpEF patients' neutrophils. On the resting neutrophil surface, the CD55 protein level is low, but that is highly expressed upon neutrophil activation (25). Finally, multiple transcripts (*CDA*, *ALOX5AP, IL6R*) with relatively high abundance in neutrophils were up-regulated in the HFpEF group as well. However, their pathophysiological relevance with cellular activation remains obscure yet.

DISCUSSION

The main findings from the present HFpEF study were as follows: (1) the patients in Southeast China were lean and who had comorbidity burden and worse cardiac structure and function; (2) the high NLR was predictive to HFpEF and independently associated with hs-CRP, NT-proBNP, and mitral E/e' ratio; (3) the heightened serum NE levels correlated with the systemic inflammation and mitral E/e' ratio in HFpEF patients; (4) multiple molecules that drive neutrophil degranulation and inflammation were transcriptionally up-regulated in neutrophils of HFpEF patients.

The clinical HFpEF is frequently bound with a broad of comorbidities (1, 4, 26). Among those comorbidities, obesity is highly prevalent in Western patients. The obese patients (BMI>30 Kg/m^2) exhibit comorbidity-driven microvascular inflammation, HF severity, and fibrosis (27). By comparison, recent epidemiologic studies suggest a unique lean phenotype of HFpEF in Asia. The lean HFpEF patients have a high

TABLE 3 | Multivariate linear regression analysis with hs-CRP, NT-proBNP, and E/e' as dependent variables, respectively.

Regression variables	*B*	VIF	95%CI	*p*	R^2	*P**
hs-CRP					0.164	<0.01
Constant term	−0.010					
NLR	0.677	1.095	(0.286 to 1.067)	<0.001		
Age	0.001	1.140	(−0.009 to 0.010)	0.908		
Female	−0.224	1.145	(−0.469 to 0.021)	0.730		
BMI	0.015	1.102	(−0.011 to 0.042)	0.250		
NYHA class	0.053	1.263	(−0.128 to 0.234)	0.562		
Diabetes	0.149	1.116	(−0.111 to 0.409)	0.258		
NT-proBNP					0.197	<0.001
Constant term	2.517					
NLR	0.514	1.107	(0.186 to 0.843)	<0.01		
Age	0.003	1.165	(−0.004 to 0.010)	0.358		
Female	−0.032	1.156	(−0.218 to 0.153)	0.731		
BMI	0.000	1.064	(−0.020 to 0.019)	0.987		
NYHA class	0.154	1.243	(0.022 to 0.287)	<0.05		
Diabetes	−0.191	1.114	(−0.385 to 0.004)	0.055		
E/e'					0.166	<0.05
Constant term	1.750					
NLR	0.136	1.057	(0.014 to 0.258)	<0.05		
Age	0.000	1.115	(−0.007 to 0.007)	0.914		
Female	0.167	1.089	(−0.012 to 0.347)	0.067		
BMI	0.016	1.060	(−0.002 to 0.035)	0.087		
NYHA class	0.112	1.223	(−0.015 to 0.239)	0.083		
Diabetes	0.015	1.166	(−0.175 to 0.205)	0.873		

The values were logarithmic transformed before analysis. Multivariate linear regression analysis was conducted in the HFpEF group (n = 172), with hs-CRP, NT-proBNP, and E/e' as dependent variables, respectively. The p < 0.05 was considered to indicate statistical significance. CI, confidence interval; VIF, variance inflation factors. Other abbreviations as in ***Table 1****. P*, p-values of ANOVA test for individual models.*

TABLE 4 | The concentration of inflammatory biomarkers in the circulation of non-HF control individuals and HFpEF patients.

	Non-HF (*n* = 42)	HFpEF (*n* = 30)	*p*-Value
Laboratory			
TNFα, pg/ml	10.20 (2.42, 12.74)	12.38 (5.75, 19.70)	<0.05
IL-1β, pg/ml	3.46 (2.53, 4.97)	17.93 (5.45, 21.93)	<0.05
IL-6, pg/ml	1.72 (1.09, 2.18)	3.79 (1.25, 7.53)	<0.05
IL-10, pg/ml	0.26 (0.12, 0.35)	0.38 (0.22, 1.16)	<0.05
MMP9, ng/ml	270.1 (180.2, 390.6)	621.3 (302.5, 915.4)	<0.05
sICAM, ng/ml	401.5 (257.2, 536.8)	583.2 (337.6, 721.5)	<0.05
NE, ng/ml	83.28 (60.5, 134.3)	121.5 (90.6, 374.0)	<0.05

Data are given as median (IQR). Mann-Whitney test for unpaired observations was applied, and p < 0.05 was considered to indicate statistical significance.
IL, interleukin; MMP9, matrix metalloproteinase 9; NE, neutrophil elastase; sICAM-1, soluble intercellular adhesion molecule-1; TNFα, tumor necrosis factor alpha.

prevalence of diabetes and worse quality of life (28). Our HFpEF cohorts were lean, with an average BMI below 30 Kg/m^2, in line with findings from the China HF registry (29). Besides the high prevalence of comorbidities, such as hypertension (71.5%), arrhythmia (64.5%), and coronary artery diseases (51.7%), diabetes was also found to be relatively common in HFpEF patients (33.7%) than that in non-HF cohorts (17.9%). In terms of pharmacological therapies, to date, the evidence-based therapies for HFpEF are scant. As such, current management of HFpEF is primarily directed toward associated cardiovascular comorbidities and control of hypervolemia (26). We found most HFpEF patients were on diuretics, ACEI/ARBs, and beta-blocker therapies. Although the evidence that beta-blockers improve symptoms in HFpEF patients is lacking, these medications were frequently prescribed to our HFpEF patients (87.2%) in order to lower cardiac oxygen demand and prolong diastolic filling time. The high rate of beta-blocker use is also found in HFpEF patients from the Asian-HF registry (78.9%), CHECK-HF registry (78%), and EMPEROR-Preserved trial (86%) (28, 30, 31). There has been compelling evidence to support the prominent role of inflammation in the pathogenesis and progression of HFpEF (5–7, 32, 33). Mechanically, pro-inflammatory molecules augment oxidative stress, impair nitric oxide bioavailability, reduce cyclic guanosine monophosphate activity but raise cardiomyocyte hypertrophy and passive stiffness. Microvascular inflammation drives the proliferation and activation of myofibroblasts. Abnormal extracellular matrix turnover triggered by pro-inflammatory molecules contributes to

TABLE 5 | The interrelation between neutrophil elastase, inflammatory biomarkers, and mitral E/e' ratio.

		TNFα	IL-1β	IL-6	IL-10	MMP9	sICAM-1	NE	E/e'
HFpEF ($n = 30$)									
NE	***r***	0.735	0.636	0.809	0.250	0.302	0.546	—	0.562
	p	<0.001	<0.001	<0.001	0.219	0.134	<0.01	—	<0.01
E/e'	***r***	0.673	0.547	0.670	0.171	0.235	0.561	0.562	—
	p	<0.01	<0.01	<0.001	0.405	0.248	<0.01	<0.01	—

The values were logarithmic transformed before analysis. Spearman's coefficients were computed to describe the correlation between the two variables. The significant differences were accepted when the $p < 0.05$, all abbreviations as in ***Tables 1***, ***4***.

cardiac interstitial fibrosis (5, 8, 34). Our lean HFpEF patients had high levels of inflammatory biomarkers in circulation, including hs-CRP, TNFα, IL-1β, IL-6, IL-10, and sICAM-10. At present, identifying which organ(s) or cells are inflamed in patients is still tricky.

Systemic inflammation and immune cell homeostasis are two interlinked processes that constantly emphasize each other. The important role of monocytes and macrophages in cardiovascular inflammation has been historically appreciated (35). By contrast, neutrophils have been neglected in the context of cardiovascular research for a long time. Currently, our understanding of the pleiotropic roles of neutrophils in chronic inflammation has been advanced (9, 36). Aberrant neutrophils in circulation can stratify the risk of patients hospitalized with HFpEF or predict the poor prognosis of patients (11–13). In cardiac specimens of both HFpEF patients and animals, the neutrophil infiltration is found to be associated with inflammatory and fibrotic damages that result in LV stiffness (14, 15). In lean HFpEF patients, we observed an apparent rise in NLR ratio and serum levels of NE. Multivariate regression analysis revealed a clear association between the NLR and hs-CRP, NT-proBNP, and mitral E/e' ratio. Moreover, NLR was likely to be predictive of the increased risk of HFpEF. Of note, such associations were independent of the effect of age, gender, BMI, NYHA class, and diabetes. Meanwhile, the elevated serum NE levels in HFpEF patients significantly correlated with multiple pro-inflammatory biomarkers. Both of them also displayed a close correlation with the mitral E/e' ratio of patients. These interrelations collectively indicated the pathological potential of activated neutrophils in aggravating systemic inflammation and diastolic dysfunction of HFpEF patients. To date, the pathophysiological mechanisms responsible for neutrophils' detrimental effects on heart tissues remain to be elucidated yet. In the future study, it is of significant interest to resolve this doubt by investigating the cardiac phenotypes and systemic inflammation levels in our lean HFpEF mice after the genetic depletion of neutrophils (15).

So far, a number of risk factors have been proposed to drive granulopoiesis, including metabolic alternations (hypercholesterolemia and hyperglycemia), inflammasome pathways, aging, stress, and disturbed lifestyle (9). We noted that the distribution of age and gender was comparable between HFpEF patients and non-HF control individuals. The comorbid hyperlipidemia was neither prevalent in HFpEF patients (11.6%). By comparison, diabetes was found to be relatively common in HFpEF patients. Hyperglycemia directly induces proliferation and expansion of bone marrow myeloid progenitors (37, 38). Under chronic inflammation, some cytokines function as critical pro-inflammatory "emergency" signals to drive myeloid differentiation. IL-1β directly accelerates myeloid differentiation of hematopoietic stem cells *via* precocious activation of a PU.1-dependent gene program (39). Myocardial infarction results in rapid recruitment of neutrophils to the infarct. The infiltrated neutrophils release IL-1β, which may contribute to the cytokine pool. As a consequence, IL-1β acts with hematopoietic progenitor cells in the bone marrow and further stimulates granulopoiesis in a cell-autonomous manner (40). Alternatively, other inflamed tissues or cells may produce cytokine that accelerates myelopoiesis and neutrophil production, leading to neutrophil recruitment in heart tissues. However, the risk factors that drive neutrophilia in lean HFpEF patients remain unknown yet.

Although neutrophils are traditionally considered to be transcriptionally silent, the transcriptional plasticity of neutrophils upon sterile stimulation and microbial insults has been unraveled (41). We further found the transcriptional signatures of neutrophils of HFpEF patients were distinctive to that of non-HF control individuals. Beyond our expectation, circulating neutrophils from HFpEF patients do not show robust transcriptomic changes of the classical pro-inflammatory cytokine found in primed neutrophils *in vitro* (42). However, we noted multiple molecules that drive neutrophil degranulation and inflammation were transcriptionally up-regulated in neutrophils of HFpEF patients. Of note, among 134 genes with differential expression, all genes enriched in the neutrophil degranulation pathway were up-regulated in HFpEF patients' neutrophils, consistent with an increased level of neutrophil-derived NE in patients' blood. Moreover, genes encoding a small calcium-binding protein family (S100A8/A9/A12) were transcriptionally up-regulated in neutrophils of HFpEF patients. S100A8/A9 functions as neutrophil-derived alarmins that can activate CD11b and induce neutrophil adhesion to fibrinogen, leading to neutrophil migration to inflammatory sites (43). Hyperglycemia can increase the release of S100A8/S100A9 from neutrophils, and this protein complex interacts with the receptor for advanced glycation end products on myeloid progenitor cells and enhance myelopoiesis (37, 38). In infarct myocardial tissues, S100A8/S100A9 released from neutrophils can bind to Toll-like receptor (TLR) 4 and prime the nod-like

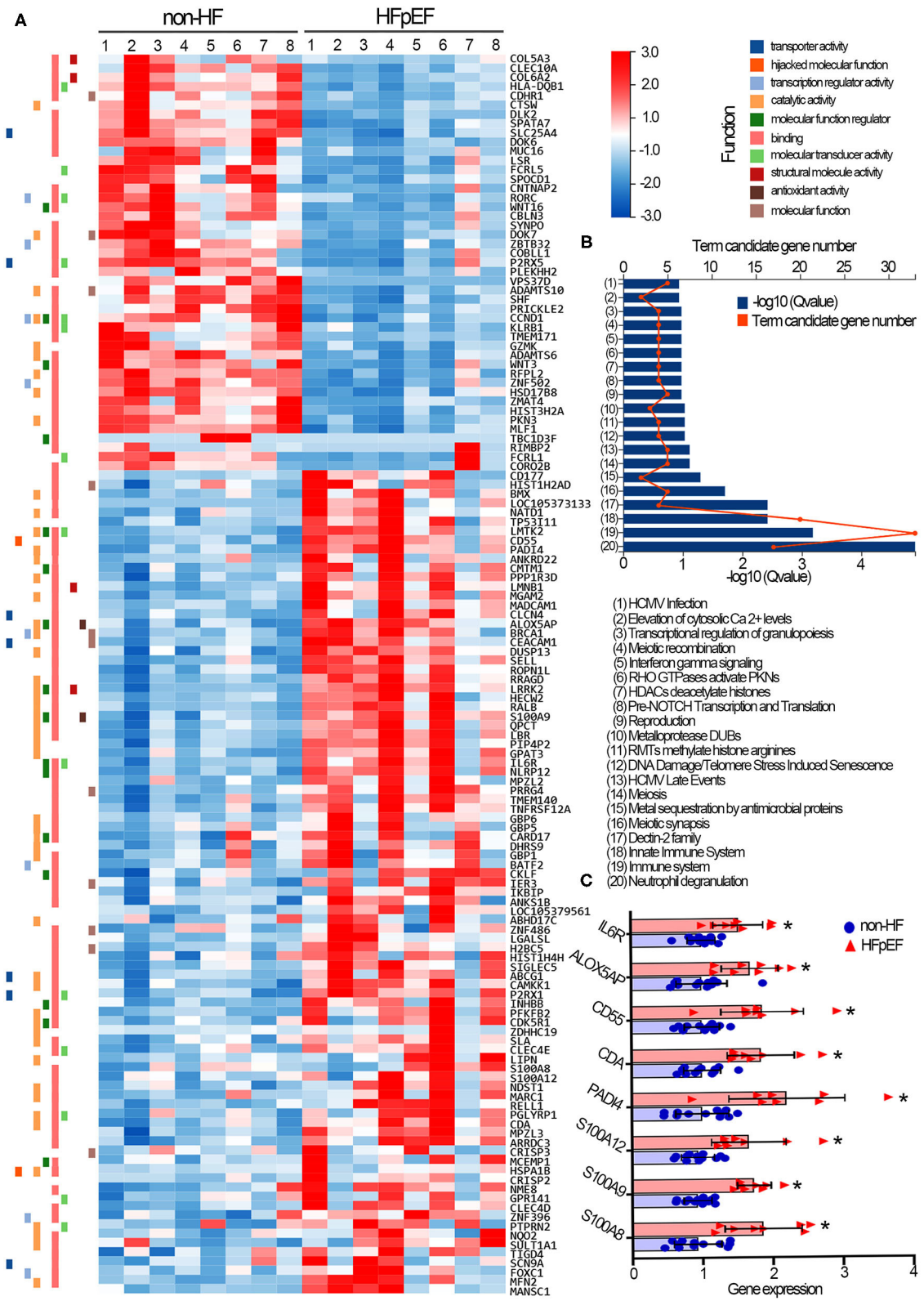

FIGURE 2 | Transcriptomic profile of circulating neutrophils collected from non-HF control individuals and HFpEF patients. **(A)** The representative heatmap of the 134 genes that were differentially expressed in neutrophils of non-HF control individuals and HFpEF patients. Red indicates relative gene up-regulation, and blue indicates

(Continued)

FIGURE 2 | relative gene down-regulation. **(B)** The Reactome enrichment analysis was performed to characterize signaling pathways that genes participate. **(C)** Gene expression levels of *S100A8*, *S100A9*, *S100A12*, *PADI4*, *CDA*, *CD55*, *ALOX5AP*, and *IL6R* were compared in neutrophils of two groups. Fold change was calculated for comparison and was presented with means and SD. *$p < 0.05$, compared with non-HF control individuals. Non-HF, non-heart failure controls ($n = 12$); HFpEF, heart failure with preserved ejection fraction ($n = 8$).

receptor family pyrin domain-containing 3 inflammasome in naive neutrophils, resulting in IL-1β-driven granulopoiesis (40). It is particularly worth noting that *PADI4* is up-regulated substantially in neutrophils of HFpEF patients. PAD4 critically regulates chromatin de-condensation and NETs formation (24). The pathogenic potential of NETs in cardiovascular inflammation has so far been well-documented (44). NETs license macrophages to turn on transcriptional regulation of IL-6 and pro-IL-1β via TLR2/4 in atherosclerosis (45). NETs stimulate human lung fibroblasts to a myofibroblast with elevated α-smooth muscle actin expression (46) and mediates extracellular matrix remodeling (47). The cytotoxic histone and deoxyribonucleic acid bound to NETs induces organ fibrosis in aged mice (48). However, in clinical HFpEF patients, it is still difficult to determine whether neutrophils with high expression of *PADI4* are prone to form NETs in failing heart tissues. In lean HFpEF mouse hearts, we observed the presence of NETs and increased PAD4 protein levels, which was paralleled with cardiac inflammation and fibrosis (15). Our ongoing study further demonstrated neutrophils from lean HFpEF mice were prone to form NETs. The NETs-containing media significantly enhanced alpha-smooth muscle actin expression in co-cultured myocardial fibroblasts, suggesting a pro-fibrotic action of NETs (unpublished data).

Given the significant roles of neutrophils in cardiovascular inflammation, the specific intervention of neutrophils may open the door for the development of a novel therapeutic strategy. Interestingly, metformin, a drug representing a worldwide cornerstone in anti-diabetes therapy, can exert inflammation-inhibitory effects independently from glucose control (49). Metformin can inhibit NETs *in vitro* (50), decrease NLR in the diabetic population, and suppress plasma cytokine levels in the non-diabetic heart failure cohort (51). It is recently reported that, in patients who are infected with coronavirus disease 2019, metformin users have a lower level of neutrophil counts but a higher level of lymphocyte counts in the blood. Meanwhile, serum inflammatory factors (CRP, IL-6, TNF-α) and cardiac injury indicators (NT-proBNP) are marked lower in the metformin group (52). Therefore, we think repurposing metformin to inflammation-driven chronic HFpEF would be an active field investigated in the future.

STUDY LIMITATIONS

Several limitations should be considered when interpreting the results of the present study. This was a small single cohort study. There was, therefore, a potential lack of power. The roles of neutrophils contributing to systemic or myocardial inflammation should be investigated in larger HFpEF cohorts. In addition, our investigation was not exploratory but based on the published hypothesis that inflammation is a critical pathogenic stimulus in HFpEF. We observed several correlations between neutrophil activation, systemic inflammation, and ventricular functional impairment in HFpEF patients. However, the tissue or cellular source of inflammatory molecules and their interrelation with neutrophilia in patients remain uncertain yet. Given the limitation to obtain heart specimens from clinical patients, the mechanisms by which neutrophils and NETs impair cardiac function need to be addressed by more intensive animal and *in vitro* studies.

CONCLUSION

The high NLR coupled with transcriptional activation of neutrophils correlates with systemic inflammation and functional impairment in HFpEF patients, which may suggest a causative role of neutrophils in the pathogenesis of the disease.

DATA AVAILABILITY STATEMENT

The data used for the transcriptomic analysis were deposited in the NCBI Sequence Read Archive (SRA) database. The data are accessible via the SRA accession: PRJNA717666.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committees of Shenzhen Second People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BB, HC, and YX were responsible for the study design and manuscript writing. BB, MC, LJ, and JX contributed to the acquisition and analysis of the data. All authors gave final approval and agreed to be accountable for all aspects of work to ensure integrity and accuracy.

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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.2021.614757/full#supplementary-material

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ORIGINAL RESEARCH
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Embryonic Stem Cell-Derived Exosomes Attenuate Transverse Aortic Constriction Induced Heart Failure by Increasing Angiogenesis

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Background: Although there are concerns regarding their clinical use, embryonic stem cells (ESCs) hold a great promise for cardiac repair. Exosomes deriving from ESCs constitute a promising alternative for heart restoration. However, their effects in hypertension-induced heart failure are still unknown.

Objective and Methods: To investigate the effects of ESCs-derived exosomes on hypertension-induced heart failure and the underlying mechanisms, sustained transverse aortic constriction (TAC) was performed on 8-week-old C57BL/6 male mice. After 1 months, ESCs-derived exosomes were isolated and injected intravenously once a week for 6 weeks. Echocardiography, wheat germ agglutinin (WGA), Masson staining, immunohistochemistry, and tube formation assays were all involved in our study.

Results: Proteomics analyses revealed that ESC-derived exosomes contain FGF2 protein. Tube formation induced by these exosomes could be inhibited by FGF2R siRNA interference. ESCs-derived exosomes evidently attenuated TAC-induced heart failure, improving cardiac function and promoting myocardial angiogenesis which can be attenuated by selective FGF2 inhibitor AZD4547.

Conclusions: ESC-derived exosomes attenuate TAC-induced heart failure mostly by promoting myocardial angiogenesis. FGF2 signaling plays a vital role in the myocardial angiogenesis induced by ESC-derived exosomes.

Keywords: embryonic stem cells, exosomes, angiogenesis, transverse aortic constriction, heart failure

INTRODUCTION

Compensatory adaptation occurs early in response to high blood pressure (1). However, persistent high blood pressure results in cardiac remodeling, which eventually leads to heart failure (2, 3). Inadequate blood supply accelerates the transition from compensatory cardiac hypertrophy to heart failure (4, 5). Adequate myocardial angiogenesis is important to maintain myocardial function in response to sustained hypertension. Previous studies have demonstrated the potential of embryonic stem cells (ESCs) in rescuing injured hearts, which is due to their considerable differentiation ability (6–8). However, ESCs pose some challenges for clinical use with respect

to immune tolerance and cell retention. ESC-derived exosomes, which carry donor-specific microRNAs and proteins, may be a promising alternative for heart failure treatment (9, 10).

In this study, we found that systemic administration of ESC-derived exosomes attenuated transverse aortic constriction (TAC)-induced heart failure by promoting myocardial angiogenesis. Furthermore, we found that fibroblast growth factor-2 (FGF2) signaling played a vital role in this process.

MATERIALS AND METHODS

Cell Culture

We cultured human umbilical vein endothelial cells (HUVEC) and human embryonic stem cells (ESC) in our laboratory for this experiment. Our culture protocols for these two cells strictly followed the description of Chen et al. (10).

Isolation and Identification of Exosomes From ESCs

The culture medium of the ESCs was collected, and exosomes were isolated using the methods described previously (11). After ultracentrifugation, the exosomes were fixed in the fixative and their morphology was observed by transmission electron microscope (TEM; Hitachi H-7650). The size distribution and particle concentration of exosomes were measured using the qNano platform (iZON® Science, UK). Expression of the exosomal markers CD9 (1:1,000; Epitomics) and Alix (1:1,000; Epitomics) was analyzed using Western blotting.

Proteomic Analysis of ESC-Derived Exosomes

ESC-derived exosomes were lysed in 8 M urea and 100 mM Tris solution (pH 7.6). After reduction by dithiothreitol and alkylation by iodoacetamide, the protein solution was digested by trypsin at 37°C for 18 h. Then, the peptide solution was transferred to a solid phase extraction cartridge for desalting and clean-up of the sample. The samples were analyzed with a QExactive HF mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a Nanospray Flex source (Thermo Fisher Scientific). Each sample was separated by an in-house micro-tip C18 column (75 × 200 mm) packed with ReproSil-Pur C18-AQ 3.0-mm resin (Dr. Maisch GmbH, Germany) on an Easy-nLC 1200 nanoflow HPLC system (Thermo Fisher Scientific). The MS1 full scan was performed at a resolution of 60,000 @ m/z 200, followed by "top 15" MS2 scans generated by HCD fragmentation at a resolution of 15,000 @ m/z 200. The normalized collision energy (NCE) was set at 28%, and the dynamic exclusion time was 45 s. Mass spectrometric data were analyzed using MaxQuant 1.6 against the human UniProt database containing 172,418 sequences. Carbamidomethyl cysteine was searched as a fixed modification. Oxidized methionine and protein N-term acetylation were set as variable modifications. Enzyme specificity was set to trypsin. The maximum number of missing cleavage sites was set to 2. The tolerances of the first search and main search for peptides were set to 20 and 4.5 ppm, respectively. The minimum peptide length was set to 7. The false discovery rates (FDRs) of peptides, proteins, and sites were all set to <0.01.

FGF2R siRNA Transfection

The transfection operation was carried out according to the instructions of siRNA kit was purchased from OBIO Biotechnology Co. Ltd. Shanghai, China. Before transfection, inoculate $2*10^5$ HUVEC in each well of the 24-well plate and add 400 ul anti-free medium. At the time of transfection, the cell density reaches 30–50%. Dilute siRNA with 50 ul Opti-MEM to a final transfection concentration of 50 nM. After mixing, let it stand for 5 min. Subsequently, the volume (ul) of added PEI was three times the mass (ng) of siRNA. After violent shaking, let it stand again for 15–20 min. Add the transfection mixture to non-antibiotic medium and place it in a 37°C cell incubator. After 4–6 h, change to complete medium and incubate for 48 h.

Endothelial Cell Culture and Tube Formation Assay

Matrigel (50 μl; BD Biosciences) was added to every well of a 96-well plate on ice and solidified at 37°C. HUVECs (3×10^4 cells/100 μl) were mixed and cultured on solidified Matrigel plugs in DMEM at 37°C in humidified air with 5% CO_2 for 6 h. Exosomes ($1*10^9$) was added to medium as treated groups. The dosage and usage of siRNA were completely in accordance with the instructions of siRNA. Tube structures were counted in 3 randomly selected fields at 10× magnification.

Animal Study

Totally, thirty-six 8-week-old C57BL/6 male mice were used in this study. TAC was performed on thirty mice as previously described (12). One month later, exosomes ($3*10^{10}$) were injected into the tail vein of experimental mice three times a week for 6 weeks as exosome group ($n = 10$). AZD4547 (2 mg/kg/day) was administered via intra-peritoneal injection at the same time besides exosomes administration as TAC+exosome+AZD4547 group ($n = 10$). TAC mice received the same volume of PBS intravenously ($n = 10$) at the same timelines as exosome administration. Control mice did not receive TAC procedure after anesthesia ($n = 6$).

Measurement of Cardiac Function

Echocardiographic images were obtained with a VisualSonics Vevo System (VisualSonics Inc., Canada) after 6 weeks treatment. The mice were anesthetized, and the heart rates were maintained between 450 and 500 beats per min. Both B- and M-mode images were acquired, and the left ventricular internal diastolic diameter (LVIDD), left ventricular interval systolic diameter (LVIDS), left ventricular ejection fraction (EF), and left ventricular fractional shortening (FS) were measured. All measurements were completed by two blinded experienced technicians. A total of 5–8 mice were analyzed per group.

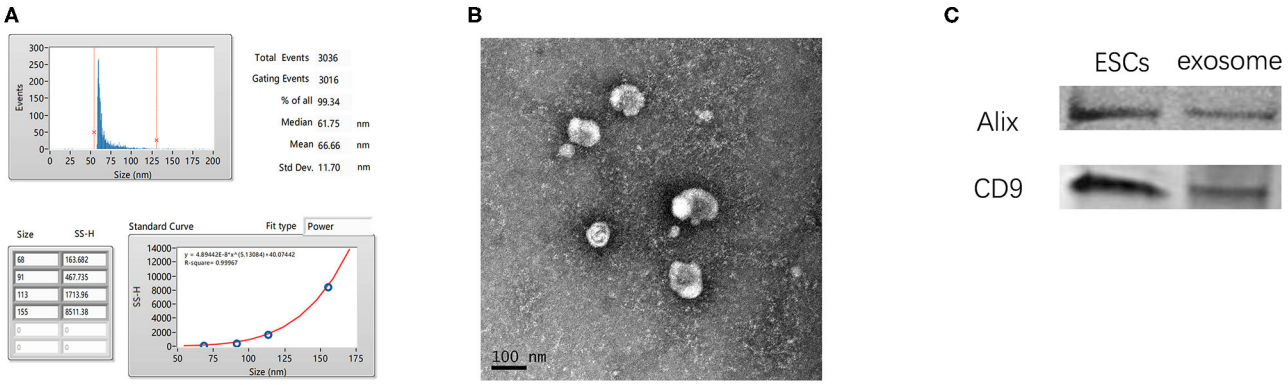

FIGURE 1 | Characterization of ESC-derived exosomes. **(A)** Particle size distribution of ESCs-derived exosomes. **(B)** Morphology of ESCs-Exosomes observed by TEM (scale bar, 100 nm). **(C)** Western blotting showed the presence of exosome markers CD9 and Alix.

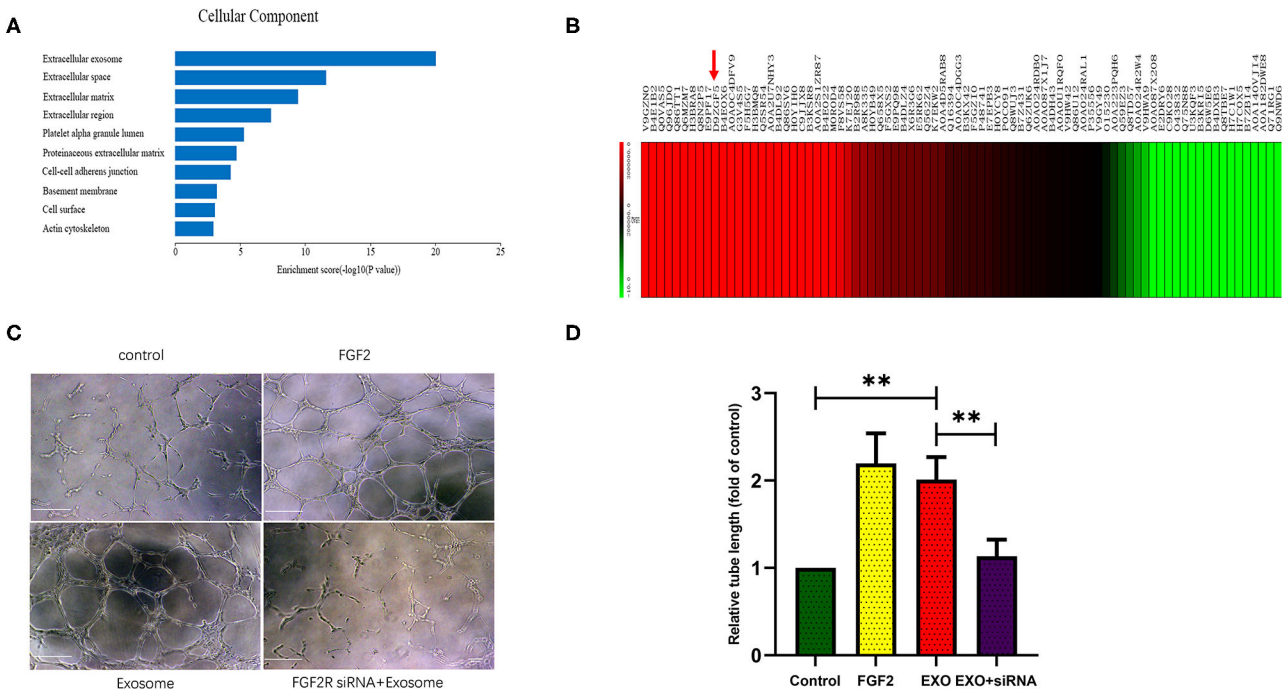

FIGURE 2 | ESCs-derived exosomes stimulate angiogenesis in a FGF2 related way. **(A,B)** Proteome analysis showed ESCs-derived exosomes contains hundreds of proteins in which FGF2 was high abundant and ranked in the top ten of all the proteins in the exosomes. **(C,D)** Exosomes treated HUVECs formed greater tube length compared with control group. FGF2R siRNA interference impaired the exosomes induced angiogenesis *in vitro* (scale bar, 100 um). $^{*}p < 0.05$, $^{**}p < 0.01$.

Histopathological Examination of Mouse Hearts

After the echo examination, the heart was harvested and the left ventricle was sliced from the apex to the base at 6-μm thickness for the evaluation of morphology and interstitial fibrosis. Sections were stained with Masson's trichrome. The percentage of LV fibrosis was determined using a previously described method (13). FITC-conjugated wheat germ agglutinin (WGA) was performed for further determination of cell size. Quantitative digital image analysis system (Image-Pro Plus 6.0) was used in image measurement. Capillary density was quantitatively measured microscopically at ×400 magnification for three randomly chosen fields. The ratio of CD31-positive cells to the total area was calculated by image analysis software. Samples from 4 to 6 mice per group were analyzed.

Statistical Analysis

All values are expressed as the means ± standard errors. Comparisons between groups were performed using an unpaired *t* test. Two-way ANOVA was used to test for differences among groups. A $p < 0.05$ was considered statistically significant.

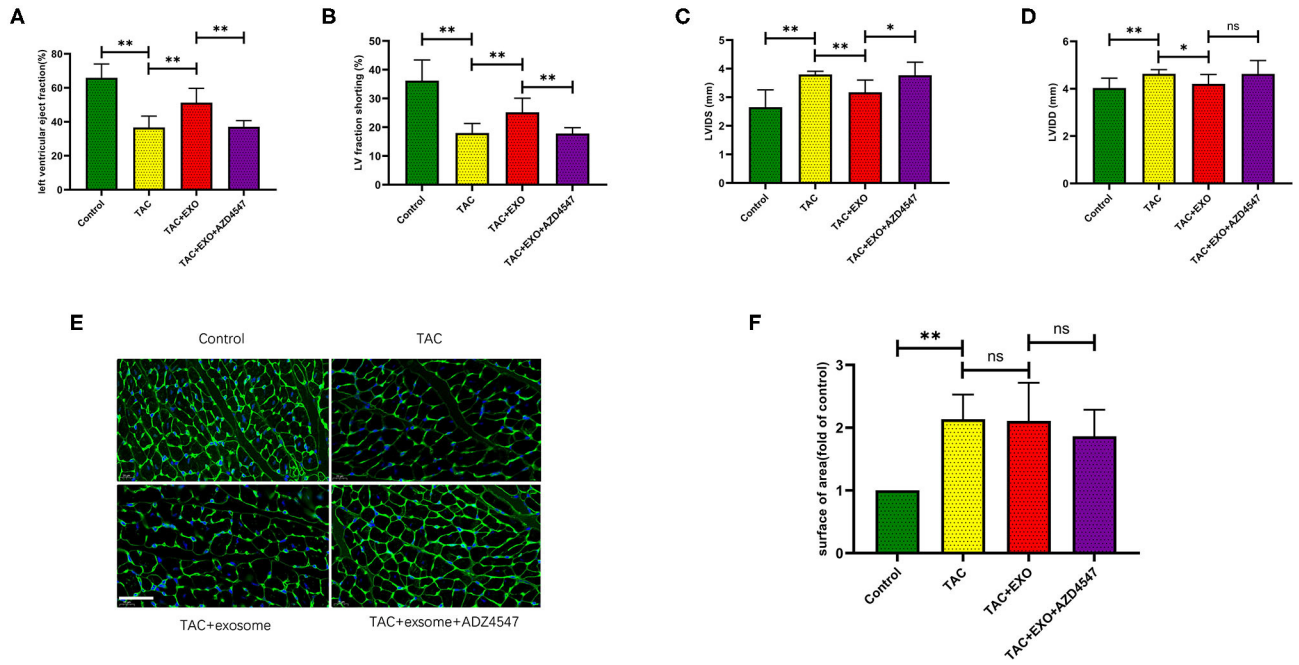

FIGURE 3 | ESCs-derived exosomes effectively rescued heart failure after TAC. **(A–D)** Exosomes treatment significantly rescued the heart function after TAC which was attenuated by AZD4547 administration. **(E,F)** TAC induced myocardium cell cross section area increasement significantly while exosomes treatment had no effect on cell size. ($n = 5-8$, scale bar, 100 um). $^{*}p < 0.05$, $^{**}p < 0.01$.

RESULTS

Characterization of ESC-Derived Exosomes

Exosomes were isolated from the cultured medium of ESCs. qNano analysis showed that most exosomes were in the range of 50–125 nm in size (**Figure 1A**). TEM imaging (**Figure 1B**) revealed exosomes with characteristic ball-shaped morphology. Additionally, vesicles from ESCs expressed the exosome marker proteins CD9 and Alix (**Figure 1C**).

ESC-Derived Exosomes Contained Abundant FGF2

Proteomic analysis of ESC-derived exosomes showed that EC-derived exosomes contained hundreds of proteins, among which FGF2 was highly abundant, ranking in the top ten of all proteins detected within exosomes (**Figures 2A,B**).

The FGF2R siRNA Impaired Exosome-Induced Angiogenesis *in vitro*

Tube formation assays showed that exosome-treated HUVECs formed greater tube length than the control HUVECs ($p < 0.01$), as FGF2 treated HUVECS. The FGFR2 siRNA impaired exosome-induced tube formation ($p < 0.01$) (**Figures 2C,D**).

ESC-Derived Exosomes Rescued Heart Failure After TAC Which Can Be Attenuated by AZD4547 Administration

Compared to PBS, exosome treatment significantly rescued LVEF and Fraction shorting (FS) ($p < 0.01$) in mice after TAC (**Figures 3A,B**). Both LVIDD ($p < 0.05$) and LVIDS ($p < 0.01$) were significantly reduced in the exosome-treated mice group relative to the control group (**Figures 3C,D**). All the above effects were attenuated by AZD4547 administration (**Figures 3A–D**).

ESC-Derived Exosomes Had No Effect on Cardiac Fibrosis and Myocardium Cell Cross Section Area

TAC induced increased cell area (**Figures 3E,F**, $p < 0.01$) and marked myocardial fibrosis (**Figures 4A,B**, $p < 0.01$) as shown by WGA and Masson staining. Exosome administration had no significant effect on TAC-induced myocardial fibrosis and cell area enlargement.

ESC-Derived Exosomes Alleviated Cardiac Microvessel Impairment in TAC Mice Which Can Be Attenuated by AZD4547 Administration

Microvessel density was markedly decreased in the TAC group relative to the control group which could be obviously rescued by exosome administration. This effect was significantly attenuated

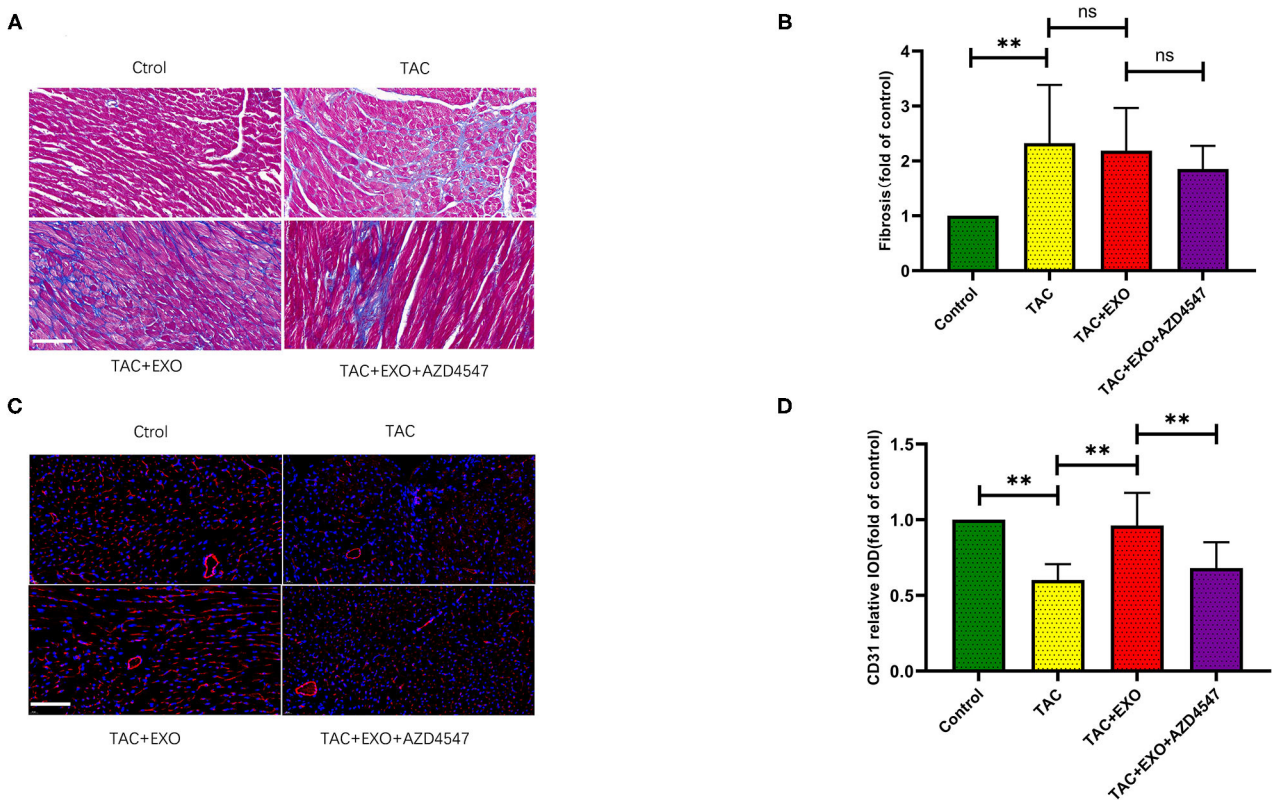

FIGURE 4 | ESCs-derived exosomes increased cardiac angiogenesis and have no effect on the interstitial fibrosis. **(A,B)** TAC induced marked myocardial fibrosis, as evidenced by Masson staining. However, exosomes administration had no significant effect on the TAC induced myocardial fibrosis (scale bar, 100 um). **(C,D)** CD31 expression was markedly decreased in the TAC group which could be obviously rescued by exosomes administration ($n = 5$–8). *$p < 0.05$,**$p < 0.01$.

by AZD4547 administration (**Figures 4C,D**, $p < 0.01$). These findings indicated that ESC-derived exosome administration can promote myocardial angiogenesis and mitigate the reduction in micro-vessel density induced by TAC in a FGF2 dependently way.

DISCUSSION

Here, we provide compelling evidence that (1) ESC-derived exosomes significantly attenuate TAC-induced heart failure by promoting myocardial angiogenesis and (2) FGF2 signaling plays vital roles in the myocardial angiogenesis induced by ESC-derived exosomes.

Our study revealed that FGF2 was highly enriched in ESC-derived exosomes. FGF2 has been confirmed to stimulate the proliferation of mesenchymal cells such as fibroblasts, endothelial cells, and smooth muscle cells (14). Endogenous FGF2 has a significant cardioprotective effect against ischemia-reperfusion injury (15). Deletion of FGF2 has been shown to result in decreased endothelial proliferation and vascular density in the infarcted myocardium of mice (16). FGFR1 and FGFR2 DKO mice, which exhibit endothelial cell-specific disruption of FGF2 function, have been shown to have significantly worsened cardiac function than controls after ischemia-reperfusion injury as well as significantly decreased vessel density (17). Our study provides the first demonstration that the administration of ESC-derived exosomes containing a high abundance of FGF2 can promote myocardial angiogenesis after aortic banding.

As ESCs-derived exosomes contain many proteins and microRNAs in addition to FGF2, we performed *in vitro* experiments to investigate the role of FGF2 in the angiogenesis induced by ESCs-derived exosomes. We found that ESC-derived exosomes can significantly increase tube formation and that this effect could be largely mitigated by FGF2R siRNA. All the above findings suggested that FGF2-FGF2R signaling played a vital role in the myocardial angiogenesis induced by ESC-derived exosomes.

FGF2 stimulates fibroblast and matrix production (16). Schultz and colleagues reported that FGF2-KO mice exhibited reduced interstitial fibrosis after aortic banding (18). However, in our study, administration of exosomes containing abundant FGF2 did not increase myocardial interstitial fibrosis. Fibroblast growth factor 2 (FGF-2) can be categorized as high molecular weight (20 kDa) or low molecular weight (18 kDa) which exert distinct biological activities: low molecular weight FGF-2 promoted sustained cardioprotection and angiogenesis, while high molecular

weight FGF-2 promoted myocardial hypertrophy and reduced contractile function (19). Our Proteomic analysis showed that ESC-derived exosomes contained rich of low molecular weight (18 kDa) FGF2 instead of high molecular weight (20 kDa). This may explain the absent of increased myocardial interstitial fibrosis in the exosome treated group in our study. Another possible explanation was that other signaling molecules than FGF2 in exosomes may also be involved in the ventricular remodeling process and offset FGF2-induced interstitial fibrosis.

CONCLUSIONS

ESC-derived exosomes attenuated TAC-induced heart failure by promoting myocardial angiogenesis. FGF2 signaling may played vital roles in the myocardial angiogenesis induced by ESC-derived exosomes.

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 below: ProteomeXchange, accession no: PXD015449.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of the First Affiliated Hospital of Soochow University.

AUTHOR CONTRIBUTIONS

XL and ZW designed the study. YP, MM, and DW wrote the manuscript and performed experiments. XW and JX analyzed these data. ZW, LH, and XL revised the article. All authors have read and approved the final version of the manuscript, and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

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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.2021.638771/full#supplementary-material

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frontiers in Cardiovascular Medicine

REVIEW
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Pulmonary Hypertension in Patients With Heart Failure With Mid-Range Ejection Fraction

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Pulmonary hypertension (PH) is common in patients with heart failure (HF). The role of PH in patients with HF with reduced (HFrEF) and preserved (HFpEF) left ventricular ejection fraction (LVEF) has been extensively characterized during the last years. In contrast, the pathophysiology of HF with mid-range LVEF (HFmrEF), and in particular the role of PH in this context, are largely unknown. There is a paucity of data in this field, and the prevalence of PH, the underlying mechanisms, and the optimal therapy are not well-defined. Although often studied together there is increasing evidence that despite similarities with both HFrEF and HFpEF, HFmrEF also differs from both entities. The present review provides a summary of the current concepts of the mechanisms and clinical impact of PH in patients with HFmrEF, a proposal for the non-invasive and invasive diagnostic approach required to define the pathophysiology of PH and its management, and a discussion of future directions based on insights from mechanistic studies and randomized trials. We also provide an outlook regarding gaps in evidence, future clinical challenges, and research opportunities.

Keywords: pulmonary hypertension, heart failure, post-capillary, left ventricular ejection fraction, mid-range, mildly reduced, right heart catheterization

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INTRODUCTION

Pulmonary hypertension (PH) in patients with left heart diseases is the most common form of PH (1). The presence of PH in this context typically reflects an advanced disease stage with exhausted compensatory mechanisms, which is associated with exercise intolerance and a poor prognosis (2). Thus, PH is a manifestation of heart failure (HF). In patients with HF with reduced left ventricular ejection fraction (LVEF; HFrEF) PH is a common feature in the decompensated state that is often reversible following appropriate therapy. In patients with advanced HFrEF, PH may become chronic and thereby is a marker of poor prognosis (3, 4). Intense research during the last decade has revealed that PH may be even more common in patients with HF with preserved LVEF (HFpEF) (2). There is increasing evidence from recent studies using invasive hemodynamics with or without exercise in combination with detailed echocardiographic assessments that the pathophysiology underlying PH in HFpEF is complex and differs from that in HFrEF (5). In contrast, our understanding of the relatively new disease entity of HF with mid-range LVEF (or "mildly reduced" LVEF; HFmrEF) is still evolving, and the pathophysiology and clinical impact of PH in this context have not been defined yet (6). In this review, we discuss the potential role of PH in HFmrEF, highlight the diagnostic challenges, propose a clinical approach, and briefly summarize the therapeutic options in these patients with an outlook to potential future developments. We

have to acknowledge that there is still a paucity of data on PH in HFmrEF. Before HFmrEF was clearly defined as a distinct entity, these patients were often included in HFrEF or HFpEF studies on pathophysiology and therapy. We therefore also discuss HFmrEF in the context of concepts regarding PH in HFrEF vs. HFpEF.

DEFINITION OF HFmrEF

The relatively new entity of HFmrEF has been introduced by the 2016 European Society of Cardiology (ESC) guidelines on the diagnosis and management of HF (7). In these guidelines, HFmrEF includes the LVEF range between 40 and 49% while patients with LVEF <40% by definition have HFrEF and those with LVEF ≥50% have HFpEF (**Table 1**). A slightly different definition of HFrEF and HFmrEF has been proposed in the recently published Universal Definition and Classification of Heart Failure (10): HFrEF: LVEF ≤40% rather than <40%, HFmrEF: LVEF 41–49% rather than 40–49%. The definition of HFpEF remains unchanged: LVEF ≥50%. In this review, we adopt this new definition. However, when discussing studies specifically looking at HFmrEF, we must be aware that often the "old" LVEF range of 40–49% was applied. The rationale underlying the creation of the HFmrEF category had been as follows: on the one hand, the established HFrEF pharmacotherapy is based on studies that included patients up to an LVEF of 40% (not up to 50%), and on the other hand, it has been realized that in the large randomized "HFpEF studies," which included patients with LVEF ≥40%/45% (rather than only ≥50%), those with LVEF <50% responded differently to several pharmacological interventions when compared to those with LVEF ≥50% (7). The 2016 ESC guidelines state that apart from the LVEF criteria the same additional criteria are required for the diagnosis of both HFpEF and HFmrEF (**Table 1**) (7). More recently, a new algorithm for the diagnosis of HFpEF was proposed in an ESC position paper (HFA-PEFF score) (8), and a somewhat different diagnostic score (H2FPEF score; gold standard: invasive exercise hemodynamics) was published around the same time by the HFpEF experts from the Mayo clinic (9). Whether or not these approaches for the diagnosis of HFpEF can also be applied to make a diagnosis of HFmrEF, has not been explicitly addressed. In 2021, new ESC guidelines on HF are expected, and some of these aspects may be described more clearly.

Notably, in both the new ESC HFA-PEFF score (8) and the Mayo clinic H2FPEF score (9) a measure of PH is an item contributing to the diagnosis of HFpEF. This highlights that PH is a common feature in HFpEF. However, the non-critical use of this criterion may be misleading in certain situations. The rationale to use PH as a marker of HFpEF is based on the fact, that this typically is a reflection of post-capillary PH in the context of advanced left ventricular diastolic and left atrial (LA) dysfunction. However, sometimes this assumption may not be correct, and a preserved or mid-range LVEF may co-exist with a form of PH that is unrelated to a left heart pathology.

HEMODYNAMIC DEFINITION OF PULMONARY HYPERTENSION IN HF

In patients with left heart disease, PH is most often a reflection of elevated LA pressure and pulmonary artery wedge pressure, respectively, i.e., post-capillary PH (group 2 PH) (1, 2). According to the 2015 ESC/European Respiratory Society (ERS) guidelines, any PH is defined as a mean pulmonary artery pressure (mPAP) ≥25 mmHg. Post-capillary PH is defined by a mean pulmonary artery wedge pressure (mPAWP) >15 mmHg (pre-capillary PH: mPAWP ≤15 mmHg) (11). If PH is driven by mPAWP elevation alone (no relevant pulmonary vascular disease), this is referred to as isolated post-capillary PH (IpcPH), which is defined as mPAP ≥25 mmHg, mPAWP>15 mmHg, pulmonary vascular resistance (PVR) ≤3 Wood units (WU), and/or diastolic pressure gradient (DPG) <7 mmHg. If there is an associated pulmonary vascular component of PH (typically as a reaction of the pulmonary vasculature to a longstanding and substantial mPAWP elevation), this is referred to as combined pre- and post-capillary PH (CpcPH), which is defined as mPAP ≥25 mmHg, mPAWP>15 mmHg, and PVR >3 WU and/or DPG ≥7 mmHg (11). It has been recognized that DPG values are often low and even negative and discordant to PVR, which leads to many unclassifiable patients when applying the original 2015 ESC/ERS criteria. In addition, in contrast to PVR data on the prognostic value of the DPG have been inconsistent. Therefore, the PVR criterion is preferred (12).

The 2018 PH World Symposium has proposed a new PH definition, which aims to overcome the above-mentioned limitations of the 2015 definition and to consider new data on the normal range of pulmonary pressures. This new definition is under intense discussion, however, and there are no new PH guidelines yet. According to this approach, pre-capillary PH is defined as mPAP >20 mmHg (new cut-off), mPAWP ≤15 mmHg, and PVR ≥3 WU (new compulsory criterion) (13). Post-capillary PH is defined as mPAP >20 mmHg (new cut-off) and mPAWP >15 mmHg (IpcPH: PVR <3 WU, CpcPH: PVR ≥3 WU; i.e., the PVR criterion has been slightly modified, and the DPG criterion has been dropped for the above-mentioned reason) (14). The rationale for this new PH definition is as follows: (1) studies have shown that the upper limit of a normal mPAP is approximately 20 mmHg, and mortality is already increased in patients with mPAP >20 mmHg. (2) The introduction of the PVR ≥3 WU criterion for the definition of pre-capillary PH makes sure that there is really pulmonary vascular disease rather than increased flow. (3) A single criterion (i.e., PVR ≥3 WU, no DPG criterion) for the differentiation between IpcPH and CpcPH ensures an unequivocal definition in case of discordant PVR and DPG criteria (13, 14).

PREVALENCE OF PH IN HFpEF AND HFmrEF

In the largest study from a catheterization laboratory database (n = 10,023), 46% of all patients undergoing right heart catheterization had post-capillary PH (74% of all patients with

TABLE 1 | Definition of heart failure (HF) with mid-range (HFmrEF) vs. HF with reduced (HFrEF) and HF with preserved (HFpEF) left ventricular ejection fraction.

	HFrEF	HFmrEF	HFpEF
LVEF[a]	≤40%	41–49%	≥50%
Definition ESC guidelines 2016 (7)	Symptoms ± signs	1. Symptoms ± signs 2. NT-proBNP >125 ng/l or BNP >35 ng/l 3. LV hypertrophy/LA dilation or significant LV diastolic dysfunction: LVMI ≥115 g/m^2 (males) or 95 g/m^2 (females), LAVI 34 ≥ml/m^2, E/e′ ≥13, e′ (average from septal and lateral annulus) <9 cm/s	
Definition ESC position paper 2019 (8)		Not explicitly included	HFA-PEFF score:[b] ≥5 points: HFpEF ≤1 points: HFpEF unlikely 2–4 points: functional test: non-invasive diastolic stress test or invasive stress test (Gold standard: mPAWP ≥15 mmHg at rest or/and ≥25 mmHg on exercise)
Definition Mayo 2018 (9)		Not included	H2FPEF score:[c] doubling of the probability of HFpEF with each one-point increase

[a] *LVEF cut-offs adopted from the 2021 Universal Definition and Classification of Heart Failure (10).*

[b] *Heart Failure Association (HFA)-PEFF: score: composed of (a) septal or lateral peak early diastolic mitral annular velocity by tissue Doppler (e′), ratio of peak early diastolic transmitral velocity by pulsed wave Doppler (E) to e′ (E/e′), peak tricuspid regurgitant velocity, estimated systolic pulmonary artery pressure (sPAP), or global longitudinal strain, (b) left atrial volume index (LAVI), left ventricular (LV), mass index (LVMI), or relative wall thickness, and (c) B-type natriuretic peptide (BNP), or N-terminal proBNP (NT-proBNP). Cut-offs depend on age (<75 vs. ≥75 years) and cardiac rhythm (sinus rhythm vs. atrial fibrillation). Values between 0 and 6.*

[c] *H_2FPEF score: composed of: Heavy: body mass index >30 kg/m^2 (two points), hypertensive: two or more antihypertensive drugs (1 point), atrial fibrillation: paroxysmal or persistent (three points), pulmonary hypertension (sPAP) >35 mmHg (1 point), elder: age >60 years (1 point), and filling pressure: E/e′ >9 (1 point). values between 0 and 9. mPAWP, mean pulmonary artery wedge pressure.*

PH) (15), and 39% of them had HFrEF, 56% had HFpEF, and in 5% the LVEF was not recorded. In this study, the LVEF cut-off for the differentiation between HFrEF and HFpEF was 45%, i.e., the HFmrEF group was not separated (15). Although there is a bimodal distribution of LVEF in HF (16), it is likely that there was a sizeable group of patients with PH in the context of HFmrEF. Cohort studies looking at unselected HF patients, i.e., patients with HF but not necessarily PH, revealed an HFmrEF prevalence of 13–24% (17–20). Mortality of HFmrEF patients was intermediate between HFrEF and HFpEF in some (20) and similar to HFrEF but better than in HFpEF in other studies (17). The proportion of HFmrEF patients among group 2 PH patients is unknown, and the prevalence of PH among unselected patients with HFmrEF and the prognosis of patients with HFmrEF and PH not known either. The estimation of the prevalence of PH in HF is difficult because a reliable diagnosis of PH by echocardiography is not possible in cross-section studies, and all invasive studies suffer from a very substantial referral bias since the indication for right heart catheterization in these patients most likely was based on evidence of PH in the echocardiogram.

In one study using a non-invasive PH definition [systolic pulmonary artery pressure (sPAP) >35 mmHg, i.e., peak tricuspid regurgitant velocity (TRV) ≈2.9 m/s assuming a normal central venous pressure], a high PH prevalence of 83% was found among 244 HFpEF (LVEF ≥50%) patients from a community based study (21). Many of the large HFpEF intervention studies also included patients who now meet the definition of HFmrEF. In the Prospective Comparison of ARNI With ARB Global Outcomes in HF With Preserved Ejection Fraction (PARAGON-HF) trial, the LVEF cut-off for inclusion was ≥45%. Study inclusion was based on the LVEF derived from a screening echocardiogram (LVEF reported by the study site). In the echo substudy of the trial (n = 1,079), the median LVEF according to secondary core lab analysis was 59%, and LVEF was ≥50% in 79%, 40–50% in 18%, and <40% in 3% of patients. The prevalence of PH (defined as peak TRV >2.9 m/s) in this PARAGON-HF subgroup was 31% (22). The mean estimated sPAP was 34 mmHg (peak TRV 2.7 m/s, plus a value for the estimated central venous pressure) (22). This was similar to the echo substudies of the Irbesartan in Heart Failure With Preserved Ejection Fraction (I-PRESERVE) (≈37 mmHg) (23) and Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist (TOPCAT) (≈38 mmHg) studies (24) where the same LVEF cut-off of ≥45% was used as inclusion criterion. It is obvious that all these large "HFpEF trials" included a certain proportion of HFmrEF patients but peak TRV values were not reported separately for patients with LVEF ≥50 vs. 45–49%. Such data were shown however in an analysis of the Trial of Intensified Medical therapy in Elderly patients with Congestive Heart Failure (TIME-CHF), where the mean peak TRV in HFrEF (n = 289), HFmrEF (n = 82), and HFpEF (n = 85) were ≈2.9, ≈2.9, and ≈3.0 m/s, respectively (no difference) (25). Thus, assuming a normal distribution of peak TRV values 50% of patients in all LVEF strata formally had an at least intermediate probability of PH in TIME-CHF and may have had some degree of PH. This is probably an overestimation as a peak TRV of 2.9 m/s without indirect signs of PH represents the lower margin of the intermediate probability stratum. Still, the data suggest that PH is equally common in HFmrEF as in HFpEF and HFrEF. On the other hand, it must be realized, however, that the TIME-CHF population was a highly selected one. All patients had been hospitalized before inclusion, and high N-terminal-pro-B-type natriuretic peptide (NT-proBNP) values (>400 ng/l for patients with age 60–74 years, >800 ng/l for those age >75 years) were

required for study inclusion (26) [cf. PARAGON study: >200 ng/l for patients in sinus rhythm, and >600 ng/l for those in atrial fibrillation (27)]. Given that pulmonary pressures are related to natriuretic peptides (28) this inclusion criterion may have led to a selection of patients with high likelihood of PH. Accordingly, up to 30–50% of patients with HFmrEF may have some form of PH.

CLINICAL, ECHOCARDIOGRAPHIC, AND BIOCHEMICAL CHARACTERISTICS OF PATIENTS WITH HFmrEF

The primary driver of PH in any type of left heart disease is an elevation in LA pressure, which in turn depends on the properties of the left ventricle, the function of the mitral valve, and the compliance of the left atrium. Data examining this pathopathopysiology specifically in HFmrEF are sparse. To understand the mechanism of PH in HFrEF, we first discuss available studies looking at the clinical characteristics and echocardiographic features in HFmrEF, and then look at the mechanistic studies on PH in HFrEF and HFpEF as a basis to speculate about the situation in HFmrEF.

In terms of clinical characteristics, HFmrEF patients more closely resemble the HFrEF rather than the HFpEF group (younger age, less females, more ischemic heart disease, less atrial fibrillation) (20). The available data on cardiac structure and function in HFmrEF suggest that these patients exhibit a phenotype, which is overall intermediate between HFrEF and HFpEF (25, 29–31). However, such data is limited, and it is actually unknown which different cardiac pathologies associated with a mid-range LVEF the patients really had who were included in the larger cross-sectional studies. In addition, the LVEF range of 41–49% is relatively narrow, and the HFmrEF group includes patients with stable LVEF but also patients with HFrEF and improved LVEF and patients with HFpEF and worsened LVEF (32). Notably, this trajectory of LVEF is important in terms of prognosis (18, 19, 32); in particular, the change from HFmrEF to HFrEF is a marker of an adverse outcome (19). In this context, the presence of coronary artery disease has been found to be an important mechanism related to a reduction in LVEF and change in LVEF category (33).

In the well-characterized TIME-CHF population, the ischemic HF etiology was similarly common in HFmrEF as in HFrEF, and the atrial fibrillation (AF) prevalence was similar in HFmrEF and HFpEF (25). Left ventricular dimension, mass and geometry in HFmrEF patients were intermediate between HFrEF and HFpEF. Despite differences in LVEF by definition, right ventricular (RV) function, and the peak TRV were similar in all three LVEF categories (25). In a study by Ghio et al. (34) the left ventricular end-diastolic volume index and the prevalence of significant mitral regurgitation (MR) in HFmrEF were similar as in patients with HFrEF and thereby larger/higher than in HFpEF. In contrast, right ventricular function expressed as tricuspid annular plane systolic excursion (TAPSE) was somewhat lower in HFmrEF and HFpEF compared to HFrEF.

The biomarker profile in HFmrEF is also characterized by intermediate plasma concentrations of natriuretic peptides and a pattern of biomarkers that includes features of both HFpEF and HFrEF, i.e., markers of both inflammation and cardiac stretch, whereas in HFpEF, biomarkers were related to inflammation, and in HFrEF, biomarkers were related to cardiac stretch (35).

PATHOPHYSIOLOGY OF PH IN HFmrEF

There is evidence for substantial differences in the pathophysiology of PH between patients with HFpEF and HFrEF (5). In HFpEF, concentric remodeling/hypertrophy and increased diastolic stiffness represent the hallmarks of the pathophysiology. Many HFpEF patients have diabetes, obesity, and hypertension, and it has been suggested that these comorbidities activate pro-inflammatory pathways leading to increased collagen deposition (36). In contrast, HFrEF patients are characterized by eccentric remodeling/hypertrophy and high wall stress. Patients with HFmrEF have intermediate left ventricular volumes, mass, and relative wall thickness, and values for the peak early mitral annular velocity (e′) (25). Left atrial dysfunction is the key mechanism contributing to LA pressure and mPAWP and mPAP elevation. Left atrial remodeling differs between patients with HFpEF and HFrEF with less atrial dilation but higher atrial stiffness in HFpEF (37). Left atrial volume index is highest in patients with HFrEF, lowest in those with HFpEF, and intermediate in HFmrEF (30), suggesting an intermediate type of remodeling. Patients with HF irrespective of LVEF exhibit a significantly reduced LA strain at rest and during exercise when compared to patients with dyspnea of non-cardiac origin (30, 38). In a HF population with a broad LVEF spectrum there was overall an inverse correlation between higher LA volume index and lower LA strain, which was relatively moderate however. There was also correlation between lower LA strain during exercise and lower peak exercise cardiac output and peak oxygen consumption (30). The HFmrEF patients had the highest LA strain at rest when compared to HFpEF and HFmrEF but a blunted response to exercise with exercise with LA strain values being intermediate between HFpEF and HFrEF (30).

There are two factors with an important interaction with LA function and thereby promoting PH: MR and AF. In HFrEF, various degrees of functional MR are common and predict prognosis (39). In these patients, MR results from an imbalance between tethering and closing forces in the context of the dilatation and distorted geometry the left ventricle (40). In contrast, HFpEF patients are characterized by "atrial" functional MR, i.e., MR due to annulus dilatation in the context of LA dilation, typically in the context of AF (41). Mitral regurgitation can be dynamic in both HFrEF and HFpEF as shown in exercise studies (31, 41). In HFmrEF both forms of functional MR likely play role, depending on the predominant type of LV remodeling. In a recent study, significant MR at rest was found in 15% of patients with HFpEF, in 27% of those with HFmrEF, and in 47% of those with HFrEF. Importantly, exercise elicited worsening of MR in all HF categories with at least moderate MR in 35, 41, and 60% of HFpEF, HFmrEF, and HFrEF patients during exercise (31). In any type of HF, there is vicious cycle between MR and LA remodeling. The same applies for AF and LA

remodeling and MR, respectively. In HFmrEF, AF is similarly common as in HFpEF and more prevalent than in HFrEF (20). Importantly, presence of AF (either by AF per se and/or mediated by the AF-associated structural changes) has substantial impact on hemodynamics, in particular on the relationship between LVEDP and mPAWP (42–44). The AF burden (paroxysmal vs. permanent) is a marker of the hemodynamic derangement in HFpEF (45), and the same may apply for HFmrEF. In sinus rhythm, LVEDP is typically similar or somewhat higher than mPAWP because MR is typically mild, LA function is only moderately reduced, and there is an effective atrial contraction. In contrast, in AF substantial LA dysfunction, higher degrees of MR and absence of LA contraction lead to high V waves and higher mPAWP than LVEDP. Patients with AF typically have worse hemodynamics and those in sinus rhythm with higher mPAWP, mPAP, and PVR and higher prevalence of PH and CpcPH (44).

Apart from differences in the mechanisms of LA pressure and mPAWP elevation, there is evidence for important LVEF-dependent differences in the pathobiology of the pulmonary vasculature in response to a certain LA pressure and mPAWP, respectively (46). In a cross-sectional study, patients with HFpEF have been shown to have a higher PVR for a given mPAWP, i.e., a higher likelihood of CpcPH, than patients with HFrEF (46). The anatomical substrate for the pre-capillary component of PH in CpcPH in HF is still not well-defined. It has been assumed that there are similar vascular changes as in pulmonary arterial hypertension. However, a recent post-mortem analysis of lung specimens from patients with HFrEF ($n = 55$) and HFpEF ($n = 53$) with PH (all with documented sPAP ≥40 mmHg; 30 with right heart catheterization data: mPAP = 40 mmHg, mPAWP = 25 mmHg, PVR = 3.9 WU) has revealed global (arteries, veins, indeterminate vessels) pulmonary vascular remodeling (47). There was substantial intimal thickening and medial hypertrophy of pulmonary veins ("pulmonary vein arterialization") resembling the changes seen in pulmonary veno-occlusive disease, and the extent of medial hypertrophy in the pulmonary arteries was related to the extent of venous intimal thickening but not arterial thickening suggesting that arterial medial hypertrophy may develop secondary to venous remodeling. The medial thickness of arteries and the intimal thickness of arteries and veins tended to be more severe in HFpEF vs. HFrEF, and intimal thickness of veins

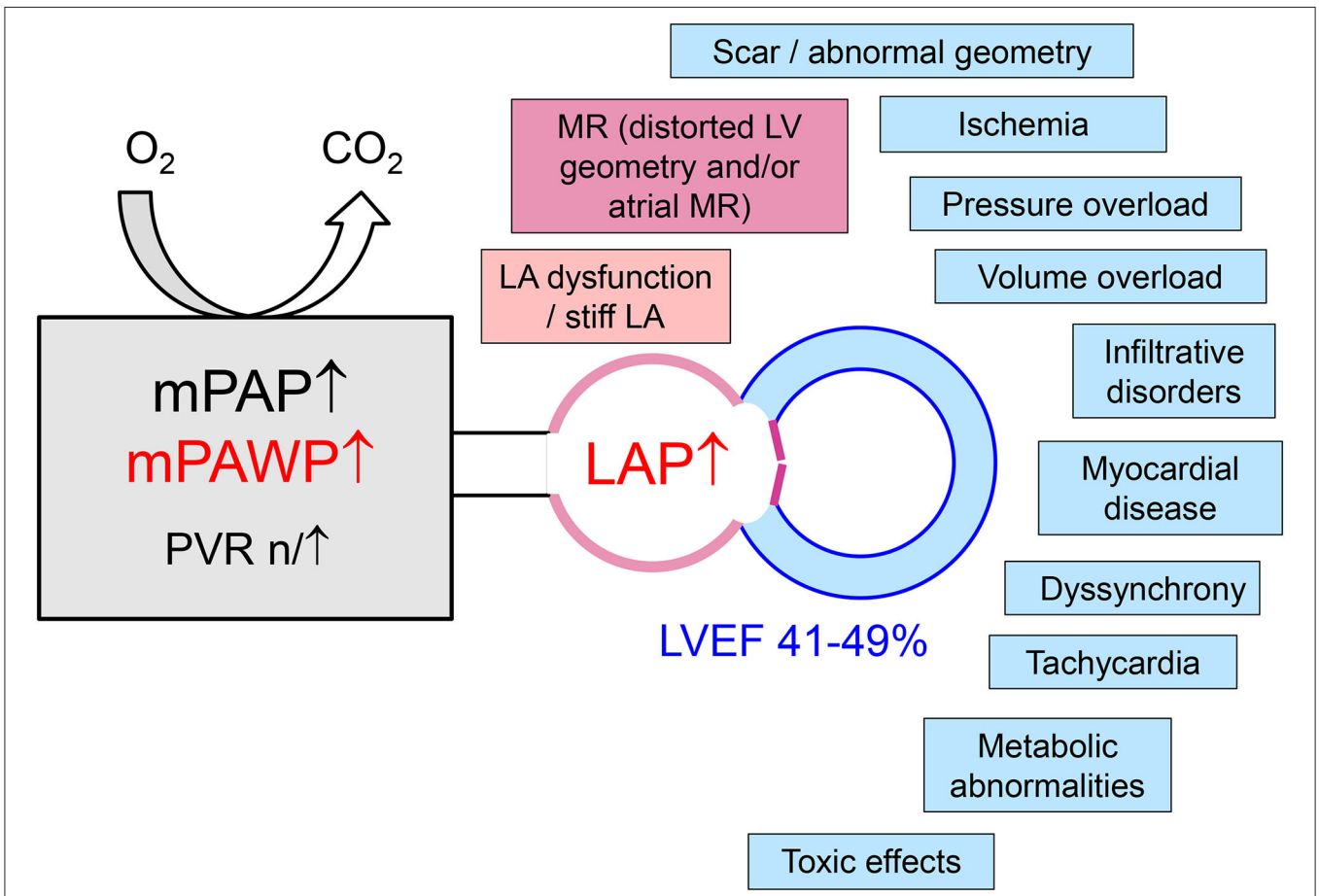

FIGURE 1 | Mechanisms of left atrial pressure (LAP) elevation in patients with pulmonary hypertension in the context of left heart disease with a left ventricular ejection (LVEF) in the mid-range of 41–49%. LA, left atrial/atrium; LV, left ventricular; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; MR, mitral regurgitation; PVR, pulmonary vascular resistance.

TABLE 2 | Pulmonary hypertension (PH) in Heart Failure with mid-range left ventricular Ejection Fraction (HFmrEF): different disease entities and mechanisms (please also see text).

	Disease characteristics	PH mechanism	Diagnostic approach	Treatment
"Lone HFmrEF"	Classical form of HFmrEF in the context of obesity, hypertension and diabetes	LA pressure elevation due to systolic and diastolic LV dysfunction, functional (atrial) MR, LA dysfunction	• TTE including tissue Doppler/strain: anatomy, extent of LV systolic and diastolic dysfunction, LA dimensions, MR mechanism/severity • RHC	• Anticoagulation in atrial fibrillation • Loop diuretics, ARB, or ARNI (typically in women), MRA • Intraatrial shunt device for selected patients
Coronary artery disease	LV dysfunction after a previous infarct or due to chronic ischemia with hibernating myocardium, typically with functional MR	LA pressure elevation due to systolic and diastolic dysfunction, moderate/severe functional MR	• TTE, TOE: regional LV function, extent/mechanism of MR • Cardiac MRI: myocardial viability • coronary angiography: treatable ischemia • RHC	• Revascularization if possible • ARB (ARNI), MRA, betablocker, loop diuretic
Hypertrophic cardiomyopathy	LV hypertrophy and dysfunction with/without dynamic LVOT obstruction, functional MR, atrial fibrillation	LA pressure elevation due to systolic and diastolic LV dysfunction, functional MR, LA dysfunction	• TTE including tissue Doppler/strain: anatomy, extent of LV systolic and diastolic dysfunction, LVOT obstruction, MR • Coronary angiography: options for alcohol ablation • RHC	• Betablockers, verapamil, diltiazem • Surgical myectomy/alcohol ablation in presence of significant LVOT obstruction • Careful use of diuretics • If available: mavacamten (cardiac myosin inhibitor)
Specific cardiomyopathy, e.g., amyloidosis, sarcoidosis, scleroderma	LV infiltration and/or scarring with systolic and diastolic dysfunction, LA dysfunction, atrial fibrillation	LA pressure elevation due to systolic and diastolic LV dysfunction, LA dysfunction, functional (atrial) MR, secondary, and/or primary pulmonary vascular disease	• TTE including tissue Doppler/strain: anatomy, extent of LV systolic and diastolic dysfunction, MR • Search for specific etiologies using cardiac MRI, bone scintigraphy, positron emission tomography	• ARB (ARNI), MRA, betablocker, loop diuretics • Specific treatment of underlying disease (e.g., immunosuppression, tafamidis)
Tachycardia-mediated cardiomyopathy	LV dysfunction due to sustained tachycardia, LV and LA dilatation	LA pressure elevation due to systolic and diastolic dysfunction, functional (atrial) MR, LA dysfunction	• TTE including tissue Doppler/strain: anatomy, extent of LV systolic and diastolic dysfunction, LA dimensions, MR • Cardiac MRI: myocardial viability, evidence for specific disease • RHC • Coronary angiography in selected cases	• Anticoagulation • ARB (ARNI), MRI, Betablocker • Rhythm control (amiodarone, cardioversion, catheter ablation)
Valvular heart disease (after correction of valve stenosis/ regurgitation)	Persistent LV systolic and diastolic dysfunction late after correction of valve stenosis/regurgitation with/without pulmonary vascular disease	LA pressure elevation due to systolic and diastolic dysfunction, pulmonary vascular disease (elevated PVR)	• TTE including tissue Doppler/strain: anatomy, extent of LV systolic and diastolic dysfunction, LA dimensions • TOE for the exclusion of paravalvular leak etc. • RHC	• ARB (ARNI), MRA, betablocker, loop diuretic
Aortic stenosis	Advanced form of chronic severe aortic stenosis with reduced LVEF	LA pressure elevation due to systolic and diastolic dysfunction, MR, LA dysfunction, secondary pulmonary vascular disease	• TTE: severity of AS, LV systolic and diastolic dysfunction, LA size, MR • RHC • Coronary angiography	• Diuretics • ACE inhibitor • Aortic valve replacement if truly severe aortic stenosis
Mitral regurgitation	Advanced form of severe primary MR with reduced LVEF	LA pressure elevation due to systolic and diastolic dysfunction, and severe MR	• TTE and TOE: severity and mechanism of MR, LV dimensions, systolic and diastolic dysfunction, LA size • RHC • Coronary angiography	• Diuretics, ACE inhibitor, ARB • Mitral valve repair if severe primary MR

ACE inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor; LA, left atrial; LV, left ventricular; LVOT, left ventricular outflow tract; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; MR, mitral regurgitation; MRA, mineralocorticoid receptor blocker; MRI, magnetic resonance imaging; PVR, pulmonary vascular resistance; RHC, right heart catheterization; TOE, transoesophageal echocardiography; TTE, transthoracic echocardiography.

was significantly more severe in HFpEF compared to HFrEF. The severity of PH expressed as transpulmonary gradient and PVR was correlated most strongly with venous and small indeterminate vessel intimal thickening as was the impairment in diffusion capacity of the lung (47). In that study, HFrEF was defined as LVEF <50%, and HFpEF as LVEF ≥50%. Thus, HFmrEF was included in the HFrEF group. The 75th percentile for LVEF in HFrEF was 35%, and thus some patients with HFmrEF may have been included. The overall similar pattern of pulmonary vascular remodeling in HFpEF and HFrEF suggest that these observations likely also apply for HFmrEF. The underlying pathophysiology in humans is not clearly defined but endothelial injury due to barotrauma (alveolar-capillary stress failure) and subsequent remodeling under the influence of several mediators seems to be of paramount importance (5). There is evidence from a rat HFpEF model that the metabolic syndrome may promote the development of pulmonary vascular disease in HFpEF (48). Given the similar prevalence of Diabetes in HFpEF and HFmrEF (20, 25) this may be relevant to the pathophysiology of pulmonary vascular disease also in HFmrEF.

Right ventricular dysfunction is a strong predictor of prognosis in HFrEF (49) and HFpEF (49). The RV is very sensitive to pressure overload. Therefore, adaption of the RV to PH is crucial (50). This seems to be particularly relevant for HFpEF and HFmrEF, while in HFrEF intrinsic RV dysfunction also plays an important role. In important study by Ghio (34), ischemic HF etiology, non-sinus rhythm, and high heart rate were related to TAPSE in HFrEF, while in HFpEF pulmonary pressure was the strongest predictor of TAPSE, and the same was true for patients with HFmrEF. In this context, the concept of RV to pulmonary artery (PA) coupling is of critical importance, i.e., ability of the RV to cope with the increased afterload. Classically, RV to PA coupling is described by RV pressure volume analysis, which is a cumbersome technique that is rarely applied in clinical practice. The RV to PA coupling is defined as the ratio between RV end-systolic elastance (Ees; end-systolic RV pressure divided by end-systolic volume) and arterial elastance (Ea; RV end-systolic pressure divided by stroke volume). Normally, Ees/Ea [which can also be expressed as RV ejection fraction/(1-RV ejection fraction)], is around 1.5 and can be reduced to approximately 0.8 before RV dilatation occurs ("uncoupling") (50). For clinical practice, the ratio of TAPSE to estimated sPAP (TAPSE/sPAP) has been proposed as non-invasive surrogate for Ees/Ea (51). In a large HFpEF population undergoing detailed non-invasive and invasive hemodynamic evaluation, those in the lowest TAPSE/sPAP tertile had the worst hemodynamics including the worst RV function, the highest right atrial pressure, mPAP, and PVR, and the highest proportion of CpcPH (52). Similarly, another study found lower TAPSE/sPAP ratio in CpcPH vs. IpcPH in both patients with HFrEF and HFpEF (53). This study used an LVEF cut-off of 45% to differentiate between HFrEF and HFpEF (53). Thus, patients with HFmrEF were included but separate data are not available. However, in an exercise echocardiography study LA dynamics expressed as changes in LA strain during exercise were correlated to TAPSE/sPAP not only in HFrEF and HFpEF but also in HFmrEF (30) suggestig that TAPSE/sPAP may be marker of RV dysfunction and high mPAP, mPAP, and PVR due to LA myopathy and functional MR with high pulsatile load also in HFmrEF.

PHENOTYPES OF HFmrEF WITH PH

The HFmrEF group is a difficult one since the LVEF spectrum is very narrow (41–49%), and assessment of LVEF in clinical practice is associated with substantial variability (6). There is a large number of disease entities potentially presenting with a HFmrEF phenotype and also PH. Principally, most of the specific HFpEF etiologies listed in the most recent ESC position paper on HFpEF (8) can also result in HFpEF. Coronary artery disease with a previous moderate myocardial infarction is a relatively common etiology of HFmrEF, and the documented change from HFpEF to HFmrEF in the context of coronary artery disease is a marker of an unfavorable prognosis (33). Apart from coronary artery disease, a large number of non-ischemic etiologies may

TABLE 3 | Clinical features echo findings favoring pre-capillary or post-capillary pulmonary hypertension (PH).

	Pre-capillary PH	Post-capillary PH
Clinical features		
Atrial fibrillation[c]	No	Yes
Obesity/Diabetes[c]	No	Yes
Coronary artery disease	No	Yes
Echocardiography		
LV+LA area < RV+RA area[b]	Yes	No
Apex-forming RV[b]	Yes	No
RV end-diastolic area[c]	↑	↓
LV mass[c]	↓	↑
LV eccentricity index (degree of LV "D-shape")[b]	↑	∼1.0
E/e'[a,b]	↓	↑
LA area (apical for chamber view)[c]	↓	↑
LA anteroposterior diameter (parasternal long axis view)[a]	<3.2 cm	>4.2 cm
Mitral regurgitation	No/little	Little to severe
Peak TRV/VTI RVOT	↑	Normal/↓
Mid-systolic notch in pulmonary artery pulsed-wave Doppler signal or acceleration time <80 ms[a]	Yes	No
IVC diameter >20 mm without inspiratory collapse (≤50%)[b]	Yes	No

E/e', ratio of the peak early diastolic transmitral velocity to the peak early diastolic mitral annular velocity (ideally assessed at the lateral annulus); IVC, inferior vena cava; LA, left atrium; LV, left ventricle/ventricular; RA, right atrium/atrial; RV, right ventricle/ventricular; TRV, tricuspid regurgitation velocity; VTI RVOT, velocity time integral in the right ventricular outflow tract. ↑, large/high; ↓, small/low.

[a] *Parameters included in the score by Opotowsky et al. (59).*

[b] *Parameters included in the score by D'Alto et al. (60).*

[c] *Parameters included in the score by Berthelot et al. (61).*

play a role including infiltrative diseases and hypertrophic cardiomyopathies. In this context, a frank reduction in LVEF (as opposed to "only" reduced tissue Doppler/strain) represents an advanced disease stage. A more detailed discussion of these specific entities is beyond the scope of the present review however. Although cohort studies suggest that overall HFmrEF patients are characterized by a structural and pathophysiological phenotype, which is intermediate between HFrEF and HFpEF (25, 29), the existence of a number of different phenotypes is very likely but this has not been analyzed in detail so far. Still, the importance of the different mechanism contributing to PH as discussed in the previous section may vary. A schematic representation of different entities/mechanisms leading to LA pressure elevation in PH in HFmrEF is shown in **Figure 1**. An incomplete list of some proposed distinct and important entities summarized under the HFmrEF umbrella and the possible mechanisms of PH is presented in **Table 2**. In contrast to previous more restrictive diagnostic criteria for HFpEF (54), the most recent ESC consensus explicitly states that the HFpEF spectrum not only includes the classical "lone" HFpEF form but also specific etiologies (e.g., cardiomyopathies) and patients with primary valve disease as long as the definition criteria are met (**Table 1**) (8). Patients with primary valve disease (i.e., typically severe aortic stenosis or severe organic MR) who have an LVEF between 41 and 49% and evidence of PH are in an advanced disease stage with relevant "cardiac damage" (**Table 2**). Evaluation and management of such patients will not be discussed in this review article but this can be found elsewhere (55, 56).

DIAGNOSTIC APPROACH

Patients with a mildly reduced LVEF, i.e., between 41 and 49%, and evidence of possible PH represent a diagnostic challenge because a broad spectrum of disease mechanisms and hemodynamic patterns can be hidden behind this constellation (**Figure 1**, **Table 2**). In any case, the presence of PH is a marker of a serious problem, be it the consequence of the left heart disease or an independent entity (57), and therefore always requires a careful evaluation. The non-invasive diagnosis of PH by echocardiography remains difficult (1, 2). The peak TRV cannot always be measured in a reliable manner, and even if so, the correlation with the true sPAP is limited at least in certain settings (58). Guidelines recommend estimating the probability of PH using both peak TRV and indirect signs of PH (i.e., RV dilatation, flattening of the interventricular septum, short RV outflow tract acceleration time, and/or midsystolic notching,

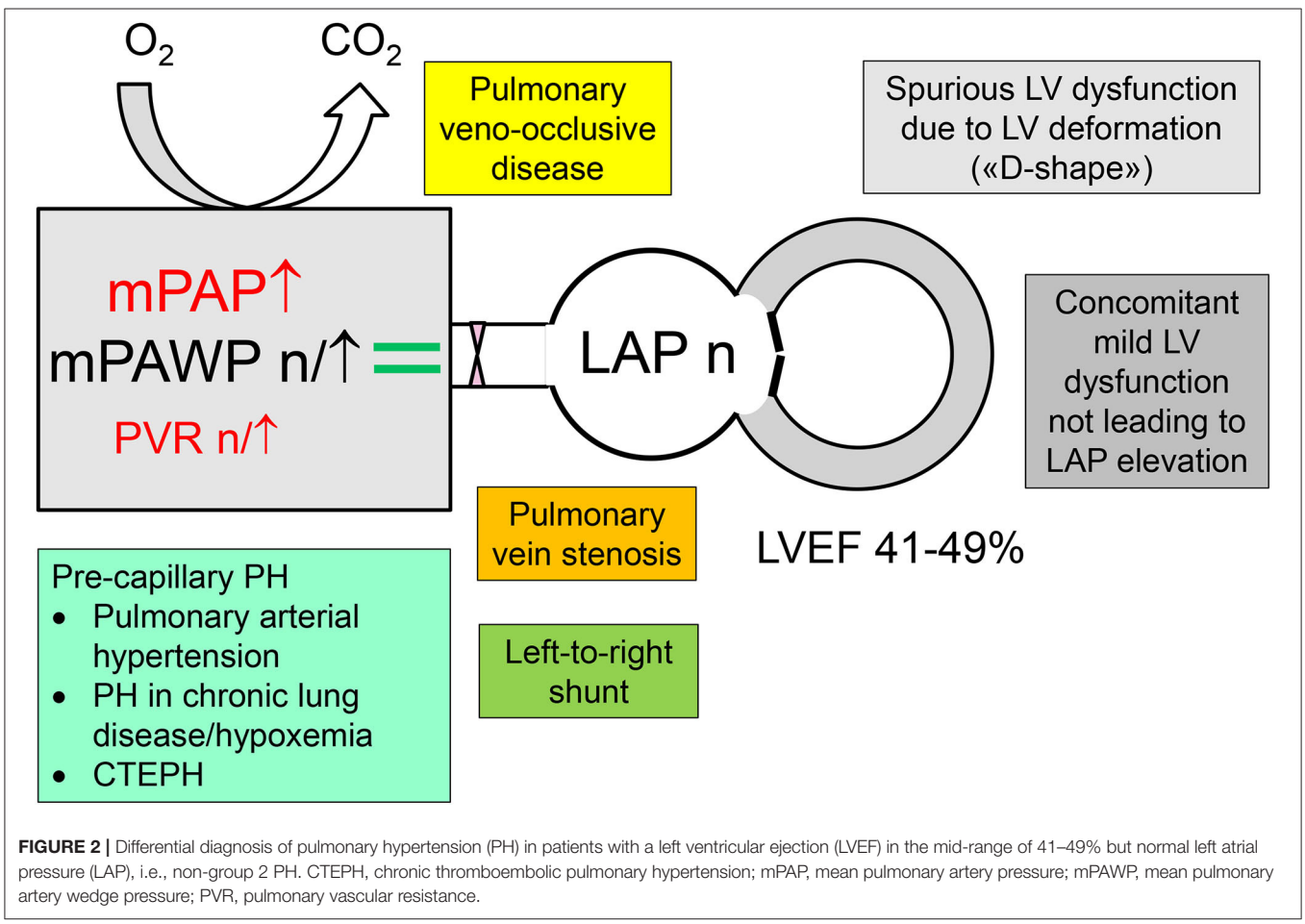

FIGURE 2 | Differential diagnosis of pulmonary hypertension (PH) in patients with a left ventricular ejection (LVEF) in the mid-range of 41–49% but normal left atrial pressure (LAP), i.e., non-group 2 PH. CTEPH, chronic thromboembolic pulmonary hypertension; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; PVR, pulmonary vascular resistance.

elevated early diastolic pulmonary regurgitation velocity, dilated inferior vena cava with reduced collapsibility, increased right atrial size): low probability of PH if peak TRV ≤2.8 m/s or not measurable and absence of indirect signs of PH, intermediate probability of PH if peak TRV ≤2.8 m/s or not measurable but indirect signs of PH or peak TRV 2.9–3.4 m/s but absence of indirect signs of PH, and high probability if peak TRV 2.9–3.4 m/s in combination with indirect signs of PH or peak TRV >3.4 m/s with or without indirect signs (11). This approach is accepted in the context of the "old" ESC/ERS 2015 PH definition as the Gold standard (PH: mPAP ≥25 mmHg). It is currently unknown whether a re-calibration is required when using the new PH definition (PH: mPAP >20 mmHg + additional criteria, see above).

There are algorithms composed of clinical parameters and non-invasive findings for the discrimination between pre- and post-capillary PH (59–61). **Table 3** summarizes features favoring pre-capillary or post-capillary PH. A mildly reduced LVEF per se is no proof for post-capillary PH, and therefore attention must be given to markers of high left sided-filling pressures such as left ventricular diastolic dysfunction and LA dilatation/dysfunction. A high peak early mitral inflow velocity to peak early mitral annular velocity (E/e′) has turned out as a useful marker of a post-capillary pathology although studies on the correlation between E/e′ and left ventricular end-diastolic pressure or mPAWP in patients with preserved LVEF have revealed mixed results (62). Overall, the best predictors of pre-capillary PH include a small left LA (59, 61), a dilated RV (60, 61), a clearly visible D-shape of the left ventricle (60), a notch in the PW Doppler signal of the PA or a short acceleration time of <80 ms (59). The areas under the curve for these scores to predict pre-capillary PH range from 0.76 (60) to 0.93 (61). Still, only right heart catheterization can definitely make a diagnosis of PH and establish the underlying hemodynamic constellation (pre-capillary vs. post-capillary PH).

In a patient with LVEF 41–49% and intermediate or high likelihood of PH, PH can be the consequence of LV dysfunction

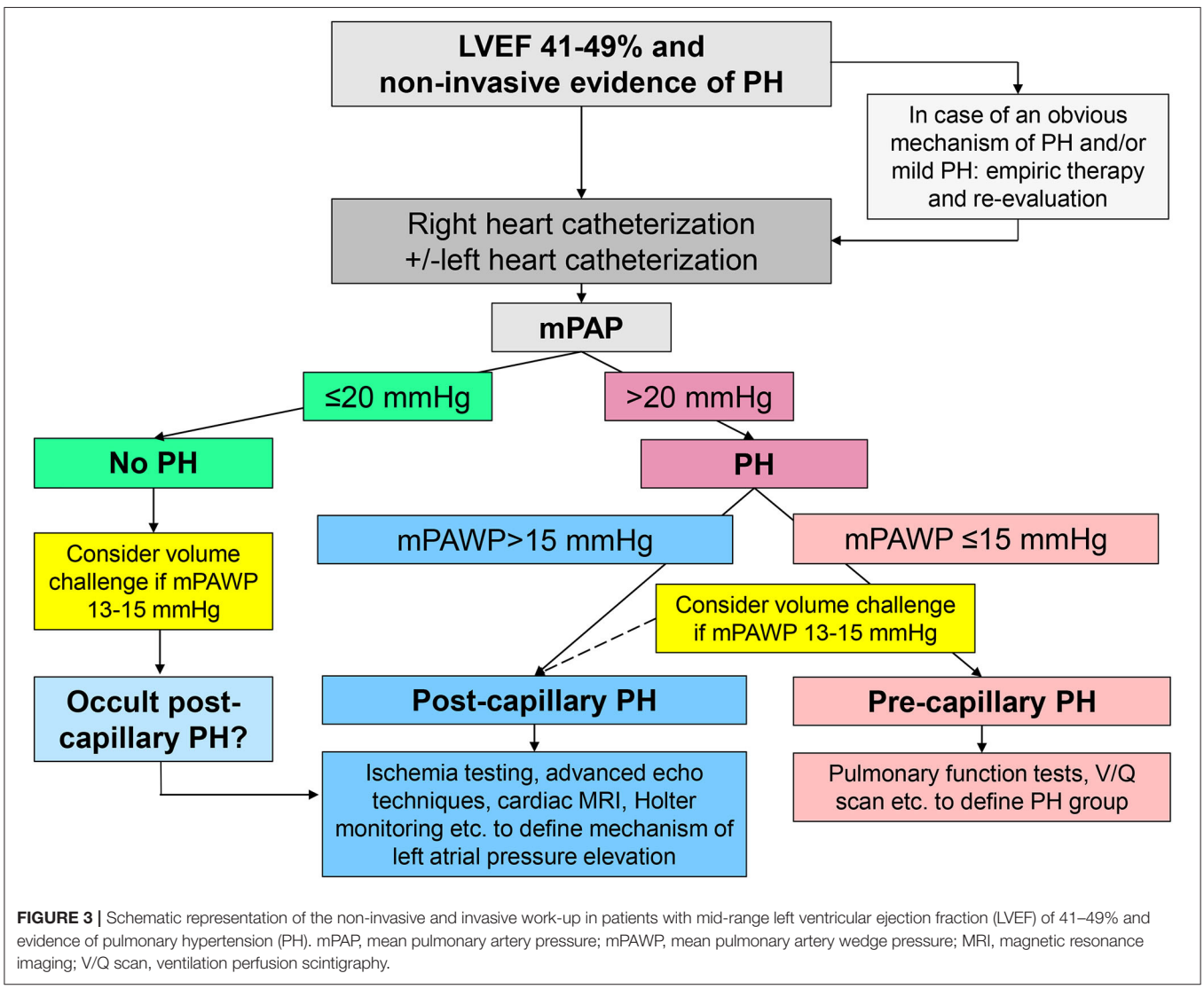

FIGURE 3 | Schematic representation of the non-invasive and invasive work-up in patients with mid-range left ventricular ejection fraction (LVEF) of 41–49% and evidence of pulmonary hypertension (PH). mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; MRI, magnetic resonance imaging; V/Q scan, ventilation perfusion scintigraphy.

with LA pressure elevation, i.e., HFmrEF with group 2 PH, or this maybe a non-group 2 PH that co-exists with mild left ventricular dysfunction (**Figures 1**, **2**). Measurement of natriuretic peptides will often not be helpful for discrimination, because elevated B-type natriuretic peptide (BNP) or N-terminal-proBNP (NT-proBNP) plasma concentrations can be the consequence of increased left ventricular wall stress (63) and thereby point toward left ventricular disease as the driver of symptoms (i.e., HFmrEF), but can also result from RV stress in case of pre-capillary PH (28).

The classical class I indication for right heart catheterization in patients with group 2 PH is in the context of transplant evaluation (11). Guidelines state that right heart catheterization may also be considered (class IIb indication) in patients with left heart diseases and suspected PH to assist in the differential diagnosis and support treatment decisions (11). If non-invasive imaging clearly points to group 2 PH, treatment can be established, in particular euvolemia must be achieved. Depending on the context and the extent of the suspected PH, right heart catheterization may still be performed early in the diagnostic pathway to clarify the hemodynamic constellation, and additional tests will be performed depending on the result (pre- vs. post-capillary PH) (**Figure 3**). In patients with a borderline hemodynamic constellation (i.e., mPAWP 13–15 mmHg), there may be occult post-capillary PH following prolonged fasting or diuretic therapy, and a volume or exercise challenge may be required to unmask group 2 PH (14). In patients with post-capillary PH, the key mechanism of LA pressure and mPAWP elevation, respectively, has to be identified as a basis for appropriate therapy (**Table 2**). In **Figures 4–6**, three examples of patients

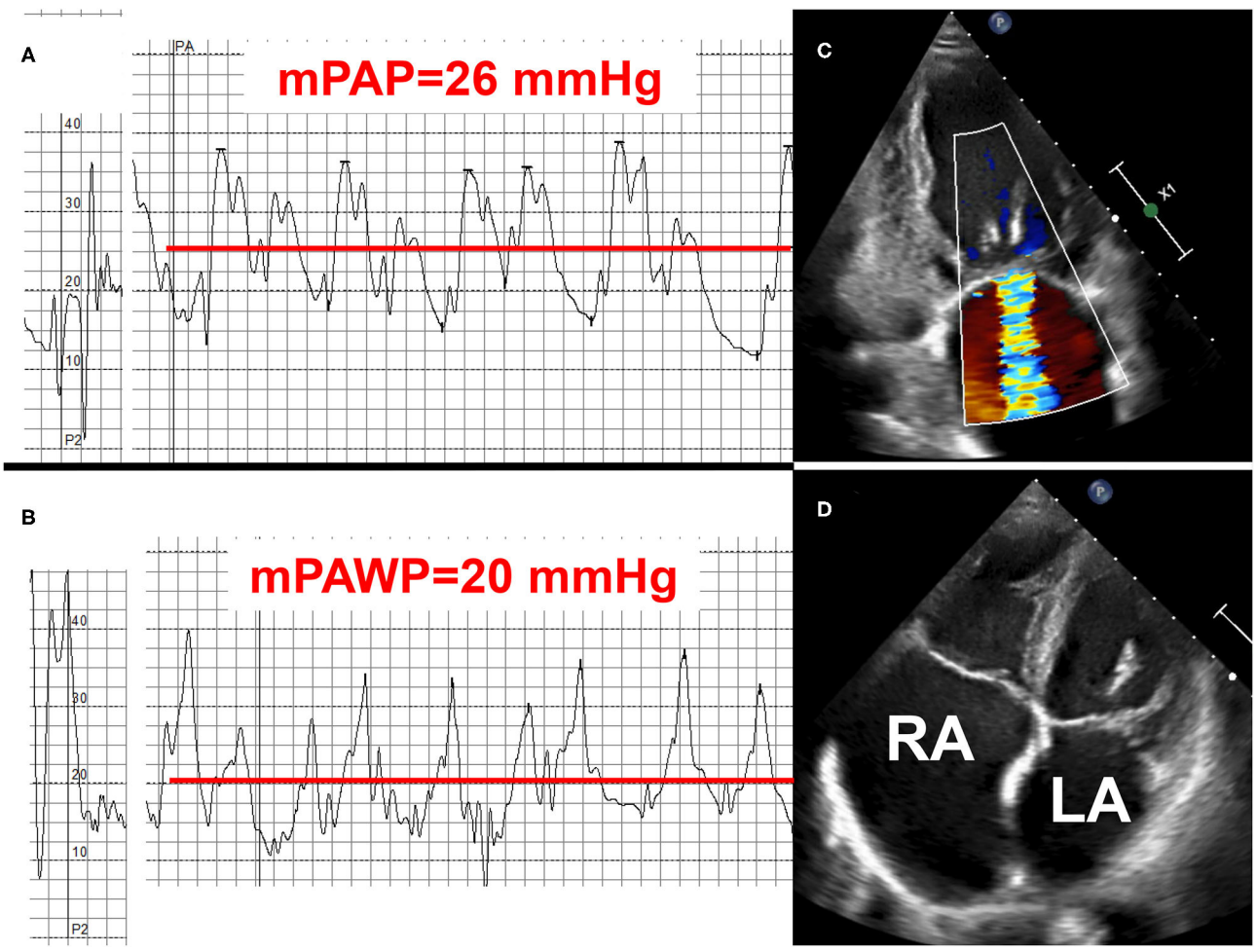

FIGURE 4 | Example 1 of a patient with a mid-range left ventricular ejection fraction (LVEF) and pulmonary hypertension (PH): 78-year old female with permanent atrial fibrillation and coronary artery disease with previous myocardial infarction and percutaneous intervention of the occluded left circumflex artery (LCX). NYHA class II. LVEF 48%, moderate mitral regurgitation (MR), biatrial dilatation (LA: left atrium, RA: right atrium). Mean pulmonary artery pressure (mPAP): 26 mmHg **(A)**, mean pulmonary artery wedge pressure (mPAWP) 20 mmHg **(B)**, pulmonary vascular resistance: 1.7 Wood units. Left atrial pressure elevation and isolated post-capillary PH, respectively, are most likely multifactorial [left ventricular dysfunction, functional MR due to distorted left ventricular geometry after LCX infarct and atrial/annulus dilatation **(C)**, left atrial dysfunction in the context of atrial fibrillation **(D)**]. Management with loop diuretics, spironolactone, candesartan or sacubitril/valsartan, and betablocker. Rhythm control of atrial fibrillation may be considered but may not be successful; no evidence-based indication for mitral valve repair.

with HFmrEF and PH are presented. These very different cases highlight the heterogeneity within the HFmrEF population and the challenges associated with diagnosis and therapy.

Notably, most patients with an LVEF between 41 and 49% (typically after myocardial infarction) seen in daily practice are not symptomatic from HF, and therefore mechanisms for dyspnea other than the mildly reduced LVEF have to be carefully looked for (**Table 4**). Patients with pre-capillary PH and LVEF 41–49% need a work-up to define the underlying PH group (pulmonary arterial hypertension, PH in context of lung disease/chronic hypoxia, chronic thromboembolic PH) as this has may have direct therapeutic consequences. The mildly reduced LVEF in these cases typically results from LV deformation due to RV pressure overload ("D-shape") or represents a concomitant mild LV disease, which however is not hemodynamically predominant.

TREATMENT OF PH IN HFmrEF

The general principle applying to the treatment of PH in HF is to treat the underlying cardiac disease and its risk factors, particularly the metabolic syndrome, and to identify and treat co-morbidities that may also lead to PH such as chronic obstructive lung disease and obstructive sleep apnea (1, 2, 11). For patients with HFrEF, treatment is well-defined and includes several drugs with different mechanisms of action with established effect on symptoms and prognosis (7). Wireless pulmonary artery pressure monitoring data have shown that PA pressure can be effectively lowered by guideline-directed disease-modifying therapy and diuretics (64). In contrast, there is still no treatment, which has been shown to improve prognosis in patients with HFpEF (7). Diuretics are recommended for the management of congestive symptoms in these patients (7). However, given the

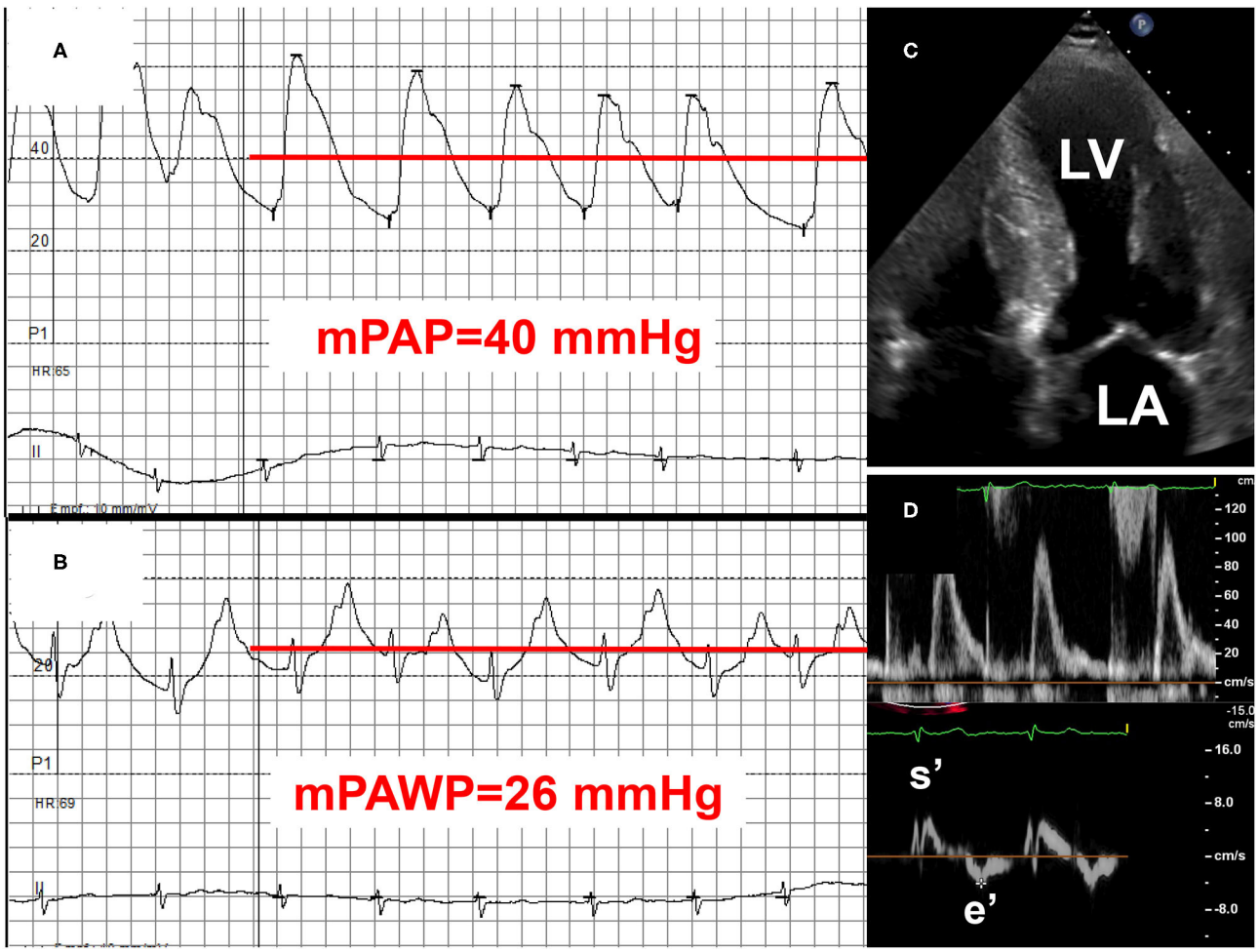

FIGURE 5 | Example 2 of a patient with a mid-range left ventricular ejection fraction (LVEF) and pulmonary hypertension (PH): 75-year old man with transthyretin cardiac amyloidosis (positive technetium pyrophosphate scan). NYHA class III. LVEF 46%. Mean pulmonary artery pressure (mPAP): 40 mmHg **(A)**, mean pulmonary artery wedge pressure (mPAWP): 26 mmHg **(B)**, pulmonary vascular resistance: 2.5 Wood units. Left atrial pressure elevation is due to myocardial amyloid deposition **(C)** with significant systolic [long axis function, reduced systolic mitral annular velocity (s')] and diastolic dysfunction [markedly reduced peak early diastolic mitral annular velocity (e'); **(D)**]. Management primarily with loop diuretics and spironolactone; tafamidis may be considered but may have limited effect in this advanced disease stage (NYHA III). LA, left atrium; LV, left ventricle.

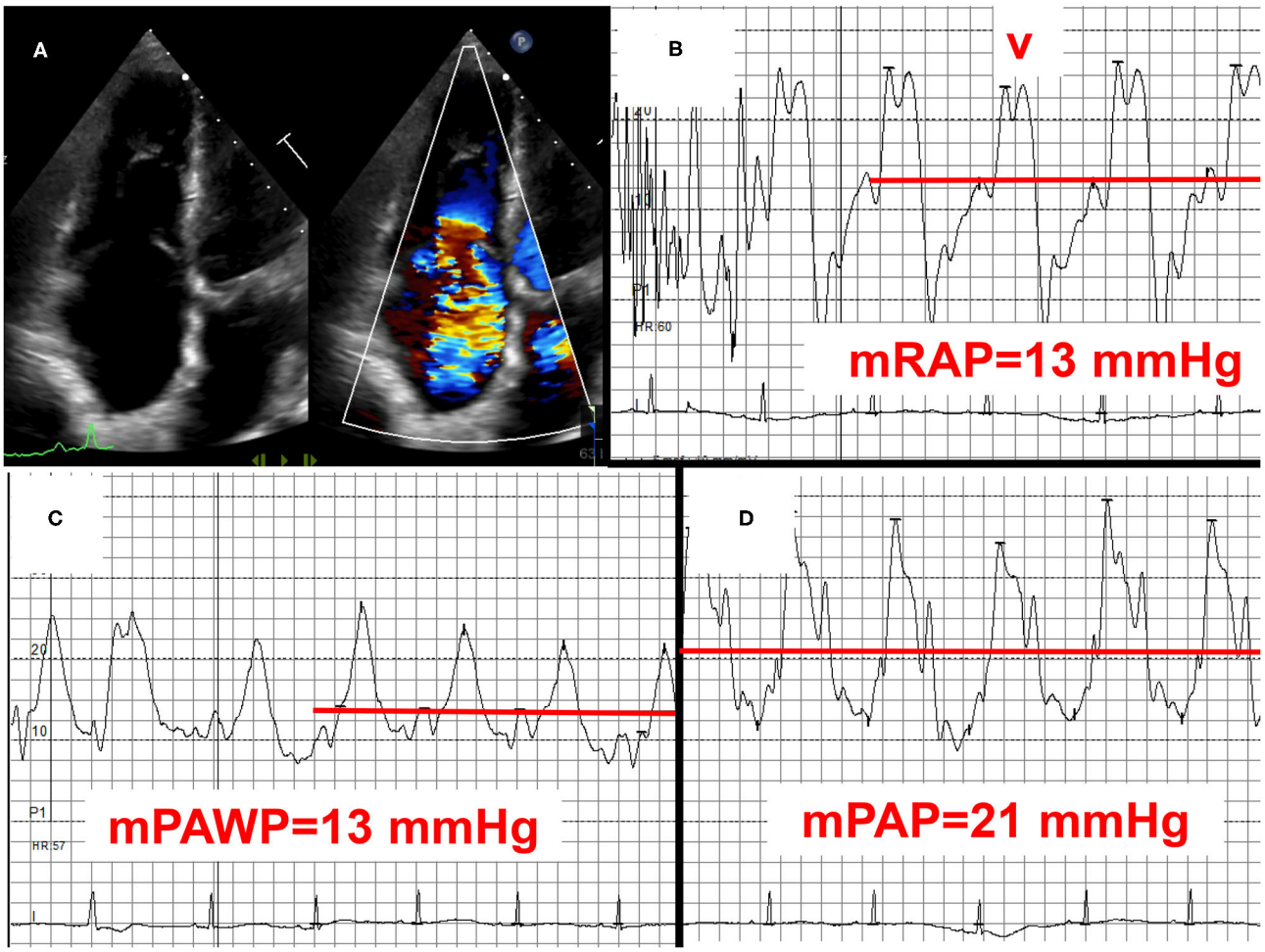

FIGURE 6 | Example 3 of a patient with a mid-range left ventricular ejection fraction (LVEF) and pulmonary hypertension (PH): 83-year old female with permanent atrial fibrillation, previous aortic valve replacement, and coronary artery disease. LVEF 45%, normally functioning aortic bioprothesis, mild to moderate mitral regurgitation, and severe tricuspid regurgitation **(A)** with signs of right heart failure and high right atrial pressure with high V waves **(B)**. Mean pulmonary artery wegde pressure (mPAWP): 13 mmHg **(C)**, mean pulmonary artery pressure (mPAP): 21 mmHg **(D)**. After coronary angiography (50 ml of contrast) rise in mPAWP to 18 mmHg and mPAP to 26 mmHg. The patient has occult post-capillary PH (2016 ESC/ERS definition)/mild post-capillary PH (2018 definition) in the context of left ventricular systolic and diastolic dysfunction. The relatively mild extent of PH does not fully explain right ventricular dilation and severe tricuspid regurgitation. Severe tricuspid regurgitation is most likely the effect of atrial fibrillation predominantly affecting the tricuspid annulus. Management with loop diuretics and spironolactone. The role of transcatheter tricuspid valve repair/replacement has not been defined yet. mRAP, mean right atrial pressure.

typically small left ventricular volumes (concentric remodeling) and the steep end-diastolic pressure-volume relationship there is a relatively narrow therapeutic window for the use of diuretics. Diuretics will efficiently reduce LVEDP, LAP, mPAWP, and mPAP but these patients are also at risk for overtreatment with hypotension and renal failure (1). In patients with "true" HFpEF (i.e., LVEF ≥50%), studies testing drugs with proven survival benefit in HFrEF (inhibitors of the renin-angiotensin system, spironolactone) have failed to show any benefit (1). For HFmrEF patients, no specifically designed trials have been performed (6), and treatment of these patients is currently not well-defined. However, subgroup and *post-hoc* analyses of three large "HFpEF studies" using variable LVEF cut-offs for inclusion and evaluating the effect of candesartan vs. placebo (LVEF >40%) (65), spironolactone vs. placebo (LVEF ≥45%) (66), and sacubitril/valsartan vs. valsartan alone (LVEF ≥45%) (27) have revealed that patients fitting into the current HFmrEF range (i.e., LVEF 40–49 or 45–49%) may benefit from these three drugs. In addition, a recent meta-analysis found evidence of a benefit of betablocker therapy in HFmrEF patients (67). Thus, we suggest that patients with HFmrEF and post-capillary PH should be treated with these drugs and loop diuretics as needed. The recommendations of the 2021 ESC HF guidelines on HFmrEF are not published yet and may be more reluctant. Still, we think that the use of these potentially effective drugs should be considered if there PH, i.e., a manifestation of advanced HF. In addition, specific mechanisms of LA pressure and mPAWP elevation must be targeted, e.g., tachycardia, atrial fibrillation,

TABLE 4 | Differential diagnosis of pulmonary hypertension (PH) and mid-range/"mildly reduced" left ventricular ejection fraction (LVEF).

	Characteristics	Hemodynamics	Diagnostic approach
HFmrEF with group 2 PH (cf. **Table 1**)	PH as consequence of HFmrEF	2015: mPAP ≥25 mmHg and mPAWP >15 mmHg 2018: mPAP >20 mmHg and mPAWP >15 mmHg	Identification of treatable mechanisms of HF: ischemia, atrial fibrillation, primary valve disease, systemic disease; RHC if hemodynamic constellation unclear
Group 1 PH and LVEF 41–49%	Pulmonary arterial hypertension with concomitant unrelated mild LV disease (or "only" LV deformation due to flattening of the interventricular septum)	2015: mPAP ≥25 mmHg and mPAWP ≤15 mmHg 2018: mPAP >20 mmHg, mPAWP ≤15 mmHg, and PVR ≥3 WU	RHC, ventilation/perfusion scintigraphy, lung function, sleep study, evaluation of specific etiologies (liver disease, connective tissue disease, etc.)
Group 3 PH and LVEF 41–49%	PH in the context of chronic lung disease/chronic hypoxemia combined with mild LV dysfunction (e.g., previous myocardial infarction)	2015: mPAP ≥25 mmHg and mPAWP ≤15 mmHg 2018: mPAP >20 mmHg, mPAWP ≤15 mmHg, and PVR ≥3 WU	Lung function tests including CO diffusion, CT scan, sleep study. RHC only in selected cases Identification of the concomitant cardiac disease, e.g., cardiac MRI and coronary angiography in case of suspected coronary artery disease
Group 4 PH and LVEF 41–49%	Chronic thromboembolic PH combined with mild LV disease (or "only" LV deformation due to flattening of the interventricular septum)	2015: mPAP ≥25 mmHg and mPAWP ≤15 mmHg 2018: mPAP >20 mmHg, mPAWP ≤15 mmHg, and PVR ≥3 WU	RHC, ventilation/perfusion scintigraphy, pulmonary angiography
Left-to-right shunt with/without mild LV disease	Atrial septal defect or abnormal pulmonary venous drainage	2015: mPAP ≥25 mmHg, pulmonary blood flow↑↑ 2018: mPAP >20 mmHg, pulmonary blood flow ↑↑	TTE and TEE and CT scan to identify the shunt, RHC
High output HF	Liver disease, severe anemia, or other high-output condition associated with mild LV dysfunction	2015: mPAP ≥25 mmHg, mPAWP >15 mmHg, and cardiac index↑↑ 2018: mPAP >20 mmHg, mPAWP >15 mmHg, PVR <3 WU, and cardiac index↑↑	Internistic work-up, TTE, RHC

LV, left ventricular; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RHC, right heart catheterization; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography. ↑↑, substantially increased.

myocardial ischemia, infiltrative diseases, and functional MR. Several mechanism may contribute to LA pressure elevation (**Figure 1**), and careful non-invasive and invasive diagnostic evaluation is prerequisite for a tailored therapy (**Table 2**) (27, 65–71). The role of AF seems to be particularly important. Atrial fibrillation seems to be a key factor in the pathophysiology of PH in HFpEF and HFmrEF as discussed above and is associated with the combined endpoint of all-cause mortality and heart failure hospitalizations in HFpEF and HFmrEF but not in HFrEF (72). A recent study has shown a favorable effect of successful catheter ablation on exercise hemodynamics (reduction in peak exercise mPAWP) and quality of life in patients with HFpEF (71). It is speculated that rhythm control of AF may be a very important strategy to treat or prevent PH in patients with HFpEF und HFmrEF.

In contrast to patients with HFrEF, evidence for the utility of device therapy for the treatment of HFmrEF and HFpEF is scarce. This refers to defibrillators (except for secondary prevention), cardiac resynchronization, and transcatheter mitral valve repair. In transplant candidates with advanced HFrEF (mean LVEF 18%) and CpcPH without acute reversibility, left ventricular unloading by implantation of an assist device has been shown to result in a reduction in PVR from 5.1 to 2.0 WU within 6 weeks (73). It is unknown how the pulmonary vasculature is remodeling in this context, and whether this approach would also be successful in HFpEF and HFmrEF. However, the latter two groups are rarely candidates for transplantation. As an important exception regarding the applicability of devices, the concept of an intraatrial shunt device for LA decompression has been successfully tested in HFpEF and HFmrEF (74, 75). In patients with LVEF ≥40%, this device led to a similar reduction in exercise mPAWP (driven by the pre-implant exercise mPAWP to right atrial pressure gradient) in patients with HFpEF and HFrEF with mPAWP >15 mmHg at rest or >25 mmHg on exercise (76). The mean resting mPAWP and mPAP in the study population were 17 and 24 mmHg indicating that the population included a relevant number of patients with post-capillary PH (74, 76). Interestingly, a *post-hoc* analysis of hemodynamics in 79 patients treated with the intraatrial shunt device (mean LVEF 47%; 68% of patients with LVEF 40–49%, mean mPAP and mPAWP 26 and 18 mmHg, respectively) revealed that the 27% increase in pulmonary flow at rest was accompanied by a 17% reduction in PVR and a 24% increase in pulmonary artery compliance (77). Similar changes were observed during exercise. It was speculated that the increase

TABLE 5 | Studies on pulmonary arterial hypertension targeted therapeutics in patients with heart failure (HF) with preserved (HFpEF) or mid-range (HFmrEF) left ventricular ejection fraction (LVEF) with or at risk for pulmonary hypertension.

	Population	Intervention	Main results
Andersen et al. (78)	Inclusion criteria: Recent myocardial infarction, revascularized, LVEF ≤45%, E/e′ ≥8, LAVI ≥34 ml/m^2, Hemodynamics: mPAP ≈ 20 mmHg, mPAWP ≈ 13 mmHg ($n = 70$)	Sildenafil 3 × 40 mg vs. placebo for 9 weeks	Trend toward exercise mPAWP reduction CO↑ and SVR ↓
Guazzi et al. (79)	Inclusion criteria: LVEF ≥50% sPAP>40 mmHg ($n = 44$) Hemodynamics: mPAP ≈ 37 mmHg, mPAWP ≈ 22 mmHg, PVR ≈ 3.5 WU	Sildenafil 3 × 50 mg vs. placebo for 6 months	mPAP↓ mPAWP↓ Cardiac index↑ Right ventricular function↑
Redfield et al. (80)	Inclusion criteria: LVEF ≥50%+elevated NT-proBNP or non-invasive evidence of elevated filling pressures ($n = 216$) Hemodynamics: not measured	Sildenafil 3 × 20 mg for 12 weeks, then 3 × 60 mg vs. placebo for 12 weeks	No effect on peak VO_2 and 6-min walking distance
Hoendermis et al. (81)	Inclusion criteria: LVEF ≥45%, mPAP >25 mmHg, mPAWP >15 mmHg Hemodynamics: mPAP ≈ 35 mmHg mPAWP ≈ 20 mmHg	Sildenafil 3 × 60 mg vs. placebo for 12 weeks	No effect on mPAP, mPAWP, CO, and peak VO_2
Belyavskiy (82)	Inclusion criteria: LVEF >50%, sPAP >40 mmHg, PVR >3 WU and/or transpulmonary gradient >15 mmHg (all assessed by echocardiography) Hemodynamics: not measured	Sildenafil 3 × 25 mg for 3 months, followed by 3 × 50 mg for 3 months vs. placebo	Improvement in NYHA class and 6 min walking distance, reduction in sPAP
Bonderman et al. (83)	Inclusion criteria: LVEF>50%, mPAP ≥25 mmHg, mPAWP >15 mmHg ($n = 39$) Hemodynamics: mPAP ≈ 35 mmHg mPAWP ≈ 20 mmHg	Single dose of Riociguat of 0.5 mg, 1.0 mg, or 2.0 mg vs. placebo	No effect on mPAP after 6 h Stroke volume↑ Systolic blood pressure↓ Right ventricular end-diastolic area
Bermejo et al. (84)	Inclusion criteria: PH post valve surgery but no significant valvular dysfunction, mPAP >30 mmHg Hemodynamics: mPAP ≈ 38 mmHg mPAWP = 23 mmHg PVR ≈ 3.3 WU ($n = 200$)	Sildenafil 3 × 40 mg (3 × 20 mg for selected patients) vs. placebo for 6 months	Worse composite clinical score (death, hospitalization for HF, change in functional class, patient global self assessment) in the sildenafil treated patients
Zile et al. (85)	Inclusion criteria: LVEF ≥50% + evidence of concentric remodeling and/or LV diastolic dysfunction E/e′ 14, peak TRV 2.7 m/s ($n = 192$) Hemodynamics: not measured	Sitaxsentan 100 mg/d vs. placebo for 24 weeks (2:1 randomization)	Increase in treadmill time, no effect on quality of life, death, HF hospitalization
Koller et al. (86)	Inclusion criteria: HFpEF (ESC 2016 definition) and mPAP ≥25 mmHg, mPAWP >15 mmHg Hemodynamics: mPAP ≈ 38 mmHg mPAWP ≈ 21 mmHg PVR ≈ 4.2 WU ($n = 20$)	Bosentan 2 × 62.5 mg for 4 weeks, 2 × 125 mg for 8 weeks vs. placebo	Higher pulmonary artery and right atrial pressure (echo) and worsening 6 min walking distance in Bosentan group
Vachiery et al. (87)	Inclusion criteria: Combined pre-capillary and post-capillary PH (mPAP ≥25 mmHg, mPAWP >15 mmHg but <25 mmHg, DPG ≥7 mmHg and PVR >3 WU), LVEF ≥30% (≥50%: 81%, <50%: 19%) Hemodynamics mPAP ≈ 47 mmHg, mPAWP ≈ 20 mmHg, PVR ≈ 5.8 WU) ($n = 63$)	Macitentan 10 mg vs. placebo for 12 weeks	Trend toward more fluid retention in the Macitenan group No effect on mPAWP and PVR

DPG, diastolic pressure gradient; ESC, European Society of Cardiology; LAVI, left atrial volume index; LV, left ventricular; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; NYHA, New York Heart Association; peak VO_2, peak oxygen consumption; PVR, pulmonary vascular resistance; sPAP, systolic pulmonary artery pressure; E/e′, ratio of peak early diastolic transmitral velocity to peak early diastolic mitral annular velocity; TRV, tricuspid regurgitant velocity.

in pulmonary flow and oxygen content may have led to beneficial effects on the pulmonary vasculature. Thus, this therapeutic approach may be relevant for the management of patients with PH in the context of HFmrEF. Importantly, the mean PVR was 1.5 WU, and patients with a PVR 4 ≥WU were excluded (77).

Patients with HFmrEF and PH (i.e., post-capillary PH) may benefit from a particularly aggressive use and combination of the available treatments including diuretics. There are, however, no established drugs specifically targeting PH in HF in general and also in HFmrEF. Only a few studies have studied the effect of specific pulmonary arterial hypertension-targeted therapeutics in patients with HFpEF or HFmrEF and PH (**Table 5**) (78–87). In general, the use of pulmonary vasodilatators did not improve hemodynamics or exercise capacity. The most promising substance in this context is the phosphodiesterase inhibitor sildenafil. In a study among patients with HFpEF or HFmrEF (LVEF ≥45%) and IpcPH or mild CpcPH (mPAWP = 20 mmHg, PVR = 2.6 WU; 35% with PVR >3 WU) sildenafil compared to placebo exerted no effect on mPAWP, cardiac output and functional capacity (81). However, Guazzi et al. (79) reported a substantial reduction in mPAWP and PVR as well as an improvement in TAPSE in HFpEF patients (LVEF cut-off for inclusion: ≥ 50%) with somewhat higher PVR (around 3.6 WU) and poor RV function (TAPSE of 11 mm). A second study in patients with HFpEF and CpcPH found a benefit of sildenafil vs. placebo in terms of NYHA class, 6-min walking distance, and sPAP (82). However, this was a non-invasive study, and both the hemodynamic inclusion criteria and the endpoint (sPAP) were assessed by echocardiography. At the moment, it remains unclear whether patients with HFmrEF (and HFpEF) and more severe CpcPH (i.e., higher PVR) and RV dysfunction may benefit from specific pulmonary arterial hypertension-targeted therapeutics, in particular phosphodiesterase inhibitors. The PASSION trial evaluating the impact of tadalafil on clinical endpoints in patients with HFpEF (LVEF ≥50%) and CpcPH (mPAP ≥25 mmHg, mPAWP >15 mmHg, PVR >3 WU) is ongoing and will provide relevant information with potential implications for HFmrEF patients (50).

FUTURE PERSPECTIVES

Intense research will be required to define the key mechanism underlying the pathophysiology of (a) HFmrEF and (b) PH in HFmrEF. This will lead to a refinement of the definition, the diagnostic criteria and the therapeutic approach. Very recently, a universal definition and classification of HF has been proposed (10). In this position paper issued by all of the important HF societies, a fourth HF class has been suggested: HF with improved LVEF, i.e., HF with an initial LVEF ≤40% and an improvement by at least 10 percent points to an LVEF >40% (10). Whether or not this group of patients requires a different treatment than patients with (stable) HFmrEF or HFpEF will have to be shown. The 2021 ESC HF guidelines are about to be published and will define the diagnostic criteria and thereby probably follow the universal definition and classification of HF (10). For the treatment of HFmrEF in general and most likely also PH in HFmrEF the data on the effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors will be very important (88, 89). Mechanistic studies suggest that SGLT2 inhibitors exhibit favorable effects on cardiac inflammation and fibrosis and thereby cardiac remodeling also in subjects with preserved LVEF (90). Importantly, significant hemodynamic effects, i.e., reduction in mPAWP (91) and PA pressures (92) have been demonstrated for SGLT2 inhibitors, most likely indirectly via beneficial effects on cardiac structure and function but also directly via the diuretic properties (93) of these drugs. The baseline characteristics of the Empagliflozin Outcome Trial in Patients With Chronic Heart Failure With Preserved Ejection Fraction (EMPEROR-Preserved) have already been published (89): the LVEF cut-off for study inclusion was ≥40%, and the mean baseline LVEF is 54 ± 9% indicating that the trial also included a relevant number of HFmrEF patients (89) and that the results of this trial will be highly relevant for the setting of HFmrEF in general and also HFmrEF with PH. The three cases presented in **Figures 4–6** highlight however, that management of these patients is challenging, that a clear guideline-based recommendation will not available for all scenarios, and that treatment must always be tailored based on a careful non-invasive and often also invasive assessment.

Apart from the treatment of the underlying left heart pathology (i.e., HFmrEF) there is currently intense research investigating novel treatments targeting the pulmonary vasculature directly (5, 94). Approaches currently under study for patients with PH in the context of HF include among others the β3 adrenergic receptor agonist mirabegron, the antifibrotic agent PBI 40–50, the rho kinase inhibitor fasudil, the calcium sensitizer levosimendan, oral sodium nitrite, and catheter-based pulmonary artery denervation (5, 94). It is likely that only certain pulmonary vascular phenotypes with PH in HFmrEF or HFpEF will derive benefit from such an approach. Only studies with detailed clinical, biochemical, and hemodynamic phenotyping will be able to define whether there is a subset of patients with PH in the context of HFmrEF who will benefit from specific pulmonary arterial hypertension-targeted therapeutics.

CONCLUSIONS

Heart failure with mid-range LVEF in general, and PH in HFmrEF in particular, are entities that have been incompletely characterized. Cross-sectional studies suggest that HFmrEF patients are overall characterized by a left heart phenotype which is intermediate between HFrEF and HFpEF. With regards to the pathophysiology of PH the available data suggest that there are many similarities with HFpEF. In clinical practice, patients with shortness of breath, an LVEF in the mid-range of 41–49% and evidence of PH represent a diagnostic challenge. First, a careful differentiation between post- and pre-capillary PH is required. Second, in patients with post-capillary PH the predominant mechanism of LA pressure elevation has to be identified as this will represent the primary target for therapy. In terms of medical therapy, there is some evidence for a

benefit of classical HFrEF therapeutics, i.e., angiotensin receptor blockers, spironolactone, sacubitril/valsartan, and betablockers for HFmrEF and presumably also for HFmrEF with PH. However, at the moment, this is still speculative, and substantial additional research will be required to define the optimal management of these patients.

AUTHOR CONTRIBUTIONS

MM: conception, collection of data, writing of first draft, and finalization of paper. LW, MB, RB, LJ, and HR: critical revision and final approval. All authors contributed to the article and approved the submitted version.

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REVIEW
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Heart Failure With Mid-range Ejection Fraction: Every Coin Has Two Sides

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This review summarizes current knowledge regarding clinical epidemiology, pathophysiology, and prognosis for patients with HFmrEF in comparison to HFrEF and HFpEF. Although recommended treatments currently focus on aggressive management of comorbidities, we summarize potentially beneficial therapies that can delay the process of heart failure by blocking the pathophysiology mechanism. More studies are needed to further characterize HFmrEF and identify effective management strategies that can reduce cardiovascular morbidity and mortality of patients with HFmrEF.

Keywords: heart failure, heart failure with reduced ejection fraction, heart failure with mid-range ejection fraction, heart failure with preserved ejection fraction, left ventricular ejecting fraction

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In 2013, the American College of Cardiology/American Heart Association defined "Heart failure with borderline ejection fraction" as heart failure with typical clinical symptoms and LVEF of 41–49%. In 2016, the European Society of Cardiology (ESC) firstly classified heart failure into three categories based on the LVEF: heart failure with reduced ejection fraction (HFrEF, LVEF<40%), heart failure with mid-range ejection fraction (HFmrEF), and heart failure with preserved ejection fraction (HFpEF, LVEF≥50%). The LVEF range of HFmrEF is 40∼49%. The new classification encourages further researches on HFmrEF, as it reflects a median phenotype between HErEF and HFpEF, and the subtype of heart failure may correspond to different stages during the development, which is inconsistent with the results of current clinical studies. Does HFmrEF represent an independent type or a transitional stage between HFrEF and HFpEF? Do the targeted therapies known to be efficacious for HFrEF patients have beneficial effects on patients with HFmrEF? This article summarizes the current understanding of the HFmrEF and discusses how to better manage patients with HFmrEF.

DEFINITION AND DIAGNOSIS

Modern treatment of HF is primarily dependent on the objective evaluation of LVEF, which can predict adverse outcomes even in the absence of symptoms. In the past, patients with heart failure (HF) had been categorized into heart failure with reduced ejection fraction (HFrEF, LVEF<40%) and heart failure with preserved ejection fraction (HFpEF, LVEF≥50%), while patients with an LVEF value in the range of 40–49% were considered in the "gray area." In 2016, ESC defined patients with LVEF in the range of 40–49% as HF with mid-range ejection fraction (HFmrEF) in order to stimulate researches on the underlying characteristics, pathophysiology, and treatment of this subtype of patients (1).

For a precise diagnosis and treatment, the introduction of this new classification is understandable and reasonable. 2017 ACC/AHA guidelines for the management of heart failure and Chinese guidelines for the diagnosis and treatment of heart failure in 2018 adopt the same definition (2, 3). And the need for identifying this new subgroup has made HFmrEF a new research hotspot.

Although HFmrEF was first introduced into the guidelines in 2016, the "gray area" between HFrEF and HFpEF had already been mentioned in 2012 ESC guidelines (4). Therefore, the guidelines merely legitimized this "gray area" as a distinct entity by giving it a name (5). The primary purpose for defining this new group is to highlight its importance and stimulate researches relevant to these patients populations, as they are typically excluded from both HFpEF and HFrEF trials. As a result, it also confused many physician, including clinical presentation, management, and outcomes of HFmrEF, which partially overlaps with HFrEF and HFpEF. OPTIMIZE-HF and ADHERE studies have begun to explore the characteristics, treatment patterns, and clinical outcomes of patients with a mild decrease in LVEF, finding that these patients may be significantly different from HFrEF and HFpEF populations (6, 7).

EPIDEMIOLOGICAL CHARACTERISTICS

Prevalence

More than 6.5 million people have been diagnosed with HF in the United States (8). Relevant research has shown that HFmrEF accounts for 13–24% of HF, meaning that there are about 1.6 million HFmrEF patients in the United States (5, 9). From 2005 to 2010, the proportion of HFpEF patients increased from 33 to 39%, the proportion of HFrEF patients decreased from 52 to 47%, and the proportion of HFmrEF patients was relatively stable (increased from 13 to 15%) (10). The PINNACLE registration study, the largest descriptive analysis of HFmrEF patients to date, determined that 36.1% of all is HFrEF patient, 7.5% is HFmrEF, and 56.5% is HFpEF (11). Continuous hospitalization data from a multicenter ADHF in Japan showed that 651 (17.1%) of 3,572 patients were categorized with HFmrEF. Of 3,580 patients with heart failure in a recent Spanish report, HFmrEF patients were found in 14% (12). The unimodal distribution of LVEF deciles shows that a large number of patients fall within the "middle zone" of LVEF; the prevalence rate of this medium-range is estimated to be 10–20%, as most patients have no clinical symptoms of heart failure according to CHARM reports (13).

Readmission Rate and Mortality

The readmission rate of HFmrEF is between those of HFrEF and HFpEF. In the GWTG-HF registry, all-cause readmission rates of HFmrEF patients were 20.9 and 63.2% within 30 days and 1 year, respectively. These numbers are similar to those of HFpEF patients (20.5 and 62.5%, respectively) and slightly higher than those of HFrEF patients (19.7 and 59.6%, respectively). However, the readmission rates for cardiovascular events in patients with HFmrEF (11.3% within 30 days, 41.6% within 1 year) were higher than those of the HFpEF group (9.9 and 37.4%, respectively) and close to those of the HFrEF group (12.9 and 42.4%, respectively) (14). Compared to HFrEF and HFpEF patients, the specific HF readmission rate for HFmrEF patients was intermediate. The GUIDE-IT trial used NT-pro-BNP to guide the treatment of patients with HFrEF; however, the study was terminated prematurely due to inefficacy (15).

Of all HF patients, the HFrEF group had the highest mortality, and the mortality of the HFmrEF group was similar to that of the HFpEF group. In the OPTIMIZE-HF study, the overall in-hospital mortality rate of HFrEF patients was 3.9%, in comparison to 3.0% for HFmrEF patients and 2.9% for HFpEF patients (6). A Canadian study of HF inpatients showed that the untreated mortality rate of HFmrEF patients was 5.1% within 30 days and 21.3% within 1 year, which were intermediate compared to those of HFpEF patients (5.3 and 22.2%, respectively) and HFrEF patients (7.1 and 25.5%, respectively), but the differences were not statistically significant (16). A meta-analysis of individual data from nearly 40,000 HF patients showed that, for patients with LVEF<40%, mortality increased gradually with every 5–10% decrease in LVEF, but there was no significant difference in LVEF>40% group (17).

A recent study by the Swedish Heart Failure Registry (Swede-HF) found that chronic kidney disease in patients with HFmrEF and HFrEF was a stronger predictor of mortality than HFpEF (18). In another study, HFmrEF patients over the age of 85 and those with the chronic obstructive pulmonary disease had a higher risk of death within 1 year after discharge than similar patients in other HF groups. Based on GWTG-HF registrations from 2005 to 2010, although the unadjusted hospital mortality rate of HFpEF patients decreased from 3.32 to 2.35%, the mortality rates of HFmrEF patients (2.69–2.88%) and HFrEF patients (3.03–2.83%) were relatively stable (10). Within each HF group, physiological factors, and concurrent disease contribute to 1-year mortality rates to varying degrees. Age over 85 years old and co-occurrence of COPD were more strongly correlated with 1-year mortality in HFmrEF patients (19).

Ethnic Characteristics

A retrospective cohort of large urban centers in the United States studied many HFmrEF patients representing blacks, Hispanics, and whites. From 2008 to 2012, cases of adult patients with HFmrEF were collected from Montefiore Medical Center in the Bronx, New York based on hospitalization echocardiography showing LVEF between 40 and 49%. A total of 1,852 HFmrEF patients (56% male with an average age of 67 years) participated in the study, including 493 non-Hispanic whites (26.6%), 541 non-Hispanic Blacks (29.2%), 489 Hispanics (26.4%), and 329 participants from other ethnic groups (17.8%). Of these groups, white patients tend to be older and less likely to take guide drugs. Compared with the rest of the population, the prevalence of myocardial infarction is lower in Black people. After adjusting for age, gender, and comorbidities, Hispanic individuals had more chronic diseases, but also higher survival rates, than whites and Blacks. There were also significant differences in clinical characteristics between different races/ethnic groups in the HFmrEF group. Non-Hispanic whites with HFmrEF had the highest prevalence of atrial fibrillation. The incidence rate of atrial fibrillation in non-Hispanic whites has been found

to be higher than that of non-Hispanic Blacks, Asians, and Hispanics, but the reason for this difference is unclear. As reported, the presence of a large left atrium is associated with a higher prevalence of atrial fibrillation. Many studies have shown that Blacks are more likely to develop coronary heart disease. Compared with whites and Hispanics, Blacks have the lowest levels of NT-proBNP. According to aggregate results from several large community research registries, plasma NT-proBNP levels of Blacks are significantly lower than those of whites due to genetic variation (20).

PATHOPHYSIOLOGICAL CHARACTERISTICS

Studies have shown that HFrEF and HFpEF are two different pathophysiological syndromes. HFrEF is usually characterized by systolic dysfunction, while HFpEF is characterized by diastolic dysfunction; however, they often overlap to varying degrees. The pathogenesis of heart failure involves three pathophysiological changes: abnormal activation of neurohormonal mechanisms (4, 21), the disorder of metabolization-inflammatory pathways (22), and dysregulation of cellular signaling mechanisms (23). Based on randomized clinical trials, the following drugs have been shown to reduce cardiovascular endpoints in patients with HFrEF: neurohormone antagonists, SGLT2 inhibitors, cellular, and cGMP-PKG signaling regulators (24). Drugs used to treat HFpEF by reducing EAT inflammation and improving diastolic function include SGLT2 inhibitors, metformin, GLP-1 receptor agonists, and statins. Drugs that can activate cGMP-PKG signaling pathways in HFpEF therapies include Vericiguat (24) and ARNI (25). Further clinical research on the pathogenesis of heart failure caused by metabolic-inflammatory mechanism disorder is required.

The latest VICTORIA study, presented at the 69th Annual meeting of the American College of Cardiology (ACC2020), adds new evidence to inform drug treatment of high-risk HFrEF patients. Deficiency of cyclic guanosine monophosphate (cGMP) derived from soluble guanylate cyclase (sGC) can lead to myocardial dysfunction and endothelium-dependent vasomotor dysfunction. NO-sGC-cGMP signal pathway has been an important therapeutic target for heart failure. Vericiguat is a novel sGC agonist that directly stimulates sGC production independent of the binding site of nitric oxide, thereby enhancing cGMP level and sensitizing sGC to endogenous nitric oxide (26) (**Figure 1**).

In fact, in the OPTIMIZE-HF study and other studies, LVEF showed a moderate bimodal distribution in HF inpatients, indicating the presence of two different disease processes (6). As a variable, LVEF shows dynamic change; however, it is by no means arbitrary. Clinical and basic researches suggest that it is appropriate to take such a tangent point of this variable, at least under existing conditions. The TIME-CHF study also showed that left ventricular hypertrophy was caused by concentric remodeling in the HFpEF and HFmrEF groups (albeit to a mild extent), but was caused by eccentric hypertrophy in HFrEF patients (27). The University of Washington Heart Failure Registry compared the degree of diastolic dysfunction between the HFmrEF recovery and deterioration groups, and found the presence of diastolic dysfunction in the deterioration group, indicating that the pathophysiological mechanisms of HFmrEF are heterogeneous (19). In 2016, ESC reported that patients with HFmrEF might present with mild systolic and diastolic dysfunction. The critical question is whether HFmrEF represents a unique clinical entity or just a "transition phase" between HFrEF and HFpEF.

In a study of 110 patients with HFpEF and 61 patients with HFmrEF, two-dimensional speckle tracking echocardiography (2D-STE) was used to evaluate LA phase function. Peak atrial longitudinal strain (PALS), peak atrial systolic strain (PACS), and PAL-PACS were measured to reflect the storage, pumping, and catheter functions of LA, respectively. In patients with normal LA size, LA reserve and pump function were still low for those with HFmrEF. PALS and PACS levels were negatively related to brain natriuretic peptide, LA volume, Emax A, Emax E', systolic blood pressure, and diastolic dysfunction of the pulmonary artery in both groups. Studies have shown that the phase function of LA, as measured by 2D-STE, is worse in patients with HFmrEF than in patients with HFpEF, though the two groups were similar in left atrial size and left ventricular diastolic function as measured by traditional echocardiography (28).

CLINICAL MANIFESTATIONS

Etiology and Inducement

The Spanish REDINSCORII study shows that the most common risk factor for HFmrEF patients is hypertension, and the most common cause of HF is ischemic heart disease (29). Japanese research groups have suggested that ischemic heart disease is a common cause of HFmrEF. Compared to patients in the HFpEF and HFrEF groups, HFmrEF patients tend to be older, female, anemic, and marked by atrial fibrillation. However, earlier studies suggested that the age, sex, and prevalence of atrial fibrillation and anemia in patients with HFmrEF were intermediate to HFpEF and HFrEF groups.Whereas the prevalence of ischemic etiology was similar to that of HFrEF and higher than that of HFpEF (30).

Clinical Features

At present, a few results have been reported from trials on HFmrEF patients. These studies only partially include patients with LVEF>45%, while some studies completely exclude patients with LVEF>50%. Nevertheless, insights gained from cohort and enrollment studies help to clarify the clinical characteristics of this group. In 2007, OPTIMIZE-HF studied 41,267 HF patients and analyzed the frequency of hospitalization, demographic characteristics, clinical symptoms, complications, laboratory results, and short-term prognosis based on different LVEF values. This analysis found that patients with LVEF values of 40–50% were more similar to HFpEF patients (6). These results are similar to those of the 2008 ADHERE registration study of patients with LVEF of 40–55%. ADHERE compared the clinical characteristics to those of the other two HF groups and found that the HFmrEF cohort was more similar to the HFpEF cohort

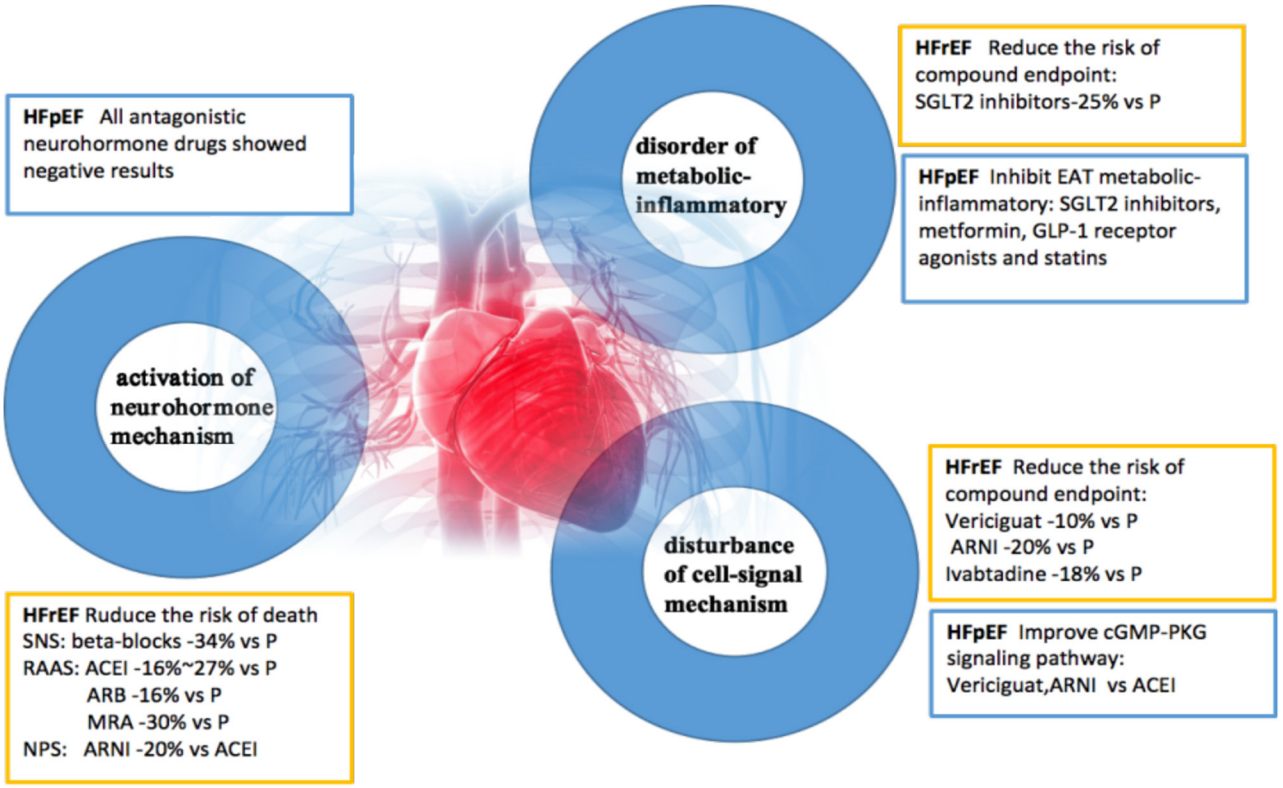

FIGURE 1 | A new understanding of pathogenesis and therapeutic benefits of heart failure. HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system; NPS, natriuretic peptide system; EAT, epicardial adipose tissue; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor antagonist; MRA, aldosterone receptor antagonist; P, placebo; ARNI, angiotensin receptor enkephalinase inhibitor; SGLT2, sodium-glucose cotransporter 2; GLP-1, glucagon-like peptide-1.

in terms of advanced age, female bias, presence of complications [hypertension, COPD, Diabetes Mellitus (DM)], abnormal laboratory indicators (creatinine, B-type natriuretic peptide, troponin) and drug use [beta-receptor blocker, angiotensin-converting enzyme inhibitor (ACEI), angiotensin II receptor antagonist (ARBs)]. However, coronary artery disease was more similar between the HFmrEF group and the HFrEF group. The ADHERE registry also reported different risk factors for these groups. Patients with LVEF>55% are less likely to develop hyperlipidemia, while U.S. patients with atrial fibrillation were more likely to have reduced LVEF. It was also reported that HF patients with LVEF of 40–55% had a higher incidence of myocardial infarction and DM than those with LVEF>55%, and cardiovascular health studies reported that HFmrEF patients had higher rates of diabetes (31).

Arrythmias

The VIP-HF was an investigator-initiated, prospective, multicentre, observational study of patients with HF and left ventricular ejection fraction (LVEF) >40%. Patients underwent extensive phenotyping, and an implantable loop recorder was implanted later. The primary aim of the VIP-HF study was to examine the incidence of sustained ventricular tachyarrhythmias (VTs) in HF with HFmrEF or HFpEF. Secondary aims were to examine the incidence of non-sustained VTs, bradyarrhythmias, HF hospitalizations, and mortality. It enrolled 113 of the planned 250 patients (mean age 73 yrs, 51% women, New York Heart Association class II/III 54/46%, median NT-proBNP 1,367 pg/ml and mean LVEF 54%; 75% had LVEF >50%). Eighteen percent had non-sustained VTs and 37% had atrial fibrillation on Holter monitoring. During a median follow-up of 657 days, the primary endpoint of sustained VT was observed in one patient. The incidence of the primary endpoint was 0.6 per 100 person-years. The incidence of the secondary endpoint of non-sustained VT was 11.5 per 100 person-years. Five patients developed bradyarrhythmias [3.2 per 100 person-years], three were implanted with a pacemaker. Despite the lower than expected number of included patients, the incidence of sustained VTs in HFmrEF/HFpEF was low. Clinically relevant bradyarrhythmias were more often observed than expected (32).

Although some post myocardial infarction (post-MI) and dilated cardiomyopathy (DCM) patients with HFmrEF face an increased risk for arrhythmic sudden cardiac death (SCD), current guidelines do not recommend an implantable cardiac

defibrillator (ICD). They stratified hospitalized HFmrEF patients for SCD with a combined non-invasive risk factors (NIRFs) guiding to programmed ventricular stimulation (PVS) two-step approach. Forty-eight patients underwent a NIRFs screening first-step with electrocardiogram (ECG), Echocardiography and 24-h ambulatory ECG (AECG). Patients were classified as either low risk, moderate risk, and high risk. All in Group 3 received an ICD. After 41 months, 9 of 48 patients, experienced the major arrhythmic event (MAE) endpoint (clinical VT/fibrillation, 3; appropriate ICD activation, 6). The endpoint occurred more frequently in Group 3 than in Group 1 and 2. In hospitalized HFmrEF post-MI and DCM patients, a NIRFs guiding to PVS two-step approach efficiently detected the subgroup at increased risk for MAE (33).

Echocardiography

All three HF subsets present a similar clinical picture, and the distinction between HFrEF, HFpEF, and HFmrEF ultimately requires an echocardiogram. LVEF is an important index to evaluate the cardiac function of patients with heart failure, and it is closely related to mortality and rehospitalization. However, LVEF is an unstable indicator that may change with treatment and over time. Therefore, LVEF can be regarded as a risk marker of heart failure, but it is by no means the cause of heart failure. The left ventricular cavity and left ventricular myocardial mass gradually increase from HFpEF to HFrEF. In previous studies, LVM was considered as an indicator of cardiovascular events and a prognostic risk factor in HF patients (34). Japanese studies have found that higher LVM may be associated with poor prognosis for patients with HFrEF. TIME-CHF studies showed that left ventricular hypertrophy in HFpEF can be characterized as centripetal hypertrophy, while HFmrEF features mild concentric hypertrophy, and HFrEF features eccentric hypertrophy (27).

In ambiguous cases, a stress test or invasively measured elevated LV filling pressure may be required to confirm the diagnosis. It has been clearer that LVEF may not be the most sensitive parameter to study cardiac function, but may be more accurate to measure myocardial deformation. Although echocardiography is convenient, the measurement of LVEF by echocardiography has inherent issues of variability. Cardiac magnetic resonance imaging is the gold standard for evaluating volume and function (35). Despite these problems, LVEF remains an effective method for HF classification, and previous clinical studies have shown that patients with HFrEF would benefit from the classical treatment of HF compared with the other two HF subgroups (21, 36).

TREATMENT AND PROGNOSIS

Drug Treatment

At present, one of the main treatments for patients with HF is a combination of the enkephalin inhibitor sacubitril and valsartan. The PARAGON-HF trial, a global, multicenter, randomized, double-blind, active-controlled trial, included 4,822≥50-year-old HFpEF patients from 43 countries with symptoms and signs of HF, LVEF≥45%, NYHA scores of II-IV in the past 6 months, evidence of structural heart disease, elevated NT-pro-BNP levels and current treatment with diuretics. The purpose of this trial was to investigate the efficacy and safety of ARNI in patients with chronic HFpEF (LVEF≥45%) compared with valsartan. The results showed that, compared with valsartan, ARNI reduced the risk of the primary endpoint by 13%, although it did not reach statistical significance ($P = 0.059$). This study confirmed for the first time clinical benefits existed for some specific subgroups of HFpEF patients, especially those with LVEF<57%. The curative effect also showed population heterogeneity, and the main compound endpoint events in the female subgroup decreased by 27%. In terms of safety, the PARAGON-HF study demonstrated that ARNI is safe and tolerable. The proportion of patients with elevated serum creatinine clearance and enhanced incidence of hyperkalemia were significantly lower than for the control group (37). PIONEER-HF compared ARNI treatment with enalapril treatment in patients with ADHF after hemodynamic stabilization. Compared with the control group, 8-week treatment in the ARNI group significantly reduced the compound risk of severe clinical end events by 46% (HR:0.54, $P = 0.001$), mainly reflected in decreased readmission rate, decreased mortality, and further reduced NT-pro-BNP (38). Therefore, the therapeutic effect of ARNI in HFmrEF patients is promising.

On February 16, 2021, based on data from Phase 3 clinical trials (PARAGON-HF) and Phase 2 clinical trials (PARAMOUNT), as well as on phase 3 HFrEF clinical trial data, FDA formally approved extended indications for chronic heart failure with Sacubitril Valsartan Sodium Tablets (Entresto) to reduce the risk of cardiovascular death and hospitalization in adult patients with chronic heart failure. This decision is a boon for patients with chronic heart failure, resolving the situation that there was no recommended treatment for HFpEF. Both HFrEF and HFpEF patients might benefit from ARNI treatment. The approval of extended indications for ARNI opens a new avenue for the treatment of HFpEF and diastolic heart failure and expands the options for the overall management of chronic heart failure. Why does ARNI expand the indications of chronic heart failure? This drug has two key characteristics. One is that it effectively antagonizes the neurohormone mechanism, the RAS system, and reduces the risk of cardiovascular death and heart failure hospitalization. The other is that it effectively regulates the cardiomyocyte cGMP-PKG signal pathway by protecting natriuretic peptides, improving ventricular diastolic function, and reducing the risk of hospitalization and cardiovascular death from heart failure.

The VICTORIA study explored the efficacy and safety of Vericiguat in high-risk HFrEF patients (24). The results showed that, in addition to the standard treatment of HFrEF, Vericiguat significantly reduces the risk of cardiovascular death or heart failure in high-risk HFrEF patients. Furthermore, Vericiguat is safe and well-tolerated. Administration does not require renal function monitoring or electrolytes and is taken once a day. It was easy to titrate and showed satisfactory drug compliance in the study. As the first sGC agonist, Vericiguat showed positive results in patients with worsening chronic heart failure with decreased ejection fraction, providing a new treatment for patients with

HFrEF, and having important theoretical significance and high clinical application value (24).

Complication Management

As previously mentioned, the clinical manifestations of complications in HFmrEF patients are more similar to HFpEF patients, and LVEF decrease is more similar to CAD and HFpEF patients (39). Non-cardiogenic comorbidities (COPD, CKD, DM, etc.) are common in HF patients and influence overall incidence. Compared with other groups, uncontrolled hypertension was the most potential cause of readmission in patients with HFmrEF. The usage of ARBs or aldosterone antagonists in patients with HFpEF reduced the readmission rate, possibly by controlling blood pressure and decreasing the risk of LVEF decline in the HFmrEF population (5). Additionally, the DAPA-HF trial confirmed that dapagliflozin, an SGLT-2 (sodium-glucose transporter 2) inhibitor for the treatment of patients with HFrEF, met the preset primary composite endpoint with statistical and clinical significance for a significant reduction in cardiovascular death or worsening of heart failure (40). These drugs provide options for HF patients with DM. From these findings, we boldly infer that SGLT-2 may also be of great significance in improving the symptoms of HFmrEF, especially in delaying the transition from HFmrEF to HFrEF. However, the specific effects require further study (**Figure 2**).

Prognosis

At present, research on the prognosis of HFmrEF remains controversial (39). HFmrEF patients may be actually classified as having HFrEF or HFpEF. These distinctions are based on the size and shape of the heart—that is the pathologically morphological characteristics of myocardial remodeling. The main features of HFrEF are cardiac enlargement (especially of the left ventricle), and centrifugal changes in the thinning of the left ventricular wall and interventricular septum. However, the changes of HFpEF usually appeared with normal heart size (or only left atrial enlargement), left ventricular wall, and interventricular septum thickening and concentric hypertrophy conversely. Therefore, if the heart of a patient with HFmrEF is significantly enlarged, this patient may represent an "improved HFrEF"; in other words, the value of LVEF may have increased from <40 to 40–49% after treatment. If the heart size (especially the left ventricle) is normal, this case should be judged as "progressive HFpEF"; that is, the LVEF of the disease has been reduced from ≥50 to 40–49%, indicating that the disease may continue to develop in the future, the heart will expand, and LVEF may be reduced to <40%, making the transition from HFpEF to HFrEF.

Studies of ESC-HF-LT heart failure have found no significant differences in all-cause mortality between HFmrEF, HFrEF and HFpEF (42). The CHART-2 study reported that, for HFmrEF patients, ischemic etiology is related to the decrease of LVEF in the first year, while LVEF is negatively related to death. Therefore, the treatment of CAD may be key to improve the prognosis of patients with HFmrEF (9). According to a Japanese multicenter study, about 1/6 of patients with acute heart failure have HFmrEF (including all-cause death and HF readmission). The combined endpoint and all lethal points were comparable during the 724-day interim follow-up in HFpEF, HFmrEF, and HFrEF patients. Many factors, such as increased age, anemia, hyponatremia, elevated blood urea nitrogen, chronic

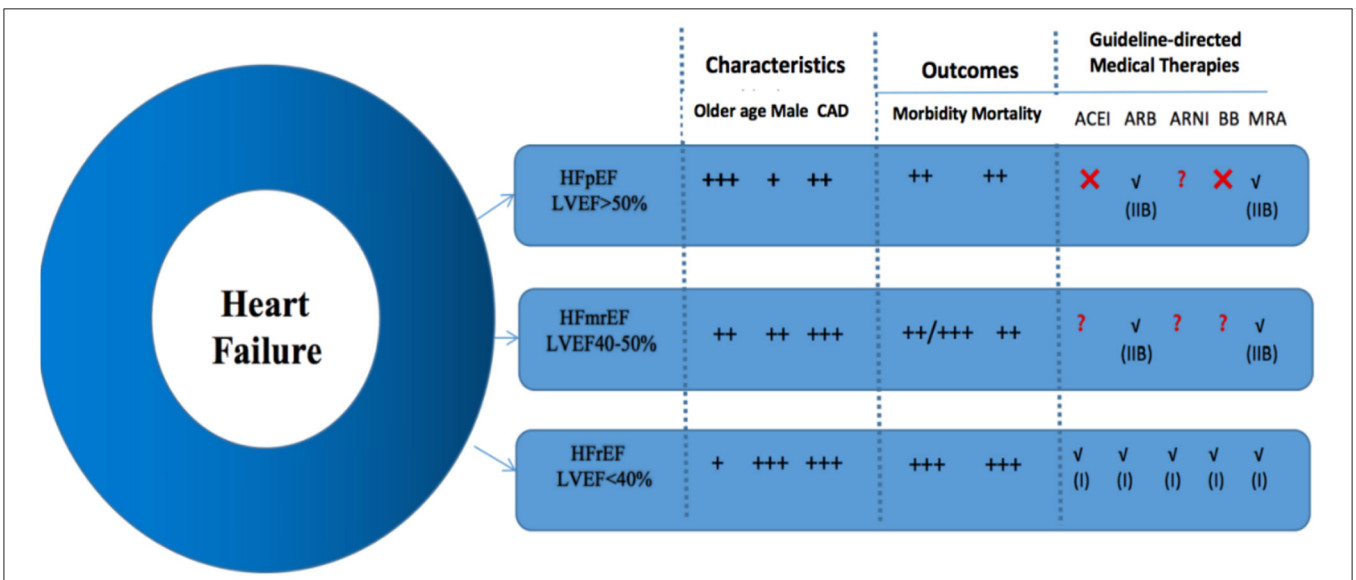

FIGURE 2 | General comparisons of the clinical characteristics, outcomes, and guideline-directed medical therapies for each heart failure group. Class of recommendation is denoted in parentheses, if applicable. ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor-neprilysin inhibitor; BB, beta-blockers; CAD, coronary artery disease; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; MRA, mineralocorticoid receptor antagonist.

TABLE 1 | Comparisons of clinical characteristics among the different phenotypes of HF.

	Characteristics*						Prognosis			
	Age	Sex	CAD	DM	HBP	AF	HOSP§	HOSP-HF	DEATH§	CV DEATH
HFpEF	+++	+	++	+++	+++	+++	?	+++	++	+++
HFmrEF	++	++	+++	+++	++	++	?	+++	++	++
HFrEF	+	+++	+++	++	+	+	?	+++	+++	++

*CAD, coronary artery disease; DM, diabetes mellitus; HBP, high blood pressure (hypertension); AF, atrial fibrillation; HOSP, hospitalization; HOSP-HF, hospitalization for HF; DEATH, death from all causes; CV-DEATH, cardiovascular death; *References; (10.21), §References (41).*

nephropathy, and increased plasma brain natriuretic peptide levels, have critical prognostic value for HFmrEF patients (43).

The PINNACLE Registry study, a descriptive analysis, found that patients with HFmrEF had more diseases, including coronary and peripheral artery diseases, myocardial infarction, percutaneous coronary intervention, and coronary artery bypass surgery, compared to patients with HFrEF or HFpEF (all $P < 0.001$). Patients with HFmrEF were also more likely to develop chronic kidney disease, diabetes, and atrial fibrillation/flutter. Additionally, these patients generally had a history of smoking (all $P < 0.001$). By LVEF assessment before the analysis, it showed that 4.8% of HFrEF patients converted to HFmrEF, and 32.9% of patients who previously had HFpEF later developed HFmrEF (11). Patients who transition from HFpEF to HFmrEF have a much more complex and less aggressive treatment than those with stable HFmrEF (**Table 1**).

FUTURE PROSPECTS

Further, LVEF may decline over time in patients with HFpEF due to myocardial infarction or inadequate treatment of concurrent cardiovascular disease, and LVEF may be reduced to a lower category. Therefore, HFmrEF is likely to be a heterogeneous category, including patients from different sources and with different clinical characteristics. HFmrEF represents a mixed subcategory that can be divided into HFiEF, HF with stable EF, and HF with deteriorating EF. However, more pathophysiological studies, prospective studies, and retrospective data analysis are needed to further refine these concepts. At present, circulating blood biomarkers and various advanced cardiac imaging models promise to advance research in this field and may guide future treatment options.

Furthermore, due to variability in LVEF measurements based on echocardiography, HF patients may be assigned to the incorrect heart failure groups, confounding the assessed efficacy of treatments in previous studies. Therefore, the LVEF-based classification system and further refinement of specific HF causes (such as ischemia, familial, hypertension) are limited, and detailed phenotypic analysis may help maximize the discovery of more effective treatment strategies. It remains unclear whether the EF classification adopted in the latest version of the guidelines has contributed to further understanding of HF development and improved therapeutic levels.

In the era of precision medicine, the treatment of HFmrEF may include identifying the characteristics of each HF patient, helping to further refine risk stratification beyond individual predictions of LVEF. Advanced imaging models can also identify high-risk patients in the HFmrEF group. Late gadolinium enhancement in CMR could predict death or appropriate (ICD) discharges of implantable cardioverter-defibrillators in patients with heart failure and LVEF>30% (44). A recent study showed that medium-term gadolinium-enhanced CMR is a strong predictor of sudden cardiac death and cardiac arrest (HR35.9) complex endpoints in 40% of patients with dilated cardiomyopathy compared to patients with LVEF of 40–50%, more predictive than LVEF itself (45). Therefore, while studies have demonstrated the potential value of cardiac magnetic resonance imaging evaluation in patients with HFmrEF and HFpEF, further studies are needed to determine whether these late gadolinium-enhanced subgroups can benefit from implanted defibrillators.

In addition, expanding the tools clinicians can use to evaluate hemodynamic variables may improve the prognosis of HFmrEF patients. The application of implantable MEMS pressure sensors in the pulmonary artery can guide the management of patients with heart failure and reduce the rate of hospitalization related to heart failure (46). Additionally, assessment of the biomarkers of patients with heart failure continues to be an active research area.

SUMMARY

Following the definition of HFmrEF by ACC/AHA and ESC, more studies are needed to explore the mysteries of the "gray area" in HF, including its prevalence, clinical features, and outcomes. Like HFpEF, there are no treatment guidelines for improving the condition of HFmrEF patients. The effective treatment of HFmrEF patients and the special attention to HFmrEF patients may lead to more promising results. The dynamic trend of ejection fraction and other new technology will be provided in the future, thereby confirming whether HFmrEF represents an independent type or a transitional stage between HFrEF and HFpEF.

AUTHOR CONTRIBUTIONS

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

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Cardiac Shock Wave Therapy Ameliorates Myocardial Ischemia in Patients With Chronic Refractory Angina Pectoris: A Randomized Trial

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Background: Cardiac shock wave therapy (CSWT) is a non-invasive new option for the treatment of chronic refractory angina pectoris (CRAP). This study aimed to evaluate the safety and efficiency of CSWT in the treatment of CRAP.

Methods: Eighty-seven patients with CRAP were randomly allocated into CWST group ($n = 46$) and Control group ($n = 41$). Canadian Cardiovascular Society (CCS) grade of angina pectoris, Seattle Angina Questionnaire (SAQ) score, 6-min walk test (6MWT), weekly dosage of nitroglycerin, and myocardial perfusion on D-SPECT were determined at baseline and during the follow-up period. Adverse events were also evaluated.

Results: CSWT was well-tolerated in the CSWT patients. CSWT significantly improved the CCS grade, SAQ score, and 6MWT ($p < 0.05$). Imaging examinations showed that the ischemic area was reduced after CSWT. However, no significant changes were observed in the Control group.

Conclusions: CSWT may improve the myocardial perfusion and reduce clinical symptoms without increasing adverse effects in CRAP patients. It provides a non-invasive and safe clinical therapy for CRAP patients.

Clinical Trial registration: www.ClinicalTrials.gov, identifier: NCT03398096.

Keywords: angiogenesis, randomized trial, angina, refractory angina pectoris, cardiac shock wave therapy

INTRODUCTION

Coronary artery disease (CAD) is one of the common and vital cardiovascular diseases. There are several options for the treatment of CAD, including pharmacotherapy (nitrates, beta-blockers, calcium antagonists, trimetazidine, and ivabradine), percutaneous coronary intervention (PCI), and coronary artery bypass surgery (CABG). Although interventional techniques have been widely used for the management of CAD, a few patients who are not suitable for interventional therapy suffer from chronic refractory angina pectoris (CRAP) (1, 2). It has been reported that the mortality rate of refractory angina is around 3–4% at 1 year (3, 4).

Several studies have investigated some new alternative therapeutic methods of refractory angina, including percutaneous myocardial laser revascularization, transmyocardial revascularization, and stem cell therapy. Nevertheless, these treatments are still underdeveloped, and most of them are invasive (5–7). There is evidence showing that microvascular dysfunction is one of the causes of refractory angina. This condition is likely much more common than previously reported, and many patients are experiencing microvascular angina due to infrequent assessment of microcirculatory physiology in clinical practice (8, 9).

Ultrasound-guided cardiac shock wave therapy (CSWT) is a new treatment of CAD and offers an alternative to revascularization by stimulating angiogenesis. Clinical trials have shown that CSWT can reduce the symptoms of myocardial ischemia and improve the cardiac function in patients with severe CAD (10–12). Therefore, CSWT seems to be a new non-invasive and effective therapy for chronic refractory angina.

In a variety of studies, nitroglycerin consumption, Canadian Cardiovascular Society (CCS) grade of angina pectoris, Seattle Angina Questionnaire (SAQ) scores, and New York Heart Association classification (NYHA class) are widely employed to evaluate the efficacy of CSWT (13). In only a few studies, the single photon emission computed tomography (SPECT) is used to assess the improvement of myocardial perfusion in cardiac ischemic patients. Myocardial perfusion imaging (MPI) has been well-established in the diagnosis of CAD and monitoring of therapeutic response and risk stratification in patients with known or suspected CAD. However, SPECT has limitations in the quantitative assessment. Dynamic single photon emission computed tomography (D-SPECT) imaging using multidetector SPECT systems and kinetic modeling of ^{99m}Tc-teboroxime has been shown to be much better to detect the microsphere-determined blood flow than traditional SPECT. In addition, it has superior sensitivity and specificity and allows a significant reduction in the administered dose of ^{99m}Tc-labeled tracers. This study was undertaken to investigate the safety and efficacy of CSWT in the treatment of CRAP.

PATIENTS AND METHODS

Study Design and Population

The present study was registered in the clinicaltrials.gov (NCT 03398096). This was a prospective, randomized, and controlled clinical trial. The study was undertaken according to the 1975 Declaration of Helsinki and approved by the ethics committee of our hospital.

Patients and Grouping

All patients were fully informed of the study protocol, and informed consent was obtained from each patient before the study. The inclusion criteria were as follows: (1) patients were 18–80 years old; (2) all patients were diagnosed with CAD as demonstrated by >50% stenosis on coronary angiography or multislice CT coronary angiography; (3) the patients were treated by revascularization with more than 70% of coronary artery stenosis; (4) patients had refractory angina (defined as CCS angina grading II–IV after pharmacotherapy with or without revascularization); (5) more than 1 month after acute myocardial infarction (AMI) and 1 month after PCI surgery.

The exclusion criteria were as follows: (1) patients had AMI, PCI, or CABG within 4 weeks prior to the study; (2) patients had a history of heart transplantation; (3) patients had a history of metal valve replacement; (4) patients had intracardiac thrombus; (5) patients had left ventricular ejection fraction (LVEF) <30% and unstable hemodynamics; (6) patients had arrhythmia with heart rate <40 bpm or >120 bpm; (7) patients had skin ulceration or infection at the treatment area; (8) patients had severe obstructive lung disease.

A total of 100 patients were recruited. According to the above criteria, 13 patients were excluded: four patients did not meet the inclusion criteria, eight patients declined to participate in this study, and one patient was excluded due to inconvenient transportation. Eighty-seven patients were randomly divided into CSWT group ($n = 46$) and Control group ($n = 41$). Patients in the CSWT group were treated with optimal drugs (including antiplatelet drug, statins, and antianginal drugs) + CSWT, and those in the Control group were treated with optimal drugs alone. Care providers and physicians who followed up the patients (parameters of this study) were blind to the grouping. In the CSWT group, CSWT was performed with an equipment (Modulith SLC; Storz Medical, Switzerland) according to the recommended protocol developed by the Tohoku University of Japan with respect to the shockwave output and the number of shots implemented to each spot and the protocol developed by the University of Essen, Germany (11, 14). CSWT was performed thrice weekly (first, third, and fifth days) in a course, and there was a 3-month interval between two courses. Patients received CSWT for 3 months, and a total of nine CSWTs were performed. Patients in the Control group did not receive CSWT.

Laboratory Examinations

Blood samples of peripheral venous were collected at baseline and follow-up. Myocardial marker [creatine kinase phosphate-isozyme (CK-MB)] and hepatorenal function indexes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (SCr)] were measured.

Imaging Examinations

Myocardial perfusion was evaluated using a ^{99}mTc-labeled tracer SPECT (D-SPECT, Spectrum Dynamics Company) at baseline and 6 months after the first treatment in the CSWT group. One-day rest–stress method was used for adenosine load protocol with tracer injection. Summed stress score (SSS) and summed rest score (SRS) were analyzed semiquantitatively in a blind manner: SSS/SRS <4, normal; 4–8, mild abnormality; 9–13, moderately abnormal; >13, severe abnormality.

Follow-Up

Patients were followed up at months after the first treatment by clinical examinations, quality of life assessment (Minnesota Living with Heart Failure), 6-min walk test (6MWT), echocardiography, and ^{99}mTc-MIBI-labeled tracer D-SPECT. Clinical examinations included the CCS grading of angina,

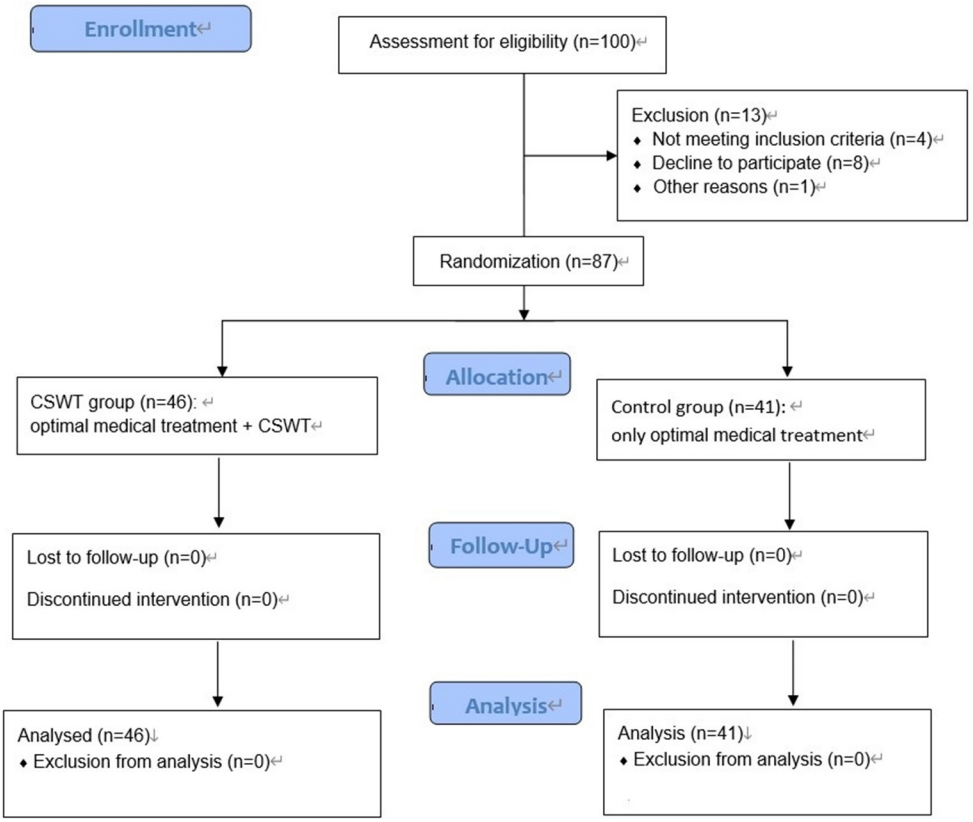

FIGURE 1 | Flowchart of patient recruitment. CSWT, cardiac shock wave therapy; D-SPECT, dynamic single photon emission computed tomography.

NYHA functional classification, SAQ scores, and nitroglycerin dose (times/week). Echocardiography was performed on a Vivid 9 (GE Vingmed, Horton, Norway). The images were stored digitally and analyzed offline by an experienced physician.

Statistical Analysis

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., USA). Continuous data with normal distribution are expressed as mean ± standard deviation and compared using paired *t*-test at baseline and follow-up. Categorical data are expressed as frequency (n) or ratio (n/N) and compared using chi-square test. Rank data were tested using non-parametric rank sum test. A value of two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics

A total of 87 patients were included in the final analysis, and the flowchart in the recruitment of these patients is shown in **Figure 1**. Patients' characteristics are shown in **Table 1**. There were 46 patients in the CSWT group and 41 patients in the Control group. The average age was 68.1 ± 6.7 years in the CSWT group and 68.9 ± 6.6 years in the control group, with no significant difference between the two groups. The average body mass index (BMI) in the CSWT group and control group was 24.7 ± 3.8 and 24.9 ± 3.7, respectively, with no significant difference. There were no significant differences in the history of hypertension, diabetes, or hypercholesterolemia between the CSWT group and Control group ($p > 0.05$). There were no significant differences in therapeutic drugs between the CSWT group and Control group ($p > 0.05$). No patients were lost in the follow-up.

Biochemical Parameters

The myocardial ischemia markers (CK-MB), hepatorenal function (AST and ALT), and renal function (SCr) were detected at baseline and 6 months after treatment. Results showed no significant differences in these parameters. It is indicated that the procedure of CSWT is safe and did not result in damage to the myocardium (**Table 2**).

Clinical Parameters

There were significant differences in the majority of clinical parameters between the two groups at 6 months. The clinical symptoms (chest tightness and chest pain) were all improved in the CSWT group. The symptoms were evaluated by CCS class scores, SAQ scores, and 6MWT. At baseline, there were no significant differences in the CCS score, SAQ score, and results from 6MWT between the two groups ($p > 0.05$). However,

6 months after treatment, these parameters were significantly improved in the CSWT group as compared to the Control group ($p < 0.05$). The average CCS class scores were 2.90 ± 0.57 at baseline and 2.10 ± 0.32 at 6 months in the CSWT group. The average SAQ scores were 63.3 ± 15.3 at baseline and 75.6 ± 10.5 at 6 months in the CSWT group. The result of 6MWT was 331.7 ± 62.3 and 403.1 ± 61.2 at baseline and 6 months, respectively, in the CSWT group. Furthermore, nitrate consumption in the CSWT group decreased as compared to the Control group (0.90 ± 0.68 vs. 1.60 ± 0.52, $p = 0.01$) (**Table 3**).

Imaging Examination

D-SPECT showed that the ischemic area was significantly reduced on the stress procedure at 6 months after CSWT in the CSWT group as compared with that at baseline. In addition, CSWT improved the myocardial perfusion in the treated area as evaluated by D-SPECT in the adenosine stress protocol. In the CSWT group and the Control group, the SSS was 16.27 ± 7.64 and 16.45 ± 5.05, respectively, and the SRS was 7.17 ± 2.62 and 7.06 ± 3.86, respectively, at baseline. However, both SSS and SRS were reduced in the CSWT group at 6 months. The SSS was 13.64 ± 6.69 and 16.82 ± 6.83 in the CSWT group and Control group, respectively ($p > 0.05$). The SRS was 6.73 ± 1.86 and 7.08 ± 2.64 in the CSWT group and control group, respectively, at 6 months ($p > 0.05$). The SSS remained unchanged after CSWT in the CSWT group ($p < 0.05$) (**Table 4**, **Figure 2**).

Echocardiography was performed on a Vivid 9 (GE Vingmed, Horton, Norway). Echocardiography was done to illustrate the left ventricular end-diastolic dimension (LVDd) and left ventricular ejection fraction (LVEF). As shown in **Table 4**, the LVEF was similar at baseline between the two groups, and it remained unchanged after treatment (6 months). The LVDd was also comparable between the two groups at baseline, and it remained unchanged after treatment (6 months). This reflected that LV function had no signs of deleterious LV remodeling.

Adverse Effects

CSWT was well-tolerated in all the subjects. No complications or adverse effects were noted in the patients of the two groups.

DISCUSSION

In the present study, results showed that the CCS grade of angina, SAQ score, 6MWT, and nitrate consumption were all improved

TABLE 1 | Characteristics of patients in two groups.

	CWST group (*n* = 46)	Control group (*n* = 41)	*P*
Age, years	68.1 ± 6.7	68.9 ± 6.6	0.507
Male, *n* (%)	32 (70)	29 (71)	0.547
BMI, kg/m^2	24.7 ± 3.8	24.9 ± 3.7	0.688
Smoking, *n* (%)	17 (37)	14 (34)	0.826
Hypertension, *n* (%)	27 (59)	23 (56)	0.831
Diabetes, *n* (%)	24 (52)	23 (56)	0.830
Hypercholesterolemia, *n* (%)	20 (43)	18 (44)	0.570
Aspirin, *n* (%)	41 (89)	36 (88)	0.554
Calcium channel blockers, *n* (%)	15 (33)	14 (34)	0.530
ß Blockers, *n* (%)	24 (52)	23 (56)	0.521
ACEI/ARB, *n* (%)	22 (48)	18 (44)	0.830

Data are shown as Mean ± standard deviation. CSWT, cardiac shock wave therapy; BMI, body mass index; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

TABLE 3 | CCS, SAQ, 6MWT and nitroglycerin consumption at baseline and follow-up.

	Parameters	CSWT (*n* = 46)	Control (*n* = 41)	*P*
Baseline	CCS (grade)	2.90 ± 0.57	2.80 ± 0.79	0.15
	SAQ (score)	63.3 ± 15.3	65.6 ± 14.6	0.53
	6MWT (m)	331.7 ± 62.3	319.3 ± 69.3	0.45
	Nitrate consumption (times/week)	2.40 ± 1.26	2.10 ± 1.02	0.45
Follow-up	CCS (grade)	2.10 ± 0.32	2.90 ± 0.57	0.002
	SAQ (score)	75.6 ± 10.5	67.3 ± 13.3	0.03
	6MWT (m)	403.1 ± 61.2	336.7 ± 71.1	0.0001
	Nitrate consumption (times/week)	0.90 ± 0.68	1.60 ± 0.52	0.01

Data are shown as mean ± standard deviation. CSWT, cardiac shock wave therapy; CCS, Canadian Cardiovascular Society grade of angina pectoris; SAQ, Seattle Angina Questionnaire score; 6MWT, 6-minute walking distance test.

TABLE 2 | Biochemical parameters at baseline and follow-up.

Group	*n*	Time	ALT (U/L)	AST (U/L)	CK-MB (ng/ml)	SCr (μmol/L)
CSWT	46	Baseline	28.9 ± 7.9	35.4 ± 7.3	27.8 ± 10.6	67.4 ± 28.4
		Follow-up	31.6 ± 8.5	28.6 ± 8.7	26.9 ± 12.5	78.4 ± 26.4
Control	41	Baseline	26.4 ± 8.2	27.9 ± 6.5	24.5 ± 8.9	59.2 ± 28.8
		Follow-up	30.8 ± 8.6	26.4 ± 8.3	26.7 ± 9.4	66.8 ± 24.3

Data are shown as mean ± standard deviation. There were not significant differences between CSWT group and Control group at baseline and follow-up in these parameters ($P > 0.05$). CSWT, cardiac shock wave therapy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK-MB, creatine kinase phosphate-isozyme; SCr, serum creatinine.

in the CSWT group. Furthermore, the ischemic area on D-SPECT was also reduced after CSWT. However, there were no significant changes in the outcomes in the Control group. These results suggest that CSWT can achieve a favorable clinical efficacy as well as a better quality of life for patients with CRAP.

Increasing clinical studies on CSWT have been published since 1999. In the majority of CSWT-related studies, results indicate that nitroglycerin consumption is reduced; angina frequency is decreased; CCS grade, SAQ score, and NYHA class score are improved; and exercise capacity is increased significantly after CSWT (13). Myocardial perfusion can be assessed by conventional SPECT, but the low sensitivity and low temporal resolution of conventional SPECT limit its wide use in clinical practice further assessment (15, 16). In the present study, D-SPECT was employed to evaluate myocardial perfusion. As a new technique, D-SPECT has better sensitivity and specificity and can provide more accurate professional information.

CSWT is suitable for patients with refractory CAD, CCS angina grade of III/IV, nonresponse to two or three anti-anginal drugs within at least 8 weeks, recurrent angina pectoris after PCI/CABG, or severe CAD unsuitable for interventional revascularization. In 2003, at the European Society of Cardiology, Nishida et al. (17) for the first time reported the therapeutic effect of CSWT in animal models of CAD and CAD patients. CSWT has been used in clinical trials and scientific studies in nine countries including Germany, Japan, Switzerland, Italy, and China and has achieved good clinical efficacy.

The mechanism underlying the therapeutic effects of CSWT is complex. Yip et al. (18) investigated the effect of shock wave on the femoral bone of adult male Sprague–Dawley rats and found that the shock wave could induce the formation of vascular endothelial growth factor (VEGF) and increase the CD31-positive cells (an endothelial phenotype), which accelerated the

TABLE 4 | Imagine Findings at baseline and follow up.

	Parameters	CSWT ($n = 46$)	Control ($n = 41$)	P
Baseline	SRS (score)	7.17 ± 2.62	7.06 ± 3.86	0.326
	SSS (score)	16.27 ± 7.64*	16.45 ± 5.05	0.781
	LVDd	45.17 ± 8.03	46.90 ± 8.47	0.628
	LVEF	48.80 ± 6.47	47.83 ± 7.78	0.758
Follow up	SRS (score)	6.73 ± 1.86	7.08 ± 2.64	0.069
	SSS (score)	13.64 ± 6.69*	16.82 ± 6.83	0.057
	LVDd	48.60 ± 5.62	48.82 ± 6.27	0.813
	LVEF	53.75 ± 5.85	48.50 ± 6.96	0.069

*Data are shown as mean ± standard deviation. CSWT, cardiac shock wave therapy; SSS, Summed stress score; SRS, summed rest score; LVEF, left ventricular ejection fraction. *P < 0.05, Baseline vs. Follow-up in the CSWT group.*

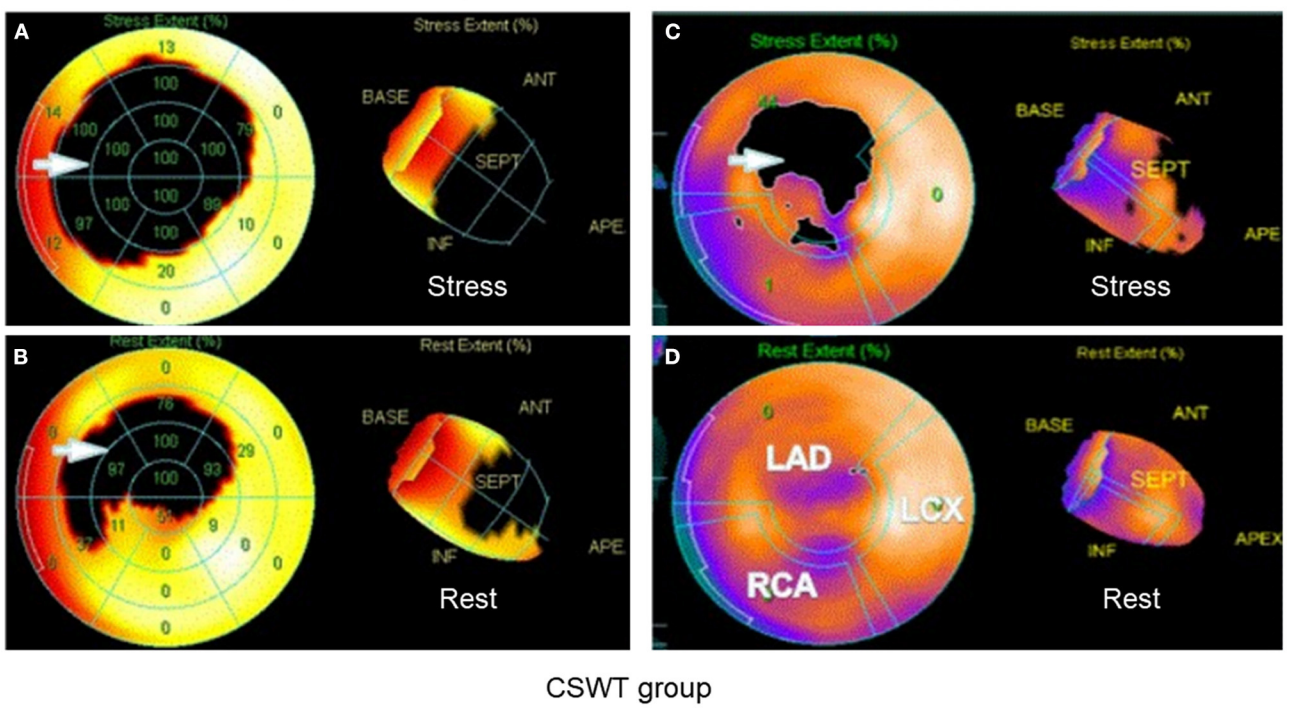

FIGURE 2 | D-SPECT of a patient. After CSWT, the ischemic myocardium at rest and stress procedure was significantly reduced at 6 months after treatment as compared to that before CSWT. **(A)** Before treatment at stress procedure. **(B)** Before treatment at rest procedure. **(C)** After treatment at stress procedure. **(D)** After treatment at rest procedure. Arrow: ischemic area. CSWT, cardiac shock wave therapy; D-SPECT, dynamic single photon emission computed tomography; LAD, left anterior descending artery; LCX, left circumflex coronary artery; RCA, right coronary artery.

differentiation of bone marrow cells into endothelial cells (16). In addition, CSWT may serve as an alternative to revascularization by stimulating angiogenesis in the ischemic myocardium, which ameliorates myocardial ischemia (17, 19, 20). Studies have shown that shock wave may also affect the expression of chemokines and matrix metalloproteinases to confer anti-inflammatory effects, activate Ras, stimulate nitric oxide (NO) synthesis, and upregulate VEGF and its receptor, *fms*-like tyrosine kinase (Flt)-1 (17, 19, 21–25). Whether all these effects contribute to the improvement of cardiac function is still unclear. However, it has been confirmed that CSWT can stimulate angiogenesis in the ischemic myocardium by upregulating VEGF expression. VEGF is an angiogenic factor. Nishida et al. (17) found that CSWT could upregulate mRNA expression of VEGF both *in vitro* and *in vivo*. In a porcine model of chronic myocardial ischemia, the LVEF, wall thickening fraction, and regional myocardial blood flow of the ischemic region were completely improved significantly in 4 weeks after shock wave treatment as compared to control animals.

In the CSWT, the patient is asked to lie in a supine position and relax, and the electrocardiogram, blood pressure, and blood oxygen saturation are monitored. Several studies have indicated that three CSWT sessions within 1 month may achieve the same efficacy as 3-month CSWT (10, 26, 27). This finding is encouraging, but whether a shorter term or less frequent CSWT can achieve a similar therapeutic effect is still unclear, and more clinical studies are needed to elucidate this issue.

One of the strengths in the present study is the use of D-SPECT in the evaluation of ischemic myocardium. Through D-SPECT, the cardiac perfusion and cardiac function can be objectively assessed by radionuclide myocardial perfusion imaging. Great progress has been achieved in the D-SPECT due to the development of imaging equipment. As compared to the traditional SPECT with sodium iodide (NaI) crystals, the latest D-SPECT uses the most advanced all-digital, cadmium zinc telluride (CZT) solid-state detector, which increases the sensitivity by 10 times, the resolution by two times, and the scanning speed by 10 times. In our study, D-SPECT was employed to evaluate the cardiac perfusion in the ischemic area, which may be helpful for the assessment of the therapeutic efficacy of CSWT. In previous studies, traditional SPECT was mainly used to determine the improvement of myocardial perfusion in myocardial ischemia patients. Hence, the use of D-SPECT in our study is a novelty.

The combination of CSWT and D-SPECT in clinical practice may benefit patients with CRAP because CSWT can improve ischemic symptoms and myocardial perfusion in patients non-responsive to interventional therapy, and D-SPECT is a safe and simple examination that can objectively and reliably assess the ischemic area after CSWT.

However, there were limitations in the present study. First, only short-term follow-up was administered in our study, and the long-term efficacy of CSWT should be further confirmed. Second, treadmill exercise test can be employed to evaluate the exercise tolerance of patients. It is a non-invasive examination and can also be used to assess the clinical improvement of patients after CSWT.

CONCLUSIONS

The present study indicates that CSWT can improve CCS grade of angina, SAQ score, 6MWT results, and nitrate consumption in patients with CRAP. Furthermore, D-SPECT shows that the myocardial ischemic area is reduced after CSWT. The significant improvement of angina symptoms may be associated with the reduction of ischemic myocardium. Therefore, CSWT is a non-invasive, safe, and easy-to-use treatment for patients with CRAP and may serve as a good alternative for the treatment of CRAP, achieving favorable clinical therapeutic efficacy and better quality of life.

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 Shanghai Shen Kang Hospital Development Center. 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

LW: design, definition of intellectual content, literature search, and statistical analysis. FXi: literature search, clinical studies, data acquisition, and manuscript preparation and editing. SJ: data acquisition and literature search. ZM and FXu: data acquisition. XY: concept, design, definition of intellectual content, manuscript review, and gain the grant. All authors contributed to the article and approved the submitted version.

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Association Between Arterial Stiffness and Heart Failure With Preserved Ejection Fraction

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Cardiovascular diseases are the leading cause of mortality in the world. Heart failure with preserved ejection fraction (HFpEF) accounts for about half of all heart failure. Unfortunately, the mechanisms of HFpEF are still unclear, leading to little progress of effective treatment of HFpEF. Arterial stiffness is the decrement of arterial compliance. The media of large arteries degenerate in both physiological and pathological conditions. Many studies have proven that arterial stiffness is an independent risk factor for cardiovascular disorders including diastolic dysfunction. In this perspective, we discussed if arterial stiffness is related to HFpEF, and how does arterial stiffness contribute to HFpEF. Finally, we briefly summarized current treatment strategies on arterial stiffness and HFpEF. Though some new drugs were developed, the safety and effectiveness were not adequately assessed. New pharmacologic treatment for arterial stiffness and HFpEF are urgently needed.

Keywords: arterial stiffening, heart failure, HFpEF, aging, HFmrEF—heart failure with mid-range ejection fraction

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INTRODUCTION

Cardiovascular diseases are the leading cause of mortality in the world. Heart failure (HF), as the consequence of so many factors that damage the heart, is a progressive and serious condition with high rate of mortality (1). Heart failure with preserved ejection fraction (HFpEF) accounts for about half of heart failure patients. The prevalence of HFpEF significantly associated with age, and age-related diseases like hypertension and coronary artery disease (2). In people over or equal to 60 years old, ∼5% of them are with HFpEF (3). Moreover, the estimated 5-year survival rate is only about 50% (4). HFpEF has been a global health problem, and there is an urgent need for physicians to understand the pathology and treatment of HFpEF.

Arterial stiffness is the decrement of arterial compliance. A lot of parameters were used in clinical practice to assess arterial stiffness (5), for example, pulse pressure (PP) and its amplification (PPA), pulse wave velocity (PWV), augmentation index (AIx), etc. For large arteries like aorta, the media of these arteries degenerate in both physiological conditions like aging and pathological conditions like chronic inflammation, oxidative stress, hypertension, diabetes, etc., resulting in the stiffness of these arteries (6). Numerous studies have proven that arterial stiffness is an independent risk factor for cardiovascular diseases, events, and mortality. Moreover, the relationship between arterial stiffness and left ventricular (LV) diastolic function is verified by dozens of observational studies (7).

Because arterial stiffness is closely related to LV diastolic function, physicians are paying great attention to the contribution of arterial stiffness to HFpEF. In this perspective, we summarized current understanding of the relationship between arterial stiffness and HFpEF, the mechanism of arterial stiffness contributing to HFpEF, and the treatment and potential future directions of HFpEF focusing on arterial stiffness.

DOES ARTERIAL STIFFNESS RELATE TO HFpEF?

Many conditions were reported to be associated with HFpEF, for example, aging, hypertension, obesity, diabetes. Diastolic dysfunction is one of the most important contributor to HFpEF (8), and arterial stiffness is a well-established factor that accelerates the development of diastolic dysfunction. A meta-analysis which included 27 studies showed that, parameters of arterial stiffness especially brachial-ankle PWV, were significantly associated with diastolic dysfunction indicators recorded by echocardiography (9). Pulse pressure is another indicator for arterial stiffness. Data from Mayo Clinic showed that, both central and brachial pulse pressure were significantly associated with diastolic dysfunction assessed by echocardiography (10). Apart from these indirect links, many studies also showed direct or independent associations between arterial stiffness and HFpEF. Compared with hypertensive controls, HFpEF patients were with reduced total arterial compliance (11). A study included 60 HFpEF patients and 51 non-HFpEF controls showed that, compared with patients without HFpEF, brachial-ankle PWV was higher in HFpEF patients. Besides, arterial stiffness was strongly associated with cardiovascular events during the median follow-up period of 54 months in this study (12). Apart from hard endpoints, a study conducted in Japan showed that cardio-ankle vascular index, another arterial stiffness parameter, was independently and significantly associated with hospitalization of HFpEF patients after adjustment for hypertension, diabetes, and renal function (13). It seemed that the difference of arterial stiffness in patients with/without HFpEF was more likely to be observed during exercise (14, 15).

Although many studies reported the associations among arterial stiffness, diastolic dysfunction, and HFpEF, till now there is very few evidence to prove that arterial stiffness is a key factor which drives the development of HFpEF. A study conducted by Wan et al. (16) showed that, in 488 hypertensive patients with HFpEF, two arterial stiffness parameters, arterial pressure volume index (API) and arterial velocity pulse index (AVI), were both significantly associated with the onset of HFpEF. Another case control study which included 77 matched pairs demonstrated that, participants with decreased aortic distensibility were more easily to develop HFpEF with asymptomatic diastolic dysfunction (17). However, negative association between arterial stiffness and HFpEF was also reported. In the Health ABC study, the authors divided 2,290 elderly participants into three groups based on the tertiles of PWV measured at baseline. This study demonstrated that, after adjustment for conventional cardiovascular risk factors, compared to participants with low PWV (tertile-1), participants with high PWV (tertile-3) were not significantly associated the high risk of HFpEF with the mean follow-up time of 11.2 years (18). More large prospective studies are warranted to further investigate the relationship between arterial stiffness and HFpEF.

In conclusion, though there is a little controversy, arterial stiffness is more likely to be regarded as a harmful factor for LV diastolic function and HFpEF. Especially for the arterial stiffness induced by metabolic disorders, it may play a major role in the development of HFpEF.

HOW DOES ARTERIAL STIFFNESS CONTRIBUTE TO HFpEF?

As we described previously, many studies have demonstrated that the magnitude of arterial stiffness in HFpEF is significantly increased compared to those without HFpEF. This accelerated arterial stiffness leads to the increment of arterial pulse pressure and LV afterload (19, 20). This increment was further amplified in specific conditions like hypertension, diabetes, and exercise (15). Several mechanisms were related to the contribution of arterial stiffness to HFpEF, including: (1) the vascular effect, (2) the ventricular–vascular interaction effect, (3) the effect on arterial hemodynamics, and (4) the linkage between renal function, arterial stiffness, and HFpEF. Cellular and molecular mechanisms are focusing on endothelial cells currently.

The vascular effect of arterial stiffness on HFpEF is similar to other organ damage induced by arterial stiffness. The former leader of the vascular group of Framingham study, Professor Mitchell, proposed a theory of "arteriosclerosis-related organ damage" (21). In this theory, pulse pressure becomes high owning to arterial stiffness. Since stiff arteries cannot adequately absorb the pulse energy from blood pressure, target organs suffered more redundant energy from pulse pressure, and subsequently got damaged from this energy. Small arteries in heart were also damaged in this way. Besides, the diastole is the most important time duration for coronary flow, and the leading force for coronary perfusion is diastolic pressure. When arterial stiffness occurs, diastolic pressure become lower than the normal (22). This low diastolic pressure is not enough for coronary perfusion. Thus, systolic pressure may become the major force for perfusion, which makes the heart more sensitive to systolic disorders (23).

The change of arterial hemodynamics plays a key role in HFpEF. Arterial stiffness is a determinant of pulsatile afterload, which is one of the two major components of arterial load. Arterial wave reflections increases with arterial stiffness, leading to the increment of mid-to-late systolic load, and subsequent left ventricular abnormalities including concentric remodeling, myocardial fibrosis, contractile dysfunction, and ejection duration reduction. These changes further contribute to the increment of mid-to-late systolic load, resulting in a vicious circle (24). There might be a critical linkage between renal dysfunction, arterial stiffness, and HFpEF. Renal dysfunction results in the calcification of arterial wall and the stiffness of arteries. Tremendous studies have proved that renal dysfunction are closely related to arterial stiffness, and it has good value in prediction of mortality (25). However, the direct evidence among HFpEF and renal dysfunction needs obtaining in the future.

The ventricular–vascular interaction effect is more complicated. In HFpEF, not only arteries but also left ventricle become stiff. The end-systolic elastance (Ees, defined as the slope and intercept of end-systolic pressure and the difference of end-systolic volume with initial volume) and arterial elastance (Ea, defined as the slope of end-systolic pressure and stroke

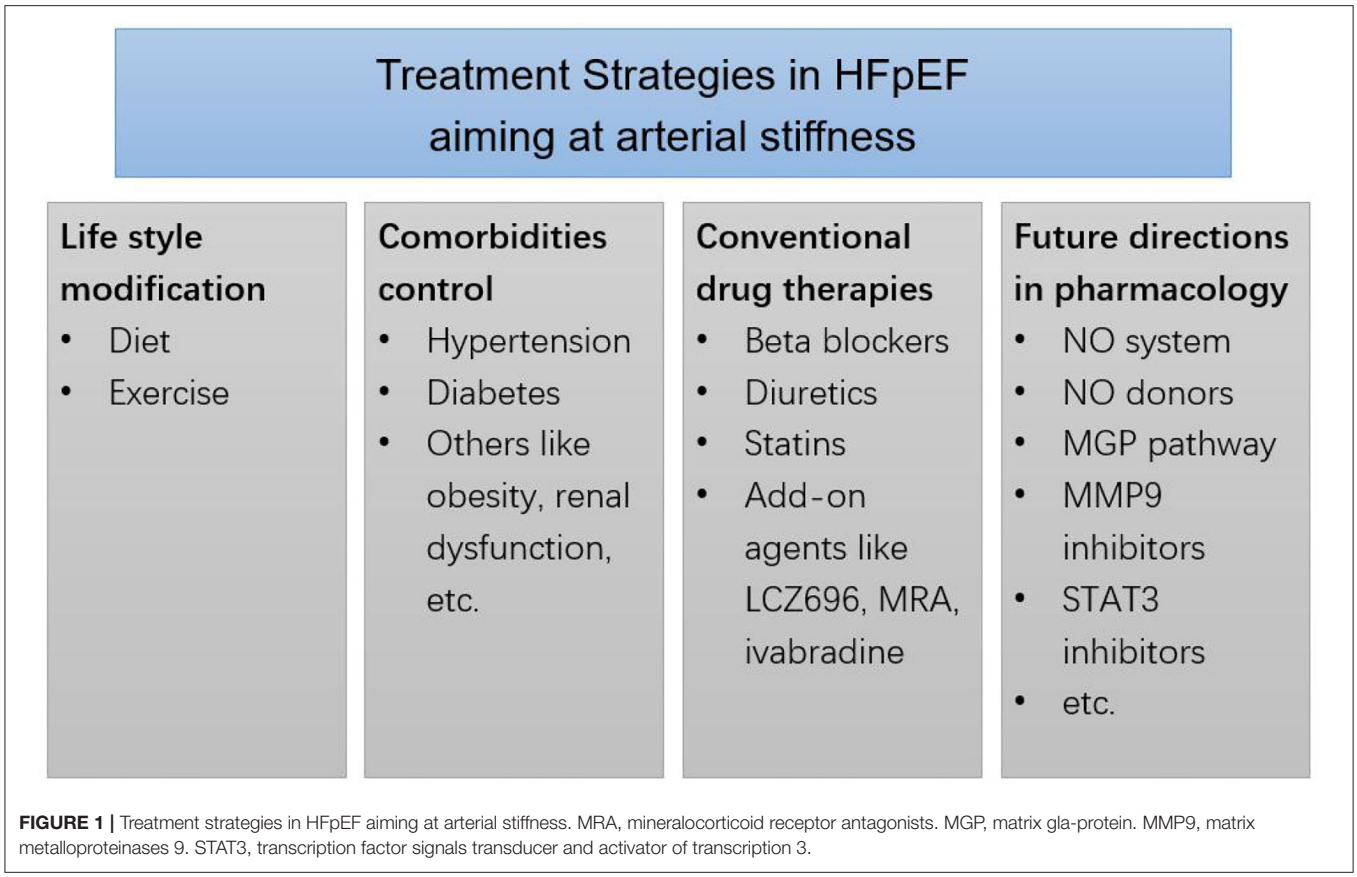

FIGURE 1 | Treatment strategies in HFpEF aiming at arterial stiffness. MRA, mineralocorticoid receptor antagonists. MGP, matrix gla-protein. MMP9, matrix metalloproteinases 9. STAT3, transcription factor signals transducer and activator of transcription 3.

volume) are the indicators for left ventricle systolic stiffness and arterial stiffness, respectively. In resting situations, though both Ees and Ea elevated in HFpEF, stroke volume and pulse energy are close to physiological conditions. However, since the slopes of Ees and Ea are sharp, a small change in blood pressure may lead to a dramatic change in Ees and Ea, leading to the mismatch between left ventricle and arteries (26). This ventricular-vascular interaction effect may explain why exercise amplified the clinical measurements of arterial stiffness in patients with/without HFpEF (27).

Endothelial function plays an important role in arterial stiffness and HFpEF. The relationship between endothelial dysfunction and HFpEF is well-established by a lot of studies (28). Endothelial function not only tightly associates with HFpEF, but also strongly predicts events in HFpEF patients (29). This is because endothelial cells: (1) participate in anti-oxidative and anti-inflammatory activities in arteries; (2) interact with extracellular matrix (Elastic and collagen fibers) to regulate vascular elasticity; (3) directly affect vascular tone by synthesizing and releasing nitric oxide (30). The Sirtuin family, especially sirt1 and sirt3, play key roles in the regulation of endothelial function in HFpEF (31). Functions of other cells like vascular smooth muscle cells and macrophages may be involved in arterial stiffness and HFpEF, but more evidences are needed (32).

DOES TREATMENT FOR ARTERIAL STIFFNESS BENEFIT IN HFpEF?

Unlikely to the treatment of heart failure with reduced EF, there is no dramatic progress in the treatment of HFpEF, and the survival rate of HFpEF patients is not significantly improved (33). Thus, looking for the new approach for the management of HFpEF is necessary. Because of the contribution of arterial stiffness to HFpEF, therapies aiming at arterial stiffness may be helpful to HFpEF. Here, we briefly discussed the treatment of arterial stiffness and HFpEF from five aspects, that is, lifestyle management, comorbidities control, conventional anti-hypertensive treatment, recent advances, and future directions (**Figure 1**).

Lifestyle management is fundamental to HFpEF patients. Proper diet and exercise are of critical importance. The continuities between good diet and cardiovascular health are obvious. For example, the sodium-restricted diet was able to improve ventricular-arterial coupling together with the arterial elastance of hypertensive HFpEF patients (34). Exercise is helpful to treat HFpEF and arterial stiffness. The beneficial effects of exercise may be owing to the improvement of oxygen utilization and exercise capacity (35, 36). Diet and exercise can also modulate risk factors like obesity, hypertension, diabetes apart from the direct beneficial effects to the heart and arteries.

The control of comorbidities is also important in arterial stiffness and HFpEF. We take hypertension and diabetes as examples. Hypertension is a major risk factor of HFpEF. According to the recommendations of guidelines, blood pressure control of HFpEF hypertensive patients should be strict and even aggressive (33). Long-term blood pressure lowing therapy is able to reduce arterial stiffness and cardiovascular events (37). Given the fact that diabetes is related to both arterial stiffness and HFpEF, the effect of diabetic control should be considered. Currently, several kinds of anti-diabetic drugs can affect arterial stiffness, for example, glucagon-like peptide-1 receptor agonist (GLP-1 RA), and sodium-glucose cotransporter-2 inhibitors (SGLT-2i). GLP-1 RA and SGLT-2i have been proven to protect against cardiovascular events (38).

Conventional anti-hypertensive agents include renin-angiotensin-aldosterone system (RAAS) blockades, beta-blockers, calcium channel blockers, and diuretics. No matter which mechanism is, arterial stiffness parameters like PWV could be reduced by most kinds of anti-hypertensive drugs. However, these drugs have their own features. RAS blockades are reported to protect from the change of vascular structure and subsequent arterial stiffness, other drugs may reduce PWV because of their influence on hemodynamics (39). But effects of RAS blockades on HFpEF is controversial. Neither angiotensin converting enzyme inhibitor (ACEI) perindopril (40) nor angiotensin receptor blocker (ARB) irbesartan (41) improved mortality in HFpEF patients. Thus, the use of ACEI or ARB for direct treatment of HFpEF is not supported by evidence. A recent meta-analysis which included 10 trials investigating beta-blockers showed that, beta-blockers might reduce cardiovascular mortality in HFpEF patients, but the evidence certainty was low (42). Till now, there is no large prospective trials focusing on calcium channel blockers in HFpEF. One ongoing clinical trial that tests the effect of nifedipine on HFpEF is found in clinicaltrials.gov (NCT01157481). Diuretics might be necessary in HFpEF patients. Despite their significant effects on symptoms in HFpEF patients, results from the ALLHAT trial suggested that, compared to amlodipine, lisinopril, or doxazosin, chlorthalidone significantly reduced the occurrence of new-onset HFpEF (43). Even these drugs did not show great superiority in HFpEF treatment, current studies did not find any disadvantages of anti-hypertensive agents for hypertensive HFpEF patients at least. And as we mentioned before, blood pressure lowing therapy has a lot of beneficial effects. The optimal and individualized anti-hypertensive strategy should be applied to accomplish effective blood pressure control. Besides, it should be pointed out that, though some drugs have effects on both arterial stiffness and HFpEF, it is unclear whether these drugs affect HFpEF through arterial stiffness.

Some advances were made in recent years. Though ACEI/ARB did not show much protective effect on HFpEF, the inhibition of RAAS is still an important approach to the management of HFpEF patients. Sacubitril/Valsartan (LCZ696) is a new superstar in the management of heart failure and hypertension. Two recently-released independent studies showed that sacubitril/valsartan inhibits the progress of diastolic dysfunction and arterial stiffness to HFpEF in rat models (44, 45). However, clinical trials are needed to verify the effects of sacubitril/valsartan on HFpEF patients. Mineralocorticoid receptor antagonists (MRA) are important in RAAS inhibition. Current evidence from the TOPCAT trial suggested that, spironolactone could be used as an add-on therapy rather than initial therapy for HFpEF patients, especially for those with resistant hypertension (46, 47). Heart rate control is another important issue for heart failure patients, and heart rate is closely associated with PWV. Apart from beta blockers, If-channel inhibitor ivabradine can reduce heart rate and improve diastolic function. However, Komajda et al. (48) found that ivabradine had no effect on E/e′ and NT-proBNP level in HFpEF patients compared to placebo. Statins are fundamental for patients with atherosclerosis, a meta-analysis showed that statin therapy may improve mortality rate of HFpEF patients (49).

As for the new treatment strategy for arterial stiffness, Tsai et al. (50) summarized nine directions for future research. A few of them are with limited data about the effect on heart failure currently, these are: (1) NO system (51), (2) NO donors (52), (3) the Matrix Gla-Protein (MGP) pathway (53), (4) Matrix Metalloproteinases (MMP) 9 Inhibitors (54), and (5) transcription factor signals transducer and activator of transcription (Stat) 3 inhibitors (55). However, these strategies are lack of clinical data so that cannot be used extensively [please refer the review by Athyros et al. (56) for the detail].

CONCLUSION

HFpEF accounts for about half of the total heart failure. However, the understand of HFpEF is poor. Arterial stiffness is a well-established cardiovascular risk factor, and it is able to accelerate the pathogenesis and development of diastolic dysfunction. Mechanisms of the contribution of arterial stiffness to HFpEF are owing to the vascular effect, the ventricular-vascular interaction effect, and the linkage of renal function. Endothelial cells play key roles in this process. Unfortunately, current therapy on HFpEF did not significantly improve the mortality in HFpEF patients. Therapies aiming at arterial stiffness may become a new strategy for the improvement of HFpEF treatment in the future.

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

DX: conception and design. CC and YL: manuscript writing. DX, CC, YL, and YX: final approval of manuscript. All authors contributed to the article and approved the submitted version.

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TANK Promotes Pressure Overload Induced Cardiac Hypertrophy *via* Activating AKT Signaling Pathway

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Background: TANK (TRAF family member associated NF-κB activator) acts as a member of scaffold proteins participated in the development of multiple diseases. However, its function in process of cardiac hypertrophy is still unknown.

Methods and Results: In this study, we observed an increased expression of TANK in murine hypertrophic hearts after aortic banding, suggesting that TANK may be involved in the pathogenesis of cardiac hypertrophy. We generated cardiac-specific TANK knockout mice, and subsequently subjected to aortic banding for 4–8 weeks. TANK knockout mice showed attenuated cardiac hypertrophy and dysfunction compared to the control group. In contrast, cardiac-specific TANK transgenic mice showed opposite signs. Consistently, *in vitro* experiments revealed that TANK knockdown decreased the cell size and expression of hypertrophic markers. Mechanistically, AKT signaling was inhibited in TANK knockout mice, but activated in TANK transgenic mice after aortic banding. Blocking AKT signaling with a pharmacological AKT inhibitor alleviated the cardiac hypertrophy and dysfunction in TANK transgenic mice.

Conclusions: Collectively, we identified TANK accelerates the progression of pathological cardiac hypertrophy and is a potential therapeutic target.

Keywords: TRAF family member associated NF-κB activator, AKT signal pathway, scaffold protein, pathological cardiac hypertrophy, tansgenic mice

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INTRODUCTION

With the aging of population, heart failure, as the end stage of various overload cardiomyopathies has become a worldwide public health problem (1). Hypertensive cardiomyopathies cause elevated blood pressure in the left ventricular wall, which triggers cardiac hypertrophy as an adaptive response (2). However, prolonged hypertrophy progresses to multifaceted pathological changes: cardiomyocyte enlargement, myofibrillar assembly, fibrosis accumulation, and expression of a set of genes that discriminate hypertrophic growth from normal growth (3, 4). In recent decades, numerous parallel effectors in signaling transduction have been reported to be involved in the development of pathological cardiac hypertrophy (5).

The TNF receptor associated factor (TRAF) family member associated NF-κB activator (TANK) was first identified in 1996 (6) and is also known as TRAF-interacting protein (I-TRAF). It binds to all reported TRAF members except TRAF4 (7, 8). TANK exhibited both stimulatory and inhibitory properties at different expression levels during TRAF2-mediated NF-κB activation (9). The binding

of TANK to TRAF3 promotes the phosphorylation of IRF-3 and IRF7, which is critical for the production of type 1 IFN in response to the recognition of viruses via TOLL-like receptors (TLRs) and acid-inducible gene-1 (RIG-1) (10, 11). Furthermore, TANK takes part in ubiquitination via regulating TRAF6, which acts as a ubiquitin ligase. Upon stimulation of the receptor activator of NF-κB (RANK) ligand (RANKL), markedly increased osteoclastogenesis in TANK-null cells was observed, with elevated ubiquitination of TRAF6 and activation of NF-κB (12). Apart from the interaction of TRAFs, TANK associates with the IKK-related kinases TANK binding-kinase 1 (TBK1) and IKKε, which functions as a scaffold protein (13). Several members of the TRAF family have been implicated in the development of cardiac hypertrophy, including TRAF3, TRAF5, and TRAF6 (14–16). Previous studies have also revealed that the knockout of IKKε in mice accelerates cardiac hypertrophy via activating the AKT and NF-κB signaling pathway (17). The overexpression of SIKE (suppressor of IKKε) attenuated cardiac hypertrophy by regulating the TBK1-AKT signaling pathway (18). However, the role of TANK in pathological cardiac hypertrophy has not yet been clarified.

In this study, we determined the expression of TANK in hypertrophic hearts and elucidated the potential signaling transduction pathway regulated by TANK. TANK was significantly upregulated in murine hearts subjected to aortic banding (AB). A cardiac-specific TANK transgenic (TANK-TG) mouse model showed accelerated pressure overload-induced cardiac remolding while the deletion of TANK exhibited a protective effect on cardiac hypertrophy and fibrosis. Mechanistically, TANK was involved in the activation of AKT, a central hypertrophic signaling effector. These data suggest TANK is a candidate for regulating pathological cardiac hypertrophy in response to sustained hemodynamic overload.

TABLE 1 | Information of reagents used in experiment.

Antibody	Manufacturer	Catalog number	Source of species	Dilution
TANK	CST	2141	Rabbit	1:1,000
ANP	Abclonal	A1609	Rabbit	1:1,000
β-MHC	Proteintech	22280-1-AP	Rabbit	1:1,000
p-MEK	CST	9154	rabbit	1:1,000
MEK	CST	9122	rabbit	1:1,000
p-ERK	CST	4370	rabbit	1:1,000
ERK	CST	4695	rabbit	1:1,000
p-JNK	CST	4668	rabbit	1:1,000
JNK	CST	9252	rabbit	1:1,000
p-p38	CST	4511	rabbit	1:1,000
p38	CST	9212	rabbit	1:1,000
p-AKT	CST	4060	rabbit	1:1,000
AKT	CST	4691	rabbit	1:1,000
p-mTOR	CST	2971	rabbit	1:1,000
mTOR	CST	2983	rabbit	1:1,000
p-GSK3β	CST	9322	rabbit	1:1,000
GSK3β	CST	9315	rabbit	1:1,000
p-p70S6K	CST	9208	rabbit	1:1,000
p70S6K	CST	2708	rabbit	1:1,000
GAPDH	CST	2118	rabbit	1:1,000
TGFβ1	CST	3709	rabbit	1:1,000
p-Smad2	CST	3108	rabbit	1:1,000
Smad2	CST	3103	rabbit	1:1,000
p-Smad3	CST	9520	rabbit	1:1,000
Smad3	CST	9513	rabbit	1:1,000
Flag	MB	M185	mouse	1:2,000
HA	MBL	M180-3	mouse	1:2,000

MATERIALS AND METHODS

All animal protocols were approved by the Animal Care and Use Committee of TongRen Hospital, Shanghai Jiao Tong University School of Medicine (No.2018-015).

Reagents

Detailed information regarding the reagents used can be found in **Table 1**.

Cardiac-Specific TANK Knockout Mice

Cardiac-specific TANK mice (TANK-CKO) were generated by utilizing a Cre-loxP system. First, the locations of two single-guide RNAs (sgRNA) that flanked exon 3 of the TANK gene were designed using an online CRISPR Design Tool. The target sequence of each sgRNA was sgRNA1 (AAAAATAG TGTCAAACTGTTGAC-TGG) and sgRNA2 (GCAGGGTTTC TCTGTTATAGCCC-TGG), respectively, and was transcribed using a MEGAshortscript™ Kit (AM1354, Ambion). A T7 mMESSAGE mMACHINE Kit (AM1345, Ambion) was used to transcribe the Cas9 plasmid (pST1374-NLS-flag-linker-Cas9, Addgene, 44758). Then, both Cas9 mRNA and sgRNAs were purified using an miRNeasy Micro Kit (Qiagen, 217084). Exon3 was inserted into a backbone vector pBluescript SK(+)-2loxP flanked by two mloxP sites and two homology arms as a donor vector. The donor vector was purified using the QIAquick Gel Extraction Kit (Qiagen, 28704), then the mixture which contains the Cas9 mRNA and sgRNAs (10 ng/ul) along with donor vector (2.0 ng/ul) were injected into zygotes by utilizing a microinjection system (FemtoJet 5247). The genomic DNA of mice was extracted and detected to identify founder mice that contained floxed exon3 on the same allele. The following primers were used to confirm that the two loxPs were on the same allele: TANK-loxp-NF1: GGTTTCTTCACGGAAGT TGG; TANK-loxp-NR2: GCAAGTTGCCTACTTATTGAGTTC T. After F1 offspring were obtained, heterozygotes were screened by PCR using the following primers: TANK-loxP-NF3: TT GTAGGAAATGAGGAAGTGGA, TANK-loxP-NR2: GCAAGT TGCCTACTTATTGAGTTCT. Homozygous TANK-flox mice were generated from mating between heterozygotes using the same screening technique. Flox mice are born according to the genetic laws of Mendel. Then, the $TANK^{Flox/Flox}$-*α-MHC*-MerCreMer mice were obtained by mating of TANK-Flox mice with α-MHC-MerCreMer (α-MHC-MCM) transgenic mice (The Jackson Laboratory, stock No. 005650). After 6 weeks, cardiac-specific TANK conditional knockout mice were established by

an intraperitoneal injection of tamoxifen (25 mg/kg/day, Sigma, T-5648) for five consecutive days. The control groups (α-MHC-MCM mice and TANK-Flox mice) were treated with equal doses of tamoxifen injection.

Cardiac-Specific TANK Transgenic Mice

First, we got the full-length cDNA of TANK gene from total RNA of mice by PCR. Then, the cDNA gene was cloned into the Bgl II and Hind III sites of pCAG-loxP-CAT-loxP-lacZ for expression. The vector was linearized by Sal I and purified like the donor vector described above. Subsequently microinjected into embryos (2.0 ng/ul) to generate the conditional transgenic mice. After collecting tail tissue of the 10-day offspring, founder mice were identified using DNA amplification by PCR: pcag-seq-F: CATGTCTGGATCGATCCCCG; Tank-seq-R: TCCAGAAGAA ACTTCTTGTCG. CAG-loxP-CAT-loxP-TANK/α-MHC-MCM mice were generated by crossing with α-MHC-MCM transgenic mice. Finally, conditional TANK transgenic (TANK-TG) mice were obtained after injecting with tamoxifen intraperitoneally for five consecutive days. Next, a western blot (WB) was used to evaluate the expression of TANK. α-MHC-MCM mice were used as non-transgenic (NTG) groups with the same drug regimen.

Animal Surgery

To induce cardiac hypertrophy, the mice underwent thoracic aortic banding (AB) surgery, as mentioned below (14). After being anesthetized using sodium pentobarbital via an intraperitoneal injection (80 mg/kg), the left chest of the male mice was opened to expose the thoracic aorta. Subsequently, ∼70% aortic constriction was made with a specific needle tied around the thoracic aorta using a 7-0 silk suture. Sham-operated animals underwent every step without aorta ligation.

AngII Induced Cardiac Hypertrophy

We conducted the mouse model of cardiac hypertrophy induced by Ang II infusion as previously described (16). Ang II (1.4 $mgkg^{-1}$ per day and dissolved in 0.9% NaCl) was subcutaneously infused for 4 weeks using an osmotic minipump (Alzet model 2004, Alza Corp) implanted into each mouse. The control mice group were received the same procedures as the experimental animals, with the same dose of saline infusion.

Echocardiography Assessment

A MyLab 30CV ultrasound system (Biosound Esaote Inc.) was used to perform echocardiography. The indicators were acquired from at least three consecutive cardiac cycles to evaluate cardiac function, including LV end-diastolic diameter (LVEDd), LV posterior wall thicknesses in diastole (LVPWd), LV end-systolic dimension (LVESd), end-diastolic interventricular septum diameter (IVSd), and fractional shortening (FS%). Calculation formula is FS(%) = (LVEDd-LVESd)/LVEDd×100%.

Histological Analysis

Hearts from the experimental animals of each group 4 weeks after operation were harvested and fixed in 10% formalin and embedded in paraffin. The samples were cut into sections of about 5 μm transversely. Hematoxylin and eosin (H&E) staining was used to calculate the myocyte cross-sectional area, and the collagen volume was assessed through picrosirius red (PSR) staining.

Cardiomyocyte Culture and Recombinant Vectors

Isolating neonatal rat cardiomyocytes (NRCMs) from the hearts of 1–2-day-old SD rats was performed as described previously (19). Hearts excised from newborn SD rats were cut into pieces and digested using 0.03% trypsin 0.04% collagenase type II. NRCMs were harvested and grown in DMEM/F12 medium (C11330, Gibco) with 5-bromodeoxyuridine (0.1 mM) which inhibited fibroblast proliferation, penicillin/streptomycin, and 10% fetal calf serum (FCS) for 48 h. Subsequently, the NRCMs were maintained under serum-free conditions for another 12 h. To generate TANK-overexpressing stable clones, the TANK gene was transfected into a replication-defective adenoviral vector. Cardiomyocytes infected with vectors expressing GFP were used as controls. Consistently, a replication-defective adenoviral vector with Short hairpin RNA against TANK was used to knockdown TANK. Meanwhile, AdshRNA served as a control. Finally, cells infected with adenoviruses were grown in the aforementioned medium for 24 h, and were then incubated with PBS or angiotensin II (Ang II, 1 μmol/L) for an additional 24–48 h. Adenoviruses for infection were used at a multiplicity of infection (MOI) of 100 particles/cell for 24 h.

In vivo and *in vitro* Inhibition Experiment

After AB, the solution containing LY294002 (L9908, Sigma), which is an PI3K inhibitor, was administered through an intraperitoneal injection at a dose of 50 mg/kg for 4 weeks. Meanwhile, the control groups were treated with a DMSO vehicle injection at the same volume. Transfected NRCMs treated with AKT inhibitor MK-2206 (MCE, 1 μmol/L) *in vitro* along with Ang II stimulation.

Quantitative Real-Time RT-PCR and Western Blot

In brief, mRNA was extracted using TRIzol reagent, and cDNA was synthesized by reverse transcription from RNA. Quantitative real-time PCR was performed to detect the expression of selected genes with SYBR Green (Roche). GAPDH was used as the reference gene. Protein was extracted from ventricular tissues and cardiomyocytes using RIPA lysis buffer. A BCA Protein Assay Kit (Pierce) was used to determine protein concentration. After being separated through SDS-PAGE, proteins were transferred onto PVDF membranes and incubated with primary antibodies overnight at 4°C. After the secondary antibodies were added the next day, bands were visualized using an Odyssey Imaging System (LI-COR Biosciences). The levels of specific protein were determined by standardizing with the level of GAPDH on the same PVDF membrane. Primer information could be found in **Supplementary Table 1**.

Immunoprecipitation

HEK293T cells were harvested after transfection for 24–48 h and lysed with IP buffer which consisted of 20 mM Tris-HCl (pH 7.4),

150 mM NaCl, 1 mM EDTA and 0.5% NP-40 and supplemented with a protease inhibitor cocktail (#04693132001, Roche). After incubated on ice for 20 min and centrifuged 13,000 g for 15 min, the cell lysate was obtained as the supernatant. Then, rabbit immunoglobulin G protein and A/G-agarose beads were added to the lysate and incubated at 4°C for 3 h. 500 μl cell lysate was incubated with 1 micro gram of antibody and 10 ml of protein A/G-agarose beads with gentle rocking at 4°C overnight. The precipitates were washed and acquired then subjected to WB with appropriate antibodies. Endogenous immunoprecipitation of TANK and AKT in TANK overexpressed NRCMs was performed similarly using indicated antibodies.

Construction and Transfection of Plasmids

Human TANK and AKT overexpressed plasmids were constructed first. The primers were designed and full-length CDS sequences of TANK and AKT were amplified from homo cDNA. The full-length CDS sequences of TANK and AKT were inserted into pcDNA5-Fag-vector and pcDNA5-HA using In-fusion method, respectively. Primer sequences are as follows: AKT-S:TCGGGTTTAAACGGATCCATGAGCGACGTGGCTATTGTG; AKT-AS:GGGCCCTCTAGACTCGAGTCAGGCCGTGCCGCTG; TANK-S:TCGGGTTTAAACGGATCCATGGATAAAAACATTGGCGAGC; TANK-AS:GGGCCCTCTAGACTCGAGTTAAGTCTCTCCATTGAAGTGTGAATTAAG. The constructed recombinant plasmid was transfected into 293T cells with the assistance of transfection reagent PEI. After 24 h, the cells were collected and lysed on ice with IP buffer. The supernatant proteins were removed after centrifugation, 1 μg antibody and beads were added were incubated at 4° for 3 h. Wash the beads using 150-mm and 300 mM NaCl Buffers. Finally, 20–30 μl 2× Loading Buffer was added to beads and boiled at 95°C for 10 min.

Immunofluorescence Analysis

Immunofluorescence staining was performed to determine the surface area of the cell. After being infected with the indicated adenovirus for 24 h, PBS or Ang II (1 μmol/L) were used to stimulate NRCMs for 48 h and were finally fixed with 3.7% formaldehyde. NRCMs were immunostained with an α-actinin antibody (1:100 dilution) first, then were stained with a fluorescent secondary antibody (1:200). Image-Pro Plus 6.0 software was used to measure the surface area of the cell.

Statistical Analysis

The values are represented as the mean ± SD. Comparisons between groups were performed using a two-tailed Student's *t*-test (two groups) or one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test (more than two groups). A value of $P < 0.05$ was considered to suggest a statistically significant difference.

RESULTS

TANK Expression Is Increased in Failing Murine Hypertrophic Hearts and Cardiomyocytes

First, cardiac hypertrophy mouse models were established after aortic banding for 4 and 8 weeks to determine whether TANK is involved in pathological cardiac hypertrophy. WB was used

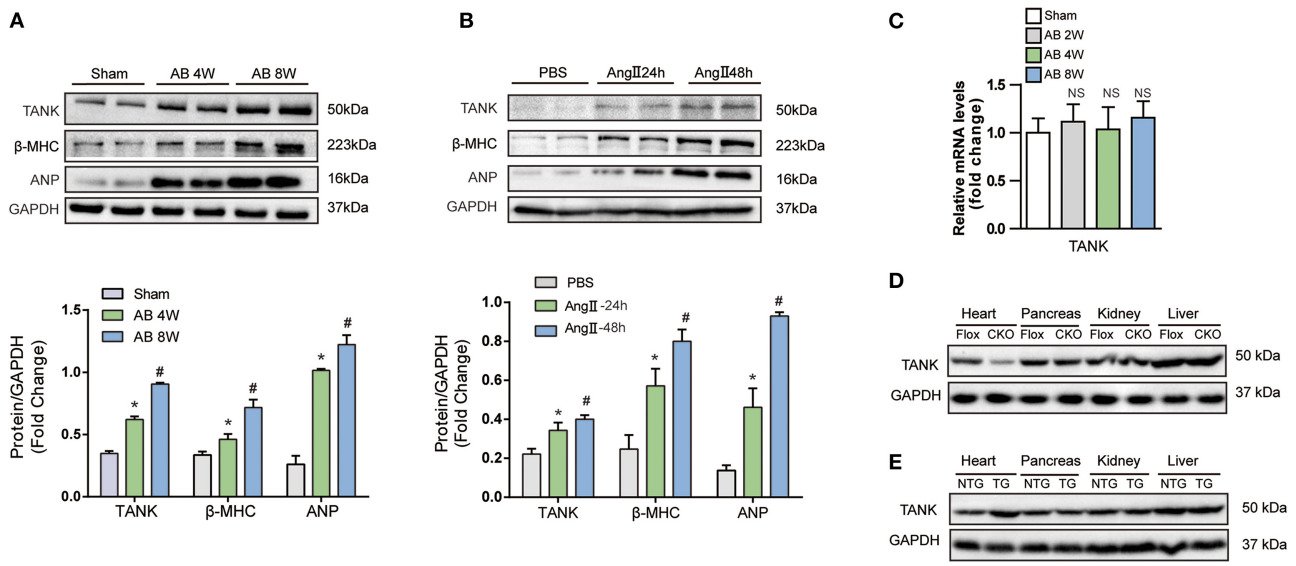

FIGURE 1 | The expression of TANK is upregulated in hypertrophic murine hearts. **(A)** Western blot analysis of TANK expression in cardiac extracts of samples from mice subjected to aortic banding at the indicated time points ($n = 6$ mice per experimental group). **(B)** TANK expression was analyzed by western blotting in extracts from neonatal rat cardiomyocytes treated with angiotensin II (Ang II; 1 μmol/L) for 24 and 48 h ($n = 3$ independent experiments). $^{*}P < 0.05$ vs. Sham or vs. PBS; $^{\#}P < 0.05$ vs. AB 2w or vs. Ang II 24 h. The data are presented as the mean ± SD. **(C)** mRNA levels of the TANK in heart samples from sham group and mice after aortic banding 2weeks, 4weeks and 8weeks ($n = 3$ per mice group). **(D)** Western blot showing TANK expression in TANK specific deleted heart, pancreas, kidney and liver. **(E)** TANK protein expression in heart samples and other tissue from TANK transgenic mice.

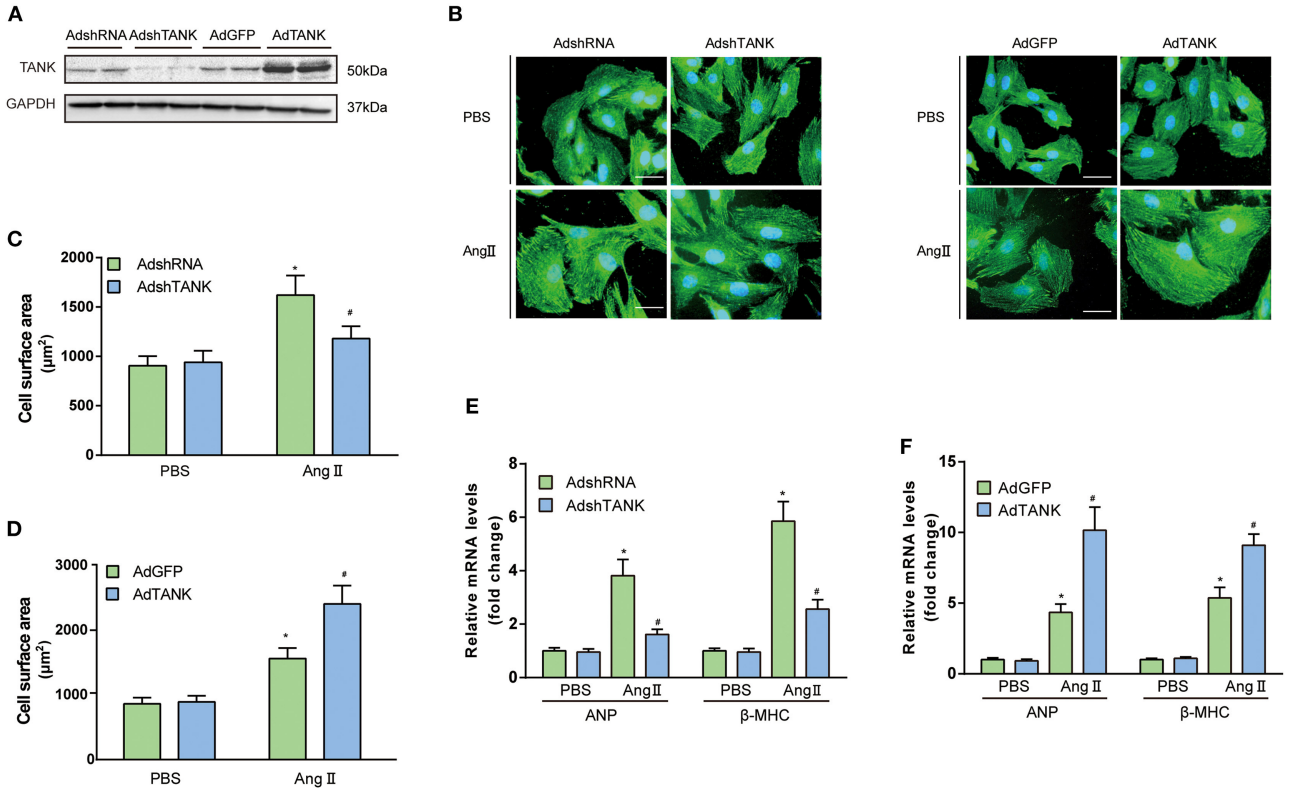

FIGURE 2 | TANK augments the Ang II-induced hypertrophic response of cardiomyocytes. **(A)** Representative Western blot showing TANK expression in neonatal rat cardiomyocytes (NRCMs) infected with AdshRNA, AdshTANK, AdGFP, and AdTANK. **(B)** NRCMs were infected with AdshTANK or AdTANK (AdshRNA and AdGFP as control) and treated with Ang II (1 μmol/L) or PBS for 48 h. Sarcomere organization was stained using anti-α-actinin antibodies (green), and nuclei were determined by DAPI staining (blue); scale bar, 20 μm. **(C,D)** Quantification of the cell surface area in AdshRNA and AdshTANK **(C)** or AdGFP and AdTANK **(D)** treated with PBS or Ang II ($n > 50$ cells per experimental group; $^{*}P < 0.05$ vs. AdshRNA/PBS or vs. AdGFP/PBS; $^{\#}P < 0.05$ vs., AdshRNA/Ang II or vs., AdGFP/ Ang II). **(E ,F)** Real-time polymerase chain reaction (PCR) analysis of hypertrophic markers atrial natriuretic peptide (ANP) and β-myosin heavy chain (β-MHC) in AdshRNA and AdshTANK **(E)** or AdGFP and AdTANK **(F)** treated with PBS or Ang II for 48 h ($n = 3$ independent experiments; $^{*}P < 0.05$ vs. AdshRNA/PBS or vs. AdGFP/PBS; $^{\#}P < 0.05$ vs. AdshRNA/Ang II or vs. AdGFP/Ang II). All the data are presented as the mean ± SD.

to examine the expression of TANK and hypertrophic markers, including atrial natriuretic peptide (ANP) and β-myosin heavy chain (β-MHC). As shown in **Figure 1A**, the levels of TANK and hypertrophic markers were markedly elevated compared to the control group. During the development of cardiac hypertrophy, the expression levels of TANK, ANP, and β-MHC were more pronounced in 8 weeks than in 4 weeks. Similar results have been observed for *in vitro* experiments. Twenty-four or fourty-eight hours after angiotensin II administration, the expression of TANK and the hypertrophic markers was upregulated in NRCMs (**Figure 1B**). These results suggest that enhanced expression of TANK is related to the pathogenesis of cardiac hypertrophy. Quantitative RT-PCR was used to quantify the level of TANK mRNA in the heart samples of Sham group and AB group at 2, 4, and 8 weeks. As shown in **Figure 1C**, the expression of TANK mRNA had no change among all groups. Besides, we performed Western blot showing TANK expression in heart, pancreas, kidney, and liver samples of cardiac-specific TANK knockout mice (TANK-CKO) and TANK transgenic mice (TANK-TG) (**Figures 1D,E**).

TANK Promotes AngII-Induced Cardiomyocyte Hypertrophy *in vivo* and *vitro*

To understand the functional role of TANK in cardiomyocytes, AdshTANK was infected to knockdown TANK. Also, AdTANK is used to overexpress TANK. the cells were then incubation with 1 μM Ang II or PBS for 48 h and immunostained with α-actin. First, the effectiveness of knockdown or overexpression of TANK in cardiomyocytes was confirmed (**Figure 2A**). As shown in **Figures 2B,C**, cardiomyocyte hypertrophy was significantly inhibited the AdshTANK group incubated with 1 μM Ang II for 48 h compared with the AdshRNA group. In contrast, the cell size of the AdTANK group was markedly increased under the stimulation of Ang II, compared to the AdGFP group (**Figure 2D**). Similarly, the mRNA levels of ANP and β-MHC decreased in the TANK-knockdown group, while the levels were upregulated after TANK overexpression, which supports the observations from cell morphology (**Figures 2E,F**). These data confirm that TANK is a positive regulator of cardiomyocyte

hypertrophy. In addition, we found similar result in TANK-TG mice when infused with Ang II (**Supplementary Figure 2**).

TANK Cardiomyocyte-Specific Deficiency Alleviates Hemodynamic Overload-Induced Cardiac Hypertrophy

To further clarify the potential role of TANK in the development of cardiac hypertrophy, a mouse model of TANK-CKO was generated (**Figures 3A–D**). TANK expression was detected and we found remarkable reduction in TANK-CKO mice compare with that in TANK-Flox mice (**Figure 4A**). M-mode echocardiograms from each group were as shown in **Figure 4B**. At baseline, there was no difference in phenotypic characteristics among groups. After AB, cardiac function was evaluated by echocardiogram and we found that the IVSd, LVPWd, and LVEDd of TANK-CKO mice were markedly decreased, and FS% increased compared with those in the control groups (**Figure 4C**). Heart weight/bodyweight (HW/BW) ratio showed a sharper decline in TANK-CKO mice than in the control groups 4 weeks (**Figure 4D**). Histological examination of the heart showed that the size of cardiomyocytes from TANK-CKO mice was decreased compared to that in the control mice after 4 weeks of aortic banding (**Figure 4E**). Consistently, expression levels of hypertrophic markers as mRNA levels of ANP, BNP, and β-MHC were decreased in TANK-CKO mice (**Figure 4F**). Cardiac fibrosis was assessed and it was found that content of collagen in interstitial and perivascular space was significant reduced in TANK-CKO mice, compared with that in control groups after AB surgery. mRNA levels of the fibrotic markers in TANK-CKO mice also decreased, including collagen Iα, collagen III, and connective tissue growth factor (CTGF) (**Figures 4G,H**). These data suggest that TANK deficiency exerts a protective effect on cardiac hypertrophy.

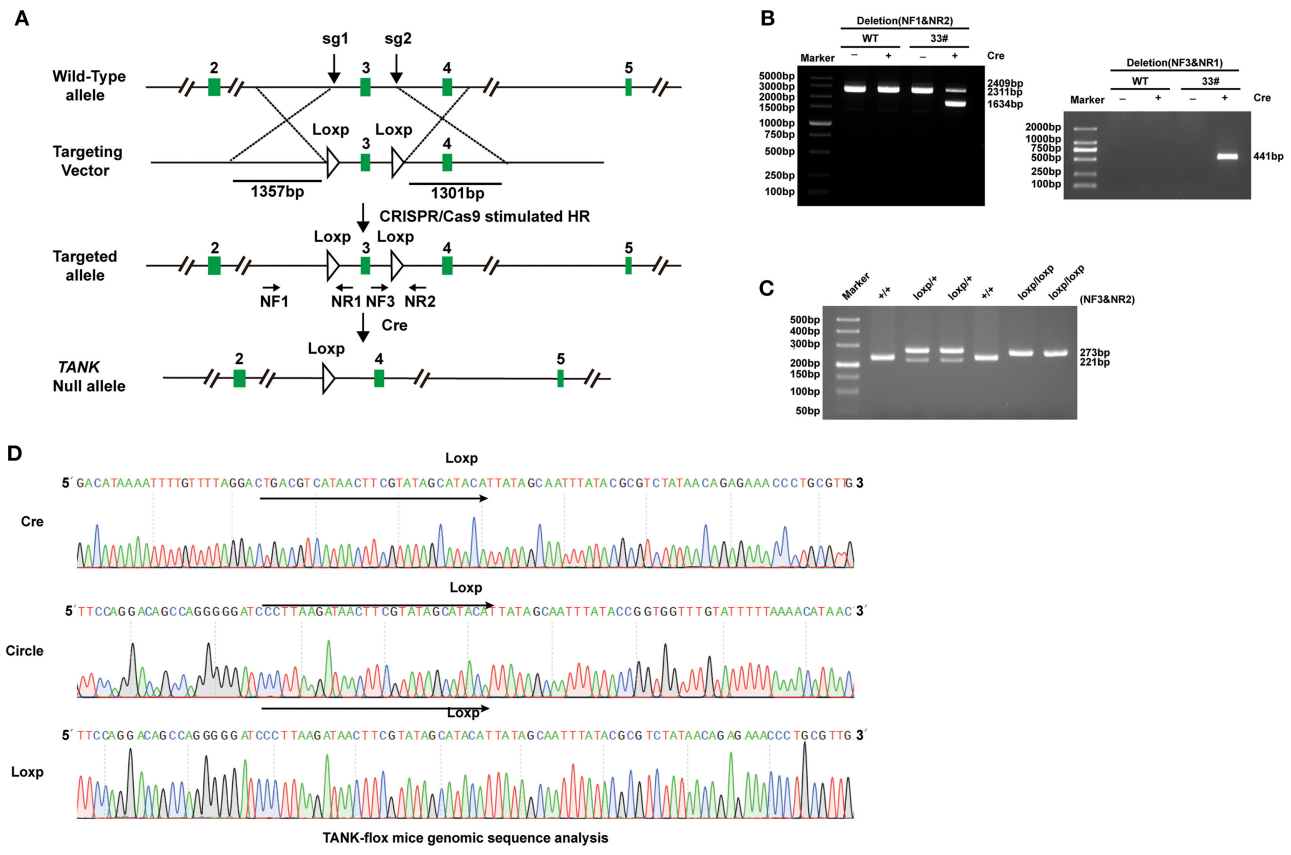

FIGURE 3 | Establishment of TANK-CKO mice. **(A)** Schematic diagram of the construction of cardiac-specific TANK knockout mice. **(B)** PCR and 3% gel electrophoresis were used to identify the recombinant TANK-gene Floxed site in founder mice mediated by Cre-loxP. Genomic DNA from founder mouse (33#) was processed using Cre recombinant enzyme and used as a template. NF1 & NR2 primers and NF3 & NR1 primers are respectively used for amplification and then gel electrophoresis was performed. A smaller band appeared in the NF1 & NR2 amplification (1634 bp), NF3&NR1, which originally extended in the opposite direction, amplified a bright band. Among all bands, 2409bp band was loxP Founder mouse genome, 2311bp band was WT mouse genome, and 441bp band was cyclization PCR product. **(C)** PCR and 3% gel electrophoresis were used to identify TANK-flox alleles, in which the single 273bp band represented homozygous insertion of loxP biallele (loxP/loxP);221bp/273bp bands represented heterozygous insertion of loxP single allele (loxP /+); and the single 221bp band represented WT alleles (+/+). **(D)** Sequencing results of truncated PCR products and cycled PCR products in **(B)** and TANK-flox allele in **(C)**.

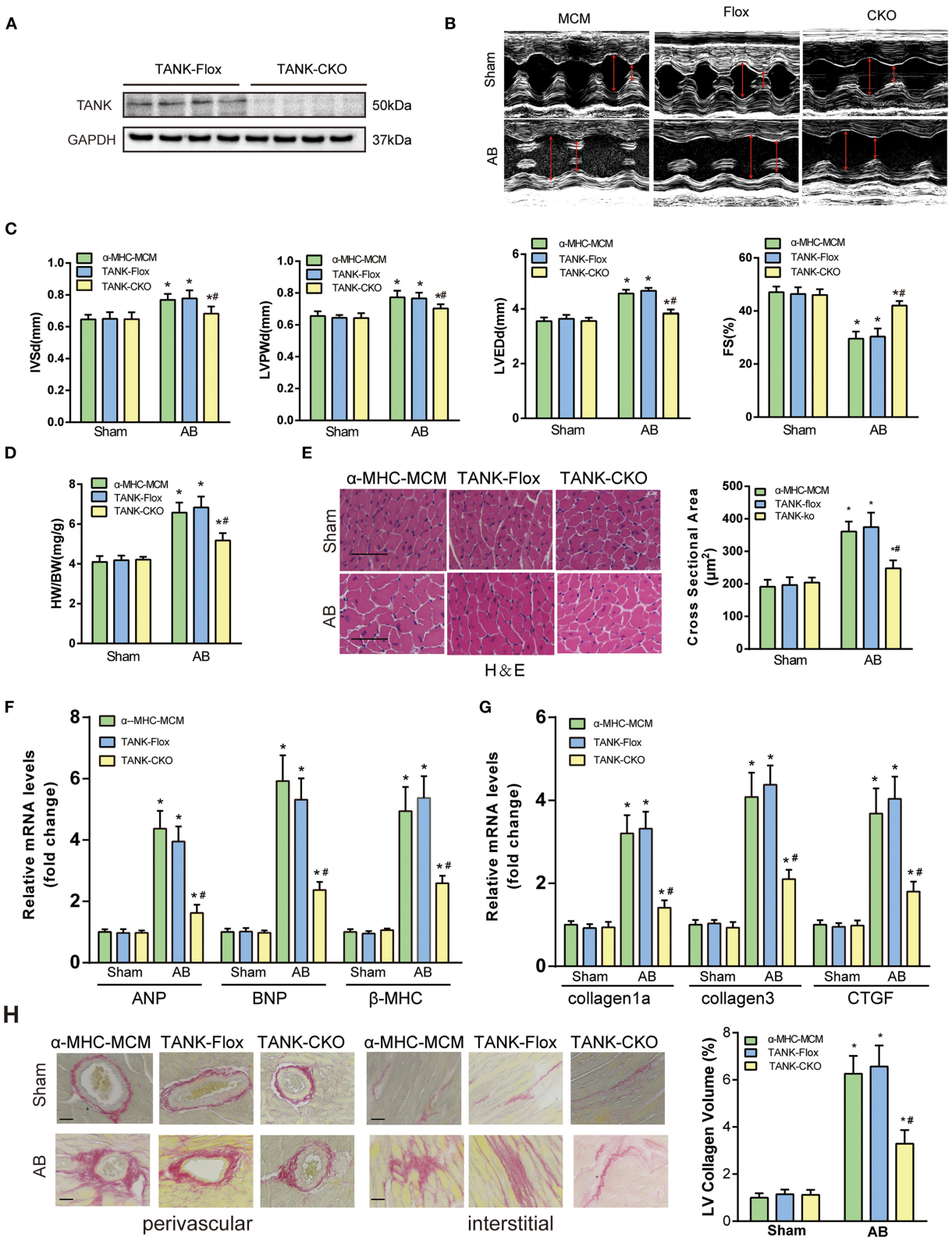

FIGURE 4 | TANK cardiac-specific deficiency ameliorates pressure overload-induced cardiac hypertrophy. **(A)** Representative western blot showing TANK expression in the TANK-Flox and TANK conditional knockout (TANK-CKO) groups ($n = 4$ mice per group). **(B)** M-mode echocardiograms from control groups and TANK-CKO

(Continued)

FIGURE 4 | mice after AB. **(C)** End-diastolic interventricular septum (IVSd), posterior wall dimensions (LVPWd), left ventricular end-diastolic diameter (LVEDd) and fractional shortening (FS%) are measured by echocardiography from α-MHC-MCM, TANK-Flox, and TANK-CKO mice (n = 12-14 mice per group). **(D)** Ratio of heart weight (HW)/body weight (BW) in the control groups (α-MHC-MCM, TANK-Flox) and TANK-CKO group subjected to sham or aortic banding for four weeks (n = 12-14 mice per group). **(E)** Left, Histological analysis of hematoxylin and eosin (H&E) staining in α-MHC-MCM, TANK-Flox, and TANK-CKO mice at four weeks after sham operation or aortic banding surgery (n = 6 mice per group). Scale bars, 50 μm. Right, Quantification of the myocyte cross-sectional area of the indicated group (n = 100+ cells per each experimental group). **(F)** mRNA expression of the hypertrophic markers ANP, BNP, and β-MHC in α-MHC-MCM, TANK-Flox, and TANK-CKO mice four weeks after the sham operation or aortic banding surgery (n = 4 mice per group). **(G)** mRNA levels of the fibrotic markers collagen Iα, collagen III, and connective tissue growth factor (CTGF) in α-MHC-MCM, TANK-Flox, and TANK-CKO mice four weeks after sham operation or aortic banding surgery (n = 4 mice per group). **(H)** Left, representative image of picrosirius red staining to detect fibrosis in α-MHC-MCM, TANK-Flox, and TANK-CKO mice four weeks after sham operation or aortic banding surgery (n = 6 mice per group); scale bars, 50 μm. Right, quantification of left ventricle (LV) collagen volume ($n \geq 40$ fields per group). All the data are presented as the mean ± SD. $^{*}P < 0.05$ vs. α-MHC-MCM/Sham or TANK-Flox/Sham or TANK-CKO/Sham; $^{\#}P < 0.05$ vs. TANK-Flox/AB or α-MHC-MCM/AB.

TANK Overexpression Results in the Exacerbation of Hemodynamic Overload-Induced Cardiac Remodeling

TANK-TG mice were established (**Figure 5A**) and the expression in different tissue was confirmed using Western blotting analysis (**Figure 1E**). The line showing the highest expression was chosen as the experimental animal group (**Figure 5B**). There was no significant distinction in morphology or pathology of the heart between TANK-TG and NTG mice. M-mode echocardiograms from NTG mice and TANK-TG mice after AB were shown in **Figure 5C**. After 4 weeks subjected to AB surgery, the TANK-TG mice exhibited higher ratios of HW/BW and HW/TL than NTG mice (**Figure 5D**). To determine if TANK-TG was associated with heart dysfunction, echocardiograms were performed. As shown in **Figures 5D,E**, TANK-TG showed an increase in LVEDd, IVSd, and LVPWd, and a decrease in FS%. The cross-sectional area and cardiomyocyte size were also analyzed by HE staining and showed a significant increase in TANK-TG mice relative to NTG mice after AB surgery (**Figure 5F**). Similarly, the overexpression of TANK resulted in up-regulation of collagen content (**Figure 5G**). Consistently, higher mRNA expression levels of hypertrophic markers were detected in TANK-TG mice, including ANP, BNP, and β-MHC; fibrosis-related markers were also elevated, such as collagen Iα, collagen III, and CTGF (**Figure 5H**). Taken together, these data demonstrate that cardiomyocyte-specific TANK overexpression aggravates the pressure overload-induced hypertrophic response.

TANK Promotes Cardiac Hypertrophy by Activating AKT Phosphorylation and Leads to Fibrosis Under Control of TGF-β1 Signaling Pathway

Since TANK is considered to promote cardiac hypertrophy, the underlying mechanism was investigated. A multitude of signaling pathways associated with hypertrophy are well-established, in which the MAPK and AKT pathways are thought to be the two most important pathways (19). First, we explored whether TANK activates MAPK signaling pathways. As shown in **Supplementary Figure 1A**, there was no obvious distinguishable activation of MEK1/2, ERK1/2, and p38 using Western Blot analysis between groups (CKO vs. Flox and TG vs. NTG). Next, the AKT signaling pathway was evaluated and it was found that TANK-deficient mice subjected to AB surgery experienced decreased AKT phosphorylation levels compared to Flox mice, while the activity of phosphorylated AKT levels was enhanced in TANK-TG mice after AB (**Figures 6A–C**). The total AKT level among all groups was not significantly different. The downstream molecules involved were detected in the same manner. In AB-treated TANK-CKO mice, the phosphorylation of mTOR and P70S6K was downregulated and phosphorylated GSK3β decreased. Conversely, TANK overexpression exhibited the opposite effect on the phosphorylation of mTOR, P70S6K, and GSK3β.

We also confirmed activation of TANK on the AKT signaling pathway in neonatal rat cardiomyocytes (**Figures 6D–F**). Analysis using Western blotting revealed that the phosphorylation of AKT/mTOR/P70S6K induced by Ang II dramatically declined in Ad-shTANK cells but markedly increased in Ad-TANK cells. These results indicate that TANK leads to hypertrophy, most likely through mediation of the AKT signaling pathway.

Furthermore, Immunoprecipitation was performed and demonstrated that TANK is able to interact with AKT (**Figures 6G,H**).

In addition, the underlying mechanism of interstitial fibrosis were also investigated using WB. As TGF-β1 is crucial for cardiac fibrosis, the expression of TGF-β1 and related molecular were detected in TANK-CKO mice and TANK-TG mice. After AB, we noted that TANK-CKO hearts had decreased amount of TGF-β1 and phosphorylated Smad2 and Smad3 (**Supplementary Figure 1B**). Besides, TANK-TG hearts show increased expression of these protein after AB compared with NTG hearts.

Blockage of AKT Signaling Reverses Cardiac Hypertrophy due to TANK Overexpression

Having shown that TANK promotes the activation of the AKT signaling pathway under hemodynamic overload condition, blockage of the AKT signaling pathway was performed to identify whether it would reverse cardiac hypertrophy. Finally, a pharmacological inhibition strategy was performed in TANK-TG mice with PI3K inhibitor LY29004 and AKT inhibitor MK2206. **Figure 7A** shows an animal experimental flowchart. LY29004-treated mice displayed less increased LVEDd, IVSd, and LVPWd as well as preserved FS% compared to mice treated with DMSO (**Figure 7B**). Similarly, TANK-TG mice

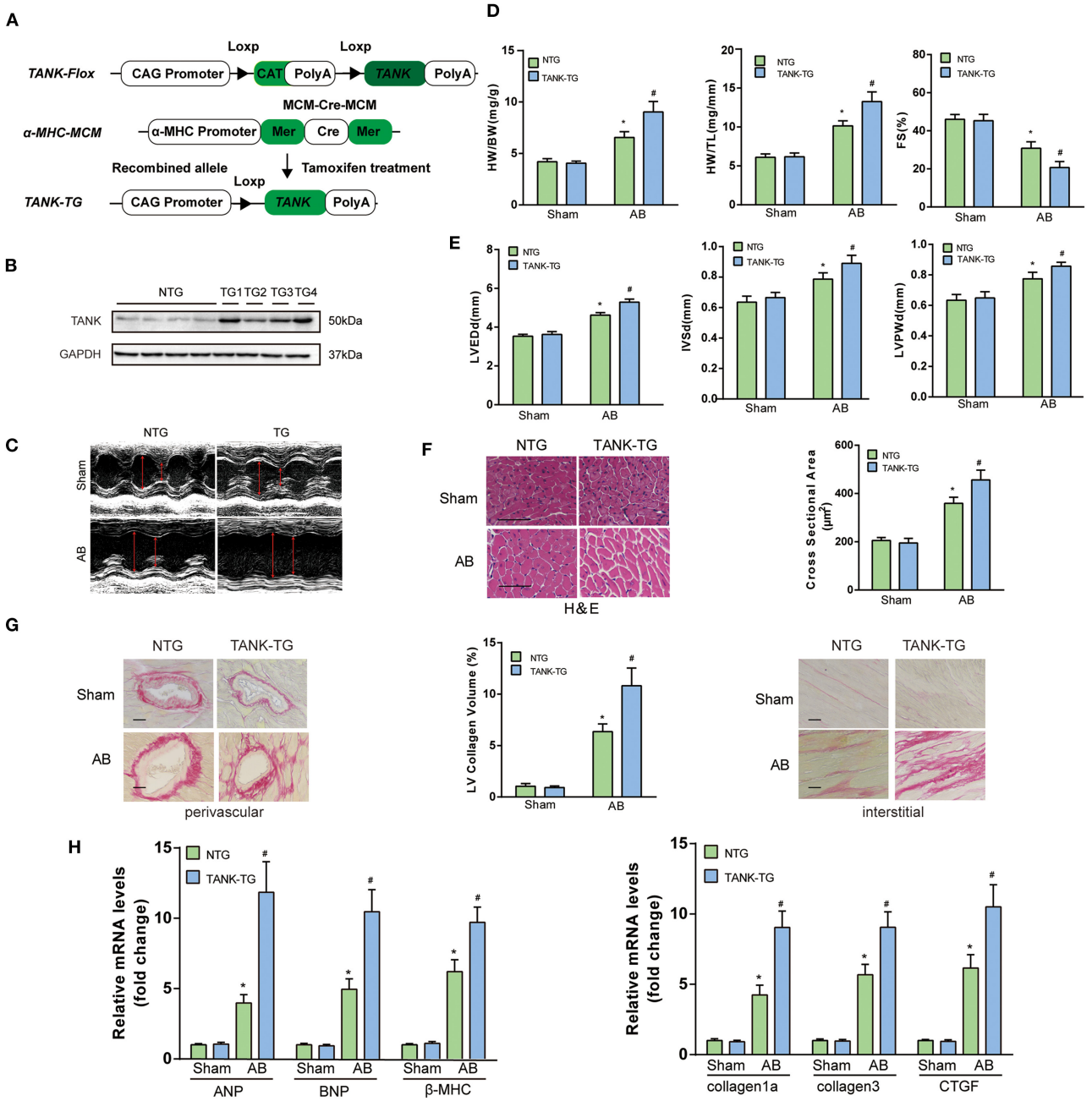

FIGURE 5 | TANK cardiac-specific overexpression results in the exacerbation of pressure overload-induced cardiac hypertrophy. **(A)** Schematic diagram of the construction of cardiac-specific TANK overexpression experimental mice. **(B)** Western blot showing TANK expression in mouse heart samples from nontransgenic (NTG) mouse hearts and cardiac-specific TANK transgenic (TANK-TG) hearts (TG1, TG2, TG3, and TG4; $n = 4$ mice per group). **(C)** M-mode echocardiograms from NTG group and TANK-TG mice after AB. **(D)** The HW/BW and HW/TL ratio determined in NTG and TANK-TG mice subjected to sham or AB treatment for four weeks ($n = 12$-14 mice per group). **(D,E)** M-mode echocardiograms of NTG mice and TANK-TG mice after AB. Measurements of LVEDd, IVSd, LVPWd, and FS% using echocardiography of NTG and TANK-TG mice subjected to sham or AB treatment for four weeks ($n = 12$–14 mice per group). **(F)** Representative histological cross-sections stained with H&E indicated concentric hypertrophy in NTG and TANK-TG mice subjected to four weeks of AB treatment ($n = 6$ mice per group) (Left). Cross-sectional area of the indicated group was quantified ($n = 100+$ cells per group) (Right). **(G)** Picrosirius red staining showing perivascular fibrosis (Left) and interstitial fibrosis (Right) in heart sections of NTG and TANK-TG mice ($n = 6$ mice per group) , Scale bars, 50 μm. Middle, Statistical results of LV collagen volume of the indicated group ($n \geq 40$ fields per group). **(H)** The relative mRNA levels of the hypertrophic markers ANP, BNP, and β- MHC (Left), or fibrotic markers collagen Iα, collagen III, and CTGF (Right) in NTG and TANK-TG mice ($n = 4$ mice per group) subjected to sham or AB treatment for four weeks. All the data are presented as the mean ± SD. $^{*}P < 0.05$ vs. NTG/Sham or TANK-TG/Sham; $^{\#}P < 0.05$ vs. NTG/AB.

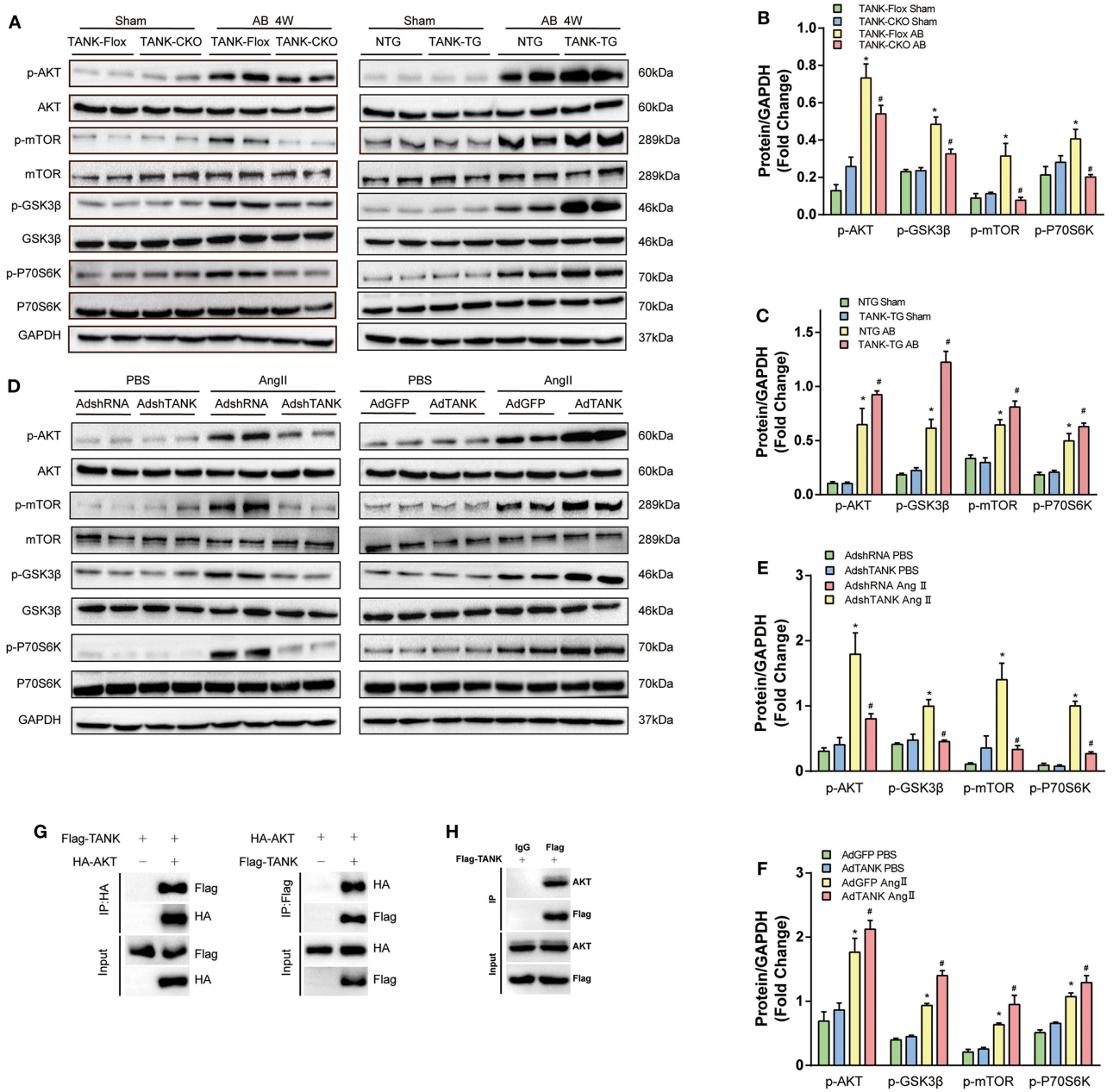

FIGURE 6 | TANK promotes AKT phosphorylation *in vivo* and *in vitro*. **(A)** Representative Western blot showing total and phosphorylated expression of AKT, GSK3β, mTOR, and P70S6K in TANK-CKO mice compared with TANK-Flox mice (Left) or TANK-TG mice compared to TANK-NTG mice (Right) subjected to sham or aortic banding for four weeks. **(B ,C)** Quantitative analysis of the phosphorylation of AKT, GSK3β, mTOR, and P70S6K in TANK-Flox and TANK-CKO mice **(B)** or in TANK-NTG and TANK-TG mice **(C)** ($n = 4$ per group; $^{*}P < 0.05$ vs. TANK-Flox/Sham or TANK-NTG/Sham; $^{\#}P <.0.05$ vs. TANK-Flox/AB or TANK-NTG/AB). **(D)** Representative Western blot for the AKT signaling-related protein from NRCMs infected with AdshRNA as the control and AdshTANK to delete TANK expression (Left) or AdGFP as the control and the AdTANK group to overexpress TANK protein (Right) treated with PBS or Ang II. **(E,F)** Quantitative analysis of phosphorylated AKT signaling-related protein in the AdshRNA and AdshTANK groups **(E)** or the AdGFP and AdTANK groups **(F)** ($n = 3$ independent experimens; $^{*}P < 0.05$ vs. AdshTANK/PBS or AdGFP/PBS; $^{\#}P < 0.05$ vs. AdshTANK/Ang II or AdGFP/Ang II). The data are presented as the mean ± SD. **(G,H)** Immunoprecipitation followed by immunoblotting revealed that TANK interact with TANK.

treated with LY294002 exhibited decreased HW/BW and HW/TL ratios 4 weeks after aortic banding, compared to DMSO-treated mice (**Figures 7C,D**). In addition, LY29004 significantly prevented against aortic banding-induced cardiac hypertrophy and fibrosis under the condition of TANK overexpression (**Figures 7E,F**). The AKT signaling-related molecules were also

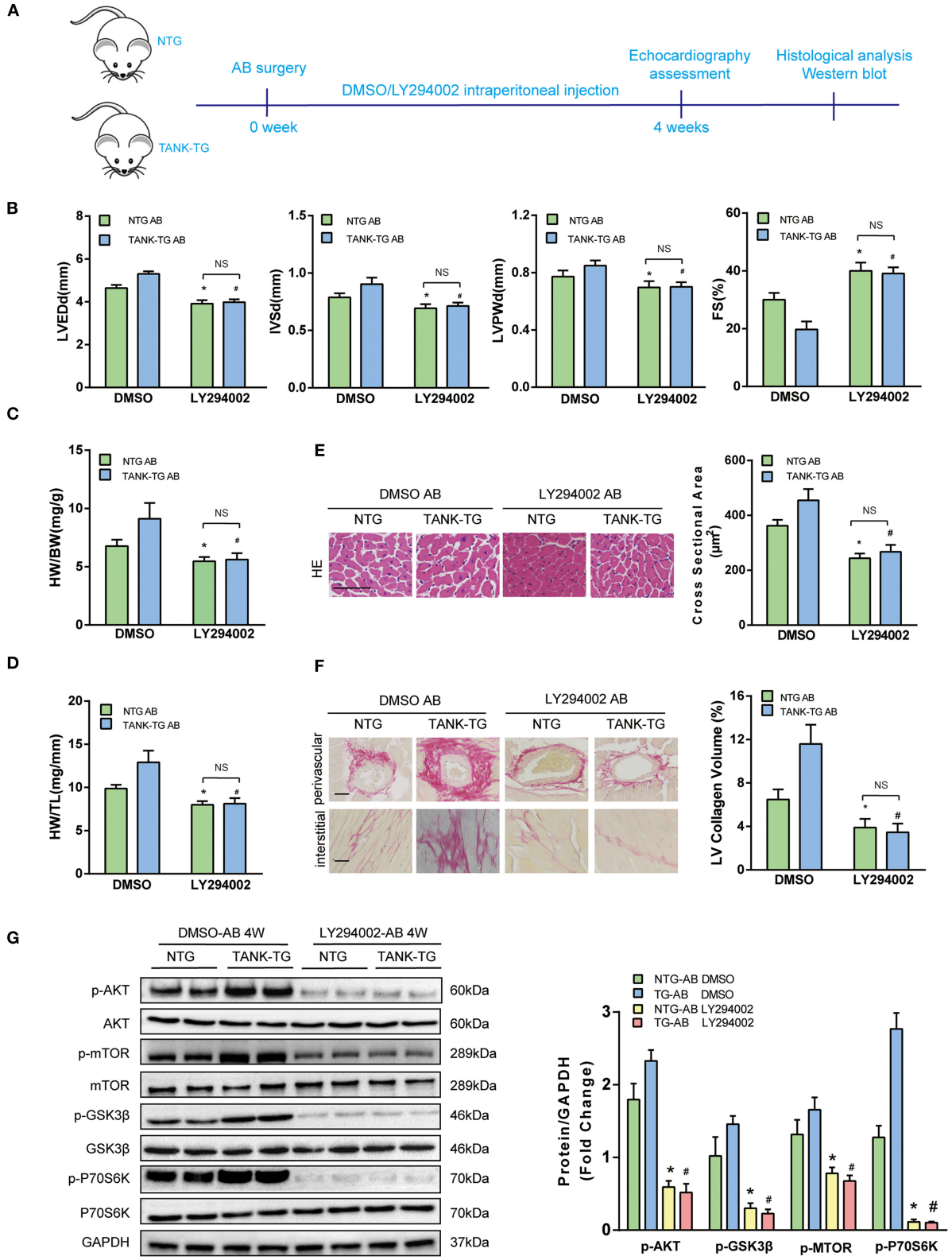

FIGURE 7 | Effect of PI3K inhibitor LY294002 on cardiac hypertrophy in TANK-TG mice. **(A)** A flowchart to illustrate animal experiments. **(B)** Echographic parameters obtained from heart samples of the NTG and TANK-TG mice injected with DMSO or LY294002 after aortic banding for four weeks ($n = 4$ mice per group).

(Continued)

FIGURE 7 | (C,D) Quantitation of HW/BW and HW/HL ratios of NTG and TANK-TG after four weeks aortic constriction and treatment with DMSO or LY294002 (n = 12 mice per group). **(E)** Left, H&E stained heart sections of NTG and TANK-TG mice after four weeks of aortic constriction and treatment with DMSO or LY294002 (n = 6 mice per group) ,Scale bars 50 μm. Right, quantification of the cross-sectional area of the indicated group ($n \geq 100$ cells each experimental group). **(F)** Left, PSR-stained heart sections of DMSO- and LY294002-treated NTG and TANK-TG mice with four weeks of aortic banding (n = 6 mice per group), Scale bars 50 μm. Right, quantification of the LV collagen volume of the indicated group ($n \geq 40$ fields per each experimental group). **(G)** Left, Western blots showing the effect of LY294002 on the phosphorylation of AKT and its substrates of NTG and TANK-TG mice after four weeks of aortic constriction. Right, quantitation of Western blot bands. All data are presented as the mean ± SD (n = 4 per group; $^{*}P < 0.05$ vs. NTG/DMSO AB; $\#P < 0.05$ vs. or TANK-TG/DMSO AB).

detected via Western blotting and are shown in **Figure 7G**. *In vitro* experiment, NRCMs overexpressed TANK exhibited larger cell size after Ang II stimulation accompanied by elevated hypertrophic makers ANP and BNP. Incubation with MK2206 can reverse myocyte hypertrophy induced by Ang II (**Figure 8**). Therefore, we demonstrated that TANK may hasten hypertrophy through the AKT signaling pathway.

DISCUSSION

TANK is a scaffold protein that binds to at least two other signaling proteins and lacks enzymatic activities (20). Scaffold proteins can function as platforms to organize signaling molecules into functional complexes, locate signaling molecules at particular sites in cells, integrate feedback signals, and prevent activation signaling molecules from being deactivated. Previous studies have noted emergence of scaffold proteins as important modifiers in the regulation of cardiac hypertrophy. IQGAP1 (IQ motif-containing GTP-ase protein (1) is key to c-Raf-MEK1/2-ERK1/2 as well as AKT signaling, and regulates pathological cardiac remodeling upon pressure overload (21). FHL1 (four-and-a-half LIM domains (1) senses biomechanical stress and promotes cardiac hypertrophy by affecting the MAPK signaling cascade (22). ANKRD (Ankyrin repeat domain1), a sarcomere scaffolding protein, induces cardiac hypertrophy by increasing the phosphorylation of ERK-GATA4 after phenylephrine (PE) stimulation (23). In this study, for the first time, to the best of our knowledge, we identified TANK as a scaffold protein activate AKT signaling in pathological cardiac hypertrophy, and provide future evidence for the IKKε-TBK1/AKT signaling pathway.

The importance of TANK in both the innate immune response and non-infectious inflammation has been observed in previous studies. TANK deficiency dampens type I interferon gene induction and enhances cell susceptibility to multiple viruses (11, 24). Besides, TANK was proven as a novel target of one type of viral protease of RNA virus named 3C protease. Encephalomyocarditis Virus 3C protease cleaved TANK not only enhanced TRAF6-induced NFκB signaling but also disrupted TANK-TBK1-IKKε-IRF3 complex, leading to a significant reduction of IFN production, and evaded the host innate immune responses (25, 26). Similarly, Seneca Valley virus (SVV) cleaved TANK via 3C protease promotes TRAF6 mediated NFκB activation and suppression of IFN mediated inflammation (27). In addition, the deletion of TANK suppressed the development of fatal glomerulonephritis caused by intestinal commensal microflora (28). In renal ischemia-reperfusion injury, the expression of TANK is also persistently upregulated, but its functional contribution has not yet been confirmed (29). Moreover, TANK plays a critical role in glioblastomas as an activator in S-phase progression and cell migration (19). Emerging evidence indicates that the expression of TANK is ubiquitously detected in various tissues, including heart tissues (29), but the expression levels of TANK under prohypertrophic stimuli remain unclear. Here, we found that TANK expression was markedly elevated in heart samples of mice subjected to aortic banding compared with that in Sham hearts. Similarly, TANK expression was progressively upregulated in NRCMs incubated with ANG II. The regulation mechanism of TANK expression has not been fully clarified. Transcription factor SOX11, a member of the SoxC family, is essential for the development of the cardiac outflow tract (30). However, there is no evidence that it is involved in the progression of cardiac hypertrophy. NFκB is considered another important modulator for TANK expression. The TNF-α signal triggers the p50-p65 heterodimer to translocate into the nucleus, and induces the expression of TANK (31). However, the increased expression of TANK during cardiac hypertrophy requires further research.

To explore the underlying mechanism of TANK involved in pathological pressure overload-induced cardiac hypertrophy, conditional transgenic mice were utilized in combination with aortic constriction, which is an effective approach for the study of hypertrophy *in vivo*. The results of this study indicate that TANK is functionally important during press overload, as TANK-CKO mice exhibit thinner ventricular walls, left chamber dilation, alleviative contractile dysfunction, and reduced reactivation of cardiac fetal genes when exposed to persistent aortic constriction. Another important detection in TANK-CKO mice is reduced fibrosis, which is a typical feature of pathologic cardiac hypertrophy (32). Compared with NTG mice, transgenic mice overexpressing TANK present exaggerated cardiomyocyte hypertrophy and interstitial fibrosis. These data provide direct evidence that TANK is an pathological hypertrophy accelerator.

Pathological hypertrophy caused by changes in signal transduction pathways responding to a series of stimuli has established MAPKs as classical proteins that are critical for cardiac hypertrophy (33, 34). Herein, we demonstrate that the altered TANK expression has no effect on MAPK signaling in the myocardium but increases the phosphorylation of AKT as well as activation of mTOR and S6K, and IP analysis revealed TANK interacts with AKT physically. AKT participates in cardiac hypertrophy ranging from cell survival to aging. Insulin-like growth factor 1 and exercise can lead to AKT phosphorylation and eventually cause physiological adaptive cardiac hypertrophy (35). However, additional experiments showed that the constitutive cardiac-specific overexpression of AKT1 cause elevated heart weight and pathological

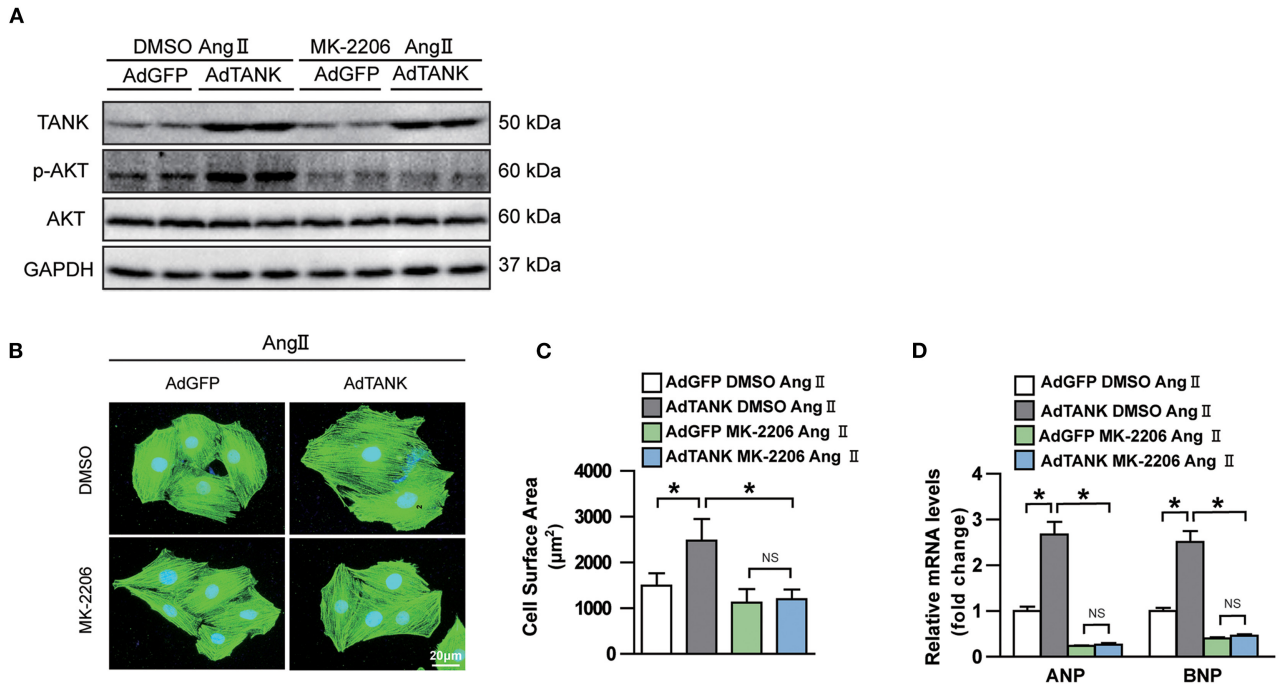

FIGURE 8 | Effect of AKT inhibitor MK-2206 on AngII-induced cardiomyocyte hypertrophy in TANK overexpression NRCMs. **(A)** Western blot showing MK-2206 inhibits phosphorylation of AKT after stimulation of AngII. **(B)** NRCMs infected with the indicated adenoviruses are incubated with MK-2206 or DMSO after stimulated with AngII. Sarcomere organization was stained using anti-α-actinin antibodies (green), and nuclei were determined by DAPI staining (blue). scale bar, 20 μm. **(C)** Quantification of cell surface area of indicated groups after MK-2206 or DMSO treatment ($n > 50$ cells per experimental group). **(D)** mRNA level of hypertrophic marker ANP and BNP in TANK overexpression cell and control group treated with MK-2206 or DMSO after stimulated with AngII ($n = 3$ independent experiments). $^{*}P < 0.05$.

hypertrophy-associated enlarged cell size, impaired contractile function, and interstitial fibrosis (36, 37). AKT3, which is functionally distinct from AKT1 in different cell types, also play a role in diseased human hearts. AKT3 transgenic mice exhibit pathological hypertrophy at 20 weeks of age (38). However, a different observation showed that AKT1-deficient mice result experience increased susceptibility to hypertrophic stimuli and more profound cardiac hypertrophy in response to aortic constriction (39). Phosphorylation is the most important post-translational determinant of AKT activity (40), PI3K is required for AKT membrane recruitment. We demonstrate that PI3K inhibitor LY294002 can reverse the phenotypic spectrum caused by aortic constriction, especially in TANK-TG mice and MK2206, a highly selective inhibitor of AKT, can reverse myocyte hypertrophy induced by Ang II.

As a Ser/Thr protein kinase, mTOR (mechanistic target of rapamycin) plays a critical role downstream of AKT. Once modulated, mTOR transduces signals to different effectors, such as P70S6K1, 4E-BP1, SREBP1, Lipin, and HIF1, and participates in protein synthesis and cell metabolism (41). mTOR is considered to be essential for pressure overload-induced pathological cardiac hypertrophy. Partial genetic deletion or pharmacological suppression of mTOR has been found to persistently ameliorate cardiac hypertrophy induced by AB (42). It is notable that mTOR activation alone is insufficient and requires coordination with other signaling pathways effectors to promote cardiac hypertrophy (43). Emerging evidence shows that epigenetic reprogramming participates in the contribution of mTOR during cardiac hypertrophy. The genetic and pharmacological downregulation of class I HDACs blunts pathological cardiac hypertrophy by inhibiting TSC2-dependent mTOR signaling (44). Chaer, a heart-enriched long non-coding RNA, interacts with PRC2 in a mTOR-dependent manner and inhibits histone H3 lysine 27 methylation at hypertrophic genes (45). Additionally, microRNA is a regulator of mTOR. MiR-99a suppresses aortic banding-induced cardiac hypertrophy targeting the mTOR/P70/S6K signaling pathway (46).

As far as we know, the interaction between TANK and AKT has not been reported before. N-terminus of TANK is essential for combination with ZC3H12A and TRAF6 (47). The Binding sites have been reported located in C-terminal and N-terminal domain of AKT. Previous lecture showed that downregulation of TANK impaired AKT phosphorylation (19). Interrupting TRIF-mediated complex formation composed of TRAF3, TANK, and IKKε leaded to downregulation of AKT phosphorylation, and eventually downregulation of inflammation (48). Also, TANK-binding kinase 1(TBK1), which form a ternary complex with TANK and TRAF2 (13), which activates AKT by direct phosphorylation (49, 50). Based on above information, we could deduce that TANK may directly or indirectly activated AKT by phosphorylation, therefore promote proliferation, inflammation etc.

Besides, cardiac fibrosis is regarded as a major factor leading to cardiac remolding and dysfunction. In our study, we also observed changes of cardiac fibrosis in transgenic mice models. TGF-β1 signaling pathway has been demonstrated correlated with cardiac fibrosis. Suppression of TGF-β1 signaling reduced cardiac fibrosis and prevent cardiac dysfunction in several models of cardiac remodeling (51). Therefore, the expression of TGF-β1 and related molecular were detected using western bolt in our experiment. After AB, TGF-β1 is upregulated in TANK-TG mice and induced increased fibrosis. Decreased TGF-β1 expression in TANK-CKO mice with pressure overload could alleviates cardiac fibrosis. According to previous studies, activation of TGF-β also could induce cardiomyocyte hypertrophy (52). In this term, inhibiting this signaling pathway may reverse the effects of TANK on cardiomyocyte hypertrophy. We found that AKT signaling pathway is involved in cardiac hypertrophy, blockage of AKT could reverse TANK overexpression induced hypertrophy. These two signaling pathways may cooperate in the process of TANK-related cardiac hypertrophy.

In latest studies, TANK was thought to respond to anti-TNF therapy in patients with autoimmune disease (53) and as a candidate gene associated with hepatitis C virus clearance in both African and European Americans (54). Our observation in this article might be a starting point for future clinical work on cardiac hypertrophy.

To the best of our knowledge, this study is the first to report TANK aggravates cardiac hypertrophy *in vitro* and *in vivo*. Moreover, we found that TANK could enhances the activation of AKT during pressure overload-induced pathological hypertrophy. With the ongoing development of new drugs, our findings have theoretical significance for the treatment of cardiac hypertrophy.

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.

ETHICS STATEMENT

The animal study was reviewed and approved by Shanghai Tongren Hospital Ethics Committee.

AUTHOR CONTRIBUTIONS

LJ conceived the study and provided financial support. YP, DW, and MM performed the experiment and collected the data. YP and XL wrote the paper. All authors contributed to the article and approved the submitted version.

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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.2021.687540/full#supplementary-material

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Epicardial Adipose Tissue Measured From Computed Tomography Predicts Cardiac Resynchronization Therapy Response in Patients With Non-ischemic Systolic Heart Failure

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Background: Epicardial adipose tissue (EAT) has been linked with the pathogenesis of heart failure (HF). Limited data have been reported about the clinical value of EAT for cardiac resynchronization therapy (CRT) in non-ischemic systolic HF. We aimed to explore the values of EAT measured from CT to predict the response to CRT in patients with non-ischemic systolic HF.

Methods: Forty-one patients with CRT were consecutively recruited for our study. All patients received both gated resting Single Photon Emission CT (SPECT) myocardial perfusion imaging (MPI) and dual-source multi-detector row CT scans. EAT thickness was assessed on both the parasternal short and horizontal long-axis views. The area of EAT was calculated at the left main coronary artery level. Left ventricular systolic mechanical dyssynchrony (LVMD) was measured by phase standard deviation (PSD) and phase histogram bandwidth (PBW). The definition of CRT response was an improvement of 5% in left ventricular ejection fraction (LVEF) at 6 months after CRT implantation.

Results: After 6 months of follow-up, 58.5% (24 of 41) of patients responded to CRT. A greater total perfusion deficit (TPD) was observed in the left ventricle, and a narrower QRS complex was observed in the nonresponse group than in the response group ($p < 0.05$). Meanwhile, the systolic PSD and systolic PBW were statistically greater in the CRT group with no response than in the response group ($p < 0.05$). Meanwhile, the baseline QRS duration, TPD, systolic PSD, systolic PBW, EAT thicknesses of the left ventricular (LV) apex, right atrioventricular (AV) groove, and left AV groove were all significantly related to the CRT response in the univariate logistic regression analysis. Furthermore, the QRS duration and EAT thicknesses of the right AV groove and left AV groove were independent predictors of CRT response in the multivariate logistic regression analysis.

Conclusions: The EAT thickness of the left AV groove in patients with non-ischemic systolic HF is associated with the TPD of LV and LV systolic dyssynchrony. The EAT thickness of the AV groove has a good predictive value for the CRT response in patients with non-ischemic systolic HF.

Keywords: epicardial adipose tissue (EAT), cardiac CT, cardiac resynchronization therapy (CRT), heart failure (HF), single photon emission computed tomography (SPECT)

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INTRODUCTION

Cardiac resynchronization therapy (CRT) is an effective option for patients with medically refractory heart failure (HF). However, one-third of patients with HF treated with CRT have a suboptimal response in clinical practice (1). As the implantation of CRT devices is associated with the risk of device implantation, periprocedural complications, and relatively high costs, optimizing the current selection of patients with CRT is essential. Research in this area has shown that the CRT response is influenced greatly by left ventricular (LV) myocardial tissue viability, left ventricular dyssynchrony (LVMD), lead implantation, and fibrosis (2). Our previous study (3) also found that higher scar burden and LV lead implantation in scarred areas were associated with a suboptimal CRT response in patients with non-ischemic systolic HF.

Epicardial adipose tissue (EAT) is speculated to be linked with microvascular dysfunction, impairment of functional myocardium, and cardiac fibrosis independent of traditional risk factors (4). Previous studies (5, 6) have found that EAT is a novel parameter for cardiovascular risk assessment in coronary artery disease (CAD), cardiac hypertrophy, and atrial fibrillation (AF). Moreover, a recent study also found that EAT, which plays a role as electrical insulation, could potentially interfere with sensing and pacing for lead design in CRT (7). Nevertheless, performing LV lead placement and electrode positioning according to EAT may lead to uncertainty.

However, little data have been obtained about the clinical value of EAT for CRT in patients with non-ischemic systolic HF. We aimed to explore the values of EAT measured from CT to predict the CRT response in patients with non-ischemic systolic HF.

METHODS

Patient Population

Forty-one patients with CRT with non-ischemic systolic HF (8) were consecutively recruited for our research from October 2016 to August 2020 at the First Affiliated Hospital of Nanjing Medical University. Patients enrolled in our study received both resting Single Photon Emission Computed Tomography (SPECT) myocardial perfusion imaging (MPI) and dual-source multi-detector row CT scans. In our study, the CRT indications were as follows: (1) sinus rhythm; (2) left ventricular ejection fraction (LVEF) $\leq$35%; (3) New York Heart Association (NYHA) functional class from II to IV; (4) completed left bundle branch block (LBBB) morphology; and (5) optimal medical treatment for HF at least 3 months before implantation. Individuals who had AF, right bundle branch block, or right ventricular pacing upgradation were excluded. Coronary artery disease (CAD) was excluded by heart CT scan, and all patients had stenosis of $<$50% in the epicardial coronary artery. This study protocol was approved by the Regional Ethics Committee (the First Affiliated Hospital of Nanjing Medical University), and written informed consent was obtained for enrolled patients.

Electrocardiography and Echocardiography

An electrocardiogram (ECG) was acquired by researchers during hospitalization. The QRS duration in 12-lead ECG was analyzed from the widest QRS complex.

Left ventricular diameter parameters and diastolic function were measured by two experienced imaging-specialized experts according to standard transthoracic echocardiography. LV parameters included left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and LVEF. The diastolic function included mitral inflow E velocity to tissue Doppler e' velocity ratio (E/e') and mitral inflow E velocity to mitral inflow A velocity ratio (E/A). Both experts were blinded to the other clinical data during the research. The biplane-modified Simpson method was used to record the LVEF.

Gated Myocardial Perfusion SPECT

The resting ECG-gated SPECT MPI scan was completed in patients before implantation. Approximately 20–30 mCi of Tc-99m sestamibi was injected, and the MPI scan was continued 60 min after injection. The MPI images were assessed by a dual-headed camera using a routine protocol (CardioMD, Philips Medical Systems, Amsterdam, Netherlands). Reconstruction and reorientation to MPI images were performed using the Emory Toolbox (Syntermed, Atlanta, GA, USA).

The resulting short-axis MPI images were performed to evaluate the LV contour parameters by inputting them into an interactive tool. These results in each cardiac frame were then put into an automatic myocardial sampling algorithm for maximal count circumferential profiles. Subsequently, the onset contraction of the left ventricle was acquired from a first-harmonic Fourier approximation (9). Global LV was measured according to the phase standard deviation (PSD) and phase histogram bandwidth (PBW) from the phase analysis. The mechanical dyssynchrony value (two SDs above the mean) was defined as systolic PSD $= 36.5^{\circ}$ and systolic PBW $= 159.6^{\circ}$.

CT Scan

All heart CT scans were acquired from a dual-source multi-detector-row scanner (Somatom Definition, Siemens Medical Solutions, Forchheim, Germany). CT scan data were as follows: detector collimation, 64 $\times$ 0.6 mm; gantry rotation time, 330 ms; pitch, 0.2–0.43 (adapted to the heartbeat); temporal resolution, 83 ms; tube current, 380–420 mAs per rotation; and tube potential, 100–120 kV depending on the body mass index (BMI). To minimize motion artifacts, all patients were requested to hold their breath during detection. We synchronized the data reconstruction by a retrospective gating technique according to the ECG signal in all cardiac phases at 40% of the left atrial volume max at a total slice thickness of 0.75 mm and a reconstruction increment of 0.4 mm. All EAT data were measured by one experienced radiologist, and the best diastolic phase images of all the patients were selected for analysis. The adipose tissue between the surface of the myocardium and epicardium was defined as EAT, and the EAT volume was automatically acquired by applying cardiac risk assessment software to the cardiac image (10) (**Figure 1A**). The EAT

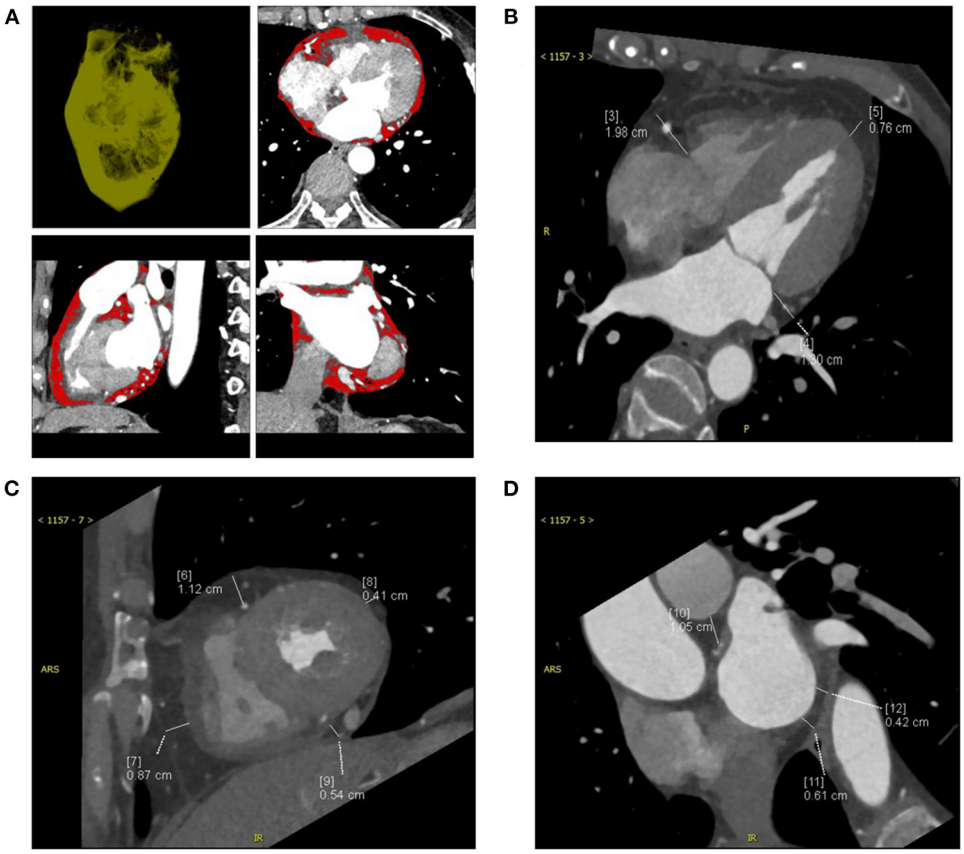

FIGURE 1 | The computed tomography (CT) measurements of a patient with CRT-responsive participating in this study were shown and epicardial adipose tissue (EAT) was measured by multidetector CT. **(A)** Contrast-enhanced CT scan presenting the region of interest (ROI) and pixels attributed to epicardial adipose tissue (red areas). **(B)** EAT thickness was measured on the parasternal short-axis view. **(C)** EAT thickness was measured on the horizontal long-axis view. **(D)** EAT area was measured at the left main coronary artery level. CRT, cardiac resynchronization therapy.

thickness in grooved segments was calculated in the horizontal long-axis plane (right atrioventricular (AV) groove and left AV groove), and the apical EAT thickness was also calculated using this image (10) (**Figure 1B**). The mean EAT thicknesses of the right ventricle (RV), lateral wall, and anterior wall were measured at two points on a parasternal short-axis view along the right ventricular (RV) free wall (**Figure 1C**). The EAT thickness over the lateral wall of the LV and posterior wall of the ventricle was also obtained on the parasternal short-axis view (11). The mean EAT thickness was calculated according to the lateral and posterior walls of the ventricle myocardial surface to the pericardium (**Figure 1D**).

CRT Implantation

All enrolled individuals underwent CRT in standard procedures. The LV lead was steadily placed in one branch vein, and the right ventricular (RV) lead was positioned in the RV apex as determined by trained electrophysiologists following standard implantation guidelines. The final position was determined based on three factors: no phrenic nerve capture, good stability, and an acceptable pacing threshold. Finally, fluoroscopic venograms were used to assess the LV lead position implantation in the left anterior oblique 45° and right anterior oblique 30° regions.

Follow-Up After CRT

Follow-up data of all the patients were collected from telephone interviews, hospital discharge summaries, and government records. The primary end point was the CRT response at 6 months after CRT implantation, which was defined as an improvement of 5% in the LVEF. All-cause mortality was the secondary end point in the whole study. Atrial arrhythmias and ventricular pacing rate during follow-up were detected and extracted from the device system. According to the follow-up data, patients were divided into two groups: the CRT response group and the CRT non-response group.

Statistical Analysis

Data were analyzed with SPSS 21 (IBM, Chicago, IL, USA). Categorical variables were summarized as counts or percentages. Continuous variables are presented as the mean ± SD. The Pearson linear correlation coefficient was used to determine whether two variables were linearly related. Baseline clinical variables associated with the CRT response were used in univariate and multivariate logistic regression analyses. All variables with $p < 0.10$ in the univariate logistic regression analysis were included in the multivariate logistic regression analysis. A receiver operating characteristic (ROC) curve

TABLE 1 | Baseline characteristics and left ventricular (LV) parameters of the enrolled patients.

Baseline characteristic	All ($n = 41$)	Non-responders ($n = 17$)	Responders ($n = 24$)	*p*-value
Age (years)	64.7 ± 10.5	65.1 ± 12.2	64.5 ± 9.4	0.860
Male (*n*, %)	31 (75.6%)	13 (76.5%)	18 (75.0%)	0.915
Hypertension	30 (73.2%)	14 (41.1%)	16 (32.0%)	0.129
Diabetes	15 (36.6%)	7 (21.0%)	8 (23.5%)	0.756
BMI (kg/m^2)	25.1 ± 2.5	25.3 ± 2.6	25.0 ± 2.4	0.672
QRS duration (ms)	171.1 ± 19.6	162.4 ± 24.6	177.3 ± 12.2	0.014
NT-proBNP	3916.64 ± 3385.6	4337.0 ± 3063.8	3618.9 ± 3630.7	0.510
History of previous hospitalization (*n*)	1.3 ± 1.6	1.8 ± 2.0	1.0 ± 1.1	0.344
NYHA class II/III/IV	21/13/7	8/7/2	13/6/5	0.931
Medication				
Sacubitril/valsartan	18 (43.9%)	8 (47.1%)	10 (41.7%)	0.110
ACE inhibitors/ARBs	17 (44.5%)	5 (29.4%)	12 (55.0%)	0.193
β-blockers	37 (90.2%)	14 (82.4%)	23 (95.8%)	0.157
Diuretics	37 (90.2%)	16 (94.1%)	21 (87.5%)	0.487
Aldosterone blocker	37 (90.2%)	16 (94.1%)	21 (87.5%)	0.487
LV parameters				
LVEDD (mm)	68.7 ± 7.6	70.9 ± 6.6	67.0 ± 8.0	0.108
LVESD (mm)	58.3 ± 7.9	60.7 ± 6.6	56.6 ± 8.3	0.102
E/e'	16.1 ± 6.5	15.7 ± 5.0	16.4 ± 7.4	0.743
E/A	1.2 ± 0.7	1.4 ± 1.0	1.0 ± 0.5	0.089
LVEF (%)	29.0 ± 8.5	30.8 ± 10.8	27.7 ± 6.4	0.268
Scar burden	29.4 ± 12.0	33.2 ± 14.0	26.8 ± 9.8	0.090
TPD	12.5 ± 9.7	16.2 ± 12.8	10.0 ± 5.8	0.042
Systolic PSD (°)	37.3 ± 18.3	44.4 ± 19.5	32.3 ± 15.8	0.034
Systolic PBW (°)	141.3 ± 79.8	174.6 ± 89.7	117.7 ± 63.8	0.022
EAT measurements				
Total volume EAT (ml)	101.2 ± 49.3	104.8 ± 55.0	98.7 ± 45.8	0.699
Anterior wall (mm)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.966
Inferior wall (mm)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.946
Left free wall (mm)	0.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	0.420
Right free wall (mm)	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.109
Ventricle apex (mm)	0.4 ± 0.2	0.5 ± 0.2	0.3 ± 0.2	0.039
Right AV groove (mm)	1.6 ± 0.5	1.8 ± 0.5	1.5 ± 0.4	0.022
Left AV groove (mm)	1.2 ± 0.3	1.4 ± 0.3	1.1 ± 0.1	0.020

Data are expressed as the mean ± SD or number (percentage); BMI, body mass index; NT-proBNP, N-terminal pro-natriuretic brain natriuretic peptide; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blocker; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; E/e', mitral inflow E velocity to tissue Doppler e' velocity ratio; E/A, mitral inflow E velocity to mitral inflow A velocity ratio, LVEF, left ventricular ejection fraction; TPD, total perfusion deficit; PSD, phase standard deviation; PBW, phase histogram bandwidth; AV, atrial ventricular; EAT, epicardial adipose tissue.

analysis was performed for the EAT variables to predict the probability value for CRT. A Kaplan-Meier analysis was performed between two groups to compare the all-cause mortality by a log-rank test. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

Table 1 displays the baseline characteristics for 41 consecutive patients (31 male, 64.7 ± 10.5 years old). Differences were not observed in medical therapy before implantation between the two groups, and these interventions included diuretics (90.2%), an aldosterone blocker (90.2%), beta-blockers (90.2%), sacubitril/valsartan (43.9%), angiotensin II antagonists, or angiotensin-converting enzyme inhibitors (44.5%). The clinical characteristics were similar between the two groups in terms of sex, age, diabetes, hypertension, history of the previous hospitalization, NYHA class, BMI, N-terminal pro-natriuretic brain natriuretic peptide (NT-proBNP), LVEDD, LVESD, E/e', E/A, and LVEF ($p > 0.05$). However, in the non-response group, there was a narrower QRS duration and more total perfusion deficit (TPD) in the left ventricle than in the response group ($p < 0.05$). Meanwhile, the systolic PSD and

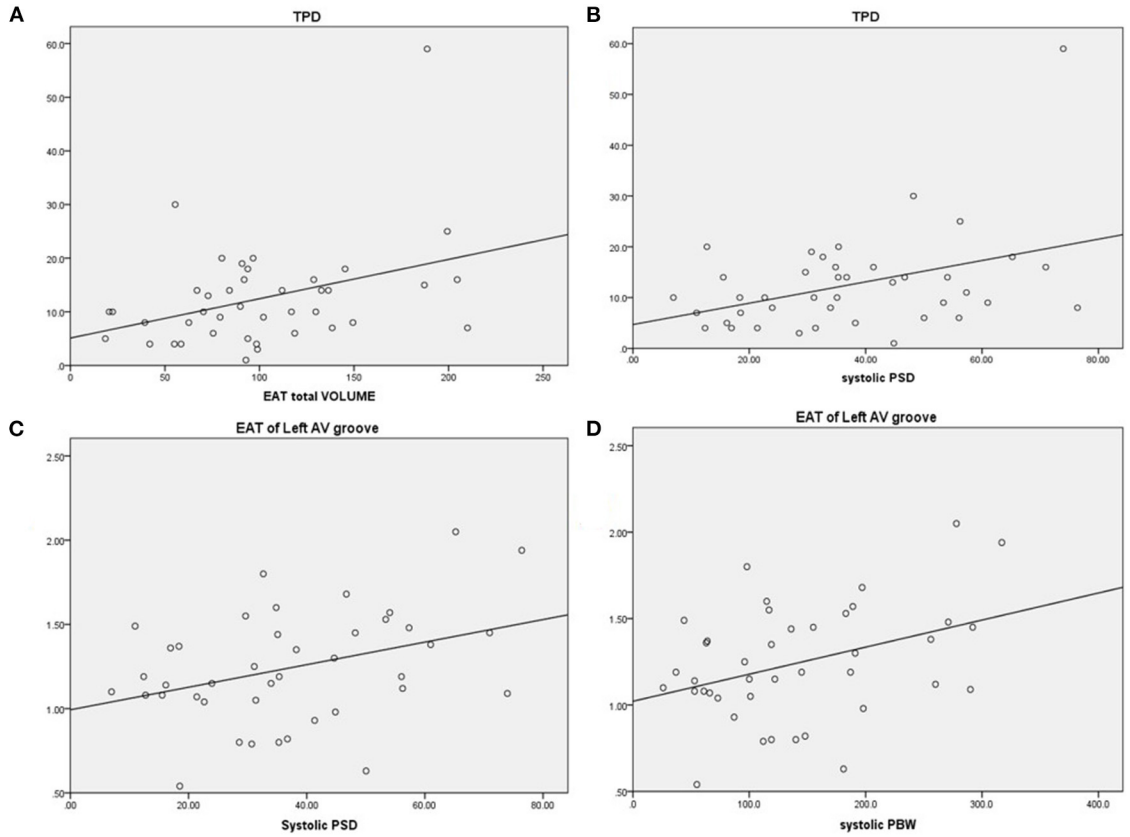

FIGURE 2 | Correlations between EAT and LV parameters. **(A)** Total perfusion deficit (TPD) was correlated with greater EAT, $r = 0.371$, $p = 0.017$. **(B)** TPD was correlated with greater systolic PSD, $r = 0.395$, $p = 0.011$. **(C)** EAT of the left AV groove was correlated with greater systolic PSD, $r = 0.367$, $p = 0.018$. **(D)** EAT of the left AV groove was correlated with greater systolic PBW, $r = 0.376$, $p = 0.016$. EAT, epicardial adipose tissue; AV, atrioventricular; PSD, phase standard deviation; PBW, phase histogram bandwidth.

systolic PBW were statistically greater in the CRT group with no response than in the response group ($p < 0.05$, for both).

In addition to the total EAT thickness values, the EAT thickness over the LV lateral wall, LV anterior wall, LV inferior wall, and the right lateral wall did not differ between subjects with and without a CRT response ($p > 0.05$ for all). Notably, EAT thickness values in the right AV groove, LV apex, and left AV groove were lower in the CRT response group than the CRT group with no response ($p < 0.05$, for all). **Figure 2** shows the relationship between the EAT volume and the LV parameters in bivariate correlation analysis. The total EAT volume was significantly correlated with the TPD levels ($r = 0.371$, $p = 0.017$). Meanwhile, a positive relationship was observed between the TPD and systolic SD levels ($r = 0.395$, $p = 0.011$). The EAT thickness of the left AV groove was positively correlated with systolic SD and systolic BW values ($r = 0.367$, $p = 0.018$; $r = 0.376$, $p = 0.016$, respectively).

Prediction of CRT Response

In total, 24 patients (58.5%) were CRT responders. LV leads were implanted in lateral, anterolateral, posterior, or posterolateral coronary veins according to the anatomic characteristics of coronary veins. In the univariate logistic regression analysis, the CRT response was significantly associated with the TPD, systolic PSD, systolic PBW, QRS duration, EAT thicknesses of LV apex, right AV groove, and left AV groove (**Table 2**). When multivariate logistic regression models were performed to test the EAT thickness parameters (LV apex, right AV groove, and left AV groove) separately, the right AV groove, left AV groove, and the QRS duration were independent predictors for the CRT response (**Table 3**). In the ROC analysis of the EAT thickness of the right AV groove, the area under the ROC curve (AUC), optimal cut off value, specificity, and sensitivity were 0.691, 1.84, 0.833, and 0.529, respectively ($p = 0.039$); meanwhile, in the ROC analysis of the EAT thickness of the left AV groove, the AUC, optimal cut off value, specificity, and sensitivity were 0.687, 1.375, 0.792, and 0.529, respectively ($p = 0.043$). According to the ROC analysis of the EAT thickness of the LV apex, the AUC, optimal cut off value, specificity, and sensitivity were 0.695, 0.415, 0.708, and 0.647, respectively ($p = 0.035$; **Figure 3**).

Follow-Up After CRT

Over the entire period of 21.5 months (IQR 6–59 months), three (7.3%) patients died of all-cause mortality, and they were all were included in the CRT non-response group ($p = 0.089$).

TABLE 2 | Univariate logistic regression models for cardiac resynchronization therapy (CRT) response.

Variables	*P*-value	Hazard Ratio	95% CI
QRS duration	0.028	1.050	1.005–1.096
Age (years)	0.856	0.994	0.937–1.056
Male	0.914	1.083	0.254–4.630
Hypertension	0.129	0.366	0.100–1.339
Diabetes	0.754	0.800	0.199–3.223
BMI (kg/m^2)	0.663	0.945	0.734–1.218
LVEDD (mm)	0.117	0.929	0.847–1.019
LVESD (mm)	0.109	0.930	0.851–1.016
E/e'	0.736	1.017	0.921–1.123
E/A	0.115	0.441	0.159–1.220
LVEF (%)	0.274	0.957	0.885–1.035
Total volume EAT (ml)	0.690	0.997	0.985–1.010
Anterior wall (mm)	0.965	1.069	0.055–20.867
Inferior wall (mm)	0.944	0.847	0.008–89.034
Left free wall (mm)	0.411	0.195	0.004–9.641
Right free wall (mm)	0.127	0.090	0.004–1.973
Ventricle apex EAT	0.050	0.028	0.001–1.000
Right AV groove EAT	0.032	0.187	0.041–0.865
Left AV groove EAT	0.031	0.079	0.008–0.792
Scar burden	0.105	0.952	0.897–1.010
TPD	0.073	0.907	0.816–1.009
Systolic PSD	0.042	0.961	0.924–0.999
Systolic PBW	0.031	0.990	0.981–0.999

CI, confidence interval; BMI, body mass index; NT-proBNP, N-terminal pro-natriuretic brain natriuretic peptide; ACE, angiotensin-converting enzyme; ARBs, angiotensin II receptor blocker; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; E/e', mitral inflow E velocity to tissue Doppler e' velocity ratio; E/A, mitral inflow E velocity to mitral inflow A velocity ratio; LVEF, left ventricular ejection fraction; TPD, total perfusion deficit; PSD, phase standard deviation; PBW, phase histogram bandwidth; AV, atrial ventricular; EAT, epicardial adipose tissue.

TABLE 3 | Multivariate logistic regression models for CRT response.

	Wald	*p*-value	Hazard Ratio (95% CI)	95% CI
Model 1-with-ventricle apex EAT				
Dyssynchrony	0.092	0.762	0.779	0.155–3.913
QRS duration	4.762	0.029	1.051	1.005–1.100
TPD	0.931	0.335	0.947	0.848–1.058
Ventricle apex EAT	2.851	0.091	0.013	0.000–2.007
Model 2-with right AV groove EAT				
Dyssynchrony	0.188	0.664	1.431	0.284–7.219
QRS duration	2.594	0.107	1.037	0.992–1.084
TPD	3.184	0.074	0.898	0.799–1.011
Right AV groove EAT	4.075	0.044	0.156	0.026–0.948
Model 3-with left AV groove EAT				
Dyssynchrony	0.290	0.590	1.571	0.304–8.118
QRS duration	3.422	0.064	1.041	0.998–1.085
TPD	2.363	0.124	0.915	0.817–1.025
Left AV groove EAT	2.947	0.086	0.089	0.006–1.409

TPD, total perfusion deficit; PSD, phase standard deviation; PBW, phase histogram bandwidth; AV, atrial ventricle; EAT, Epicardial adipose tissue.

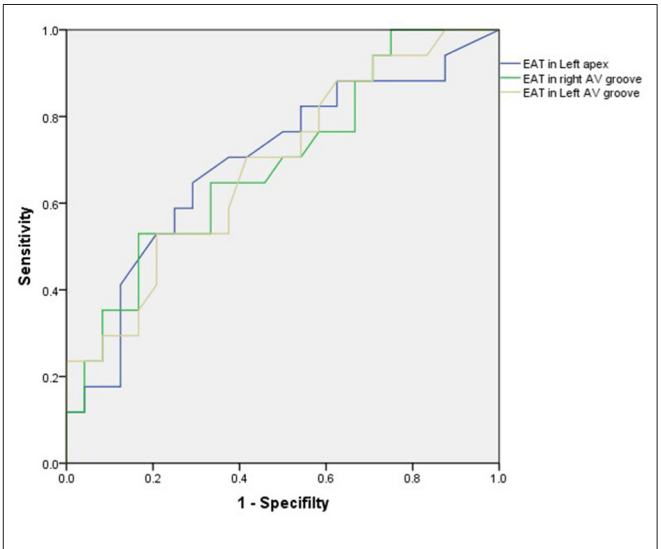

FIGURE 3 | In the ROC analysis of the EAT thickness of the right AV groove, the area under the ROC curve (AUC), optimal cut off value, specificity, and sensitivity were 0.691, 1.84, 0.833, and 0.529, respectively ($p = 0.039$); meanwhile, in the ROC analysis of the EAT thickness of the left AV groove, the AUC, optimal cut off value, specificity and sensitivity were 0.687, 1.375, 0.792, and 0.529, respectively ($p = 0.043$). In the ROC analysis of the EAT thickness in the LV apex, the AUC, optimal cut off value, specificity, and sensitivity were 0.695, 0.415, 0.708, and 0.647, respectively ($p = 0.035$). AV, atrioventricular; EAT, epicardial adipose tissue; LV, left ventricular.

There was no significant difference in the number of new AF during follow-up in the response group and the no response group (2 vs. 3, $p = 0.375$). Meanwhile, the ventricular pacing rates were 94.5 and 91.6% in the response group and the no response group ($p = 0.061$), respectively. Meanwhile, the LVEF was significantly increased compared with the baseline (29.0 ± 8.5 vs. 40.6 ± 14.4%, $p < 0.001$). In the Kaplan-Meier survival analysis, the CRT response group had a better prognosis in the long term than the non-response group (log-rank $\chi^2 = 3.971$, $p = 0.046$; **Figure 4**).

DISCUSSION

The main findings of our study are as follows: (1) the EAT thickness of the left AV groove is associated with total defect perfusion of the left ventricle and LV systolic synchrony in patients with non-ischemic systolic HF; and (2) the EAT thickness of the AV groove is predictive of CRT response at 6 months in patients with non-ischemic systolic HF.

The Clinical Explanation for the Lack of CRT Response

The CRT value has been widely evaluated in patients with HF in a large number of clinical trials. Despite moderate improvement over the past two decades, the CRT response is still rather mixed, and approximately one-third of patients with HF do not show

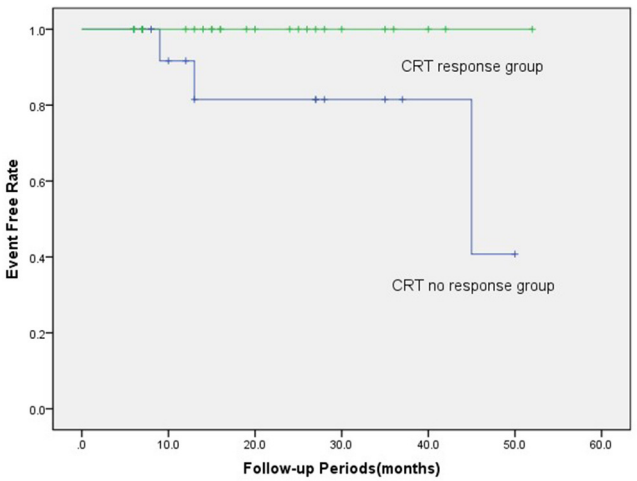

FIGURE 4 | In the Kaplan-Meier survival analyses, the CRT response group had a better prognosis than the CRT non-response group in terms of all-cause mortality (log-rank $\chi^2 = 3.971$, $p = 0.046$). There were three (7.3%) patients who died from all-cause mortality, which all were included in the CRT non-response group. CRT, cardiac resynchronization therapy.

benefits from CRT implantation. Therefore, it is imperative to enhance CRT benefits by patient selection, LV lead placement optimization, post-implantation device programs, and patient management. Numerous attempts have already been made to find reliable parameters to improve the CRT response. Different studies have reported the significance of LV myocardial scars in CRT non-response (12, 13). In addition, other studies have found that the CRT response was lower in patients with ischemic HF than in non-ischemic patients with HF because of the extensive burden on the left ventricle of scarred myocardium (14). Additionally, other researchers (15) reported that extensive scar burden in patients with CRT led to worse clinical outcomes and less improvement in LVEF. Compared with earlier findings, however, Riedlbauchova et al. (16) reported that the extent of scar burden was not predictive of CRT response or mortality, and they reported that the scar burden was higher than 40% in all groups, which appears to be much higher than that in a previous report (17, 18). In our study, a trend toward higher scar burdens was observed in the CRT non-response group, although the difference was not significant. In addition, the total deficit perfusion of the left ventricle in the CRT non-response group was lower than that in the CRT response group. However, the scar burden and TPD were not predictive of CRT response in our research. One explanation is that the higher myocardial scar burden might weaken the predictive value of these parameters in CRT (16).

In accordance with previous results, our study also confirmed that a wider QRS complex was a strong predictor of CRT response (19). When the EAT thickness parameters (LV apex, right AV groove, and left AV groove) were separately included in the multivariate logistic regression, a wider QRS complex remained an effective predictor of CRT implantation.

The use of LV dyssynchrony to predict benefits from CRT in patients with HF has been discussed in recent studies. Several recent studies found that LV mechanical dyssynchrony could predict CRT response during follow-up (20, 21). However, some reports have provided contradictory conclusions about the value of mechanical dyssynchrony in predicting CRT response, and they suggested that the baseline dyssynchrony parameters did not have a positive predictive value for CRT response in relatively large samples (22, 23). Nevertheless, our previous study found that baseline mechanical dyssynchrony from gated MPI was a significant independent predictive factor for CRT response in patients with non-ischemic systolic HF. In the present study, dyssynchrony parameters in both the PSD and PBW were significantly different in the CRT non-response groups. Meanwhile, the mechanical dyssynchrony parameters of both systolic PSD and systolic PBW were significantly associated with CRT response in the univariate logistic regression analysis. However, the mechanical dyssynchrony parameters were not significant independent predictors for CRT response in the multivariate logistic regression models. A reasonable explanation is that our population is relatively small compared with that in the previous study (3).

Significance of the EAT in CRT

A novel finding of our work is that the EAT thickness of the AV groove has predictive value for CRT implementation in patients with non-ischemic systolic HF. This study is the first to relate the volumes and distribution of EAT to CRT response. In a previous study, EAT was found to be a directly adjacent tissue to the cardiac myocardium, which is a vital regulator of the energy needs of the heart myocardium in lipid fluxes (24). However, expanding EAT becomes hypoxic and dysfunctional in cardiovascular disease related to metabolic processes, which would cause a reduction in protective cytokines, accumulation of detrimental adipocytokines, and extensive fibrosis in the myocardium (25). Up to now, the correlation of EAT with LV function is still inconsistent (4, 26, 27). A meta-analysis reported that EAT is associated with diastolic function, independent of other influential variables (28). While EAT is an effect modifier for chamber size but not systolic function. Meanwhile, van Woerden (29) revealed that the volume of EAT in patients with HF was larger than that in the controls despite a similar BMI in recent years. Furthermore, the EAT volume was associated with AF, type 2 diabetes mellitus, and myocardial injury-related biomarkers. Wu et al. (4) concluded that EAT was closely linked with the extent of myocardial fibrosis and heart dysfunction in the pathophysiology of HF. Moreover, Maimaituxun et al. (30) found that localized EAT (AV groove and left free wall) was strongly associated with LV function in preserved patients with LVEF. In our results, the EAT thickness of the left AV groove was associated with total defect perfusion of the left ventricle as measured by SPECT, and it was influenced by increases in the severity of ischemia or global fibrosis (31, 32). We further investigated the parameters of the whole heart (total volume EAT) and localized EAT (left anterior wall, inferior wall, left free wall, right free wall, apex, right AV groove, and left AV groove) in patients with non-ischemic systolic HF. The total

volume EAT was not associated with LVEF, and LV scar burden in patients with non-ischemic systolic HF and the volume of EAT was similar between the two groups. However, the thickness of the localized EAT (LV apex, right AV groove, and left AV groove) was higher in the non-response group. This conclusion is also consistent with a previous study which concluded that regional EAT is associated with alterations in local cardiac structure and function (33). More importantly, the right AV groove and left AV groove were both independent predictors of CRT response. This finding suggested that localized EAT in the right AV groove and left AV groove is related to cardiac resynchronization and thus to the CRT response. Furthermore, the strong link between localized EAT in the left AV groove and systolic LVMD (SD and BW) further clarified that evaluation of localized EAT would alter the dilemma of CRT response.

Nevertheless, the potential mechanisms between EAT and CRT response remain to be elucidated, which might partly be attributed to mechanical and paracrine processes. The physical boundary in anatomy between the EAT and ventricular myocardium is not obvious and shares the same coronary microcirculation. Therefore, factors contributed by EAT could have direct vasocrine and paracrine effects on the ventricular myocardium, which worsen myocardial fibrosis by localized EAT accumulation (34). Previous studies on EAT may explain its value in predicting CRT response. Reasonable speculation is that EAT accumulation affects myocardial fibrosis, which influences CRT response [35], especially in the LV lead position (lateral wall or posterior lateral wall). Meanwhile, localized EAT accumulation around the right AV groove and left AV groove is directly related to impaired motion (30), such as, mechanical dyssynchrony, which was widely accepted corresponding to CRT response. On the other hand, EAT is a relevant electrophysiological factor in CRT patients that may interfere with pacing and sensing (7). Meanwhile, greater EAT thickness might increase under LV lead sensing and reduce battery longevity. Therefore, the evaluation of EAT might be a novel factor for predicting the CRT response rate and selecting patients for CRT implantation. Furthermore, EAT has the potential for therapeutic use in sodium dependent glucose transporters 2 (SGLT-2) inhibitor treatment to improve the CRT response rate.

LIMITATION

First, the samples in our retrospective study were relatively small, which was a disadvantage for identifying the predictive factors for CRT response in HF. While, the present retrospective study included patients with non-ischemic systolic HF, undergoing CRT therapy, and receiving ECT and contrast-enhanced cardiac CT scans. So far, there was limited data about the association between EATs and CRT response. Second, the definition of CRT non-response was based on echocardiographic results of LVEF to measure the primary end point. We did not investigate other clinical factors, such as the 6-min walking test or quality of life. Third, the follow-up period was rather short for post-CRT implantation in the current research. Fourth, EAT parameters were limited in all patients with CRT, and our sample was not compared with an adequate control group. Finally, this study was still preliminary, and further research should be undertaken in a larger population.

CONCLUSION

The EAT thickness of the left AV groove is associated with total defect perfusion of the left ventricle and LV systolic synchrony in patients with non-ischemic systolic HF. The EAT thickness of the AV groove has predictive value for CRT response in patients with non-ischemic systolic 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 Ethical Committee of the First Affiliated Hospital of Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

H-yQ and CW performed the clinical study and drafted the original manuscript. H-yQ and D-dQ enrolled the patients and analyzed all of the clinical data. CC and M-lC reviewed and edited the manuscript. All authors contributed to the whole article and approved the final version of this manuscript.

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Hemodynamic Changes During Physiological and Pharmacological Stress Testing in Patients With Heart Failure: A Systematic Review and Meta-Analysis

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Although disease etiologies differ, heart failure patients with preserved and reduced ejection fraction (HFpEF and HFrEF, respectively) both present with clinical symptoms when under stress and impaired exercise capacity. The extent to which the adaptation of heart rate (HR), stroke volume (SV), and cardiac output (CO) under stress conditions is altered can be quantified by stress testing in conjunction with imaging methods and may help to detect the diminishment in a patient's condition early. The aim of this meta-analysis was to quantify hemodynamic changes during physiological and pharmacological stress testing in patients with HF. A systematic literature search (PROSPERO 2020:CRD42020161212) in MEDLINE was conducted to assess hemodynamic changes under dynamic and pharmacological stress testing at different stress intensities in HFpEF and HFrEF patients. Pooled mean changes were estimated using a random effects model. Altogether, 140 study arms with 7,248 exercise tests were analyzed. High-intensity dynamic stress testing represented 73% of these data (70 study arms with 5,318 exercise tests), where: HR increased by 45.69 bpm (95% CI 44.51–46.88; I^2 = 98.4%), SV by 13.49 ml (95% CI 6.87–20.10; I^2 = 68.5%), and CO by 3.41 L/min (95% CI 2.86–3.95; I^2 = 86.3%). No significant differences between HFrEF and HFpEF groups were found. Despite the limited availability of comparative studies, these reference values can help to estimate the expected hemodynamic responses in patients with HF. No differences in chronotropic reactions, changes in SV, or CO were

found between HFrEF and HFpEF. When compared to healthy individuals, exercise tolerance, as well as associated HR and CO changes under moderate-high dynamic stress, was substantially impaired in both HF groups. This may contribute to a better disease understanding, future study planning, and patient-specific predictive models.

Systematic Review Registration: [https://www.crd.york.ac.uk/prospero/], identifier [CRD42020161212].

Keywords: heart failure, stress testing, meta-analysis, HFpEF—heart failure with preserved ejection fraction, HFrEF—heart failure with reduced ejection fraction, physiologic changes, exercise testing (in) heart failure

INTRODUCTION

Mortality rates and the frequency of symptom deterioration requiring hospitalization are nearly identical between heart failure (HF) with reduced (HFrEF) and preserved ejection fraction (HFpEF) (1). Both tend to have an impaired exercise capacity causing symptoms with exertion. An impaired contractile reserve and left ventricular remodelng are the key characteristics of HFrEF (2). The origins of HFrEF comprise a broad spectrum of etiologies, of which hypertension and ischemic heart disease are the leading causes (3). Almost half of all patients suffering from HF have a preserved ejection fraction (HFpEF) (4). These individuals are typically older, more often female, diabetic, and obese, and more frequently present with renal disease and arterial hypertension combined with left ventricular hypertrophy (5, 6). HFpEF is characterized by an impaired diastolic function accompanied by vascular changes resulting in an abnormal ventricular-arterial coupling (7). As poor functional capacity reduces the quality of life and indicates a worse prognosis in both groups of HF (8), the objective quantification of exercise intolerance is of importance, especially when symptoms occur (9).

The extent to which reduced cardiac output (CO) limits exercise tolerance can be quantified by different forms of cardiac stress testing, using dynamic and pharmacological, as well as isometric stress. Combining these tools with imaging methods, such as cardiac magnetic resonance imaging (MRI) or echocardiography, allows for the analysis of hemodynamic parameters. While the diagnosis of HFrEF is unequivocal, identifying patients with HFpEF can be more challenging, especially when patients present in a stable condition, so that the diagnosis mainly relies on imaging parameters indirectly indicating elevated left ventricular filling pressures (10, 11). Computational models simulating physiological or non-physiological responses to stress have, therefore, become of interest in achieving a better understanding of both cardiovascular hemodynamic interactions and early detection (12). To develop and optimize such predictions in patients with HF, reliable and robust disease-specific reference data of hemodynamic responses are required.

We performed, therefore, a systematic analysis of the available literature that has assessed hemodynamic changes under stress testing in patients with HF. In addition to providing reference ranges for the expected changes during exercise testing, we explored the associations of these stress-induced changes to cardiovascular parameters at rest, as well as medical therapy profiles of the included studies.

MATERIALS AND METHODS

Search Strategy

A pre-established review protocol was used and registered in PROSPERO (CRD42020161212). The specific search included studies in which patients with HF performed dynamic, isometric, or pharmacological stress testing and where hemodynamic changes were assessed by MRI, ECG, or echocardiography. The search aspects are specified in the standardized scheme addressing patient population, interventions, comparators, outcomes, and study design (PICOS) in **Table 1**. Prior to our analysis, no meta-analysis has addressed this question in the HF patient population. Nevertheless, the study was built on previous study addressing this question in healthy controls (13). We conducted our search in MEDLINE (*via* PubMed) deploying pre-specified search items (**Supplementary Table 1**). No relevant deviations were found compared to an Embase query. The date of the final search was 29 February 2020.

Study Selection and Quality Assessment

If at least one of the parameters, such as heart rate (HR), stroke volume (SV), CO, or ejection fraction (EF), under resting and stress conditions was assessed in a human patient population,

TABLE 1 | The population, interventions, comparators, outcomes, and study design (PICOS) scheme.

PICOS	
Patient population	HFrEF patients undergoing stress testing combined with MRI, ECG, or echocardiography HFpEF patients undergoing stress testing combined with MRI, ECG, or echocardiography
Interventions	Dynamic exercise Dobutamine infusion Isometric exercise
Comparators	Resting state
Outcomes	Heart rate [bpm] Stroke volume [ml] Cardiac output [L/min] Ejection fraction [%]
Study design	Studies with or without a control group

studies were included. Any studies published before 1985 and publications that were not available in the English or German language, or which could not be accessed as full texts within the institutional subscriptions or the National Library license, were not considered. Studies that assessed stress conditions other than dynamic, isometric exercise, or dobutamine infusion as pharmacological stress were excluded. If less than 10 subjects were part of a study arm, these results were not included. Furthermore, we excluded review letters, comments, conference posters, and single case reports. According to these criteria, articles were screened on the title and abstract level before full texts were retrieved. Every cohort testing for several forms of stress on different intensity levels formed a separate study arm. Each article was reviewed by one reviewer (AW) before verification by a second reviewer (AB) was performed. In case of a disagreement, a third reviewer was involved in the review process (MK). Stepwise study assessment was guided by a modified version of the Downs and Black checklist (14). Studies were assessed for their reporting, external validity, internal validity, distribution, and adjustment for confounding variables, where appropriate as described previously (13) in more detail. Studies were categorized into low, moderate, and high quality based on their quality assessment scores.

Data Extraction

If available, means and standard deviations under resting and different forms of stress conditions were extracted and documented. If unable to provide information on the variance, such studies were excluded from the analysis. If studies provided indexed SV or the cardiac index and body surface area (BSA), then the absolute SV and CO would be calculated. Data extraction included information on the clinical characteristics of study cohorts, such as sex, age, BSA, body mass index (BMI), and the New York Heart Association (NYHA) functional classification. Comorbidities such as arterial hypertension, atrial fibrillation, diabetes mellitus, and coronary artery disease (CAD), as well as medication usage (beta blockers, angiotensin-converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARBs), and aldosterone antagonists), were also extracted. If available, information on cardiac resynchronization therapy (CRT) completed the baseline data. For further analyses and comparison of HFrEF or HFpEF patients with healthy subjects, we used results from a previously published analysis (13).

Intensity Classification

Studies in which dynamic stress testing was performed were categorized as light, moderate, or high intensity according to the intensity stated in watts (W) during ergometric exercise (assuming a body weight of 60–80 kg) (15), the percentage of age-specific maximal HR [HR_{max} = 220 − age (years)] (16), or the statement of the authors regarding the intensity level. In the case of incongruity between these three indicators, we complied with the statement of the study's authors for a final classification. Submaximal exercise capacity in patients with HF was commonly defined between 20 and 30 W (17–23), with load increments between 10 and 20 W and exhaustion above 30 W and was thus lower than in healthy subjects (17, 18, 24). This classification was applied for dynamic stress testing studies (**Table 2**).

TABLE 2 | Intensity levels of stress testing.

Intensity	Dynamic exercise	Dobutamine stress
Light	Ergometer: < 20 W* HR max: ≤ 54% Statement: Light	0–10 μg/kg/min
Moderate	Ergometer: 20–30 W* HR max: 55–84% Statement: Submaximal/moderate	11–20 μg/kg/min
High	Ergometer: > 30 W* HR max ≥ 85% Statement: Exhaustion/symptom-limited	> 20 μg/kg/min

**Submaximal exercise capacity in patients with HF was commonly defined between 20 and 30 W (17–23), with load increments between 10 and 20 W and exhaustion above 30 W and was thus lower than in healthy subjects (17, 18, 24). This classification was applied for dynamic stress testing studies in this table.*

We included studies that performed pharmacological stress testing using dobutamine. According to the well-established classifications, the intensity of pharmacological stress was categorized as light for a low-dose infusion of dobutamine of 0–10 μg/kg/min, as moderate for 11–20 μg/kg/min, and as high for a dose exceeding 20 μg/kg/min (25–28). Isometric stress tests were categorized as light intensity exercise tests, given that static contraction causes only a slight increase in HR or CO, mainly affecting mean arterial pressure and not being expected to reach the changes of higher levels of dynamic exercise (29). A summary of these criteria is illustrated in **Table 2**.

Heart Failure Classification

According to 2016 and in line with the 2021 ESC guidelines, patients were classified as individuals with HFpEF when left ventricular ejection fraction (LVEF) was ≥ 50% (30). The 2012 ESC guidelines defined HFrEF when LVEF is below 35%, whereas the more recent 2016 guidelines changed this definition to an LVEF below 40%, and the 2021 ESC guidelines further changed the definition to below or equal 40%. An LVEF of 35–50% was considered a "gray area" in the 2012 guidelines, whereas more recent guidelines define a new class of HF individuals with mid-range/mildly reduced ejection fraction (HFmrEF) when LVEF is 40–49% (31, 32). There was, therefore, an inhomogeneity of classification among the studies investigating stress testing in HFrEF before and after 2016. The definition and terminology of HF according to LVEF are displayed in **Table 3**. For reasons regarding the simplification of our analysis, patients with an LVEF < 50% were classified as HFrEF when no separation to HFmrEF was made.

Statistical Analysis

The analyses were executed in STATA, version 15.1 (StataCorp, College Station, Texas, United States), by using the "*metan*" package. A multivariate meta-regression model was used

TABLE 3 | Definition and terminology of heart failure (HF) related to left ventricular ejection fraction.

EF in%	< 40*	40–49	≥ 50
Classification according to ESC guidelines 2012 (31)	HFrEF (< 35%)	Gray area (35–50%)	HFpEF
Classification according to ESC guidelines 2016/2021* (32)	HFrEF	HFmrEF	HFpEF
Classification for analysis	HFrEF		HFpEF

**The 2021 ESC guidelines have changed the definition of HFrEF ≤ 40% and HFmrEF between 41 and 49. Classification for data analysis across studies from different time periods is shown in the bottom row.*

to determine variables that potentially influenced outcome parameters. Correlations were investigated through univariate meta-regression. Furthermore, a pairwise meta-analysis was conducted in studies directly comparing different types of stress. Otherwise, study arms were grouped according to stress type and stress level, with the aim to obtain pooled estimates of changes. Furthermore, results were analyzed separately for HFrEF and HFpEF patients. Mean differences of hemodynamic parameters between rest and stress conditions, with respective standard errors of the difference between means, were calculated (33). Outcomes were pooled using a DerSimonian-Laird random effects model (34).

Heterogeneity was assessed using the Q-statistic and with a visual inspection of forest plots for all interventions and outcomes. Between-study variation, due to true heterogeneity, was measured using the I^2 statistic (35), with values of 25% or higher indicating significant heterogeneity that supports the use of a random effects model (36, 37). Results are shown as absolute mean changes and with 95% confidence intervals (CIs) between resting and stress conditions, as well as a visualization in forest plots (**Supplementary Material**). A lack of overlap between the CIs of pooled changes indicated significant differences between the different stress types (37).

RESULTS

Study Characteristics

A total of 1,123 references were extracted from the database. Ten additional studies were obtained from further sources, mainly as they were referenced in other studies. After screening at the title and abstract level, 290 full-texts were extracted. Notably, 102 studies examining stress testing in HFpEF and HFrEF patients with a total of 158 study arms, 9,298 subjects, and 9,764 stress examinations could be retrieved after screening the full-texts. Of note, 7,248 stress examinations were considered for further analysis after eliminating studies in which HFpEF and HFrEF could not be clearly assigned (N = 9). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart (**Figure 1**), the network of evidence (**Supplementary Figure 1**), and the list of included studies (**Supplementary Table 2**) show details of the study selection process. Mean absolute changes for HR, SV, CO, and EF from single-arm studies are shown in **Supplementary**

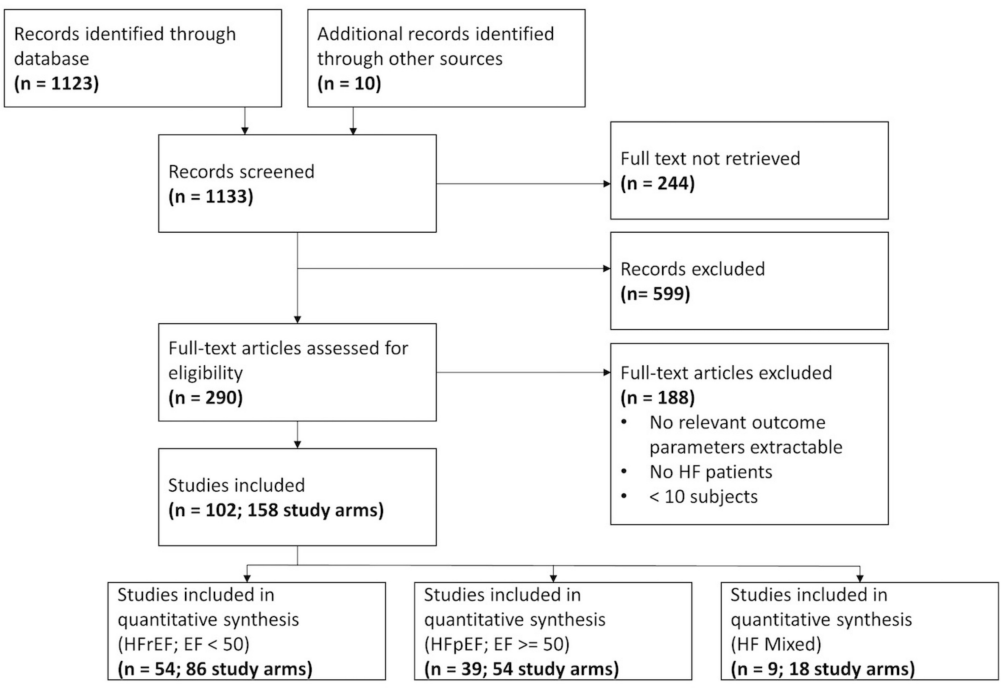

FIGURE 1 | Flow chart of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Table 3. The results of the quality assessment are shown in **Supplementary Table 4**.

Additional single study arms were included, resulting in a total of 114 study arms (5,920 stress examinations) for dynamic exercise testing, 25 study arms (1,308 stress examinations) for pharmacological stress testing, and 1 study arm (20 stress examinations) for isometric exercise testing in a pooled analysis. Only one study (3 study arms) was found to directly compare dynamic, pharmacological, and isometric stress testing in HF patients (38). Three studies (6 study arms) could be obtained that directly matched HFpEF and HFrEF individuals (39–41).

Baseline characteristics for all patients with HFpEF or HFrEF undergoing dynamic or dobutamine stress testing are listed in **Table 4**. In $N = 5$ study arms (3%), atrial fibrillation was defined as an exclusion criterion, and in $N = 15$ (9.4%), atrial fibrillation was reported with an average of 15%. Due to the low number of study arms reporting on a minority of subjects with atrial fibrillation, the parameter was excluded from further analysis. Due to a low number of study arms of individuals undergoing isometric stress tests ($N = 1$), these were not considered for further analysis. Baseline tables for those HF patients comparing dynamic and pharmacological stress testing are shown in **Supplementary Tables 5–7**. HFrEF patients were generally younger than the

TABLE 4 | Baseline characteristics for individuals with HF.

	HFrEF		HFpEF		Sign.
		Study arms reporting variable (N of tests)		Study arms reporting variable (N of tests)	*P*-value
Total N of stress tests		86 (5,027)		54 (2221)	
Age, years	62 (57–66)	86 (5,027)	67.2 (65–70)	54 (2,221)	0.0001
Male,%	80.28 (72.46–93.75)	86 (5,027)	37.46 (28.13–52.5)	54 (2,221)	0.0001
BSA, m^2	1.89 (1.86–1.94)	8 (399)	1.9 (1.71–1.99)	17 (652)	0.8613
BMI, kg/m^2	27 (26.4–28.5)	23 (1,164)	31 (29.8–33.6)	43 (1,774)	0.0001
NYHA class	2.48 (2.20–2.92)	72 (4,674)	2.27 (2.00–2.46)	30 (1,018)	0.0009
CRT,%	25.1 (12–100)	29 (1,954)			
ACE,%	82.61 (74–92)	51 (3,718)	44 (36–53)	17 (779)	0.0001
ARB,%	20 (13–26)	27 (2,338)	28 (19–33)	15 (686)	0.0275
Beta blockers,%	88 (78–93)	65 (3,866)	64 (44–71)	49 (2,086)	0.0001
Aldosterone antagonist,%	53.25 (48.1–69)	42 (3,194)	24 (9–26)	5 (171)	0.0007
Hypertension,%	42 (28–62)	30 (1,726)	80 (69–94)	48 (1,938)	0.0001
Diabetes mellitus,%	23 (17–33)	35 (1,769)	24.5 (15–36)	46 (2,059)	0.6644
CAD,%	56 (38–70)	49 (2,207)	19 (10–36)	22 (796)	0.0001
Resting HR, bpm	72 (69–78)	85 (4,925)	69 (67–75)	54 (2,221)	0.0219
Resting SV, ml	64.85 (55–82)	6 (201)	71 (65–74.1)	13 (409)	0.5686
Resting CO, L/min	3.9 (3.5–4.3)	19 (723)	5.1 (4.9–5.1)	47 (2,010)	0.0001
Resting EF,%	30.15 (26.5–35)	84 (4,958)	62 (60–63)	17 (596)	0.0001
Light intensity,%	3	3 (64)	4	2 (31)	
Moderate intensity,%	14	12 (597)	22	12 (455)	
High intensity,%	83	71 (4,366)	74	40 (1,735)	

Values are reported as medians (interquartile range). ACE, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin II Receptor Blockers; BMI, body mass index; BSA, body surface area; CAD, Coronary artery disease; CRT, cardiac resynchronization therapy; HR, heart rate; SV, stroke volume; CO, cardiac output; EF, ejection fraction.

HFpEF patients, with a lower BMI and a higher NYHA class, and were predominantly male, while HFpEF studies included more female patients. Within the dynamic exercise group of HFrEF, individuals tended to be both younger and with a better EF than the patients receiving dobutamine.

Pooled Effects of Rest-Stress Changes From Single-Arm Studies

We reported effect measures and, where multiple studies were available, pooled effects in (1) light intensity, (2) moderate intensity, and (3) high intensity:

1. Dynamic exercise with light intensity was reported in one study: Compared to resting baseline values, HR increased by 21 bpm (95% CI 0.84–41.16), SV by 40 ml (95% CI 22.03–57.97), and CO by 5.5 L/min (95% CI 3.45–7.55). Low-dose dobutamine infusion (5–10 μg/kg/min) resulted in the changes of HR by 8.9 bpm (95% CI 5.13–12.67; I^2 = 0.0%), SV 9 ml (95% CI –3.23 to 21.23; reported in one study), CO 0.97 L/min (95% CI 0.62–1.32; I^2 = 0.0%), and EF 4.65% (95% CI 2.2–7.11; I^2 = 0.0%). Pooled changes of isometric exercise were reported in one study: HR 7 bpm (95% CI –0.11 to 14.11), CO 0 L/min (95% CI –0.89 to 0.89), and –5% for EF (95% CI –8.51 to –1.49).
2. Within the moderate dynamic intensity group, pooled estimates of changes in HR were 21.23 bpm (95% CI 19.69–22.76; I^2 = 0.0), SV 6.02 ml (95% CI –0.9 to 12.94; I^2 = 67.0%), CO 1.83 L/min (95% CI 1.32–2.33; I^2 = 66.6%), and EF 4.59% (95% CI 1.08–8.11; I^2 = 0.0%). Moderate dosage of dobutamine infusion (11–20 μg/kg/min) resulted in HR changes of 18.3 bpm (95% CI 10.42–26.17; reported in one study), SV –0.61 ml (95% CI –29.02 to 27.81; I^2 = 88.8%), CO 1.65 L/min (95% CI 0.61–2.69; I^2 = 71.3%), and EF 6.06% (95% CI 3.23–8.89; I^2 = 82.5%).
3. High dynamic exercise increased HR by 45.69 bpm (95% CI 44.51–46.88; I^2 = 98.4%), SV by 13.49 ml (95% CI 6.87–20.10; I^2 = 68.5%), CO by 3.41 L/min (95% CI 2.86–3.95; I^2 = 86.3%), and EF by 3.69% (95% CI 2.49–4.89; I^2 = 52.9%). For high dosage of dobutamine infusion (11–20 μg/kg/min), changes in HR were 40.72 bpm (95% CI 33.93–47.50; I^2 = 92.7%), and changes in EF were 11.87% (95% CI 10.06–13.67; I^2 = 44.7%). There were not enough studies available investigating changes in SV and CO for high-intensity pharmacological stress testing. A detailed summary of all findings and a subgroup analysis for both HF groups is available in the **Supplementary Figures 2–4**.

Comparison Between HFrEF, HFpEF, and Healthy Subjects

We identified six categories in which changes in HR, SV, CO, or EF from single study arms could be compared between patients with HFpEF and HFrEF, where at least two studies were available for both disease groups at the same intensity level and stress type (marked in bold in **Table 5**). Those included (1) HR change by dynamic exercise at moderate intensity, (2) HR change by dynamic exercise at high intensity, (3) HR change by pharmacological exercise at high intensity, (4) SV increases by dynamic exercise at high intensity, (5) CO increases by dynamic exercise at high intensity, and (6) EF increases by dynamic exercise at high intensity.

For eligible studies, where at least two studies were available in each HF group, mean absolute changes and 95% CIs of HR, SV, CO, and EF of HF subjects, as well as in healthy controls (13), are visually summarized in **Figure 2**.

1. The changes in HR by moderate dynamic exercise in HFrEF patients were 20.02 bpm (95% CI 13.31–26.74; I^2 = 0.0), 21.29 bpm (95% CI 19.72–22.87; I^2 = 0.0) in HFpEF patients, and 49.57 bpm (95% CI 40.03–59.1; I^2 = 97.0%) in healthy controls.
2. By high dynamic exercise in HFrEF individuals, pooled estimates of changes in HR were 46.61 bpm (95% CI 45.22–48.01; I^2 = 98.8%), 45.02 bpm (95% CI 40.03–50.01; I^2 = 95.8%) for HFpEF patients, and 89.31 bpm (95% CI 81.46–97.17; I^2 = 97.6%) for healthy subjects.
3. High pharmacological stress in HFrEF patients resulted in changes in HR of 38.06 bpm (95% CI 30.36–45.76; I^2 = 93.1%), in HFpEF patients of 52.38 bpm (95% CI 43.56–61.20; I^2 = 74.8%), and in healthy subjects of 53.58 bpm (95% CI 36.53–70.64; I^2 = 98.4%).
4. High dynamic exercise in HFrEF subjects resulted in a change in SV of 12.04 ml (95% CI 7.19–16.90; I^2 = 0.0%), in HFpEF patients of 14.51 ml (95% CI 3.04–25.97; I^2 = 80.6%), and in healthy subjects of 21.31 ml (95% CI 13.42–29.21; I^2 = 91.1%).
5. High dynamic exercise in HFrEF patients resulted in changes in CO of 3.23 L/min (95% CI 2.56–3.89; I^2 = 87.9%), in HFpEF patients of 3.86 L/min (95% CI 2.82–4.89; I^2 = 84.0%), and in healthy subjects of 10.45 L/min (95% CI 8.04–12.85; I^2 = 98.9%).
6. High dynamic exercise in HFrEF patients increased EF by 3.79% (95% CI 2.56–5.03; I^2 = 55.6%) and by 1% (95% CI –4.59 to 6.59; I^2 = 0.0%) in HFpEF patients. There were no data available for changes in EF in healthy subjects.

High-intensity dynamic stress testing represented 73% of the data included for comparison. A detailed assessment of study heterogeneity and a comparison is found in **Supplementary Material**, including a visual representation as forest plots (**Supplementary Figures 5–31**).

Effects of Stress Type, Intensity Level, and Age on Stress-Induced Hemodynamic Changes

The results of a multivariable meta-regression model [$p < 0.001$, $F(6, 131) = 27.5$, adjusted R^2 = 57.91%] indicate that high-intensity level stress testing was associated with a greater absolute increase in HR, as compared to light intensity level stress testing (45.69 bpm vs. 21.0 bpm; Coef., 31.2; 95% CI 19.5–42.9; $p < 0.001$). Furthermore, age was associated with HR changes (Coef., –0.55; 95% CI –0.88 to –0.21; p = 0.002) within the combined model. No differences were found for

TABLE 5 | Available stress testing studies for HF individuals (number of stress tests).

		Light intensity		Moderate intensity		High intensity	
Parameter	**Stress type**	**HFrEF**	**HFpEF**	**HFrEF**	**HFpEF**	**HFrEF**	**HFpEF**
Heart rate change (HR)	Dynamic	0 studies	1 study (*N* = 11)	**2 studies** (*N* = 52)	**11 studies (*N* = 435)**	**38 studies (*N* = 3,671)**	**32 studies (*N* = 1,647)**
	Pharmacologic	2 studies (*N* = 64)	0 studies	7 studies (*N* = 545)	1 study (*N* = 20)	**8 studies (*N* = 591)**	**2 studies (*N* = 88)**
Stroke volume (SV)	Dynamic	0 studies	1 study (*N* = 11)	0 studies	7 studies (*N* = 250)	**3 studies (*N* = 131)**	**5 studies (*N* = 148)**
	Pharmacologic	1 study (*N* = 22)	0 studies	1 study (*N* = 46)	0 studies	0 studies	0 studies
Cardiac output (CO)	Dynamic	0 studies	1 study (*N* = 11)	0 studies	8 studies (*N* = 347)	**9 studies (*N* = 597)**	**6 studies (*N* = 198)**
	Pharmacologic	2 studies (*N* = 64)	0 studies	2 studies (*N* = 60)	1 study (*N* = 20)	0 studies	0 studies
Ejection fraction (EF)	Dynamic	0 studies	0 studies	0 studies	2 studies (*N* = 51)	**12 studies (*N* = 928)**	**2 studies (*N* = 37)**
	Pharmacologic	2 studies (*N* = 64)	0 studies	6 studies (*N* = 510)	1 study (*N* = 20)	5 studies (*N* = 346)	1 study (*N* = 47)

Studies can include multiple study arms. Six categories were identified for direct comparison where at least two studies were available for both disease groups (marked in bold).

moderate intensity compared to light intensity stress testing ($p = 0.112$) nor for pharmacological compared to dynamic stress testing ($p = 0.130$). Detailed results of the model are shown in **Supplementary Table 8**.

Pharmacological stress testing (Coef., 6.7; 95% CI 4.5–8.9; 95% CI 4.5–9; $p < 0.001$) and high-intensity level stress testing (Coef., 5.9; 95% CI 1.9–9.9; $p = 0.005$) were both associated with higher increases in EF under stress conditions [model $p < 0.001$, $F(6, 39)$ 10.26, adjusted $R^2 = 71.22\%$]. No associations of intensity level or stress type were found for SV or CO. HF group allocation (HFpEF or HFrEF) was not associated with stress-induced changes in HR, SV, CO, or EF. All subsequent analyses of HR, SV, and CO were performed across studies from both HF groups and all intervention types and included separation for different intensity levels.

Univariable meta-regression, stratified by intensity levels, showed an association with age and HR changes within the high-intensity study arm (Coef., –0.52; 95% CI –0.86 to –0.18; $p = 0.003$; Cons., 78.3; 95% CI 56.8–99.8). No other significant correlations between the age of patients within a study arm and changes in HR, changes in SV, or changes in CO were found ($p > 0.05$). HR changes for light intensity were $p = 0.734$, and those for moderate intensity were $p = 0.461$. SV changes for moderate intensity ($p = 0.680$) and high intensity ($p = 0.284$) were calculated, without data availability for light intensity. CO changes for light intensity were $p = 0.168$, those for moderate intensity were $p = 0.826$, and those for high intensity were $p = 0.565$. Graphical plots of the meta-regression models are shown in **Figure 3**.

Effects of Resting Conditions on Stress-Induced Hemodynamic Changes

Meta-regression did not show associations between the average resting HR and those reported HR changes during light ($p = 0.675$) and medium intensity ($p = 0.219$) stress testing. For high intensity, an inconclusive association was demonstrated (Coef., 0.31; 95% CI –0.004 to 0.630; $p = 0.053$; Cons., 22.6; 95% CI –0.6 to 45.8). Whereas no sufficient amount of studies was available to assess SV changes in light activity, there was a relevant inverse correlation between the reported average resting SV and SV changes during moderate-intensity stress testing (Coef., –1.3; 95% CI –2.6 to –0.04; $p = 0.044$; Cons., 98.3; 95% CI 7.0–189.7). No such correlation was found for SV changes during high-intensity stress testing.

Resting CO was not associated with CO changes under light-intensity ($p = 0.476$) and moderate-intensity ($p = 0.625$) stress testing but was correlated during high activity (Coef., 1.12; 95% CI 0.14–2.1; $p = 0.027$; Cons., –1.3; 95% CI –0.5 to 2.8). The results of these meta-regression analyses are illustrated in **Figure 4**.

Effects of Reported Treatment on Stress-Induced Hemodynamic Changes and Resting Conditions

Associations between stress testing-induced changes in hemodynamic parameters and reported treatment were analyzed for HR changes due to an insufficient amount of studies having reported data on treatment for SV and CO. No associations between reported treatment and HR changes were found for light- and moderate-intensity stress levels for either HFrEF or HFpEF patients.

Meta-regression models indicated an association between the proportion of patients taking ACEi and the stress testing-induced changes in HR among studies reporting data for HFrEF patients tested at high-intensity levels (Coef., 0.30; 95% CI 0.03–0.56; $p = 0.028$; Cons., 20.71; 95% CI –1.1 to 42.5; **Figure 5**). No such effects were seen in HFpEF patients. In patients with HFpEF,

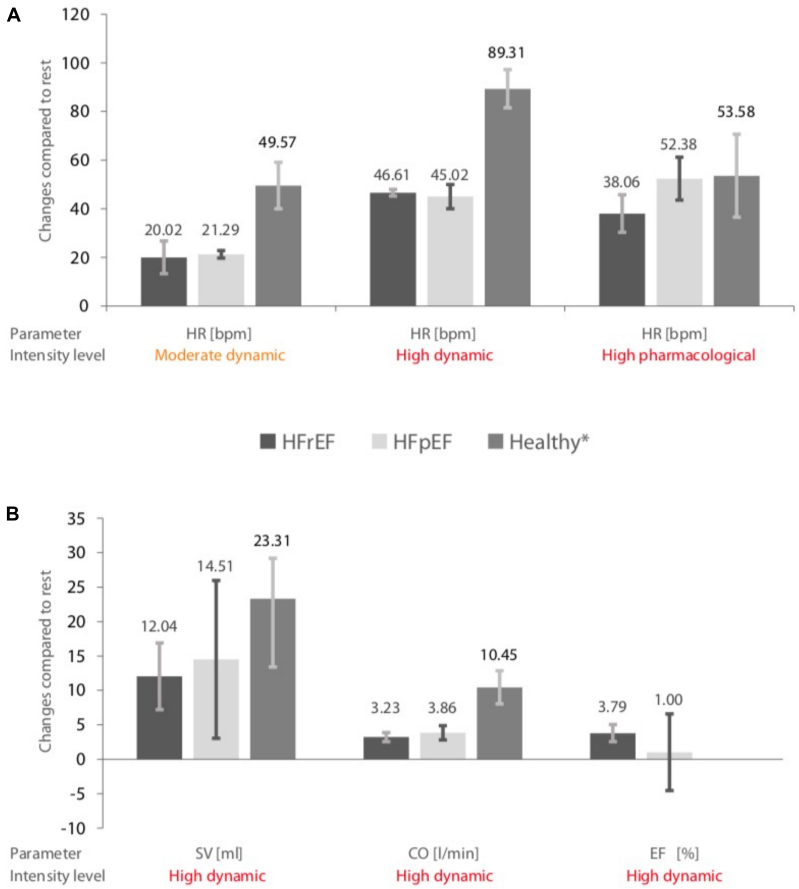

FIGURE 2 | (A) Pooled changes in heart rate (HR) during different stress levels in heart failure with preserved ejection fraction (HFrEF) patients, heart failure with reduced ejection fraction (HFpEF) patients, and healthy controls. **(B)** Changes in stroke volume (SV), cardiac output (CO), and ejection fraction (EF) during a high dynamic stress test in HFrEF, HFpEF patients, and healthy controls. The error bars indicate mean values ± standard deviations. *Data obtained from a previous meta-analysis (13). Included heart failure study arms and sample sizes are given in **Table 5**.

there was an inconclusive association between the intake of ARB and HR changes (Coef., –0.46; 95% CI –0.96 to 0.03; $p = 0.061$; Cons., 62.0; 95% CI –45.5 to 78.5); this was not seen in HFrEF. In patients with HFrEF, there was an inconclusive association between the intake of beta blockers and HR changes (Coef., –0.12; 95% CI –0.26 to 0.01; $p = 0.079$; Cons., 54.3; 95% CI –42.8 to 65.7); this was not seen in patients with HFpEF. Furthermore, there were no associations between treatment with aldosterone antagonists and stress testing-induced change in HR at any intensity level for HFrEF or HFpEF patients. No effects were found for CRT.

As treatment can impact resting HR rather than affecting changes under exercise conditions, the associations between different treatment methods and the resting HR were analyzed (**Figure 6**): resting HR was associated with reported beta-blocker intake (Coef., 0.01; 95% CI –0.12 to 0.15; $p < 0.001$; Cons., 88.5; 95% CI 82.5–94.6) in HFrEF, while this effect was not found in patients with HFpEF. Resting HR in HFpEF was associated with ARB intake (Coef., –0.27; 95% CI –0.47 to –0.07; $p = 0.016$; Cons., 79.8; 95% CI 73.2–86.4). No other associations of medical treatment and resting HR were found.

Comparative Studies

We identified one study that directly compared pharmacological, dynamic (bicycle exercise), and isometric stress testing in 20 patients (38). In this study, HR change was at 7 bpm with isometric exercise (95% CI –0.11 to 14.11), 26 bpm (95% CI 19.26–32.74) with dobutamine infusion (20 μg/kg/min), and 38 bpm (95% CI 27.12–48.88) when stressed dynamically with high intensity.

We also identified 3 studies (6 study arms) directly comparing HFpEF with HFrEF patients during high-intensity dynamic stress testing (39–41). Changes in CO and EF were analyzed in one study with no difference between HFrEF and HFpEF patients. HR changes were tested in all three studies (**Table 6**).

DISCUSSION

This systematic review and meta-analysis reports on the stress-induced changes of hemodynamic parameters in patients with HFrEF and HFpEF. Despite the limited availability of comparative studies, pooled changes of the included study arms

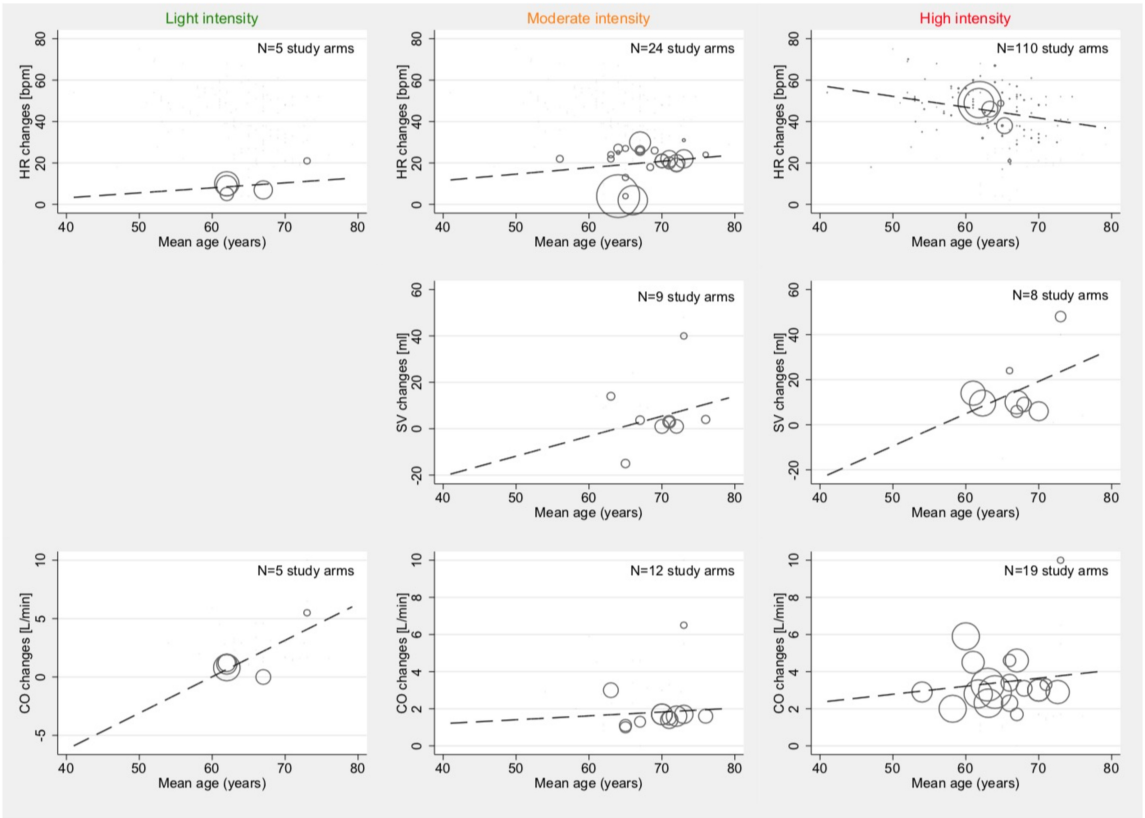

FIGURE 3 | Associations between mean age and mean absolute changes in HR (top row), SV (2nd row), and CO (bottom row) among studies reporting outcomes with light- (1st column), moderate- (2nd column), and high intensity (3rd column). Studies with both HFpEF and HFrEF patients under dynamic and pharmacological stress testing were included. Bubble size indicates the sample size of one study arm in relation to other study arms within the same category.

are presented. In the activity levels where sufficient data from both HF groups were available, the results were compared between both HF groups as well as with data previously reported in healthy populations (13). Most of the data included in this comparison were from high intensity examinations.

In studies where HF patients were tested with dynamic exercise at moderate or high intensity, smaller changes in HR and CO were found when compared to healthy controls. When stressed pharmacologically at high intensity, changes in HR were lower and 95% CIs marginally overlapped with those from healthy controls. Although CO at high dynamic stress testing was lower in HF than in controls, there were no differences found in SV between both HF groups and controls.

Whereas HR increases during exercise follow a typical pattern in healthy individuals, such regulation is commonly compromised in HF patients (42). Explanations for an attenuated HR increase, in response to dynamic exercise, include the use of HR-lowering drugs (typically beta blockers), as well as lower exercise intensity levels compared to healthy individuals. Such an impaired chronotropic reserve has been described in HF patients due to imbalances in the autonomic nervous system (43).

Thus, examining HR responses to incremental workload or to dobutamine infusion may help to identify patients with HF or to assess the severity of autonomic dysfunction. While both methods have their unique advantages and disadvantages, our results suggest that for the distinction of HF patients from healthy individuals, the evaluation of HR and CO changes in response to dynamic stress testing may be more appropriate than pharmacological stress testing. Furthermore, dynamic exercise testing is typically considered the most physiological type of stress (44). Nevertheless, dynamic stress testing includes the assessment of a personal maximum or submaximal workload, which can be substantially altered in HF. In conjunction with wearable devices, models based on such changes were recently shown to be capable of predicting the outcome of standardized 6-min walk tests in patients with heart disease (45). The adaptation of such models, as well as surveillance strategies to disease-specific aspects of HF, may help to better identify patients at risk providing data-driven approaches to patients and caregivers that can help to detect deterioration early on (46).

One study arm comprising 20 stress examinations was considered for isometric exercise testing. Additionally, and after the date of the final search, Blum et al. published a study comparing strain during handgrip exercise between HF groups in 53 patients. This recent study includes information on HR responses and addresses particular responses of HFmrEF patients to isometric exercise (47). Furthermore, no sufficient data for comparison were available in HFmrEF patients. Studies with

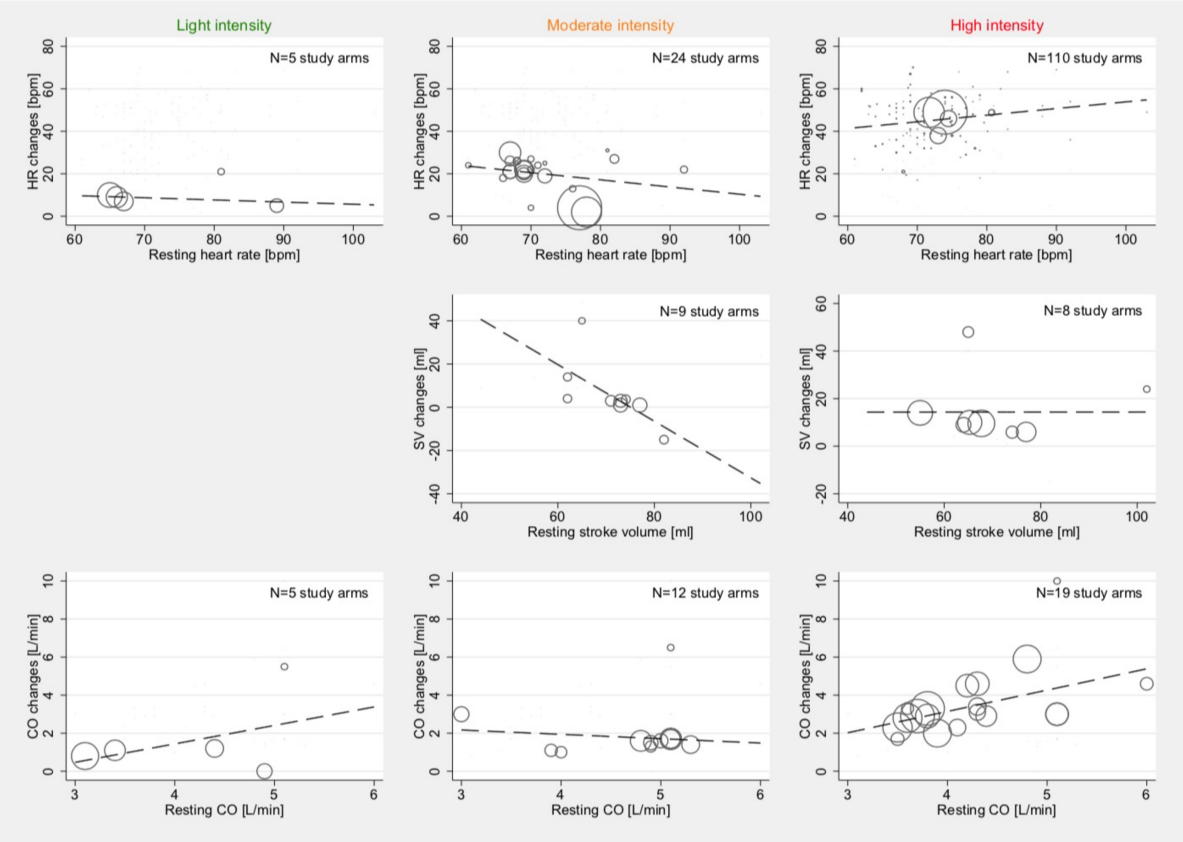

FIGURE 4 | Associations between resting conditions and changes under stress: HR (top row), SV (2nd row), and CO (bottom row) among studies reporting outcome with light- (1st column), moderate- (2nd column), and high intensity (3rd column). Studies with both HFpEF and HFrEF patients under dynamic and pharmacological stress testing were included. Bubble size indicates the sample size of one study arm in relation to other study arms within the same category.

older classifications of HFrEF may, however, include HFmrEF patients without allowing for further distinction.

The HF is often accompanied by cardiac and non-cardiac disease, as well as potential confounders, which may contribute additionally to reduced exercise capacity and which may limit the patient's ability to perform dynamic exercise testing adequately. Etiologies and treatment regimens for HFrEF and HFpEF vastly differ, and in line with these concepts, relevant group differences were found: HFpEF patients were older, predominantly female, had a higher BMI, were in a lower NYHA class, and were less frequently characterized with CAD. Only a few studies reported on a small minority of subjects with atrial fibrillation. This was in clear contrast to the existing literature where around 50% of all HF patients have been described to also suffer from atrial fibrillation and 30% of all patients with atrial fibrillation to suffer from HF (48). Medication profiles differed according to current treatment practice (main differences: ACEi were used by 83% of HFrEF patients and by 44% in HFpEF; beta blockers were used by 88% in HFrEF and by only 64% in HFpEF; aldosterone antagonists were used by 53% of HFrEF patients and only 24% in HFpEF). By including the HF group in our analysis, these differences, as well as other potentially unobserved variables, were indirectly considered.

Relevant group characteristics with a sufficient amount of studies were reported for stress type, stress intensity, age, patient group allocation, and medication. The magnitude of exercise-induced HR responses in patients with HF did not substantially differ between studies investigating dynamic stress testing and pharmacological exercise, respectively. Nevertheless, it remains open to further evaluate whether the assessment of chronotropic competency by exercise testing and pharmacological stress testing can be considered as an alternative for those patients who do not tolerate dynamic testing. Furthermore, HR changes were inversely correlated with age within high-intensity exercise study arms. No consistent reporting was found for pacemaker use, although the devices may influence HR response under stress conditions. Nevertheless, CRTs were reported in HFrEF, as no consensus for a benefit in HFpEF exists.

This meta-analysis was not designed to assess the effects of daily pharmacotherapy on stress testing in HF, as this would require comparable studies in combination with standardized stress testing protocols. Some authors, however, provide population-based medication data. The increase in HR was more pronounced, and thus, more physiological patients were treated with ACEi in HFrEF studies. The positive effects associated with the inhibition of the renin-angiotensin-aldosterone system

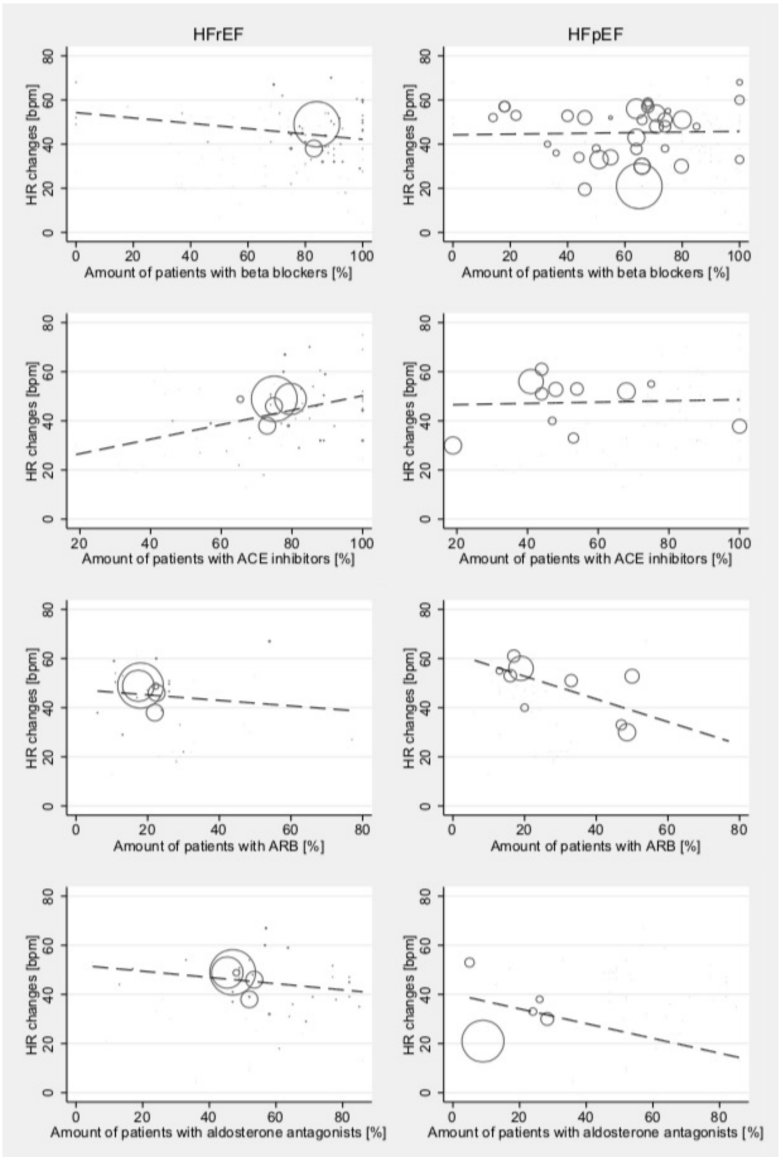

FIGURE 5 | Relationship between medical treatment and mean absolute stress testing-induced HR changes in those studies reporting outcomes for HFrEF (left) and HFpEF (right) patients at high-intensity levels. Studies include both dynamic and pharmacological stress testing. Bubble size indicates study sample size.

(RAAS) were previously shown to lead to a significant reduction in mortality and morbidity rates, and in turn, ACEi are considered first-line treatment for patients with HFrEF (49). In HFpEF, no such association of ACEi intake and HR changes under stress was found. However, as treatment with ACEi is not commonly recommended, the average number of patients taking ACEi was lower within the HFpEF study arms. The effects of RAAS inhibition in HFpEF are less well understood, and a benefit in reducing the mortality rate has not been demonstrated (50). Moreover, an inconclusive inverse association between ARB intake and HR changes under stress was observed in HFpEF but not in HFrEF. In line with these results, the findings of our analyses suggest that RAAS inhibition might be of lesser benefit in HFpEF than in the HFrEF populations. In patients who do not tolerate ACEi, ARBs are recommended and frequently used alternatives. However, it is evident that ACEi do not have the same inhibitory effect on RAAS activity and, therefore, that their beneficial effects differ from ARB (51), which could explain this discrepancy in the findings of our current study.

We did not find sufficient data on the more recently advocated combined use of ARBs and neprilysin inhibitors (i.e., valsartan + sacubitril) within the analyzed study arms, which has been proposed particularly in HF patients with symptoms under stress conditions (52). Whereas the majority of patients within HFrEF study arms were under beta-blocker therapy, the use of beta blockers in HFpEF is still under controversial discussion.

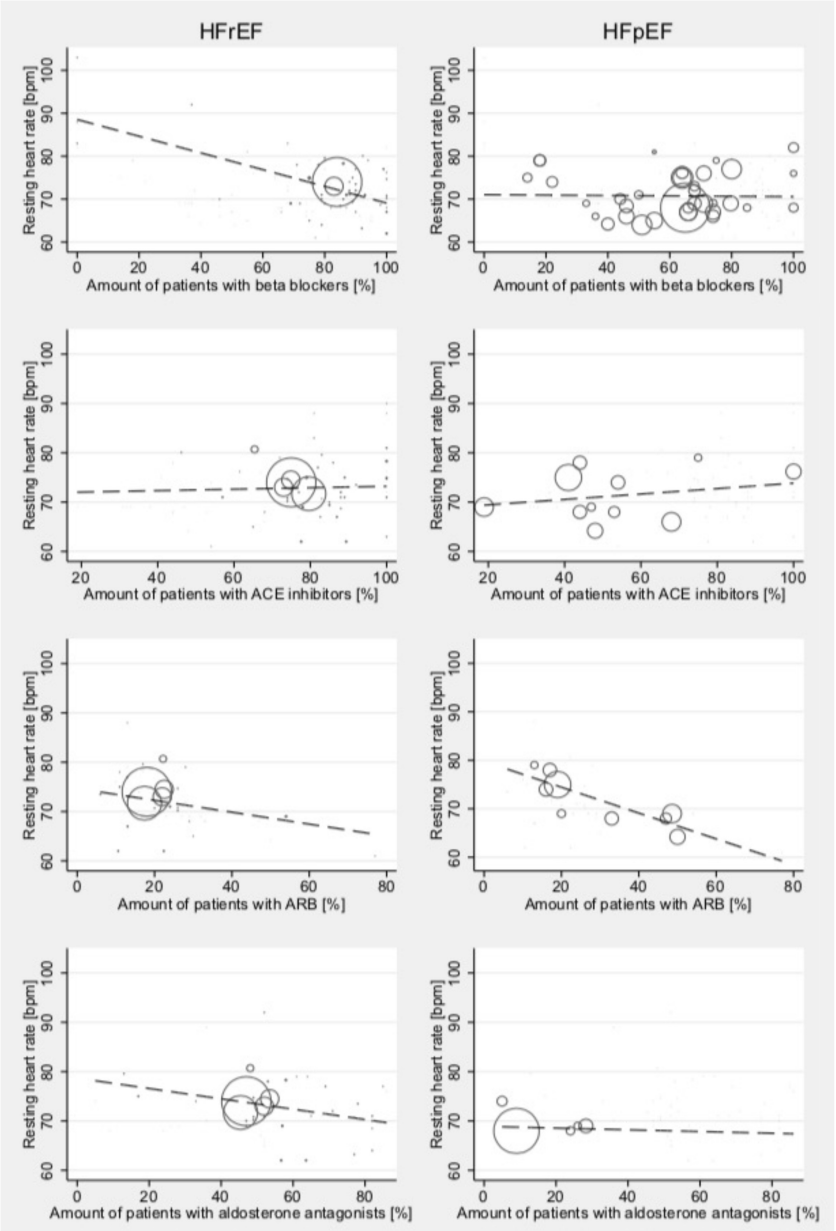

FIGURE 6 | Relationship between medical treatment and the resting HR among studies reporting outcomes for HFrEF (left) and HFpEF (right) patients test at high-intensity levels. Studies include both dynamic and pharmacological stress testing. Bubble size indicates study sample size.

In HFrEF, the resting HR was inversely correlated with the number of patients for beta-blocker intake. No such effect was seen in HFpEF. Neither relevant effects on the number of patients taking beta blockers nor aldosterone antagonists on HR changes under exercise were found. Although heterogeneously reported in the analyzed studies, the inverse correlation between resting HR and beta-blocker use can be seen as an indicator for medication intake before stress testing. Elimination of the beta-blocker effects would require withholding the drug for 5 half-lives (53). As this is known to be rarely done, current recommendations by the American Society of Echocardiography suggest that the discontinuation of beta blockers is not essential but may require intermediate (15–20 μg/kg/min) dobutamine doses (53).

Limitations

The majority of the studies were observational trials for HFpEF or HFrEF patients, and mainly, our results are based on a comparison of single study arms. Only three studies were identified which had directly compared the two HF groups. The results of these studies were in line with our

TABLE 6 | Studies directly comparing HFpEF with HFrEF patients during high-intensity dynamic stress testing.

Study	*N*	Parameter	HFrEF	HFpEF
Farr et al. (39)[a]	HFrEF: *N* = 185 HFpEF: *N* = 43	HR change, bpm	44 (40–48)	37 (30–45)
Sugimoto et al. (40)[b]	HFrEF: *N* = 49 HFpEF: *N* = 20	HR change, bpm	37 (31–43)	38 (27–49)
		CO change, L/min	2.3 (1.64–2.96)	2.9 (2.10–3.70)
		EF change	3% (–0.85–6.85)	1% (–7.73–9.73)
Wang et al. (41)[c]	HFrEF: *N* = 50 HFpEF: *N* = 80	HR change, bpm	62 (55–69)	54 (49–59)

[a]*HFrEF EF < 50%, HFpEF EF ≥ 50%.* [b]*HFrEF EF < 40%, HFpEF EF > 50%.* [c]*HFrEF EF < 50%, HFpEF EF ≥ 50%.*

findings from the single-arm analysis, confirming that exercise-induced HR changes are similar between both HF groups. Nevertheless, comparative studies of stratified HF populations, as well as HFmrEF patient populations in a standardized exercise protocol, would be highly desirable for an improved disease understanding.

For some intensity/parameter categories, only a few data were available (**Table 5**), and the uncertainty of pooled changes was consequently high. Therefore, the stress responses of HFpEF and HFrEF patients were only compared when at least two studies were available for each disease group at the same intensity level.

The intensities for dynamic stress testing used in the analyzed studies were low compared to intensities for a variety of different sports activities in healthy individuals (15). When compared to data from healthy subjects, lower HR changes during exercise found in HF patients may be explained by this effect. The classification of exercise intensities, however, was adapted according to the predefined lower submaximal exercise load targets and load increments in HF (17–22, 24). Nevertheless, large heterogeneity exists between the classification of exercise and to classifications in healthy cohorts. Subjective submaximal exercise and exhaustion, as well as symptoms, were commonly instantiated criteria as stated by authors in a majority of the studies. Due to a lack of studies that subjected HF patients to more intensive stress conditions, we were not able to systematically analyze hemodynamic changes for higher workloads.

Relevant heterogeneity can also be found in the dynamic exercise type and stress testing protocols. Responses to different protocols, load increments as well as responses to treadmill, supine, and upright bicycle exercise testing are known to differ. However, information on test protocols is not consistently available, and subgrouping according to available information did not reveal relevant differences. The study, therefore, further emphasizes the need for standardized stress testing protocols, transparent reporting, and for data-sharing initiatives to allow for more detailed network meta-analyses.

CONCLUSION AND OUTLOOK

Reference values presented in this review can help to estimate the expected range of hemodynamic and circulatory responses in patients with HF. This may contribute to a better disease understanding, future study planning, and patient-specific predictive models. Although based on different etiologies and having differing baseline characteristics, no substantial differences in chronotropic reactions, changes in SV, or CO were found between HFrEF and HFpEF. When compared to healthy individuals, exercise tolerance, as well as associated HR and CO increases under moderate-high dynamic stress, was substantially impaired within HF patients and may reflect a relevant aspect of disease burden.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are publically available. **Supplementary Table 2** includes a full overview of all included studies.

AUTHOR CONTRIBUTIONS

AB, MS-K, DF, LG, and MK conceptualized and designed the study. AW and AB conducted the study selection. AW and MK performed the quality assessment. AW extracted the data. LG and MK conducted the statistical analysis. AB and MK drafted the manuscript. KR, MS-K, LG, SK, DM, BP, FB, and TK revised the manuscript. All authors interpreted the data and approved the final version of the manuscript.

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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.2022.718114/full#supplementary-material

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