NOVEL RISK PREDICTING SYSTEM FOR HEART FAILURE

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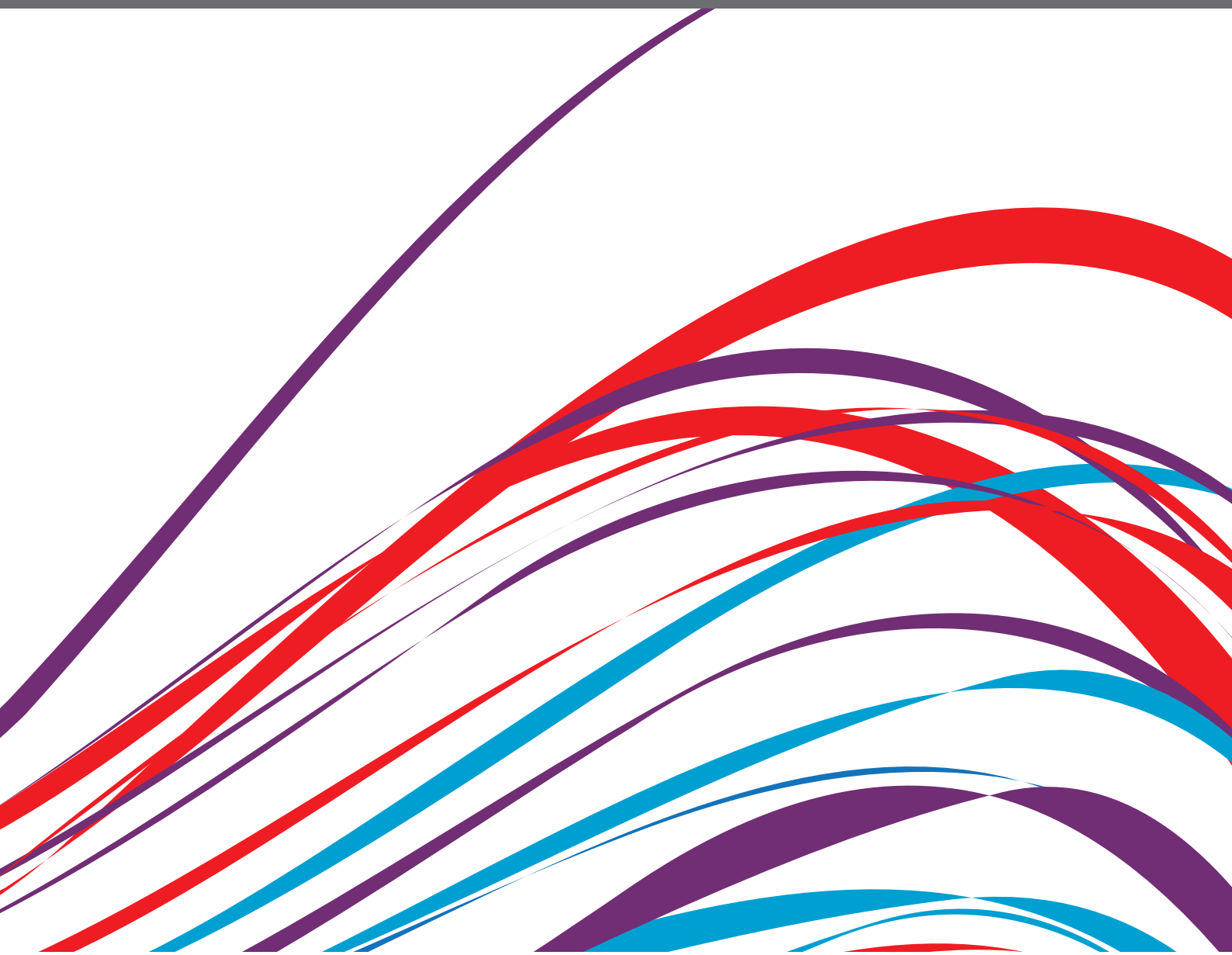

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NOVEL RISK PREDICTING SYSTEM FOR HEART FAILURE

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Table of Contents

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EDITORIAL
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Editorial: Novel Risk Predicting System for Heart Failure

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Keywords: risk prediction, imaging, genetics, heart failure, circulating biomarkers

Editorial on the Research Topic

Novel Risk Predicting System for Heart Failure

The prevalence of heart failure (HF) is a major public health problem, as its prevalence and high morbidity and mortality are increasing worldwide. To date, we are in need of biomarkers that improve the diagnosis and prognosis of HF. Within this context, this Research Topic called for research on novel HF risk factors as diagnostic and prognostic cues that may improve HF detection and prognosis. Ideally, a HF marker should reflect pathological perturbations that contribute to HF pathogenesis and progression, while enabling prediction, diagnosis, staging, prognosis or management of this condition. As represented in this Research Topic, HF biomarkers may include easily accessible clinical parameters as well as more advanced biomarkers involving (epi)genetics, imaging and blood biochemistry.

For instance, Li Y. et al. investigated the association between fluctuations in a basic clinical parameter (body weight) and the risk of adverse events in 1,691 patients with HF with preserved ejection fraction (HFpEF). In this TOPCAT cohort, higher body weight variability over time was associated with higher risk of future cardiovascular events independent of traditional risk factors and regardless of the direction of the weight change. As such, body weight monitoring may improve risk stratification in patients with HFpEF.

In search for genetic markers of HF, Li X. et al. conducted a Mendelian randomization study on inflammatory biomarkers in HF. They collected genome-wide association study data of 47,309 HF cases and 930,014 controls of European descent to identify genetic variants in inflammatory biomarkers such as C-reactive protein (CRP) and fibrinogen that may underlie HF. Overall, none of the SNPs for CRP, fibrinogen and components of the interleukin-1 and −6 signaling pathway were causally linked to HF risk. More studies are warranted to identify (epi)genetic markers associated with HF pathogenesis and progression.

Furthermore, this Research Topic includes three original research articles on the predictive value of imaging markers in a hospital setting. First, Yang et al. followed 156 patients with concomitant HF with reduced ejection fraction (HFrEF) and atrial fibrillation who underwent first-time catheter ablation. One year after ablation, left ventricular ejection fraction (LVEF) was improved in 72.3% of patients, which could only be predicted by E/e', an echocardiographic surrogate marker of LV filling pressure [$OR_{adjusted}$ 1.13 (1.03–1.24); $P_{adjusted} = 0.011$]. E/e'≥15 provided optimal prediction with poor sensitivity (38.7%) but high specificity (89.2%). E/e' may thus complement current risk prediction approaches in HFrEF patients and atrial fibrillation undergoing catheter ablation.

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Furthermore, Zhu et al. conducted a case-control study investigating non-invasive markers of myocardial work (MW, calculated from pressure-strain loops using 2D speckle tracking echocardiography). In adjusted analyses, MW indexes strongly correlated with LVEF and differed between patients with coronary artery disease (CAD) and healthy controls. Future studies should dig deeper into the additive value of MW indexes beyond conventional echocardiographic parameters of LV structure and systolic and diastolic function for risk stratification and diagnosis in CAD and HF.

Zhang et al. monitored heart-rate recovery at 1 min after exercise (HRR1) in 89 consecutive patients with inoperable chronic thromboembolic pulmonary hypertension undergoing balloon pulmonary angioplasty (BPA). In this study, HRR1 significantly improved after the procedure, suggesting the alleviation of sympathovagal imbalance upon BPA. HRR1 may thus represent an easily available and non-invasive surrogate marker to predict BPA outcome and monitor its efficacy.

Besides genetic and imaging markers, also circulating biomarkers may enable early detection of HF and pave the way to novel therapies. Two state-of-the-art reviews in this Research Topic illustrate the potential of circulating biomarkers in HF. One review on cardio-oncology provides a thorough overview of the advancements in the field of biomarkers to monitor cardiovascular toxicity of various tumor therapies. The review particularly focuses on subclinical markers of cancer treatment-related cardiac dysfunction during drug therapy and radiotherapy. Another review summarizes the current literature on the relationship between the amino-acid metabolism and gut microbiome alterations during the development of heart failure, while describing the potential prognostic and therapeutic value of the gut–amino acid–HF axis. In line, four original research studies in this Research Topic elaborated on the value of: (i) α-linolenic acid for HF risk stratification (a meta-analysis), (ii) plasma galectin-3 for diagnostic and prognosis of HFrEF, (iii) insulin growth factor-1 (IGF-1) and IGF-binding protein 1 for detection of HF subtypes, and (iv) the systemic immune-inflammation index for prediction of short-term mortality in overt HF. In addition, Xu et al. dug deeper in the molecular mechanisms underlying the development of cardiac hypertrophy. In particular, they found that Samm50 can promote cardiac hypertrophy by regulating Pink1-Parkin-mediated mitophagy. This pathway may be an interesting therapeutic target for managing cardiac hypertrophy. Each of these studies highlight the potential for circulating biomarkers to complement current strategies used in clinic for stratifying a patient's risk for HF and assessing HF prognosis. The findings on circulating biomarkers may even pave the way to novel therapeutic options for HF as illustrated by the study on Samm50. Evidently, future studies should validate the findings, investigate which and how these novel biomarkers could be implemented cost-effectively in HF clinic and investigate therapeutic strategies linked to the highlighted pathways contributing to HF pathology.

For clinical practice, multidimensional risk scores will be inevitable, as no single HF biomarker will fulfill all clinical needs (diagnosis, prediction, prognosis, monitoring, etc.) on its own. Within this context, Gong et al. tested the risk stratification strategy for pulmonary arterial hypertension as endorsed by European guidelines in 392 patients with idiopathic pulmonary arterial hypertension in China. Of note, the multidimensional risk stratification approach effectively stratified along risk, while accurately predicting mortality in these patients. Therapeutic implications of this risk grading approach remain to be resolved. Furthermore, Yan et al. trained and tested a nomogram score from eight preoperative factors to predict the risk of in-hospital mortality in HFrEF patients after coronary artery bypass grafting surgery (CABG). While the EuroSCORE-2 underestimated postoperative mortality risk, especially in high-risk patients, their nomogram provided better preoperative prediction of mortality after CABG in patients with HFrEF. This may facilitate identifying HFrEF patients at high risk of post-procedural in-hospital mortality.

In the future, optimal HF management will require integration of the most informative biomarkers, regardless of whether they form clinical, (epi)genetic, imaging or biochemical evidence of HF pathology. Thus, future studies should identify the ideal combination of biomarkers that captures the full spectrum of HF pathogenesis and progression and that adequately steers the clinical decision-making process.

AUTHOR CONTRIBUTIONS

All authors have made a substantial contribution to the concept or design of the editorial, either drafted the article or critically revised it critically for important intellectual content, approved the submitted version to be published, and agreed to be accountable for all aspects of the work.

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ORIGINAL RESEARCH
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Association of Body-Weight Fluctuation With Outcomes in Heart Failure With Preserved Ejection Fraction

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Aims: To investigate the relationship between body-weight fluctuation and risks of clinical outcomes in patients with heart failure with preserved ejection fraction (HFpEF).

Methods and Results: We measured intra-individual variations in body weight from baseline and follow-up visits in 1,691 participants with HFpEF from the Americas from the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial. The primary endpoint was any cardiovascular events (a composite of death from cardiovascular disease, non-fatal myocardial infarction, aborted cardiac arrest, or hospitalization for HF). The body-weight fluctuation was measured according to average successive variability and high variability was defined as greater than or equal to the median. After adjustment for risk factors, mean body weight and weight change, each increase of 1 standard deviation in body-weight variability was significantly associated with increased risks of any cardiovascular events (hazard ratio [HR] 1.23, 95% confidence interval [CI] 1.15–1.33, $P < 0.001$). Patients with high variability had a 47% increased risk of any cardiovascular events and 27% increased risk of all-cause death compared with those with low variability. Such association was similar among patients with New York Heart Association functional class I/II vs. III/IV, obesity vs. non-obesity, and weight loss, gain vs. stability (the *P*-values for interaction were all insignificant).

Conclusion: Among patients with HFpEF, body-weight fluctuation was associated with increased risks of cardiovascular events independent of traditional cardiovascular risk factors, and regardless of HF severity, baseline weight or weight change direction.

Clinical Trial Registration: Aldosterone antagonist therapy for adults with heart failure and preserved systolic function (TOPCAT), https://clinicaltrials.gov, identifier [NCT00094302].

Keywords: heart failure with preserved ejection fraction, body weight, fluctuation, outcome, heart failure

INTRODUCTION

Heart failure (HF) is a global pandemic affecting at least 26 million people worldwide and is increasing in prevalence (1). Body weight of patients with HF often oscillates over time, and fluctuations in weight may have negative consequences. Monitoring of body weight has been recommended in self-care for all patients by HF management guidelines (2, 3). The relationship between body weight and outcomes is complex in patients with established HF (4–7). A U-shaped distribution curve has been proved in which mortality is greatest in underweight patients, lower in normal to overweight patients, and higher again in more severely obese patients with HF (8). Weight loss may reflect cachexia status in advanced HF (9), and associates with a higher risk of mortality and cardiovascular events (10–14). Weight gain is also associated with a modestly increased mortality risk (11, 13). However, the association between body-weight fluctuation and health outcomes in patients with HF is not yet fully established. Furthermore, whether HF severity, body weight at baseline, and direction of weight change affect the association of body-weight fluctuation and outcomes also remains unknown.

Accordingly, based on the data from the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial, which involved patients with established HF with preserved ejection fraction (HFpEF), we performed a *post-hoc* analysis to explore the relationship between intra-individual fluctuations in body weight and the risk of cardiovascular events. We further explored the interaction between body-weight fluctuation and HF severity, baseline weight, and weight change direction.

METHODS

Study Population

We conducted a *post-hoc* analysis of the TOPCAT trial, a multi-center, international, randomized, double-blind, placebo-controlled trial of spironolactone in adults with HFpEF recruited from over 270 clinical sites. The design of the TOPCAT trial was described in detail previously (15). The primary results of the trial were published at NEJM.org (16). In the present study, we included patients from the Americas enrolled in the TOPCAT trial, who had at least two post-baseline measurements of body weight. 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 half year thereafter, at which times data on vital signs, including body weight, were collected. Patients were followed for a mean of 3.5 years.

The TOPCAT 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). All study participants provided written informed consent. We acquired the dataset of the TOPCAT trial from the National Heart, Lung, and Blood Institute (NHLBI) by applying to Biologic Specimen and Data Repository Information Coordinating Center (BIOLINCC, https://biolincc.nhlbi.nih.gov/). Our study was approved by the Medical Ethical Committee of the First Affiliated Hospital, Sun Yat-sen University. The TOPCAT investigators were not involved in the present study.

Measures of Body-Weight Variability

Body-weight variability was assessed using three indices: (1) standard deviation (SD), (2) variability independent of the mean (VIM), and (3) average successive variability (ASV). VIM was calculated as $100 \times$ SD/mean β, where β is the regression coefficient, based on the natural logarithm of the SD over the natural logarithm of the mean. In this study, ASV was used as the primary variability measure, defined as the average absolute difference between successive values.

Study Outcomes

The primary outcome was the occurrence of any cardiovascular events (a composite of death from cardiovascular disease, non-fatal myocardial infarction, aborted cardiac arrest, or hospitalization for HF). The secondary outcomes were individual components of the primary outcome, as well as all-cause death, myocardial infarction, and new onset of atrial fibrillation.

Statistical Analysis

We stratified patients into two groups based on the body-weight variability: high variability (greater than or equal to the median of ASV) and low variability (below the median of ASV). Categorical variables were described by frequencies with percentages, and continuous variables were described by a median with interquartile ranges. Demographic and clinical characteristics were compared between the two groups of high vs. low variability. Kruskal-Wallis test for continuous variables and chi-squared tests for categorical variables.

The relation between body-weight variability and the risk of outcomes was evaluated with the use of body-weight variability as both continuous and categorical variables. When analyzed as a categorical variable, the Kaplan-Meier survival analysis and Cox proportional hazards models were performed to evaluate the risk of outcomes between groups of high vs. low variability. When analyzed as a continuous variable, Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for outcomes associated with per increase in variability of 1 SD. Four models were used: model 1 being unadjusted; model 2 adjusting model 1 for diuretic therapy at baseline; model 3 adjusting model 2 for mean body weight and change in weight, taking directionality into account (continuous variable); and model 4 adjusting model 3 for + age, sex, race, smoking status, diabetes, atrial fibrillation, peripheral arterial disease, previous hospitalization for HF, prior myocardial infarction, known stroke, chronic obstructive pulmonary disease, New York Heart Association (NYHA) class, systolic blood pressure, heart rate, ejection fraction, estimated glomerular filtration rate, and number of weight measurement, with stepwise selection of covariates which were significant at the 0.05 level. Sensitivity analyses were conducted using other measures of variability ($\pm$SD and VIM) to evaluate the consistency of the results.

TABLE 1 | Characteristics of the patients by body-weight variability groups.

	Low variability *N* = 842	High variability *N* = 849	Total *N* = 1,691	*P*-value
Demographic				
Age, median (IQR), y	75 (68–81)	69 (62–77)	72 (64–79)	<0.001
Women, *n* (%)	459 (54.5)	378 (44.5)	837 (49.5)	<0.001
Race, *n* (%)				<0.001
White	690 (81.9)	646 (76.1)	1,336 (79.0)	
Black	103 (12.2)	177 (20.8)	280 (16.6)	
Clinical				
Randomization to spironolactone, *n* (%)	421 (50.0)	431 (50.8)	852 (50.4)	0.753
Diuretics, *n* (%)	740 (87.9)	768 (90.5)	1,508 (89.2)	<0.001
Current smoker, *n* (%)	45 (5.34)	61 (7.18)	106 (6.3)	0.18
Previous hospitalization for CHF, *n* (%)	442 (52.5)	556 (65.5)	998 (59.0)	<0.001
Previous myocardial infarction, *n* (%)	153 (18.2)	197 (23.2)	350 (20.7)	0.011
Known stroke, *n* (%)	68 (8.1)	85 (10.0)	153 (9.0)	0.168
COPD, *n* (%)	123 (14.6)	161 (19.0)	284 (16.8)	0.017
Hypertension, *n* (%)	760 (90.3)	762 (89.7)	1,522 (90.0)	0.672
Peripheral Arterial Disease, *n* (%)	90 (10.7)	112 (13.2)	202 (11.9)	0.115
Atrial fibrillation, *n* (%)	384 (45.6)	338 (39.8)	722 (42.7)	0.015
Diabetes mellitus, *n* (%)	305 (36.2)	449 (52.9)	754 (44.6)	<0.001
Previous pacemaker, *n* (%)	126 (15.0)	108 (12.7)	234 (13.8)	0.178
Previous ICD, *n* (%)	17 (2.0)	25 (2.9)	42 (2.5)	0.223
NYHA class III/IV, *n* (%)	232 (27.5)	355 (41.8)	587 (34.7)	<0.001
Heart rate, median (IQR), (bpm)	67 (60–75)	69 (62–76)	68 (61–76)	<0.001
SBP, median (IQR), (mmHg)	128 (118–138)	128 (118–139)	128 (118–138)	0.638
Body weight, median (IQR), (Kg)	82.3 (71.2–95.7)	101.6 (85.3–121.0)	90.7 (76.0–108.9)	<0.001
Ejection fraction, median (IQR)	60 (53–65)	58 (52–64)	58 (53–64)	0.177
eGFR, median (IQR)	61.8 (49.5–76.6)	60.4 (48.9–77.2)	61.3 (49.0–77.0)	0.778

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

Subgroup analyses were performed to evaluate whether the relationship between body-weight variability and outcomes differed by sex, baseline NYHA class, body-mass index (BMI), and weight change direction, by introducing a weight variability × variable interaction terms. Patients were assigned to the following subgroups: (1) men or women; (2) NYHA I/II or NYHA III/IV; (3) obesity (BMI, $\geq$30 kg/m^2) or non-obesity (BMI, <30 kg/m^2); (4) weight loss (weight witnessed a decrease of $\geq$5%), weight gain (weight witnessed an increase of $\geq$5%), or weight stability (weight change <5%). Unadjusted and adjusted models were constructed to evaluate the association of high variability in weight and the risk of the primary outcome in the above-mentioned subgroups.

All statistical analyses were conducted using SAS statistical software version 9.4 (SAS Institute Inc) and the survival plot was made using GraphPad Prism 7. All comparisons were 2-sided and $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the Patients

Among all study populations enrolled in the trial, 1,691 participants met the inclusion criteria for the present analysis. The median age was 72 years (IQR 64–79), and 49.5% were. The median baseline body weight of the patients was 90.7 kg (IQR 76.0–108.9). The median number of weight measures was 7 (range, 2–11) (**Supplementary Figure 1A** in the Supplementary Appendix). The median body-weight variability was 2.1 kg (IQR 1.4–3.1) (**Supplementary Figure 1B** in the Supplementary Appendix). The median body-weight variability was 3.1 kg (IQR 2.5–4.1) and 1.4 kg (IQR 1.0–1.7) for patients in high and low variability group. **Table 1** outlines the baseline characteristics of the study population with high- vs. low-weight variability. Compared with patients with low variability, those with high variability were younger, predominantly males, less likely to be white, had higher proportions of previous HF hospitalization or myocardial infarction, chronic obstructive pulmonary disease, and diabetes mellitus. They also had more often with NYHA class III/IV, and had higher baseline body weight.

Body-Weight Variability and Outcomes

When body-weight variability (as measured by ASV) was used as a continuous variable in the adjusted model 4, each increase in body-weight variability of 1 SD (1.88 kg) was associated with increased risks of any cardiovascular events (HR 1.23, 95% CI 1.15–1.33, $P < 0.001$), non-fatal myocardial infarction (HR 1.30, 95% CI 1.09–1.55, $P = 0.004$), hospitalization for HF (HR 1.28, 95% CI

TABLE 2 | Risk of outcomes in per 1-SD change of body-weight variability.

Outcomes	Model 1*		Model 2#		Model 3§		Model 4¶	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Any cardiovascular events	1.27 (1.19–1.35)	<0.001	1.27 (1.19–1.35)	<0.001	1.35 (1.25–1.45)	<0.001	1.23 (1.15–1.33)	<0.001
Cardiovascular death	0.97 (0.84–1.13)	0.738	0.96 (0.83–1.12)	0.633	1.11 (0.95–1.30)	0.184	1.00 (0.88–1.14)	0.980
Myocardial infarction	1.13 (0.95–1.35)	0.153	1.13 (0.95–1.35)	0.156	1.26 (1.05–1.52)	0.013	1.30 (1.09–1.55)	0.004
Hospitalization for HF	1.36 (1.28–1.45)	<0.001	1.36 (1.27–1.45)	<0.001	1.41 (1.30–1.52)	<0.001	1.28 (1.19–1.38)	<0.001
All-cause death	1.10 (1.00–1.21)	0.045	1.09 (0.99–1.20)	0.068	1.22 (1.10–1.35)	<0.001	1.05 (0.95–1.14)	0.332
New onset atrial fibrillation	1.16 (0.98–1.37)	0.074	1.15 (0.98–1.36)	0.092	1.12 (0.92–1.36)	0.275	1.09 (0.92–1.29)	0.306

**Model 1 was unadjusted; #Model 2 was adjusted for diuretics. §Model 3 was adjusted for diuretics, mean body weight, and change in weight, taking directionality into account; ¶Model 4 was adjusted for the same variables as model 3 and for age, sex, race, smoking status, diabetes status, atrial fibrillation, peripheral arterial disease, previous hospitalization for chronic heart failure, prior myocardial infarction, known stroke, chronic obstructive pulmonary disease, New York Heart Association class, systolic blood pressure, heart rate, ejection fraction, estimated glomerular filtration rate, number of weight measurement. HF, heart failure; HR, hazard ratio; CI, confidence interval.*

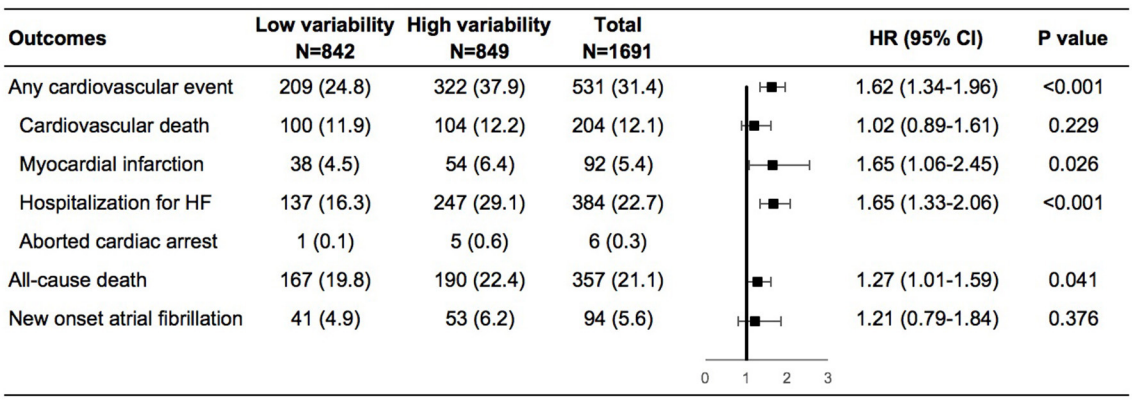


FIGURE 1 | Risk of outcomes in the high vs. low body-weight variability in multivariable model. The multivariable model was adjusted for diuretics, mean body weight, change in weight, age, sex, race, smoking status, diabetes status, atrial fibrillation, peripheral arterial disease, previous hospitalization for chronic heart failure, prior myocardial infarction, known stroke, chronic obstructive pulmonary disease, New York Heart Association class, systolic blood pressure, heart rate, ejection fraction, estimated glomerular filtration rate, number of weight measurement. HF, heart failure; HR, hazard ratio.

1.19–1.38, $P < 0.001$) (**Table 2**). Sensitivity analyses with two other indices of variability (SD, VIM) observed a consistent association between body-weight variability and risk of any cardiovascular events (**Supplementary Table 1** in the Supplementary Appendix).

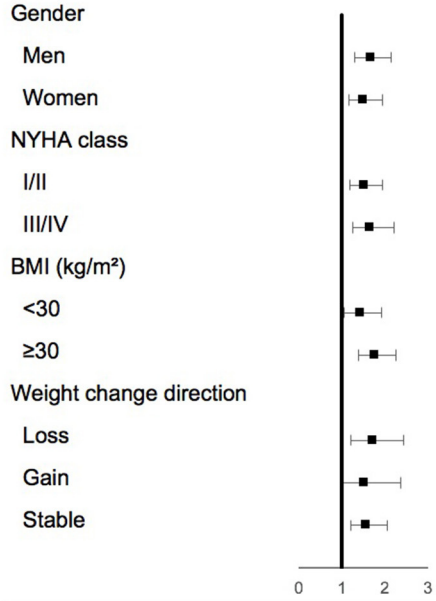
During a mean follow-up of 3.5 years, cardiovascular events occurred in 209 (24.8%) and 322 (37.9%) of patients with low and high weight variability, respectively (**Supplementary Figure 2** in the Supplementary Appendix). In the adjusted model 4, compared with patients with low-weight variability, those with high-weight variability had an increase in the risks of any cardiovascular events by 62%, non-fatal myocardial infarction by 65%, and HF hospitalization by 65%, all-cause death by 27%, and a non-significant increase in the risk of new onset atrial fibrillation of 21% (**Figure 1**).

Subgroup Analyses

Patients with high variability in body weight had significant higher risk of any cardiovascular events than patients with low variability in various subgroups including men or women, NYHA class I/II or III/IV at baseline, obesity, or non-obesity, weight loss, gain or stability during the follow-up period (**Supplementary Figures 3A–D** in the Supplementary Appendix). In the adjusted model, similar findings were demonstrated except for patients with non-obesity, in which high variability in body weight was associated with a numerically increased risk of any cardiovascular events although not significant (**Figure 2**). We further evaluated the interactions on any cardiovascular events between body-weight variability and the subgroups based on gender, baseline NYHA class, BMI, and weight change direction. As shown in **Figure 2**, none of these tests for interactions were statistically significant (the *P*-values were 0.86, 0.33, 0.22, and 0.75, respectively).

DISCUSSION

In this *post-hoc* analysis of patients with established HFpEF who participated in the TOPCAT trial, fluctuation in body weight was strongly associated with the risk of cardiovascular events and even death independent of traditional risk factors. Moreover, the



Subgroups	HR (95% CI)	P value	P for interaction
Gender			0.859
Men	1.67 (1.30-2.15)	<0.001	
Women	1.51 (1.16-1.95)	0.002	
NYHA class			0.326
I/II	1.52 (1.19-1.95)	<0.001	
III/IV	1.66 (1.25-2.20)	<0.001	
BMI (kg/m²)			0.224
<30	1.41 (1.05-1.92)	0.021	
≥30	1.76 (1.38-2.25)	<0.001	
Weight change direction			0.748
Loss	1.71 (1.20-2.44)	0.003	
Gain	1.51 (0.96-2.38)	0.075	
Stable	1.57 (1.21-2.05)	<0.001	

FIGURE 2 | Body-weight variability and risk of any cardiovascular events for various subgroups in multivariable model. The multivariable model was adjusted for diuretics, mean body weight, change in weight, age, sex, race, smoking status, diabetes status, atrial fibrillation, peripheral arterial disease, previous hospitalization for chronic heart failure, prior myocardial infarction, known stroke, chronic obstructive pulmonary disease, NYHA class, systolic blood pressure, heart rate, ejection fraction, estimated glomerular filtration rate, number of weight measurement. NYHA, New York Heart Association; BMI, body mass index; HR, hazard ratio.

associations observed were consistent among those who were at NYHA class I/II or III/IV, non-obese or obese, weight loss, gain, or stability over time.

Prior studies have explored the complex impact of baseline weight and weight change on outcomes in patients with HF. The "obesity paradox" (4–6) that more favorable prognosis in obese vs. normal-weight patients was found. Moreover, both weight loss and weight gain were associated with poor prognosis (10–14). However, another important aspect of the body weight, the variability over time (17, 18), has not been evaluated in HF. Highly variable body-weight was associated with increased total mortality and morbidity (19) and a higher incidence of HF (20) and diabetes mellitus (21) in the general population. Other studies found body-weight variability was associated with increased risks of cardiovascular events and mortality in patients with coronary artery disease (22) and type 2 diabetes (23–25). To the best of our knowledge, this analysis is the first one to demonstrate that in patients with HF, body-weight variability was also independently associated with a significant increase in the risk of cardiovascular events and death. A prior study found body-weight fluctuation was associated with increased risk of incident atrial fibrillation in the general population (26). We proved that this association also existed in patients with HF.

Fluctuation in body weight is a common phenomenon, especially in patients with HF. Weight loss is commonly prescribed as a lifestyle intervention in obese patients. However, weight loss is frequently followed by weight gain (or "weight cycling") or by other patterns of weight fluctuation. In patients with established HF, weight loss may also be caused by the higher total energy expenditure of HF in cachexia status, and rapid weight gain often appears when volume overloaded. Prior studies (27, 28) suggested simply discharge education including monitoring of body weight can improve clinical outcomes. Thus, both the American College of Cardiology/American Heart Association (2) and European Society of Cardiology (3) guidelines for HF recommend patients with HF should receive specific education to facilitate self-care, including weight monitoring. Whereas, to what extend that weight fluctuation affects HF prognosis is not known. We found that in patients with HFpEF, each 1-SD increase in body-weight variability increased the risk of any cardiovascular events by 30% and the risk of mortality by 25%. The mechanism behind such association remains unclear. In this analysis, a higher risk of new onset atrial fibrillation associated with body-weight variability may lead to acute decompensation and hospitalization for HF. The association between increased body weight variability and adverse cardiovascular events and mortality highlights the substantial importance of avoiding weight fluctuation in long-term HF care.

The associations observed in our study may be due to reasons other than causality. Moreover, higher body-weight fluctuation may be a marker of advanced HF that has a worse

prognosis. However, patients with NYHA class I/II witnessed similar results as patients with NYHA class III/IV in our study. Obvious weight loss due to chronic wasting may also proceed worse prognosis. However, patients with weight loss, gain, or stability yielded similar results, suggested the association was independent of weight change direction. Prior studies reported significant interactions with BMI in the association between weight variability and outcomes in patients with coronary artery disease (22) or type 2 diabetes (23). However, such interaction was not found in our study.

Study Limitations

The present study has certain limitations as follows. First, we acknowledge that the main aim of the TOPCAT Trial was not to determine the role of weight variation in patients with HFpEF, thus, future studies of weight variation targets may be warranted. Second, we could not determine whether the weight change was intentional or unintentional, which may have different effects on prognosis. Third, body weight was collected at certain points, variability calculated may not reflect the whole follow-up phase. Fourth, because the analytic sample was limited to patients with HFpEF with stringent inclusion and exclusion criteria, additional studies in a broad spectrum of patients with HF are required to generalize our results. Fifth, since participants were enrolled more than 10 years ago in the TOPCAT trial, this analysis may not reflect current state of art.

CONCLUSIONS

In patients with HFpEF, body-weight fluctuations were independently associated with a significant increase in the risk of cardiovascular events. The magnitude of this risk increased with greater variability in body weight and was independent of HF severity, baseline weight, or direction of weight change.

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.

AUTHOR CONTRIBUTIONS

PH, WZ, and YD design the research. YL, YY, YW, WL, BD, and RX analyse the data. YL, YY, PH, and WZ 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.2021.689591/full#supplementary-material

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Samm50 Promotes Hypertrophy by Regulating Pink1-Dependent Mitophagy Signaling in Neonatal Cardiomyocytes

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Pathological cardiac hypertrophy, the adaptive response of the myocardium to various pathological stimuli, is one of the primary predictors and predisposing factors of heart failure. However, its molecular mechanisms underlying pathogenesis remain poorly understood. Here, we studied the function of Samm50 in mitophagy during Ang II-induced cardiomyocyte hypertrophy via lentiviruses mediated knockdown and overexpression of Samm50 protein. We first found that Samm50 is a key positive regulator of cardiac hypertrophy, for western blot and real-time quantitative PCR detection revealed Samm50 was downregulated both in pressure-overload-induced hypertrophic hearts and Ang II-induced cardiomyocyte hypertrophy. Then, Samm50 overexpression exhibits enhanced induction of cardiac hypertrophy marker genes and cell enlargement in primary mouse cardiomyocytes by qPCR and immunofluorescence analysis, respectively. Meanwhile, Samm50 remarkably reduced Ang II-induced autophagy as indicated by decreased mitophagy protein levels and autophagic flux, whereas the opposite phenotype was observed in Samm50 knockdown cardiomyocytes. However, the protective role of Samm50 deficiency against cardiac hypertrophy was abolished by inhibiting mitophagy through Vps34 inhibitor or Pink1 knockdown. Moreover, we further demonstrated that Samm50 interacted with Pink1 and stimulated the accumulation of Parkin on mitochondria to initiate mitophagy by co-immunoprecipitation analysis and immunofluorescence. Thus, these results suggest that Samm50 regulates Pink1-Parkin-mediated mitophagy to promote cardiac hypertrophy, and targeting mitophagy may provide new insights into the treatment of cardiac hypertrophy.

Keywords: cardiac hypertrophy, mitophagy, Samm50, Pink1, heart failure

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INTRODUCTION

Heart failure, a complex multifactorial syndrome, has now become a worldwide problem reaching epidemic proportions. Cardiac hypertrophy is regarded as the leading cause of heart failure. The onset of cardiac hypertrophy is characterized by a fetal reprogramming of gene expression where adult genes are repressed and fetal genes are activated, resulting in an imbalance between protein synthesis and degradation (1–3). At the molecular level, pathological hypertrophy has been

associated with mitogen-activated protein kinase signaling pathways, insulin-like growth factor-I phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B mammalian target of rapamycin (mTOR) signaling pathways, calcium signaling pathway, chromatin remodeling and so on (4–8). Although considerable progress has been made in elucidating the molecular mechanism of cardiac hypertrophy, there remain many unknowns.

Over the last decade, many studies have demonstrated that autophagy participates in the pathogenesis of cardiac hypertrophy. For example, cardiac-specific deficiency of Atg5 mice with pressure overload developed cardiac dysfunction and left ventricular dilatation (9). Furthermore, the key inhibitor of the mTOR pathway rapamycin, which is a potent activator of autophagy, has been reported to prevent cardiac hypertrophy (10, 11). Mitophagy, a special autophagy, removes damaged or redundant mitochondria to maintain heart function in response to various stress and heart disease conditions. PTEN-induced kinase1 (Pink1) is a key molecule for mediating mitophagy. Basally, Pink1 is rapidly degraded when it is translocated into mitochondria. However, in any case, if Pink1 is accumulated in the outer membrane of mitochondria (OMM), it would phosphorylate Parkin leading to activating its ligase activity. OMM proteins would be ubiquitinated, and autophagic receptors are recruited. A series of events eventually lead to the mitochondria being delivered to lysosomes (12–18).

All nuclear DNA-encoded mitochondrial proteins must be transported into mitochondria through channels in the OMM. The channel-forming protein Tom40 and voltage-dependent anion channel proteins (VDACs) play key roles (19). The sorting and assembly machinery (SAM) is critical for membrane integration and assembly of Tom40 and VDACs into the mitochondrial outer membrane. SAMM50 sorting and assembly machinery component (Samm50), a member of the SAM complex, contains a β-barrel domain that is conserved in evolution from bacteria to humans (20, 21). Samm50 is considered to be an essential protein present on the outer mitochondrial membrane, and it has been confirmed that it promotes the biogenesis of ß-barrel protein by directly interacting with the TOM complex. Samm50 interact with core proteins of the mitochondrial contact site and cristae organizing system complex to regulate cristae stability (22). Long-term lack of Samm50 influences the protein quantity of all large respiratory complexes of mitochondrial coding subunits, as well as mitochondrial swollen and mitochondrial inheritance impaired (23, 24). Samm50 also plays a key role in regulating Pink1 degraded through direct interaction (25).

Although Samm50 is a key regulatory factor of Pink1 and might affect mitophagy, whether it regulate the Pink1-Parkin pathway and involve in cardiac hypertrophy remains poorly understood. In this study, we found that Samm50 was downregulated in the cardiac hypertrophy model. Samm50 knockdown or overexpression was confirmed to mitigate or aggravate Angiotensin II (Ang II) induced cardiomyocyte hypertrophy, respectively. Mechanistically, we found that Samm50 inhibit mitophagy through interacting with Pink1. Collectively, we propose that Samm50 regulates mitophagy in cardiomyocytes and mediates pathological hypertrophy.

MATERIALS AND METHODS

Cell Culture and Treatment

Mouse neonatal ventricular cardiomyocytes were separated from 1–2-day-old neonatal C57BL/6J mice using enzymatic dissociation and cultured as previously described (26). Briefly, neonatal mice hearts were cut into small pieces and digested in 0.125 mg/ml trypsin (Gibco, #15090046) at 37°C. Then the supernatant was collected with complete Dulbecco's modified Eagle medium: Nutrient Mixture F-12 (DMEM/F12, Gibco, #8120319) and centrifuged at 600 g for 5 min. After repeating this cycle 6–8 times, the cell pellets were resuspended in DMEM/F12 containing 10% fetal bovine serum (FBS, Gibico, #10099133C), passed through a 100 mm cell strainer, and plated onto 10 cm dishes for 1.5 h at 37°C in 5% CO_2. The supernatant was then obtained and plated on dishes for further experiments. For gene overexpression and knockdown studies, cardiomyocytes were infected with lentiviruses according to the manufacturer's protocol. Recombinant lentiviruses were designed and synthesized by Fubio Biological Technology. After lentiviral transduction for 72–96 h, cardiomyocytes were stimulated with 10^{-6} M Ang II (Sigma, #A9525) for the indicated time points.

HL-1 cardiac muscle cell line (HL-1 cell) was purchased from Sigma-Aldrich (#SCC065) and maintained in Claycomb medium (Sigma-Aldrich, #51800C) supplemented with 10% FBS and 2 mM L-glutamine. Plasmids were transfected according to the manufacturer's protocol. After starving for 24 h in serum-free Claycomb medium, the cells were treated with 10^{-6} M Ang II for 24 h and followed by co-immunoprecipitation (co-IP) experiments.

Plasmids and Reagents

Pink1-Flag and Samm50-influenza hemagglutinin (HA) complementary DNA (cDNA) were cloned into the pcDNA3.1 vector. Short hairpin RNA (shRNA) against Samm50 and Pink1 were performed using the pLKO vector. The target sequences of shRNA oligonucleotides were listed in **Supplementary Table 1**. The Vps34 inhibitor was purchased from MCE (#HY-12794). Lipo8000 transfection reagent was purchased from Beyotime (#C0533).

Transverse Aortic Constriction Model

C57BL/6J male mice (10–12 weeks) were subjected to transverse aortic constriction (TAC) to simulate the pressure overload model, and mice were sacrificed two weeks after surgery. After opening the chest cavity and separating the aortic arch, a 27-gauge needle was placed on the aorta between the left common carotid artery and the innominate artery, followed by ligation with 6-0 silk. Then, the needle was removed to generate aortic constriction. The Sham group mice underwent an identical surgery apart from the ligation. The animal study was reviewed and approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

RNA Isolation and Quantitative PCR Analysis

Total RNA was extracted with the TRIzol reagent (Ambion, #257401). cDNA was generated using PrimeScript^{TM} RT Reagent Kit with gDNA Eraser (Takara, #RR047A) following the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed by ChamQ Universal SYBR qPCR Master Mix (Vazyme, #Q711-02) on a Bio-Rad IQ5 multicolor detection system. The program was as follows: 5 min at 95°C followed by 40 cycles of 20 s at 95°C and 30 s at 60°C. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method (27). The primer sequences were listed in **Supplementary Table 2**.

Immunoblot and Co-immunoprecipitation Assay

Proteins were obtained from tissues and cells lysed in lysis buffer (Beyotime, #P0013B). Then, samples were separated by 10–15% SDS-polyacrylamide electrophoresis gel and transferred to PVDF membranes. After blocking in 5% BSA, blots were incubated with the primary antibodies at 4°C overnight, followed by incubation with peroxidase-conjugated rabbit secondary antibody (Thermo Fisher, #A0545, 1:5000) at room temperature for 1 h. The following antibodies were used in this study: anti-GAPDH (Proteintech, #HRP-60004, 1:10000), anti-LC3 (Cell Signaling Technology, #2775, 1:1000), anti-COX4 (Proteintech, #11242-1-AP, 1:1000), anti-Pink1 (Proteintech, #23274-1-AP, 1:1000), anti-Parkin (Proteintech, #14060-1-AP, 1:1000), anti-Samm50 (Abcam, #ab133709, 1:5000) and anti-TOM20 (Santa Cruz Biotechnology, #sc-11415, 1:1000).

For co-IP analysis, proteins were prepared with non-denaturing lysis buffer and incubated with primary antibodies against HA or isotype control immunoglobulin G (IgG) at 4°C overnight. Then, 50 μl magnetic beads (MCE, #HY-K0205) were added into the mixture and incubated for 4 h with rotation. After removing the surface attachments, samples were obtained from the bead-antibody complexes and subjected to immunoassay as above described. The following antibodies were used in this study: anti-IgG (Proteintech, #30000-0-AP, 1:50) and anti-HA (Proteintech, #51064-2-AP, 1:50).

Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.1% Triton X-100 for 5 min, and blocked in 5% BSA for 1 h. Then cells were stained with the following primary antibodies overnight at 4°C: Cardiac troponin T (Abcam, #ab8295, 1:200), Samm50 (Proteintech, #28679-1-AP, 1:50), Pink1 (Proteintech, #23274-1-AP, 1:100) and VDAC1 (Santa Cruz Biotechnology, #sc-390996, 1:50). After washing with PBS 3 times, cells were incubated with specific secondary antibodies conjugated to Alexa Fluor for 1 h at 37°C. Five minutes after co-staining with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, #D1306), the cells were observed by a fluorescence microscope, and cardiomyocyte surface area was calculated by Image-Pro Plus software.

Heart sections were deparaffinized and rehydrated and then antigenically retrieved in sodium citrate buffer for 30 min. The following procedures were conducted according to the protocol described for cardiomyocytes. Co-localization was analyzed by Image-Pro Plus software.

Measurement of Autophagy Flux

Adenovirus harboring tandem fluorescent mRFP-GFP-LC3 system (adenovirus-tf-LC3) was used to evaluate autophagy flux as previously described (28). Cardiomyocytes plated on coverslips were transfected with adenovirus-tf-LC3 at 10 MOI for 24 h and treated with Ang II for 6 h. Then cells were fixed with 4% paraformaldehyde, stained with DAPI, and observed under a fluorescence microscope. The number of GFP and mRFP dots were recorded by manual counting of fluorescent puncta from at least 50 cells. The number of DAPI-stained nuclei were recorded to represent the nuclear number.

Statistics

All data were presented as mean ± s.e.m. All statistical results were analyzed using GraphPad Prism Software. Statistical analysis of two sets was performed by Student's t-test. Multiple comparisons were conducted by one-way analysis of variance with the Newman–Keuls test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Samm50 Is Downregulated in Myocardial Hypertrophy

To determine whether Samm50 expression is associated with cardiac hypertrophy, we performed the TAC mice model (**Supplementary Figures 1A–D**) and found the mRNA, and protein levels of Samm50 were remarkably decreased in response to pressure overload (**Figures 1A,B**). Parallelly, in the isolated mice neonatal cardiomyocytes, qRT-PCR and western bolt (WB) data showed that Samm50 expression levels were significantly reduced by Ang II stimulation (**Figures 1D,E**). Interestingly, Samm50 expression was unchanged in cardiac fibroblasts under Ang II treatment (**Supplementary Figures 1E,F**). The immunofluorescence staining also showed a consistent result of decreased Samm50 levels in TAC-induced hypertrophic hearts and Ang II-treated cardiomyocytes (**Figures 1C,F**). These results revealed that Samm50 was dramatically downregulated both *in vivo* and *in vitro* in response to hypertrophic stimuli, indicating a potential role of Samm50 in the regulation of cardiac hypertrophy.

Overexpression of Samm50 Exacerbates Cardiomyocyte Hypertrophy and Inhibits Mitophagy

To investigate the potential effect of Samm50 on cardiomyocyte hypertrophy, we transfected lentivirus overexpressed Samm50 into isolated and cultured mice neonatal cardiomyocytes and verified Samm50 expression by WB and qRT-PCR analysis (**Figures 2A,B**). After Ang II administration, the expressions of hypertrophic markers such as *nppb* (natriuretic peptide B), *c-jun* (jun proto-oncogene), *c-fos* (Fos proto-oncogene), and *rcan1.4* (regulator of calcineurin 1, transcript

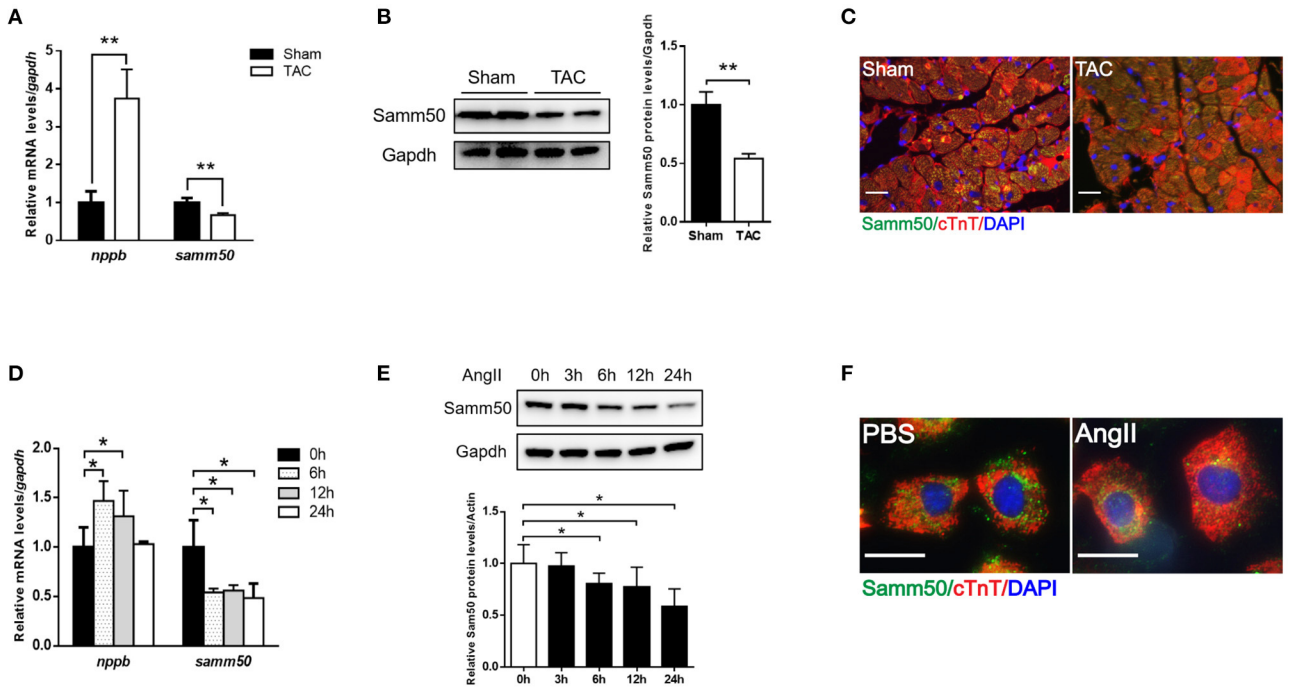

FIGURE 1 | Samm50 is decreased in cardiomyocytes and ventricular tissues. **(A)** Quantification analysis of *samm50* and *nppb* expression in hearts of Sham and TAC mouse. **(B)** Representative western bolts (WBs) images and statistical results of *samm50* expression in cardiac tissues. **(C)** Immunofluorescence staining of *samm50* in mouse ventricular tissues of Sham and TAC group. Scale bars, 20 μm. $n = 5$ mice in each group. **(D,E)** Quantification (left) and WBs (right) of *samm50* expression in mouse neonatal cardiomyocytes treated with Ang II (10^{-6} M) for the indicated time points. **(F)** Immunofluorescence staining of endogenous *samm50* in mouse neonatal cardiomyocytes treated with PBS or Ang II. Scale bars, 20 μm. $n = 3$ independent experiments. $^{**}P < 0.01$ compared with Sham or PBS group; and $^{*}P < 0.05$ compared with Sham or PBS group. All data are presented as mean ± s.e.m.

4) were obviously elevated in the Samm50-overexpressed group compared with the control group (**Figure 2C**). Furthermore, cardiomyocytes were incubated with cardiac troponin T to evaluate the cardiomyocyte surface area. As shown in **Figure 2D**, Samm50 overexpression markedly enlarged the cardiomyocyte size in response to Ang II treatment. These data indicated that Samm50 exacerbates cardiac hypertrophy.

Mitochondrial dynamics are critical to maintain cardiomyocyte function, especially under pathological stress. Given that Samm50 is associated with mitochondrial homeostasis and mitophagy (25), we explored whether Samm50 aggravates cardiac hypertrophy by affecting mitochondria function. We first evaluated mitochondrial biogenesis by detecting the expression of mitochondrial *NADH dehydrogenase 1* (*mtND1*). The results showed that *mtND1* was not altered in response to Samm50 overexpression (**Supplementary Figure 1G**). Then, autophagy markers and mitochondria protein levels were assessed by immunoblotting. Compared with the control group, overexpression of Samm50 displayed decreased LC3-II/LC3-I ratio and increased levels of TOM20 and COX4, indicating inhibition of mitophagy in cardiomyocytes (**Figure 2E**). Besides, the adenovirus-tf-LC3 was generated to evaluate autophagic flux. We monitored the number of red and green puncta by fluorescence microscope. The red puncta overlapped with the green ones are indicators of autophagosomes, while the free red puncta represent autolysosomes (29). As shown in **Figure 2F**, the yellow and red puncta were both increased in Ang II-treated cardiomyocytes, whereas Samm50 overexpression evidently decreased the number of red puncta, and most of them overlaid with the green ones in merged images, indicating Samm50 inhibits autophagic flux. Overall, these data demonstrated that Samm50 exacerbates Ang II-induced cardiomyocyte hypertrophy and inhibits mitophagy.

Samm50 Depletion Attenuates Cardiomyocyte Hypertrophy by Promoting Mitophagy

To further verify the effects of Samm50 on cardiac hypertrophy and heart failure, we performed lentivirus-delivered shRNA to ablate *samm50* expression. The efficacy of *samm50* depletion was confirmed by WB and qRT-PCR analysis, respectively (**Figures 3A,B**). In accordance with the results observed in Samm50 overexpression, Samm50 deficiency remarkably reduced the expression of hypertrophic markers and ameliorated cardiomyocyte size compared with the control group, as evidenced by qRT-PCR and immunofluorescence (**Figures 3C,D**). Knockdown of

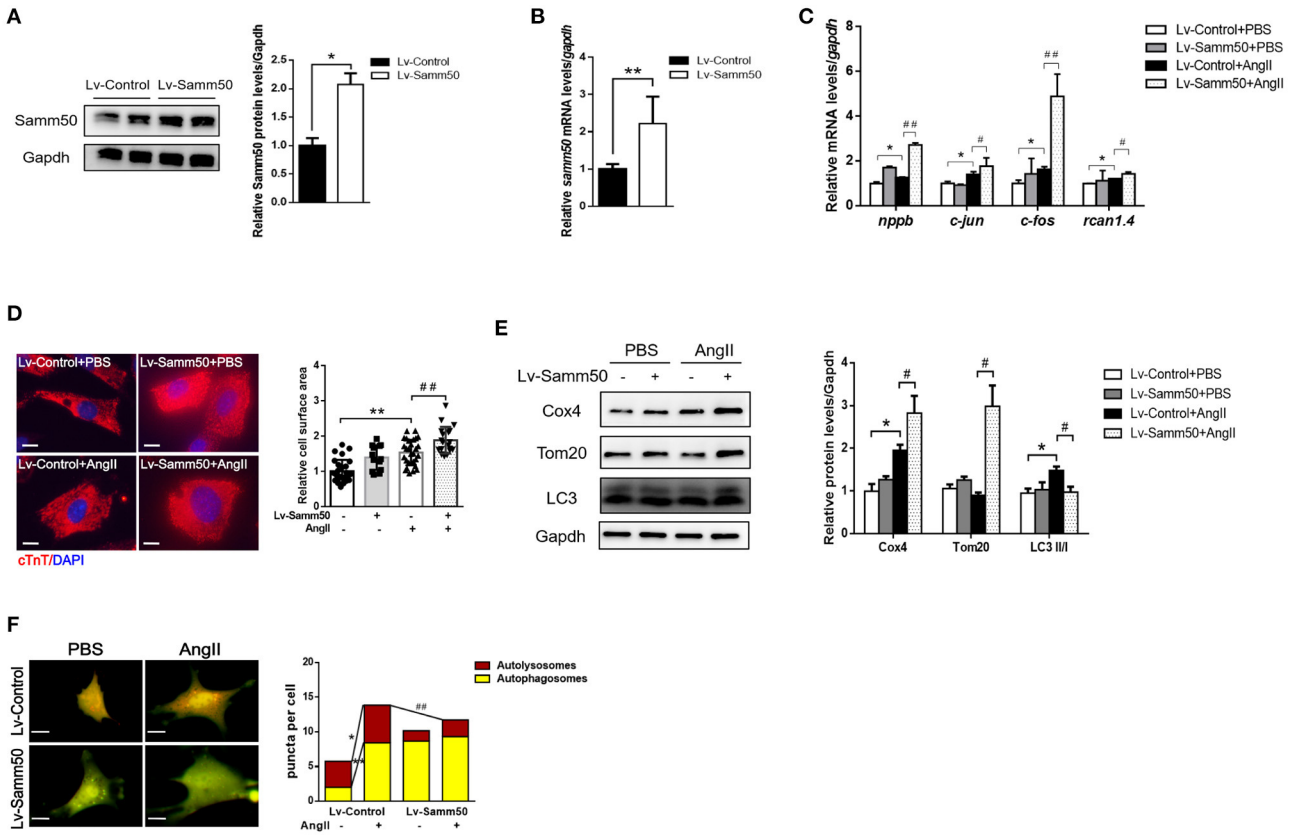

FIGURE 2 | Samm50 exacerbates cardiomyocyte hypertrophy and inhibits mitophagy. **(A,B)** WB (left) and qRT-PCR (right) analysis of Samm50 overexpression levels in mouse neonatal cardiomyocytes. **(C)** Relative mRNA levels of hypertrophic markers gene expression in Lv-Control or Lv-Samm50 cardiomyocytes with Ang II treatment. Lv-Control, lentiviral pCDH-control and Lv-Samm50, lentivirus overexpressed Samm50. **(D)** Representative images and calculated cell surface area of cardiomyocytes infected with indicated lentivirus in response to Ang II stimulation. Scale bars, 10 μm. **(E)** Immunoblotting analysis of indicated protein levels in Lv-Control or Lv-Samm50 cardiomyocytes treated with PBS or Ang II. **(F)** Representative images of fluorescent LC3 puncta and statistical analysis results, and autolysosomes per cell. $n = 6$ for each experiment group. $^{*}P < 0.05$ compared with the control group; $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$ compared with Ang II group; and $^{\#\#}P < 0.01$ compared with Ang II group. All data are presented as mean ± s.e.m.

Samm50 also exhibited increased mitophagy, manifested by an increase in LC3-II/LC3-I ratio and a decrease in TOM20 and COX4. Furthermore, in response to Ang II treatment, Samm50 ablation caused a significant accumulation of red puncta, which was greater than that in yellow puncta, indicating Samm50 increases autophagosomes more than autophagosomes and thus stimulates autophagic flux (**Figure 3F**). These results suggested that Samm50 deficiency ameliorates cardiomyocyte hypertrophy and enhanced mitophagy.

To evaluate the role of mitophagy in Samm50-mediated cardiac hypertrophy, we introduced an autophagy inhibitor into the Samm50-depleted cardiomyocyte prior to Ang II stimulation. Notably, the protective effect of Samm50-deficiency on cardiomyocyte hypertrophy was largely diminished in the presence of autophagy inhibitors, as demonstrated by the increases of hypertrophic markers and cardiomyocyte size (**Figures 3G,H**), further supporting the idea that mitophagy is required for Samm50-mediated cardiomyocyte hypertrophy.

Samm50 Regulates Pink1-Parkin-Mediated Mitophagy in Myocardial Hypertrophy

The previous study has suggested that Samm50-mediated mitophagy is dependent on the Pink1-Parkin pathway under cancer conditions (25). To further elucidate the underlying mechanism of Samm50 in the regulation of myocardial hypertrophy, we first detected the expression of Pink1 and Parkin in response to Ang II stimulation. As seen in **Figure 4A**, knockdown of Samm50 caused accumulation of Pink1 and Parkin, indicating the activation of mitophagy in cardiomyocytes. However, Pink1 and Parkin expression had no obvious difference between Samm50 overexpression and control group (**Figure 4B**). To evaluate whether Pink1 is required for Samm50-mediated cardiac hypertrophy, we interfered with the expression of Pink1 in the absence of Samm50. Our data revealed that Samm50 depletion-trigged activation of mitophagy was blunted in cardiomyocytes with simultaneous Pink1 knockout, as indicated by decreased LC3-II/LC3-I ratio and increased protein level of TOM20 and COX4 (**Figure 4C**). Consistent

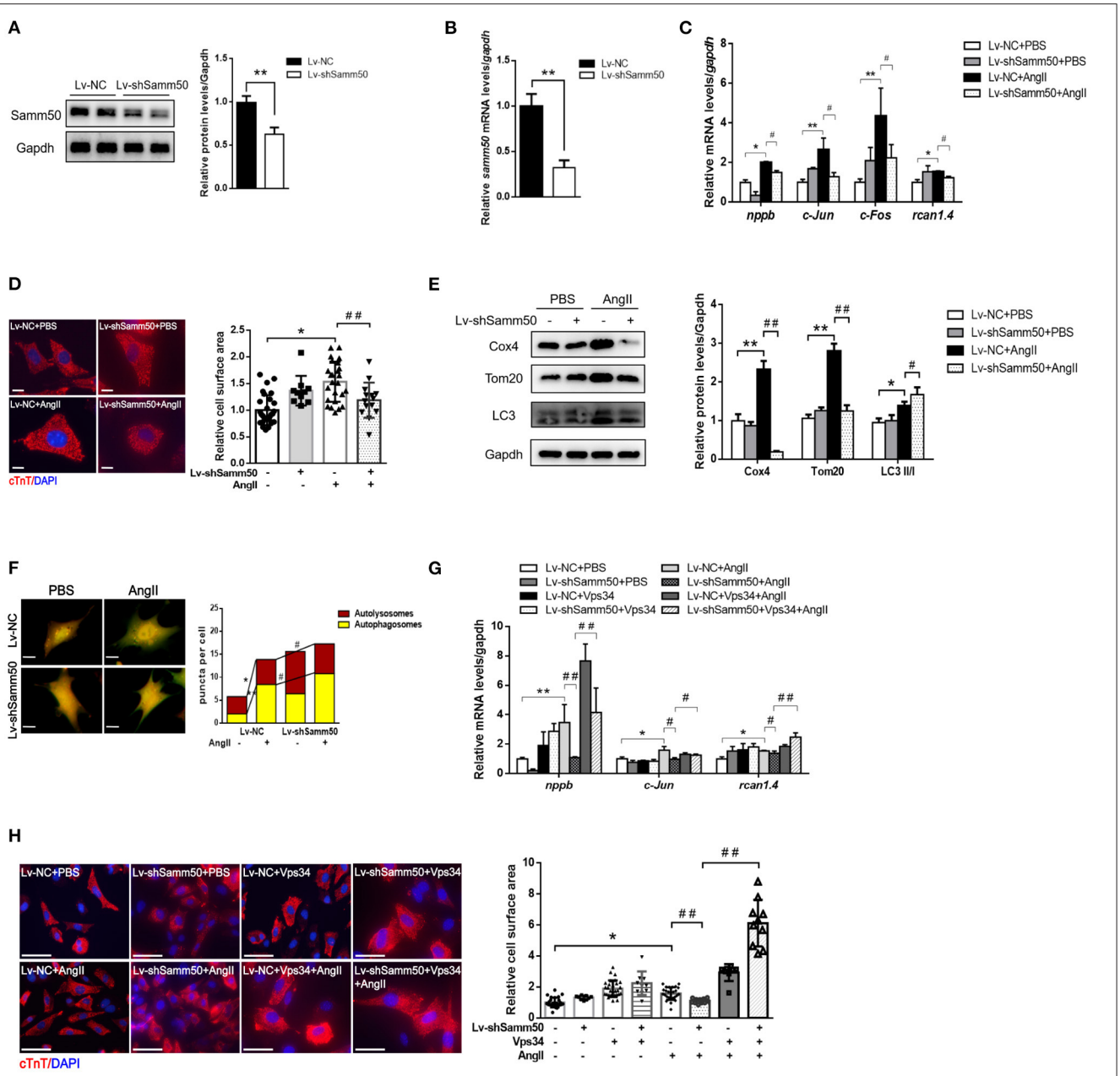

FIGURE 3 | Samm50 deficiency alleviated cardiomyocytes hypertrophy by promoting mitophagy. **(A,B)** WB and qRT-PCR analysis of Samm50 knockdown efficiency in mouse neonatal cardiomyocytes. **(C)** Relative mRNA levels of hypertrophic marker gene expression in Lv-NC or Lv-shSamm50 cardiomyocytes with Ang II treatment. Lv-NC, lentivirus with pLKO control and Lv-shSamm50, lentiviral-delivered shRNA against Samm50. **(D)** Representative images and calculated cell surface area of cardiomyocytes with Ang II treatment. Scale bars, 10 μm. **(E)** Immunoblotting and statistical analysis of mitophagy protein levels in Lv-NC or Lv-shSamm50 cardiomyocytes treated with PBS or Ang II. **(F)** Representative images of fluorescent LC3 puncta and analysis results. Scale bars, 10 μm. **(G)** Quantification of hypertrophic markers expression in cardiomyocytes infected with indicated lentivirus and treated with or without Vps34 inhibitor prior to Ang II stimulation. **(H)** Representative images and quantitative results of cell surface area in response to Ang II. Scale bars, 50 μm. $n = 6$ for each experiment group. $^{*}P < 0.05$ compared with the control group; $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$ compared with Ang II group; and $^{\#\#}P < 0.01$ compared with Ang II group. All data are presented as mean ± s.e.m.

with immunoblotting results, the protective effect of Samm50 deficiency in response to Ang II induction was blocked by Pink1 knockdown, which was manifested by enhanced hypertrophic markers and cardiomyocyte enlargement (**Figures 4D,E**).

Based on Samm50 interaction with Pink1 and regulation of its stability (25), we transfected HL-1 cells with HA-tagged Samm50 and Flag-tagged Pink1, followed by Ang II treatment and co-IP analysis to explore whether their physical interaction changes

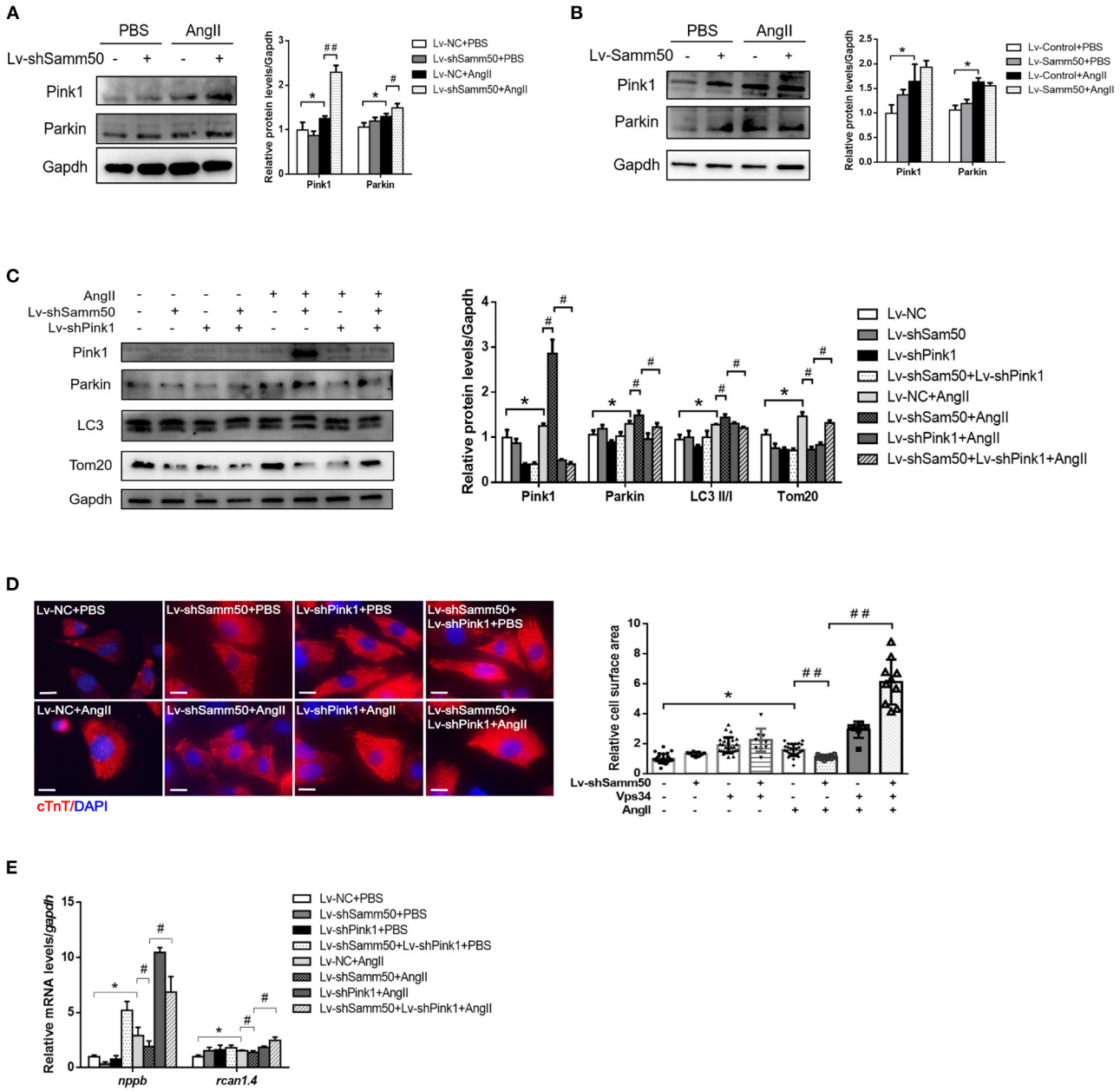

FIGURE 4 | Samm50 regulates Pink1-Parkin-mediated cardiomyocyte mitophagy. **(A)** Cardiomyocytes transduced with Lv-NC or Lv-shSamm50 lentivirus were treated with Ang II and subjected to immunoblot analysis. **(B)** WB analysis of indicated proteins in Lv-control or Lv-Samm50 cardiomyocytes in response to Ang II. **(C)** Immunoblotting analysis for indicated proteins of control and Samm50-deficient cardiomyocytes infected with control or shPink1 lentiviral particles **(D,E)** Cell surface area and quantification of hypertrophic markers expression in cardiomyocytes infected with indicated lentivirus with Ang II stimulation. Scale bars, 10 μm. $^{*}P < 0.05$ compared with the control group; $^{\#}P < 0.05$ compared with Ang II group; $^{\#\#}P < 0.01$ compared with Ang II group. All data are presented as mean ± s.e.m.

under Ang II stimulation. The co-IP of Samm50 by HA antibody exhibited an interaction between Samm50 and Pink1 in HL-1 cells, while there was no significant difference in the level of Pink1 detected in the absence or presence of Ang II, indicating the interaction between Samm50 and Pink1 was not influenced by Ang II stimulation (**Figure 5A**).

Pink1 and Parkin were recruited during mitophagy and accumulated in mitochondria (16). To further verify the activation of the Pink1/Parkin pathway in Samm50-mediated hypertrophy, we utilized the immunofluorescent staining to visualize the subcellular localization of Parkin and mitochondria in Samm50-deficient cardiomyocytes. The results showed

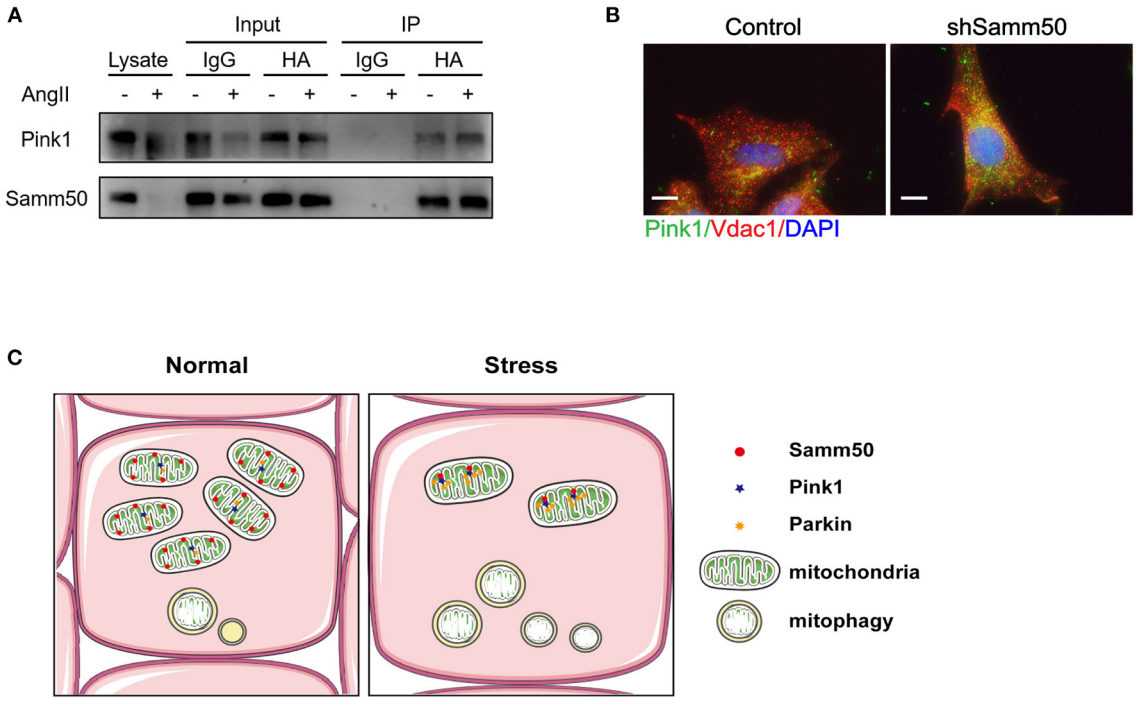

FIGURE 5 | Samm50 interacts with Pink1 to mediate cardiac hypertrophy. **(A)** Co-immunoprecipitation analysis of Samm50 and Pink1 in HL-1 cells. **(B)** Representative immunofluorescence images of Pink1 and VDAC1 co-localization in mouse neonatal cardiomyocytes infected with control or shSamm50 lentiviral particles in response to Ang II. Scale bars, 10 μm. $n = 6$ for each experiment group. **(C)** The model of Samm50 regulates cardiac hypertrophy. Under cardiac hypertrophy, Samm50 interacts with Pink1 resulted in Pink1 accumulation, Parkin recruitment, and mitophagy.

that Parkin was predominantly stained with mitochondria marker VDAC1 (**Figure 5B**), indicating the accumulation of Parkin in mitochondria. Overall, these results demonstrate that Samm50 regulates cardiomyocyte hypertrophy in a Pink1/Parkin-dependent manner.

DISCUSSION

Cardiac hypertrophy is one of the primary reasons for heart failure. Pathological gene reprogramming could develop cardiac hypertrophy. This process is governed by multiple factors that could coordinate cardiac remodeling or against remodeling. Here, we identified a member of mitochondrial SAM complex, Samm50, which positively regulates cardiac hypertrophy involving mitophagy. We first observed the downregulation of Samm50 expression in the TAC model and Ang II-treated cardiomyocytes. Using gain- and loss-of-function approaches, we determined the potential role of Samm50 on pathological cardiac hypertrophy. Our results showed that Samm50 exacerbates cardiac hypertrophy by inhibiting mitophagy.

The more recent development of signaling effectors of cardiac hypertrophy is identified using genetically modified mouse models and primary cardiomyocytes. Mechanical stress, humoral stimuli, chromatin remodeling, inflammation, redox, and Ca^{2+} signaling were discovered to be strongly involved in cardiac hypertrophy (30). Furthermore, mitochondrial dysfunction has been indicated as a potential and important player in the development of cardiac hypertrophy (31). Mitophagy is essential to mitochondria homeostasis. However, few studies focus on the relationship between mitophagy and cardiac hypertrophy. One study showed that macrophage migration inhibitory factor significantly reduced pressure overload-induced cardiac hypertrophy by activating mitophagy and autophagy (32). Another one showed that the knockdown of lysocardiolipin acyltransferase 1 upregulates mitophagy to mitigate cardiac dysfunction associated with cardiac hypertrophy (33). Given that Samm50 was reported as a regulator of mitophagy and mitochondrial function, we first explored the involvements of mitochondria biogenesis in Samm50-mediated cardiomyocyte hypertrophy. Consistent with the previous study, the mitochondrial DNA copy number was not altered in these groups (**Supplementary Figure 1G**). We further observed that Samm50 depletion attenuates cardiomyocyte hypertrophy and promotes mitophagy (**Figures 3C–F**), indicating that Samm50 might inhibit mitophagy to exacerbate cardiac hypertrophy. Thus, we investigated the effect of autophagy inhibitors on the effect of Samm50 depletion in response to Ang II. The results revealed that the Vps34 inhibitor largely diminished the protective effect of Samm50 deficiency on Ang II-induced hypertrophy (**Figures 3G,H**), indicating the

beneficial function of mitophagy in cardiac hypertrophy and that Samm50 regulates cardiomyocyte hypertrophy by inhibiting mitophagy. This result enlarged our understanding of mitophagy in cardiac hypertrophy.

Cardiac hypertrophy decompensation could be regulated by mitochondrial reprogramming. Samm50 is widely known as an essential protein of the mitochondrial outer membrane and has been verified to interact directly with the TOM complex to facilitate the biogenesis of ß-barrel protein. However, whether Samm50 participates in the cardiac hypertrophy process remains unclear. In our study, we found that the expression of Samm50 was significantly downregulated *in vivo* and *in vitro* cardiac hypertrophy model (**Figures 1A–F**), indicating that the changes of Samm50 maybe play a role in cardiac hypertrophy. To further clarify the relationship between Samm50 and hypertrophy, we conducted gain- and loss-of-function studies using lentivirus. We found that Samm50 overexpression aggravated the expression of hypertrophic markers and enlarged the cardiomyocyte size in response to Ang II (**Figures 2C,D**). Meanwhile, knockdown Samm50 could ameliorate cardiac hypertrophy (**Figures 3C,D**). Further understanding the mechanism by which Samm50 regulates mitophagy in cardiac hypertrophy is important for exploring the new strategies for treatment. Samm50 is a critical regulator of Pink1-Parkin-mediated or P62-mediated mitophagy (25, 34), whereas most mitophagy signals converge on the Pink1-Parkin-mediated pathway. Fenglei et al. revealed that Samm50 interacts with Pink1 and regulates its stability in HeLa cells (25). Agreed with the previous study, our results showed that Samm50 could directly interact with Pink1 in the cardiac hypertrophy model (**Figure 5A**). Besides, interference with Pink1 can eliminate the activation of mitophagy and the protective effect induced by Samm50 deficiency (**Figures 4D,E**). Collectively, we first discovered that Samm50-mediated mitophagy in cardiac hypertrophy was largely dependent on Pink1-Parkin signaling (**Figure 5C**). However, further experiments need to be performed to elaborate the mechanism involved in modulating Pink-Parkin by Samm50.

In conclusion, our present study shows Samm50 aggravates cardiac hypertrophy by regulating the Pink1-Parkin signaling and further clarifies the relationship between mitophagy and cardiac hypertrophy. These results may provide new insights into the treatment of cardiac hypertrophy.

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 animal study was reviewed and approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

AUTHOR CONTRIBUTIONS

ZD, YZ, and RX designed the study. RX, ZD, LK, and SW predominantly performed experiments. RX, LK, CY, and YF analyzed the data. ZD and RX wrote the manuscript. YZ revised the manuscript. All authors read 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.2021.748156/full#supplementary-material

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Development and Validation of a Novel Nomogram for Preoperative Prediction of In-Hospital Mortality After Coronary Artery Bypass Grafting Surgery in Heart Failure With Reduced Ejection Fraction

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Background and Aims: Patients with heart failure with reduced ejection fraction (HFrEF) are among the most challenging patients undergoing coronary artery bypass grafting surgery (CABG). Several surgical risk scores are commonly used to predict the risk in patients undergoing CABG. However, these risk scores do not specifically target HFrEF patients. We aim to develop and validate a new nomogram score to predict the risk of in-hospital mortality among HFrEF patients after CABG.

Methods: The study retrospectively enrolled 489 patients who had HFrEF and underwent CABG. The outcome was postoperative in-hospital death. About 70% (n = 342) of the patients were randomly constituted a training cohort and the rest (n = 147) made a validation cohort. A multivariable logistic regression model was derived from the training cohort and presented as a nomogram to predict postoperative mortality in patients with HFrEF. The model performance was assessed in terms of discrimination and calibration. Besides, we compared the model with EuroSCORE-2 in terms of discrimination and calibration.

Results: Postoperative death occurred in 26 (7.6%) out of 342 patients in the training cohort, and in 10 (6.8%) out of 147 patients in the validation cohort. Eight preoperative factors were associated with postoperative death, including age, critical state, recent myocardial infarction, stroke, left ventricular ejection fraction (LVEF) ≤35%, LV dilatation, increased serum creatinine, and combined surgery. The nomogram achieved good discrimination with C-indexes of 0.889 (95%CI, 0.839–0.938) and 0.899 (95%CI, 0.835–0.963) in predicting the risk of mortality after CABG in the training and validation cohorts, respectively, and showed well-fitted calibration curves in the patients whose predicted mortality probabilities were below 40%. Compared with EuroSCORE-2, the nomogram had significantly higher C-indexes in the training cohort (0.889 vs. 0.762, p = 0.005) as well as the validation cohort (0.899 vs. 0.816, p = 0.039). Besides, the

nomogram had better calibration and reclassification than EuroSCORE-2 both in the training and validation cohort. The EuroSCORE-2 underestimated postoperative mortality risk, especially in high-risk patients.

Conclusions: The nomogram provides an optimal preoperative estimation of mortality risk after CABG in patients with HFrEF and has the potential to facilitate identifying HFrEF patients at high risk of in-hospital mortality.

Keywords: CABG, HFREF, nomogram, prediction, mortality, EuroSCORE-2

INTRODUCTION

The most common cause of heart failure (HF) is coronary artery disease (CAD), which accounts for about 60% of all causes of HF with reduced ejection fraction (HFrEF) (1, 2). For patients with HF, severe left ventricular (LV) systolic dysfunction, and CAD suitable for myocardial revascularization, coronary artery bypass grafting (CABG) is recommended as the first revascularization strategy (3). Despite the recent advances in cardiovascular surgery, CABG among HFrEF patients is still associated with a higher risk of morbidity and mortality than other patients. Therefore, risk assessment is necessary at the time of surgery in patients with HFrEF undergoing CABG. Several risk scores have been developed to help clinicians and patients make informed decisions regarding the risks of surgery. Examples include the Society of Thoracic Surgeons (STS) (4, 5), the EuroSCORE (6), the EuroSCORE-2 (7), and the SinoSCORE risk scores (8). Although helpful, these scores were based on general cardiac surgery patients rather than patients with HFrEF. Additionally, in addition to being outdated and collected more than 10 years ago such scores were developed on western patients, they might be less generalizable to the Chinese patients.

Due to the lack of a specific and practical risk score for HFrEF patients, developing a predictive model that incorporates factors associated with mortality based on preoperative variables is needed. Therefore, this study aims to develop and validate a nomogram score to predict the risk of in-hospital mortality among HFrEF patients with CABG and compare the nomogram score's predictive value with the EuroSCORE-2.

MATERIALS AND METHODS

Study Population

We recruited retrospectively consecutive patients who had undergone CABG in state of HFrEF between January 2013 and July 2019 at Beijing Anzhen Hospital, Capital Medical University. And the HFrEF is commonly defined as a reduction in LVEF to $\leq$40%, with symptoms and/or signs of heart failure (1, 2). The inclusion criteria included the following: (1) LVEF $\leq$40% assessed by the last preoperative echocardiography (closest to the time prior to surgery); (2) Symptomatic HF (New York Heart Association [NYHA] functional class II–IV) and; (3) Underwent elective CABG, with or without mitral valve surgery due to ischemic mitral regurgitation. The exclusion criteria included the following: (1) Emergency surgery; (2) Systolic arterial blood pressure <90 mmHg when supine, sitting, or standing; (3) Hemodynamically significant stenotic valvular heart disease; (4) Non-ischemic mitral valve regurgitation caused by papillary muscle rupture, rheumatism, degeneration, infective endocarditis, and congenital heart disease and other organic diseases; (5) Complicated with aortic valve disease, primary myocardiopathy, congenital heart disease, rheumatic heart disease, macrovascular disease or other non-ischemic myocardial diseases; and (6) Cardiogenic shock. Ethical approval was obtained from the Institutional Ethics Committee of Beijing Anzhen Hospital.

Surgical Procedures

All patients underwent CABG through a midline sternotomy. The left internal mammary artery was the first choice for graft the left anterior descending artery. Saphenous veins and radial arteries were harvested with an open technique, and sequential or separate aortocoronary bypass grafting was performed in the remaining coronary arteries. A transit-time flow probe was used to assess the quality of anastomosis after grafting in all patients. The surgical procedure was jointly decided by more than two experienced surgeons after discussion for patients with mitral regurgitation or ventricular aneurysm. For isolated CABG, the choice of off-pump CABG, on-pump CABG, or On-pump beating heart CABG depended on the surgeon's habit and experience as well as intraoperative conditions.

Data Collection

Clinical characteristics, echocardiographic findings, laboratory results, and surgical characteristics were collected by trained physicians who are blind to the aim of study with a standard data collection form. In EuroSCORE-2, the critical state is an important variable that included various preoperative conditions and major adverse events. Refer to the definition of critical preoperative state in the EuroSCORE-2, the critical state was defined as a history of ventricular tachycardia or ventricular fibrillation or aborted sudden death, preoperative cardiac massage, preoperative ventilation before anesthetic room, preoperative inotropes, or end-organ damage. Recent myocardial infarction (MI) was defined as MI within 90 days. Increased serum creatinine was defined as serum creatinine measured before surgery >1.5 mg/dl. The echocardiographical parameters, including LVEF and Left ventricular internal diameter at end-diastolic (LVIDd), were extracted from the last preoperative echocardiography (closest to the time prior to surgery). BSA was calculated by Mosteller's formula (9). LVIDd/BSA $\geq$3.5 cm/m^2 indicated a moderate or serve Left ventricular

(LV) dilatation according to Echocardiography's Guidelines for Chamber Quantification (10). Combined surgery indicated operations combined more than one procedure: include major interventions on the heart such as CABG, mitral valve repair or replacement, and treatment on ventricular aneurysm.

Clinical Outcome

The primary end point was post-operative mortality during hospitalization. Mortality was defined as any death occurring after a surgical procedure during the hospital stay.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median (25th, 75th percentiles) in case of normal or non-normal distribution. The differences between the two groups were examined by independent-sample *t*-test or Mann–Whiney *U*-test, correspondingly. Categorical variables were presented as counts (percentage) and compared by Pearson chi-square test (Pearson $\chi 2$ test) or Fisher exact test, as appropriate.

The entire cohort was randomly divided into training cohort and validation cohort (7:3) base on complete data. The training cohort was used to develop the model, and the validation cohort was applied to validate the model. Univariable logistic regression analysis was used to identify the possible predictive factors. The variables with a $p < 0.15$ in univariable analysis and those consistently reported in previous studies were candidates for multivariable logistic regression analysis to identify the independent risk factors for predicting postoperative mortality. We used a backward stepwise elimination approach to simplify the model based on the Akaike Information Criterion. LASSO regression was also applied in the predictors' selection to examine the importance of predictive variables selected by stepwise regression analysis. Based on the selected predictive variables, the logistic regression model was developed and presented as the nomogram.

We assessed the predictive accuracy of the nomogram with discrimination and calibration. To quantify the discrimination performance of the nomogram, Harrell's C-index was measured. The Harrells C statistic is a measure of discrimination that is similar to the area under a receiver operating characteristic curve (ROC) (11). Calibration curves were plotted to assess the calibration of the nomogram, accompanied with the Hosmer-Lemeshow test [A significant test statistic implies that the model doesn't calibrate perfectly (12)]. To further assess model calibration, predicted probabilities for mortality were calculated for participants in the training cohort, divided into quintiles, and compared with observed mortality. The results were presented as a bar chart. To decrease the overfit bias and increase precision, the nomogram model was subjected to bootstrapping validation (1000 bootstrap resamples) to evaluate a relatively corrected C-index and calibration ability in the training cohort. To assess the performance of the nomogram in the validation cohort, the logistic regression formula developed in the training cohort was then applied in the validation cohort, with predicted postoperative mortality calculated. Finally, the C-index, the calibration curve, and the Hosmer-Lemeshow test were used.

EuroSCORE-2 online calculator (http://www.euroscore.org) was used to calculate the predicted mortality of each patient. DeLong's test was used to compare C-index between the nomogram and the EuroSCORE-2 in the training and validation cohort, respectively. Besides, we calculated the categorical net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to determine the extent to which the predictive power of the nomogram is better than EuroSCORE-2. Calibration of the two models was evaluated and compared by the Hosmer-Lemeshow statistic $\chi 2$ and $P > 0.05$ indicates the model fits well. Similarly, the two models were visualized graphically by comparing the observed probability with the predicted probability of death across quintiles of predicted risk.

The present study is reported in compliance with standard guidelines (13) for prediction models and the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) checklist is presented in **Supplementary Material**. Statistical analysis was conducted in R software (version 4.0.2; http://www.Rproject.org). C-index, calibration curve, nomogram, and bootstrapping validation were calculated or formulated using rms and riskRegression packages in R. NRI and IDI were calculated with PredictABEL packages in R. A two-tailed $p < 0.05$ indicated statistical significance. Data analysis was conducted from November 7, 2020 to February 24, 2021.

RESULTS

A total of 489 consecutive patients were enrolled in the present study. Of these, 36 patients (7.4%) died after surgery. The perioperative complications included prolonged ventilation >96 hours in 36 (7.36%) patients, reoperation for any reason in 23 (4.70%), reoperation for bleeding in 9 (1.84%), tracheostomy in 11 (2.25%), renal failure requiring continuous renal replacement therapy (CRRT) in 24 (4.91%), stroke in 12 (2.45%), cardiac arrest requiring cardiopulmonary resuscitation (CPR) in 14 (2.86%), and implantation of extracorporeal membrane oxygenation (ECMO) in 11 (2.25%).

We randomly allocated 70% (342) of patients to the training cohort and the remainder 30% (147) to a validation cohort. There were 26(7.6%) and 10(6.8%) patients who died after surgery in the training and validation cohorts, respectively. The baseline characteristics in all cohorts are listed in **Table 1**. There were no significant differences between the training and validation cohorts regarding preoperative baseline and surgical characteristics.

Univariable Analysis

The results of the univariable logistic regression analysis of predictors associated with postoperative mortality in the training cohort are presented in **Table 2**. Univariable analysis in the training cohort showed a significant association of postoperative morality with several predictors including age, critical state, diabetes on insulin, stroke, recent myocardial infarction (MI) within 90 days, CCS angina class IV, lower limb arterial stenosis, left ventricular ejection fraction (LVEF) ≤35%, LV (left

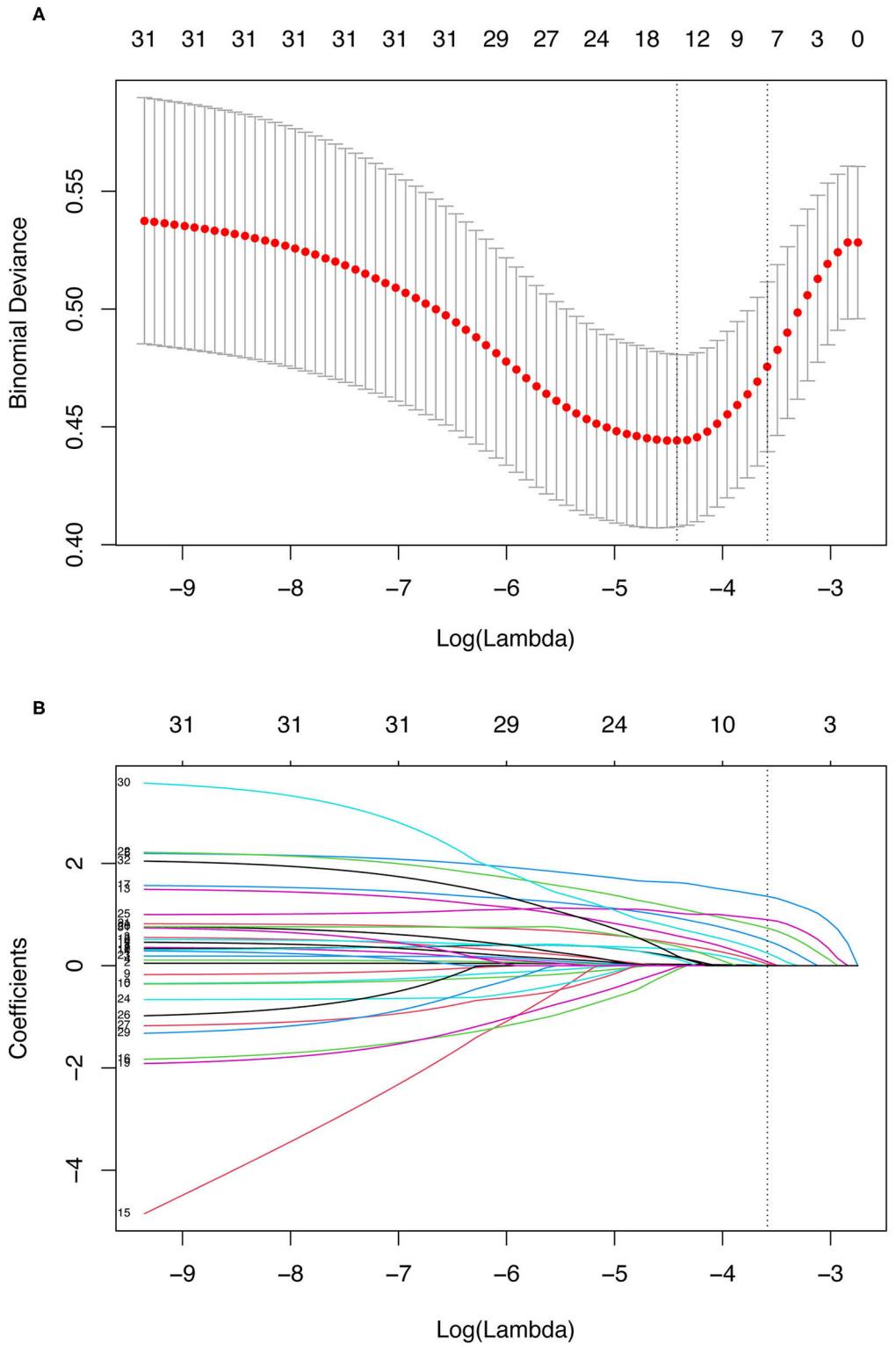

FIGURE 1 | Demographic and clinical variables selection using the LASSO binary logistic regression model. **(A)** Optimal parameter (lambda) selection in the LASSO model used tenfold cross-validation *via* minimum criteria. The binomial deviance curve was plotted vs. log(lambda). Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 SE of the minimum criteria (the 1-SE criteria). **(B)** LASSO coefficient profiles of the 31 variables. A coefficient profile plot was produced against the log(lambda) sequence. A dotted vertical line was drawn at the value selected using tenfold cross-validation, where optimal lambda (with the 1-SE criteria) resulted in eight predictive variables the same as variables selected by the stepwise regression method. LASSO, least absolute shrinkage and selection operator; SE, standard error.

TABLE 1 | Baseline characteristics of patients.

Variable	Entire cohort (n = 489)	Training cohort (n = 342)	Validation cohort (n = 147)	P-Value
Age (years)	60.0 (52.0,67.0)	60.0 (52.2,66.0)	60.0 (52.0,67.0)	0.746
Female	75 (15.3%)	53 (15.5%)	22 (15.0%)	0.990
BMI (kg/m^2)	25.2 (23.4,27.3)	25.4 (23.5,27.4)	24.9 (23.3,27.0)	0.359
Critical state	13 (2.7%)	9 (2.6%)	4 (2.7%)	1.000
Hypertension	243 (49.7%)	169 (49.4%)	74 (50.3%)	0.929
Diabetes mellitus	233 (47.6%)	163 (47.7%)	70 (47.6%)	1.000
Diabetes on insulin	82 (16.8%)	50 (14.6%)	32 (21.8%)	0.071
Hyperlipidemia	151 (30.9%)	107 (31.3%)	44 (29.9%)	0.849
Smoke	280 (57.3%)	200 (58.5%)	80 (54.4%)	0.464
Alcohol	105 (21.5%)	67 (19.6%)	38 (25.9%)	0.154
Chronic kidney disease	8 (1.6%)	6 (1.8%)	2 (1.4%)	1.000
Dialysis	4 (0.8%)	2 (0.6%)	2 (1.4%)	0.587
Chronic pulmoriary disease	19 (3.9%)	16 (4.7%)	3 (2.0%)	0.259
Stroke	74 (15.1%)	51 (14.9%)	23 (15.6%)	0.944
PCI	116 (23.7%)	84 (24.6%)	32 (21.8%)	0.582
Previous cardiac surgery	2 (0.4%)	1 (0.3%)	1 (0.7%)	0.511
Pulmonary hypertension	16 (3.3%)	12 (3.5%)	4 (2.7%)	0.786
Recent MI	126 (25.8%)	87 (25.4%)	39 (26.5%)	0.888
NYHA class III/IV	77 (15.7%)	52 (15.2%)	25 (17.0%)	0.714
CCS angina class = 4	19 (3.9%)	15 (4.4%)	4 (2.7%)	0.536
Carotid artery stenosis	150 (30.7%)	101 (29.5%)	49 (33.3%)	0.466
Lower limb arterial stenosis	174 (35.6%)	120 (35.1%)	54 (36.7%)	0.806
LVEF ≤ 35%	161 (32.9%)	109 (31.9%)	52 (35.4%)	0.515
LV dilatation	110 (22.5%)	68 (19.9%)	42 (28.6%)	0.046
Ischemic mitral regurgitation				0.151
No	253 (51.7%)	188 (55.0%)	65 (44.2%)	
Mild	111 (22.7%)	75 (21.9%)	36 (24.5%)	
Moderate	87 (17.8%)	55 (16.1%)	32 (21.8%)	
Severe	38 (7.8%)	24 (7.0%)	14 (9.5%)	
Ventricular aneurysm	135 (27.6%)	95 (27.8%)	40 (27.2%)	0.985
Increased serum creatinine	20 (4.1%)	14 (4.1%)	6 (4.1%)	1.000
CPB				0.064
OP	323 (66.1%)	236 (69.0%)	87 (59.2%)	
ONBEAT	72 (14.7%)	49 (14.3%)	23 (15.6%)	
ONSTOP	94 (19.2%)	57 (16.7%)	37 (25.2%)	
MVP	36 (7.4%)	25 (7.3%)	11 (7.5%)	1.000
MVR	18 (3.7%)	10 (2.9%)	8 (5.44%)	0.274
Intervention on ventricular aneurysm	60 (12.3%)	38 (11.1%)	22 (15.0%)	0.298
Combined surgery	104 (21.3%)	68 (19.9%)	36 (24.5%)	0.307

BMI, Body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention; NYHA, New York heart association; CCS, Canadian cardiovascular society; LVEF, left ventricular systolic function; CPB, Cardiopulmonary bypass; CABG, coronary artery bypass grafting; OP, Off-pump CABG without CPB; ONBEAT, On-pump beating heart CABG; ONSTOP, On-pump CABG; MVR, mitral valve replacement; MVP, mitral valve replacement.

ventricular) dilatation, ischemic mitral regurgitation, increased serum creatinine, and mitral valve replacement (MVR).

Multivariable Analysis

Multivariable logistic regression analysis demonstrated that age, critical state, recent MI within 90 days, stroke, LVEF ≤35%, left ventricular (LV) dilatation, increased serum creatinine, and combined surgery remained significant independent risk factors for postoperative mortality. The β-coefficients, odds ratios, 95% confidence interval (CI) and p-values for each of the variables in the multivariable analysis are displayed in **Table 3.** What's more, LASSO regression also resulted in eight predictive variables the same as the variables selected by the stepwise regression method (**Figure 1**).

Nomogram Derived From the Training Cohort

The model that integrated selected predictive factors was developed and presented as the nomogram (**Figure 2**). The C-index for death risk prediction in the training cohort was

TABLE 2 | Univariable Logistic Regression Analysis of predictors associated with in-hospital mortality in the Training Cohort.

Variables	OR (95%CI)	*P*-value
Age (years)	1.07 (1.02–1.12)	0.007
Sex, male vs. female	2.18 (0.80–5.33)	0.119
BMI (kg/m^2)	0.89 (0.78–1.02)	0.084
Critical State, yes vs. no	11.2 (2.50–47.0)	0.003
Hypertension, yes vs. no	0.74 (0.32–1.66)	0.461
Diabetes mellitus, yes vs. no	1.83 (0.81–4.32)	0.148
Diabetes on insulin, yes vs. no	3.56 (1.42–8.42)	0.008
Hyperlipidemia, yes vs. no	0.65 (0.23–1.59)	0.361
Smoke, yes vs. no	0.69 (0.31–1.56)	0.371
Alcohol, yes vs. no	0.75 (0.21–2.07)	0.608
Chronic renal insufficiency, yes vs. no	6.64 (0.79–38.1)	0.076
Dialysis, yes vs. no	12.4 (0.31–490)	0.152
Chronic pulmoriary disease, yes vs. no	1.90 (0.26–7.47)	0.460
Stroke, yes vs. no	2.84 (1.09–6.80)	0.033
PCI, yes vs. no	1.71 (0.70–3.95)	0.232
Previous cardiac surgery, yes vs. no	–	–
Pulmonary hypertension, yes vs. no	1.25 (0.05–6.91)	0.848
Recent MI, yes vs. no	3.86 (1.69–8.92)	0.001
NYHA class, III/IV vs. I/II	2.00 (0.85–4.53)	0.108
CCS angina class, IV vs. I/II/III	5.10 (1.28–16.6)	0.023
Carotid artery stenosis, yes vs. no	0.71 (0.25–1.74)	0.472
Lower limb arterial stenosis, yes vs. no	2.72 (1.21–6.33)	0.016
LVEF, ≤35 vs. >35%	3.80 (1.67–9.05)	0.001
LV dilatation		
LVIDd/BSA <3.5 cm/m^2	Reference	Reference
LVIDd/BSA ≥3.5 cm/m^2	8.01 (3.46–19.4)	<0.001
Ischemic mitral regurgitation		
No	Reference	Reference
Mild	2.31 (0.77–6.81)	0.132
Moderate	4.36 (1.56–12.4)	0.005
Severe	2.14 (0.28–9.49)	0.405
Ventricular aneurysm, yes vs. no	0.78 (0.27–1.91)	0.602
Increased serum creatinine, yes vs. no	8.11 (2.26–26.3)	0.002
CPB		
OP	Reference	Reference
ONBEAT	2.23 (0.74–5.98)	0.144
ONSTOP	1.89 (0.63–5.01)	0.240
MVP, yes vs. no	1.13 (0.16–4.19)	0.879
MVR, yes vs. no	5.85 (1.13–23.3)	0.037
Intervention on ventricular aneurysm		
No	Reference	Reference
Yes	2.08 (0.65–5.56)	0.201
Combined surgery, yes vs. no	2.32 (0.94–5.39)	0.068

OR, odds ratio; CI, confidence interval; BMI, Body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention; NYHA, New York heart association; CCS, Canadian cardiovascular society; LVEF, left ventricular systolic function; LVIDd, left ventricular internal diameter at end-diastolic; BSA, Body Surface Area; CPB, Cardiopulmonary bypass; MVR, mitral valve replacement; MVP, mitral valve replacement; CABG, coronary artery bypass grafting; OP, Off-pump CABG without CPB; ONBEAT, On-pump beating heart CABG; ONSTOP, On-pump CABG.

TABLE 3 | Multivariable logistic regression analysis of predictors associated with in-hospital mortality in the Training Cohort.

Variable	β coefficient	OR (95% CI)	*P*-value
Age	0.058	1.06(1.01–1.11)	0.016
Critical State	1.990	7.31(1.68–30.15)	0.006
Recent MI	1.601	4.96(2.12–12.09)	<0.001
Stroke	1.017	2.77(1.08–6.85)	0.029
LVEF≤35%	0.887	2.43(1.06–5.65)	0.036
LV dilatation	1.579	4.85(2.06–11.82)	<0.001
Increased serum creatinine	1.423	4.15(1.00–16.19)	0.043
Combined surgery	1.185	3.27(1.42–7.49)	0.005
(Intercept)	−8.538		<0.001

OR, odds ratio; CI, confidence interval; MI, myocardial infarction; LVEF, left ventricular ejection fraction.

0.889 (95%CI, 0.839–0.938; **Table 4**), which was confirmed to be 0.823 (the corrected C-index) *via* bootstrapping validation. The Hosmer-Lemeshow test yielded a non-significant statistic ($p = 0.535$), which suggested no departure from a perfect fit. For the patients whose predicted mortality probabilities were below 40%, the calibration curve demonstrated an optimal agreement between the prediction by nomogram and actual observation (**Figure 3A**). In addition, the calibration curve with bootstrap similarly showed good calibration in patients in whom the predicted mortality probabilities were below 40% (**Supplementary Figure 1**).

Validation of Predictive Accuracy of the Nomogram in the Validation Cohort

In the validation cohort, the C-index of the nomogram for predicting postoperative mortality was 0.899(95%CI, 0.835–0.963; **Table 4**). There was no significant difference regarding the C-index between the training and validation cohort (0.889 vs. 0.899, $p = 0.804$). The Hosmer-Lemeshow test similarly yielded a non-significant statistic ($p = 0.682$) indicating acceptable goodness-of-fit. For patients with predicted mortality probabilities below 40%, the calibration curve also showed accepted agreement between prediction and observation in the probability of mortality (**Figure 3B**). Model calibration of the nomogram was further explored by comparing the predicted and observed probabilities across predicted risk quintiles. It showed that the nomogram had an acceptable agreement between prediction and observation both in the training and validation cohort (**Figures 4A,B**). The nomogram derived from the training cohort displayed good discrimination and calibration in predicting postoperative mortality both in the training and validation cohort.

Comparison of Predictive Accuracy Between the Nomogram and EuroSCORE-2

The C-index of the nomogram was significantly higher than the EuroSCORE-2 in training (0.889 vs. 0.762, $p = 0.005$) and validation cohort (0.899 vs. 0.816, $p = 0.039$; **Table 4**

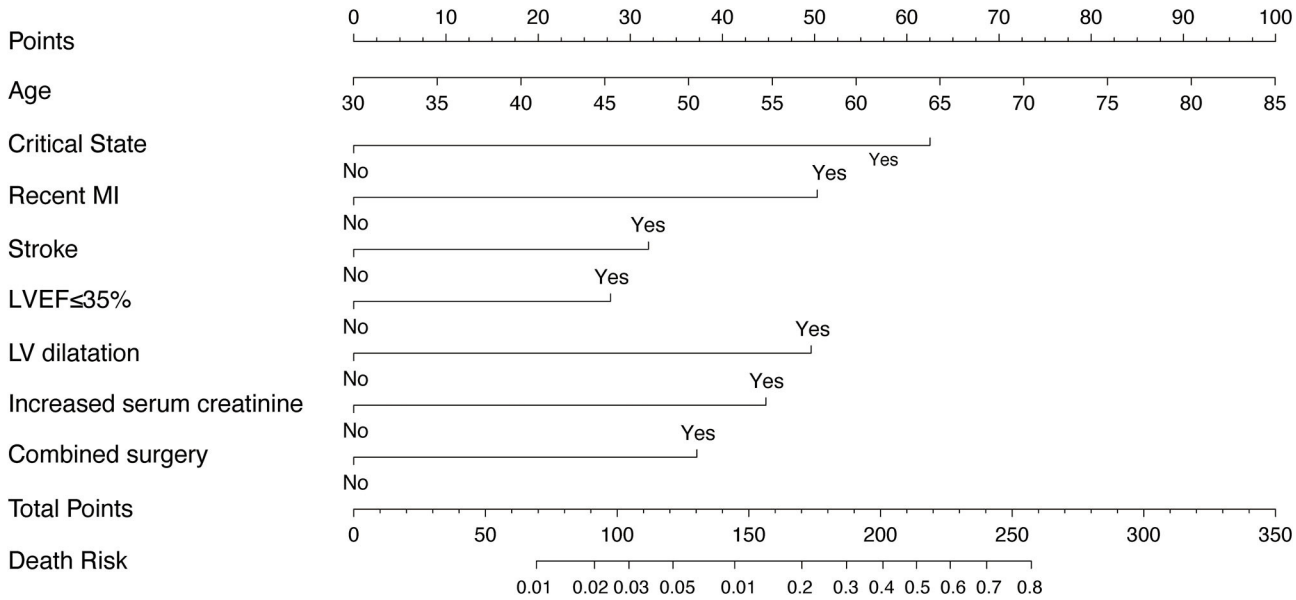

FIGURE 2 | The nomogram derived from training cohort for predicting mortality after CABG. MI, myocardial infarction; LVEF, left ventricular ejection fraction.

TABLE 4 | Comparison of the nomogram and the EuroSCORE-2.

	Training cohort ($n = 342$)			Validation cohort ($n = 147$)		
	EuroSCORE-2	**Nomogram**	***P*-value**	**EuroSCORE-2**	**Nomogram**	***P*-value**
C-index (95% CI)	0.762 (0.661–0.863)	0.889 (0.839–0.938)	0.005	0.816(0.705–0.928)	0.899(0.835–0.963)	0.039
Categorical NRI (95% CI)	Reference	0.471 (0.287–0.655)	<0.001	Reference	0.572 (0.367–0.776)	<0.001
IDI (95% CI)	Reference	0.202 (0.112–0.291)	<0.001	Reference	0.157 (0.072–0.243)	<0.001
χ-squared (Hosmer-Lemeshow test)	77.337	7.016	–	24.998	5.694	–
P-value (Hosmer-Lemeshow test)	<0.001	0.535	–	0.002	0.682	–

NRI, net reclassification improvement, IDI, integrated discrimination improvement, CI, confidence interval.

and **Figure 5**). The reclassification and discrimination ability of the nomogram was assessed. Compared with EuroSCORE-2, the nomogram showed significantly improved prediction performance in training cohort (categorical NRI: 0.471 [0.287–0.655], p <0.001; IDI: 0.202 [0.112–0.291], p <0.001) and validation cohort (categorical NRI: 0.572 [0.367–0.776], p < 0.001; IDI: 0.157 [0.072–0.243], $p < 0.001$; **Table 4**).

The nomogram had acceptable calibration in training (Hosmer-Lemeshow statistic $\chi^2 = 7.016$, $p = 0.535$) and validation cohort (Hosmer-Lemeshow statistic $\chi^2 = 5.694$, $p = 0.682$; **Table 4**). For EuroSCORE-2, the Hosmer-Lemeshow test yielded a significant statistic in training (Hosmer-Lemeshow statistic $\chi^2 = 77.337$, $p < 0.001$) and validation cohort (Hosmer-Lemeshow statistic $\chi^2 = 24.998$, $p = 0.002$), indicating that the EuroSCORE-2 does not calibrate perfectly. For patients with an expected mortality rate of <40%, the calibration curve of the nomogram indicated a good fit of predicted and observed mortality in the training and validation cohort (**Figures 3A,B**). As for EuroSCORE-2, the calibration curve showed poor agreement between prediction and observation in the probability of mortality in the training and validation cohort. The calibration curve was almost above the 45° diagonal line, which means EuroSCORE-2 underestimated the probability of mortality, especially in high-risk patients (**Figures 3C,D**). Model calibration was further explored by comparing the predicted and observed probabilities of mortality across patient predicted risk quintiles. It also shows that EuroSCORE-2 underestimated the probability of mortality in high-risk patients (**Figures 4C,D**).

DISCUSSION

To our knowledge, we developed the first nomogram model to efficiently predict the in-hospital mortality after CABG among patients with HFrEF. The nomogram risk prediction model performed well in our training and validation cohorts, and showed good discrimination and calibration in patients with predicted mortality probabilities below 40%. The model incorporates only eight preoperative variables which are easily measured and readily available: age, critical state, recent MI, stroke, LVEF ≤35%, left

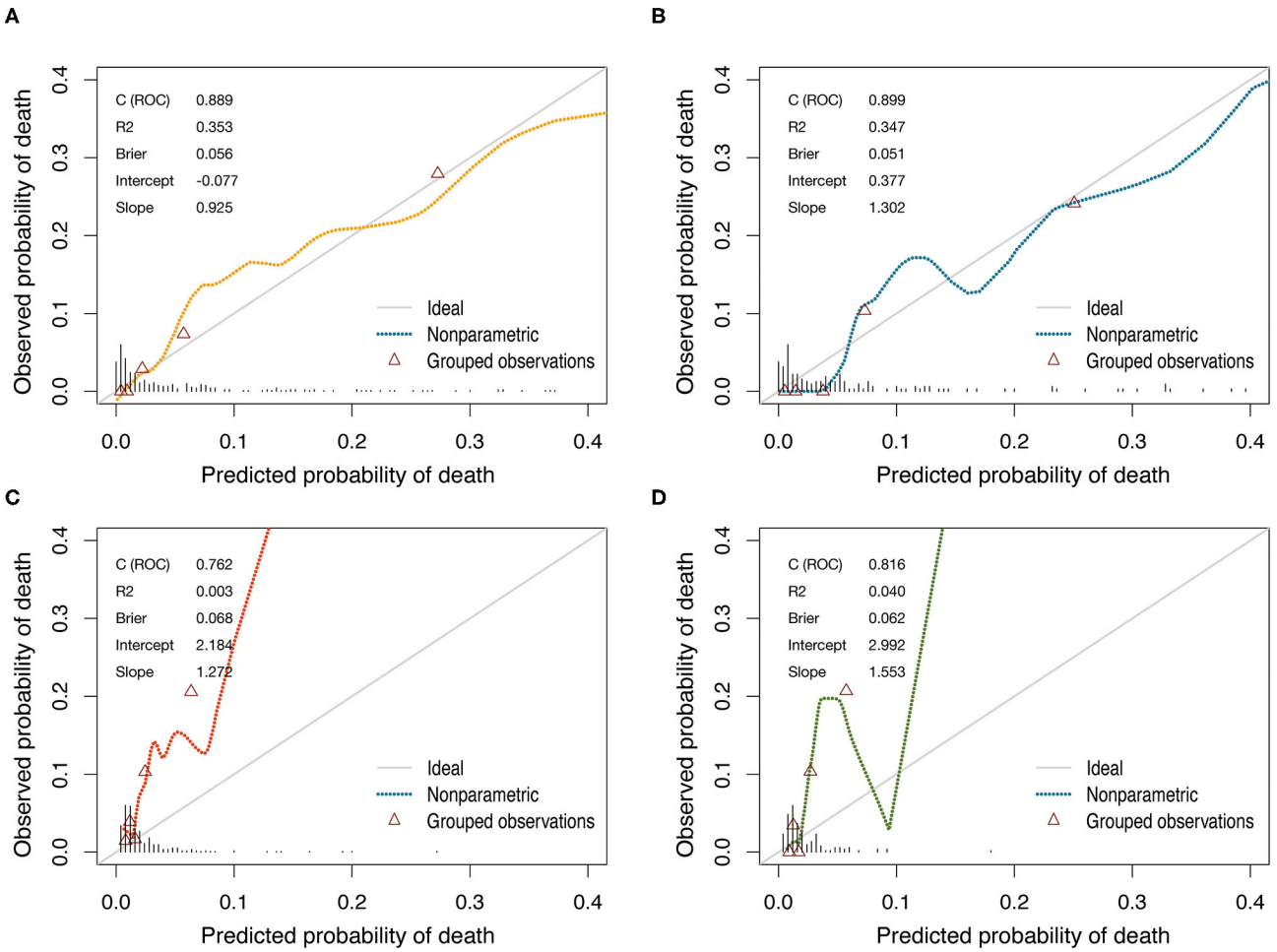

FIGURE 3 | Calibration curves of the nomogram and the EuroSCORE-2 for predicting mortality after CABG in training cohort and validation cohort. **(A)** The nomogram in training cohort. **(B)** The nomogram in validation cohort. **(C)** The EuroSCORE-2 in training cohort. **(D)** The EuroSCORE-2 in validation cohort. Diagonal line indicates perfect calibration. The triangles indicate the observed frequencies of death by the quintiles of the predicted probability.

ventricular dilatation, increased serum creatinine, and combined surgery.

Risk Factors

The nomogram incorporates only 8 variables but achieved good model performance. We can conclude that the 8 risk factors included in the nomogram are the most important variables associate with mortality in patients with HFrEF undergoing CABG. It is well-established that age independently affects post-CABG mortality, and was included in the nomogram. Contrary to commonly used risk scores, sex and BMI were not independent risk factors in the nomogram. In EuroSCORE-2, previous cardiac surgery and critical state were two risk factors given the heaviest weight. Similarly, the critical state was given the heaviest weight in the nomogram. However, previous cardiac surgery wasn't included in our model because only two patients had a history of cardiac surgery in the entire cohort and accounted for a very small proportion in our cohort. A growing number of literatures documented the effects of renal dysfunction on mortality and morbidity after CABG surgery (14–18). Serum creatinine is often used to reflect renal function because it is readily available and simple. It was reported that patients with a baseline serum creatinine of more than 1.5 mg/dl had a significantly higher 30-day mortality after CABG (15). Consistent with those reports, in our model, we defined increased serum creatinine as serum creatinine >1.5 mg/dl and similarly found it was independently associated with increased postoperative mortality in patients with HFrEF. The combined surgery not only reflects more severe lesions that need additional intervention of mitral valve or ventricular aneurysm, but also reflects a longer time of anesthesia and use of cardiopulmonary bypass (CPB). These factors increased the risk of surgery but also might have encouraged surgeons to change or simplify operative procedures to limit anesthesia time and avoid cardiopulmonary bypass.

One of the most powerful predictors of in-hospital mortality in our study was LV dilatation. Yamaguchi et al. (19) revealed that preoperative LV end-systolic volume index (LVESVI) >100 ml/m^2 predicted the development of congestive HF and late

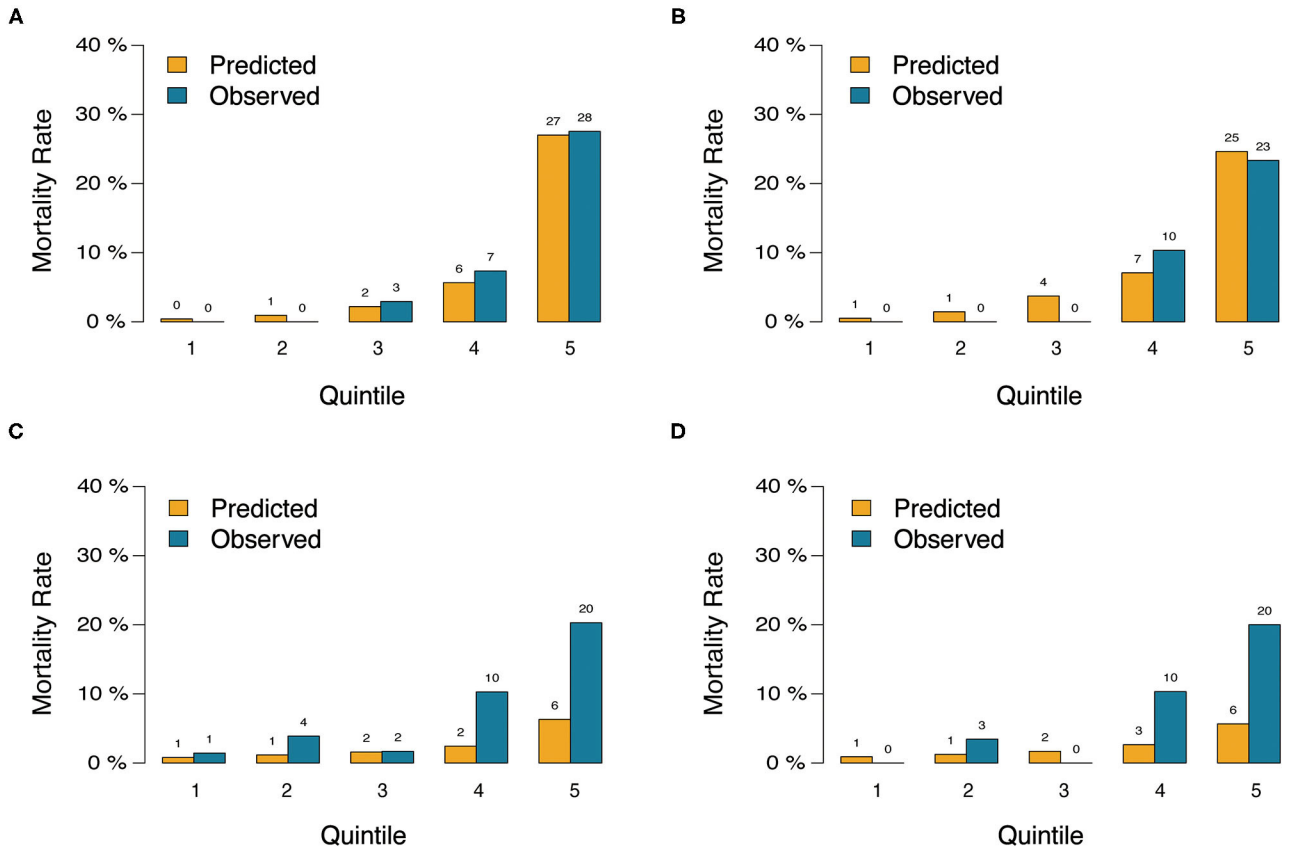

FIGURE 4 | Predicted vs. observed risk of death after CABG based on quintile of predicted risk in training cohort and validation cohort. **(A)** the nomogram in training cohort. **(B)** The nomogram in validation cohort. **(C)** The EuroSCORE-2 in training cohort. **(D)** The EuroSCORE-2 in validation cohort.

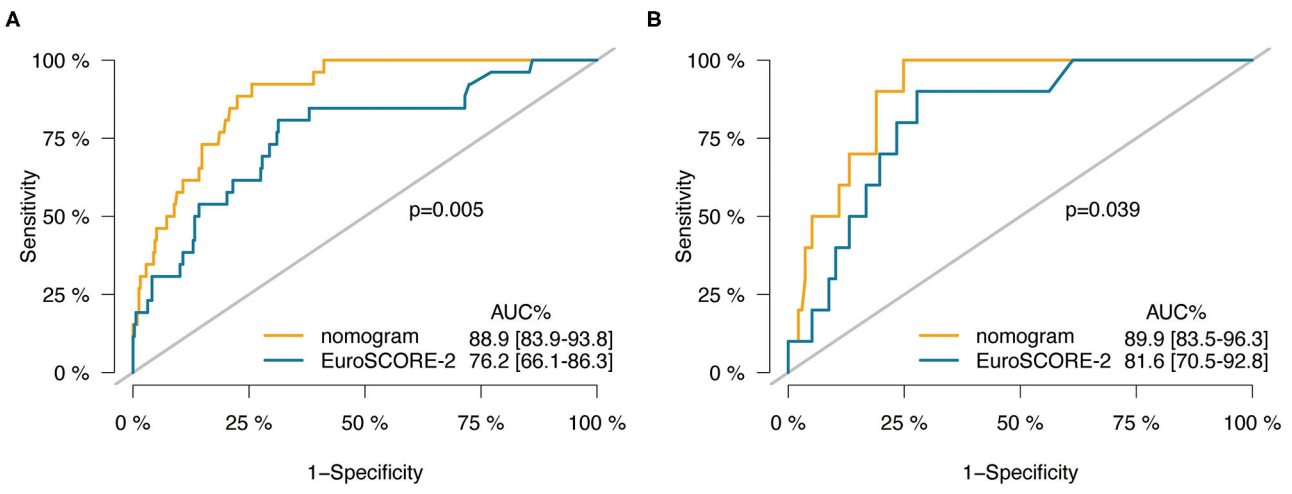

FIGURE 5 | ROC curves of the nomogram vs. the EuroSCORE-2 in training cohort **(A)** and validation cohort **(B)**.

mortality in patients with LVEF <30% undergoing isolated CABG. The results from Surgical Treatment for Ischemic Heart Failure (STICH) Trial (20) showed that, in patients with left ventricular dysfunction who underwent CABG, LVESVI was a stronger predictor of 30-day mortality than LVEF, and mortality risk increased linearly with increasing values of LVESVI.

Fukunaga et al. (21) found that LV size >5.5 cm was a significant predictor of operative mortality and major morbidity (OR 5.5 [2.0–15.7] ($p < 0.001$) in patients undergoing isolated CABG. Our study defined LVIDd/BSA $\geq$3.5 cm/m^2 as moderate or serve LV dilatation. Similarly, we found LV dilatation was a significant risk factor of in-hospital mortality and showed stronger predictive ability than LVEF. Well-accepted surgical risk scores have identified only LVEF as a powerful predictor of surgical and 30-day mortality, which may be inaccurate. A variable reflecting LV size may be a more important predictor of outcome than LVEF and should be incorporated into their risk-adjustment models.

The Advantages of Nomogram Compared With the EuroSCORE-2

EuroSCORE-2 and STS score are the most commonly used risk scores and have been proven effective in assessing postoperative risk for general patients undergoing cardiac surgery (22–24). However, these scores were based on data including only a small number of patients with HFrEF and may not be accurate to predict surgery risk in such high-risk patients. Howell et al. (25) showed that EuroSCORE-2 performed not well with a low C-statistic of 0.67 and poor model calibration (chi-square 16.5; $p = 0.035$) in high-risk patients who underwent cardiac surgery (preoperative logistic EuroSCORE $\geq$10). Several pieces of literature reported that EuroSCORE-2 or STS score had underestimated surgery mortality of CABG when applied to specific high-risk populations (26–28). Di Dedda et al. revealed that, EuroSCORE-2 significantly underestimated the mortality risk (predicted mortality 6.5%) in high-risk patients with cardiac surgery (observed mortality 11%). (26). In patients with an LVEF $\leq$35% undergoing CABG, it has been reported (29) that both the STS Score and the EuroSCORE-2 performed moderately well, but with a C-index (C statistic is <0.75), somewhat inferior to that reported for overall cardiac surgical populations (where their C statistic is >0.80). What's more, both the STS score and EuroSCORE-2 significantly underestimated mortality. The STS score appeared to consistently underestimate risk compared with the EuroSCORE-2. Consistent with these reports, in our study, EuroSCORE-2 had a moderate C-index (0.762 and 0.816 in training and validation cohort) and similarly significantly underestimated the risk of mortality after CABG in patients with HFrEF as shown in the calibration curve, especially in the high-risk group.

Unlike Western countries, China is a developing country and has different medical standards and characteristics. For example, Off-pump CABG is more common than on-pump CABG in china. Thus, based on populations in Europe and the US, EuroSCORE-2 and STS score are not suitable for Chinese patients. Moreover, the data of EuroSCORE-2 and STS score were obtained from more than 10 years ago, which could be outdated with the improvements in surgical, anesthetic and intensive care during the past decade. Consequently, a new model developed for specific Chinese patients with HFrEF undergoing CABG is urgently needed.

In this study, we established a nomogram prediction model that showed favorable discrimination with C-index consistently more than 0.8 and significantly higher than EuroSCORE-2 in the training and validation cohort. Besides, the nomogram showed a better calibration than EuroSCORE-2 in both cohorts. We thought it might be attributed to reasons as followed: First, our nomogram was specifically developed for patients with HFrEF instead of general patients. Second, the nomogram was developed using data from the last 8 years. However, EuroSCORE-2 was based on data obtained from more than 10 years ago, which could be outdated. Third, it has been reported that EuroSCORE-2 underestimated mortality in the high-risk Chinese patients undergoing CABG (27). Different from EuroSCORE-2 that based on the western population, our nomogram is more suitable for Chinese patients. Fourth, LV dilatation is a more important predictor of outcome than LVEF and was incorporated in the nomogram but not in the EuroSCOR-2. Finally, our risk model developed from single-center data with internal validation instead of external validation. The performance of the nomogram in external validation may not be that good.

Furthermore, our nomogram has unique advantages over traditional risk scores. It has only eight risk factors generally included in the medical records and was easier to calculate risk bedside in a few minutes and worthy of clinical popularizing. However, the STS score is complex, with more than 50 demographic and operative variables, and even EuroSCORE-2 has 18 variables. Despite fewer variables for prediction, our nomogram had demonstrated better predictive performance in calibration and discrimination than EuroSCORE-2. With fewer variables but achieved better model performance, this study demonstrates the utility and feasibility of using specific patient data for constructing models to improve prediction of cardiac surgery mortality in specific populations and gain additional insight into factors that modify the risk of outcomes in patients with HFrEF.

Limitation

There are several limitations in this study. First, this study is a retrospective analysis, and hence selection bias remains a possibility and prospective studies are required to confirm the results. Second, our risk model was developed from single-center data without external validation. Although we tried to overcome this limitation by internal validation in the validation cohort and additional validation with the bootstrap method, external validation in other cohorts is needed before clinical application. Third, the nomogram model was developed and validated in a small cohort with only 36 outcomes. Considering the relatively small sample size, results from this study should be interpreted with caution. The present study is a preliminary explore in predicting risk of mortality in these specific high-risk patients with CABG. And future studies with large sample size are needed to further confirm our findings. Finally, the model was based on routine clinical data, some potentially important predictor variables were not collected, such as natriuretic peptide levels. Specific markers to estimate surgery risk might further improve the accuracy of the model.

CONCLUSION

In conclusion, this study presents an easily applied nomogram that can predict in-hospital mortality in HFrEF patients undergoing CABG. This nomogram showed an improvement in the predictive accuracy when compared to EuroSCORE-2. The nomogram may help identify HFrEF patients at high risk of in-hospital mortality after CABG who might benefit from a simplified operation approach, perioperative intense attention, and more personalized treatment.

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 Ethical approval was obtained from the Institutional Ethics Committee of Beijing Anzhen Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PY, RD, TL, KZ, and JC: study concept and design. All authors acquisition, analysis, or interpretation of data, critical revision of the manuscript for important intellectual content, and read and approved the final manuscript. PY: drafting 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.2021.709190/full#supplementary-material

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Myocardial Work by Speckle Tracking Echocardiography Accurately Assesses Left Ventricular Function of Coronary Artery Disease Patients

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Objective: Myocardial work (MW) is a novel non-invasive method that uses speckle tracking echocardiography (STE) to assess left ventricular (LV) function. MW incorporates the global longitudinal strain and afterload conditions. Here we aimed to use MW to assess the LV function of patients with coronary artery disease (CAD) with or without heart failure (HF).

Methods: We enrolled a total of 150 individuals (50 each) with CAD and a normal LV ejection fraction (LVEF), CAD with HF, and healthy controls. Patients were divided into the hypertension (HTN) and normal blood pressure (no HTN) subgroups. MW was determined from the pressure-strain loop using STE. The relationships between MW indices and conventional echocardiographic parameters were evaluated, and the MW indices were compared among groups.

Results: Univariate and multivariate analyses showed that MW indices were strongly correlated with LVEF. The global work index (GWI) was increased in the CAD with normal LVEF subgroup with HTN vs. controls (1,922.3 ± 393.1 vs. 1,639.7 ± 204.6 mmHg%, $p < 0.05$) and decreased in CAD patients with HF (no HTN: 940.9 ± 380.6 vs. 1,639.7 ± 204.6 mmHg%, $p < 0.05$; HTN: 857.3 ± 369.3 vs. 1,639.7 ± 204.6 mmHg%, $p < 0.05$). Global waste work was increased in all CAD subgroups vs. controls. Global constructive work had the same tendency as GWI in patients with CAD. Global MW efficiency was decreased in all patients with CAD.

Conclusion: MW using STE accurately quantifies LV function in patients with CAD. It offers additional information about LV function with respect to disease progression, particularly in CAD patients with a normal LVEF.

Keywords: myocardial work, speckle tracking echocardiography, global longitudinal strain, coronary artery disease, heart failure, left ventricular function

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INTRODUCTION

An accurate assessment of left ventricular (LV) function is crucial in clinical decision-making regarding cardiovascular diseases. This remains a major challenge during disease development. In addition to echocardiography, cardiac magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) are used to assess heart function. However, MRI and SPECT are severely limited by their high operational cost, need for expensive and high-maintenance equipment, and radioactivity. In addition to these advantages, echocardiography can provide diagnostic results faster than MRI and SPECT. The conventional echocardiographic parameter, LV ejection fraction (LVEF), is currently recognized as the standard assessment of LV function. However, LVEF is based on movement of the endocardial border, which is less sensitive in the early stage of ischemia. Tissue Doppler imaging (TDI) was used to evaluate heart function before speckle tracking echocardiography (STE) emerged. However, TDI has the disadvantage of its angle dependence leading to inaccurate results. Global longitudinal strain (GLS), which is derived from speckle tracking imaging, is a more sensitive measure of myocardial impairment and ischemia-induced LV function damage and has gradually been used in clinical practice (1). However, GLS may be inadequately interpreted if the LV afterload is increased (2). Myocardial work (MW), which considers the GLS and afterload conditions, offsets the disadvantages of GLS alone for detecting LV dysfunction (3, 4).

Recent studies reported that MW showed distinct patterns in different afterload conditions and settings (4–8). LVEF is usually preserved in the early stages of cardiovascular diseases (9). It is difficult to quantify LV myocardial functional impairments in coronary artery disease (CAD) patients with a normal LVEF. According to Edwards et al., MW can identify early abnormalities in LV function, making it a sensitive index for assessing early LV dysfunction (10). No studies to date have investigated the MW patterns in CAD patients with a normal LVEF and reduced LVEF. The current study aimed to investigate MW indices under different afterload conditions and MW patterns in healthy individuals and CAD patients with different heart functions.

MATERIALS AND METHODS

Study Population

This study enrolled all individuals treated at Beijing Hospital between September 2018 and December 2019. The study protocol complied with the guidelines of the Declaration of Helsinki. All participants provided written informed consent. This study was approved by the Beijing Hospital Ethics Committee. The participants were divided into three groups of 50 each: controls, CAD with normal LVEF, and CAD with heart failure (HF). The control group included healthy individuals from the healthcare management center who had no cardiopulmonary symptoms, had normal electrocardiography findings, and received no medication. Patients with symptoms and diagnosed as HF were assigned to the HF group. The HF group included patients with a mid-range or reduced ejection fraction (11). The inclusion criteria for CAD patients were (1) myocardial ischemia-related symptoms (such as chest pain, chest tightness, palpitation, and shortness of breath); (2) age $\geq$18 years; and (3) sinus rhythm. The exclusion criteria for CAD patients were (1) ST-segment elevation myocardial infarction; (2) other serious heart disease (congenital cardiomyopathy, moderate to severe valvular disease, malignant arrhythmia, hypertrophic cardiomyopathy, dilated cardiomyopathy, etc.); (3) other end-stage diseases (life expectancy $<$1 year); (4) coronary angiography findings indicative of myocardial bridge without coronary atherosclerosis; and (5) poor-quality echocardiography images. The CAD patients were divided into hypertension (HTN) and no HTN subgroups. Blood pressure was recorded at the time of the echocardiography. An increased afterload was identified when the brachial systolic blood pressure (SBP) was $\geq$140 mmHg or diastolic blood pressure (DBP) was $\geq$90 mmHg. Patients were excluded if they had malicious arrhythmia, valvular disease (moderate or severe), congenital heart disease, or poor-quality echocardiographic images.

Angiography and Conventional Echocardiography

Angiography was performed after echocardiography by an experienced cardiologist. Stenosis $\geq$50% in at least one major coronary artery or its main branch was identified as CAD. Echocardiography was performed using a Vivid E95 (GE Vingmed Ultrasound, Norway) according to the guidelines of the American Society of Echocardiography (12). All images were stored in RAW format for offline analysis. LV mitral velocities were obtained using pulsed-wave TDI. The diastolic interventricular septal thickness (IVSd), diastolic posterior wall thickness (PWd), and LV diameter diastole (LVDd) were acquired using two-dimensional (2D) imaging. The LV end-diastolic volume (LVEDV) was measured on the apical four-chamber view. LVEF was calculated using Simpson's method. The transmitral E and A wave velocities were measured using pulsed-waved Doppler

GLS and MW

GLS and MW were measured using 2D STE. The indices were obtained from four-, two-, and three-chamber views at 45–75 frames/s. The offline analysis was performed using an EchoPAC V203 (GE Vingmed Ultrasound). The MW parameters were obtained using a pressure-strain loop (PSL) area module that was constructed using LV pressure values on the vertical axis and strain on the horizontal axis. Based on previous studies, the peak systolic LV pressure was assumed to be equal to the brachial SBP (3, 4).

The global work index (GWI) represents the total work within the area of the LV-PSL from mitral valve closure to mitral valve opening (**Figure 1**). Global constructive work (GCW) represents the work performed during myocardial shortening in systole and lengthening in isovolumic diastole. Global waste work (GWW) represents the work performed by myocytes during myocardial elongation in systole and shortening in isovolumic diastole. Global work efficiency (GWE) is defined as the GCW divided by the sum of the GCW and the GWW: GCW/(GCW + GWW).

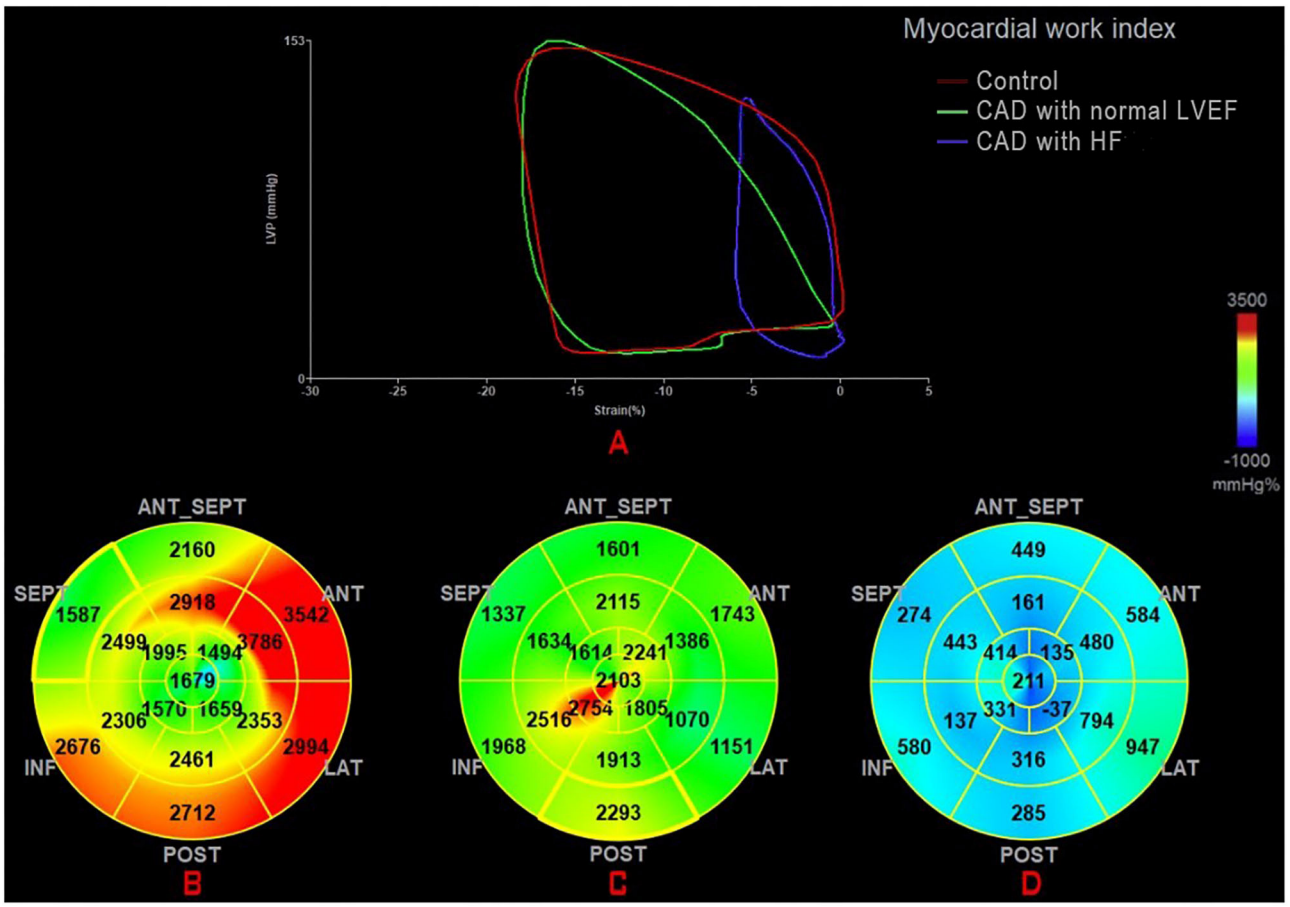

FIGURE 1 | (A) Mean PSL in the healthy individual (green) and a CAD patient with normal LVEF (red) and a CAD patient with HF (blue). Seventeen segment bull'seye representation of the MW index (GWI): **(B)** in a healthy individual; **(C)** in a CAD patient with normal LVEF; and **(D)** in a CAD patient with HF. CAD, coronary artery disease; GWI, global work index; HF, heart failure; MW, myocardial work; PSL, pressure-strain loop.

Statistical Analysis

Continuous variables are represented as mean ± standard deviation. The normality of the distribution was tested using the Kolmogorov–Smirnov test. Categorical variables are presented as numbers and percentages. Individual characteristics were compared across subgroups using the χ^2 test for categorical variables and analysis of variance for continuous variables in relation to the control group. Correlation analysis was performed using Pearson's or Spearman's correlation coefficients. Multivariable linear regression analyses were performed to evaluate the independent correlations between the MW indices and other parameters. Two-sided values of $p < 0.05$ were considered statistically significant. All analyses were performed using SPSS v25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient Characteristics and Conventional Echocardiographic Analysis

Patients in the CAD groups were significantly older than those in the control group ($p < 0.05$) (**Table 1**). The body mass index (BMI) was higher ($p < 0.05$) in the CAD patients with a normal LVEF. The SBP was significantly higher in the HTN subgroup ($p < 0.05$). The DBP was significantly higher in patients in the CAD and normal LVEF with HTN subgroup and lower in patients with CAD and HF without HTN subgroup ($p < 0.05$). The SBP and DBP values differed significantly between the CAD subgroups ($p < 0.05$).

Patients in the HF subgroup had significantly thicker interventricular septal thickness than the controls ($p < 0.05$) (**Table 2**). The PWd was higher in HF patients without HTN. The LVDd and LVEDV were significantly increased in patients with CAD and HF. The A wave value was significantly increased ($p < 0.05$) in the CAD subgroup except in HF patients with HTN. The E/A ratio was decreased ($p < 0.05$) in the CAD subgroups except in HF patients with HTN. GLS was significantly impaired ($P < 0.05$) in both HF subgroups. However, no significant differences in GLS were observed in CAD patients with a normal LVEF. The E/e′ was increased ($P < 0.05$) in all patients with CAD.

TABLE 1 | Patients' demographic characteristics.

	Control ($n = 50$)	CAD with normal LVEF group		CAD with HF group	
		No HTN ($n = 26$)	HTN ($n = 24$)	No HTN ($n = 34$)	HTN ($n = 16$)
Age (years)	37 ± 16	63 ± 8.19*	64.96 ± 9.10*	63.18 ± 9.21*	70.04 ± 11.65*
BSA (m^2)	1.73 ± 0.21	1.82 ± 0.15	1.77 ± 0.22	1.79 ± 0.17	1.70 ± 0.18
BMI (kg/m^2)	23.23 ± 3.35	25.75 ± 2.9*	26.65 ± 2.92*	24.55 ± 4.51	23.6 ± 3.51
SBP (mmHg)	115.3 ± 11.01	124.77 ± 9.98*	150.96 ± 11.03*,**	115.35 ± 13.92	144.63 ± 8.07*,**
DBP (mmHg)	73.78 ± 9.69	70.52 ± 10.01	80.11 ± 10.79*,**	75.23 ± 18.37	67.18 ± 12.99*,**
HR (bpm)	75.52 ± 11.31	73.35 ± 10.68	75.92 ± 11.44	67.97 ± 11.84*	81.31 ± 15.7
Male sex	25 (50%)	19 (73.1%)	14 (58.3%)	20 (58.8%)	13 (81.2%)
DM history	—	10 (38.5%)	12 (50.0%)	8 (23.5%)	2 (12.5%)
HTN history	—	5 (19.2%)	22 (91.7%)	21 (61.8%)	7 (43.8%)

*$p < 0.05$ vs. the control group.
**$p < 0.05$ in the no HTN vs. HTN group.
BMI, body mass index; BSA, body surface area; CAD, coronary artery disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HF, heart failure; HTN, hypertension; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure.

TABLE 2 | Conventional echocardiography parameters.

	Control ($n = 50$)	CAD with normal LVEF group		CAD with HF group	
		No HTN ($n = 26$)	HTN ($n = 24$)	No HTN ($n = 34$)	HTN ($n = 16$)
IVSd (mm)	9.18 ± 1.51	9.70 ± 1.02	9.67 ± 1.13	10.77 ± 1.90*	10.43 ± 2.21*
PWd (mm)	8.86 ± 1.43	9.74 ± 1.25	9.59 ± 1.08	10.00 ± 1.44*	9.71 ± 1.42
LVDd (mm)	43.54 ± 4.24	47.48 ± 3.54	45.86 ± 4.13	56.82 ± 8.02*	57.39 ± 8.15*
LVEDV (mL)	86.62 ± 19.47	105.43 ± 18.11	97.81 ± 21.13	157.63 ± 59.03*	166.83 ± 53.75*
LVEF (%)	64.14 ± 2.83	62.70 ± 3.64	63.52 ± 3.43	38.14 ± 13.5*	36.93 ± 8.86*
A wave (cm/s)	0.62 ± 0.14	0.83 ± 0.21*	0.84 ± 0.19*	0.78 ± 0.26*	0.70 ± 0.17
E wave (cm/s)	0.81 ± 0.13	0.73 ± 0.16	0.74 ± 0.16	0.57 ± 0.29*	0.76 ± 0.35
E/A ratio	1.38 ± 0.46	0.91 ± 0.77*	0.93 ± 0.31*	0.92 ± 0.77*	1.01 ± 0.51
E/e′	6.59 ± 1.37	10.99 ± 4.50*	10.60 ± 5.18*	15.00 ± 6.44*	15.42 ± 11.00*
GLS	−18.26 ± 2.16	−16.78 ± 2.68	−17.25 ± 2.44	−8.09 ± 3.05*	−9.04 ± 8.30*

*$p < 0.05$, significantly different from the control group.
GLS, global longitudinal strain; HF, heart failure; HTN, hypertension; IVSd, diastolic interventricular septal thickness; LVDd, left ventricular diameter diastole; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; PWd, diastolic posterior wall thickness.

Correlations Between MW and Other Parameters

GWI was strongly correlated with the LVEF and LVDd, moderately correlated with SBP, E wave, and E/e′, and weakly correlated with IVSd (**Figure 2A**; **Table 3**). The multivariable analysis revealed that GWI was significantly correlated with LVEF, moderately correlated with SBP, and weakly correlated with DBP and LVDd (**Table 3**). GWW was strongly correlated with LVEF and LVDd and moderately correlated with age, SBP, IVSd, E/e′, and PWd (**Figure 2B**; **Table 4**). GWW was weakly correlated with E/A ratio (**Table 4**). In the multivariable analysis, GWW was strongly correlated with LVDd and moderately correlated with body surface area and SBP (**Table 4**). GCW was strongly correlated with LVEF and LVDd, moderately correlated with SBP and E wave, and weakly correlated with E/e′ and IVSd (**Figure 2C**; **Table 5**). In the multivariable analysis, GCW was significantly correlated with LVEF and moderately correlated with SBP (**Table 5**). GWE was strongly correlated with LVEF and LVDd and moderately correlated with age, IVSd, PWd, E wave, E/A ratio, and E/e′ (**Figure 2D**; **Table 6**). In the multivariable analysis, GWE was significantly correlated with LVEF and moderately correlated with LVDd. GWE was weakly correlated with body surface area, BMI, SBP, and E/e′ (**Table 6**).

MW by Study Subgroup

The GWI was significantly elevated in CAD patients with a normal LVEF and HTN compared with controls (HTN: 1,922.3 ± 393.1 vs. 1,639.7 ± 204.6 mmHg%, $p < 0.05$) but not in those without HTN. In the HF group, the GWI was significantly reduced in both subgroups (no HTN: 940.9 ± 380.6 vs. 1,639.7 ± 204.6 mmHg%, $p < 0.05$; HTN: 857.3 ± 369.3 vs. 1,639.7 ± 204.6 mmHg%; **Figure 3A**).

The GWW was increased in all CAD patients with a normal LVEF vs. controls (no HTN: 124.7 ± 58.1 vs. 79.1 ± 40.3

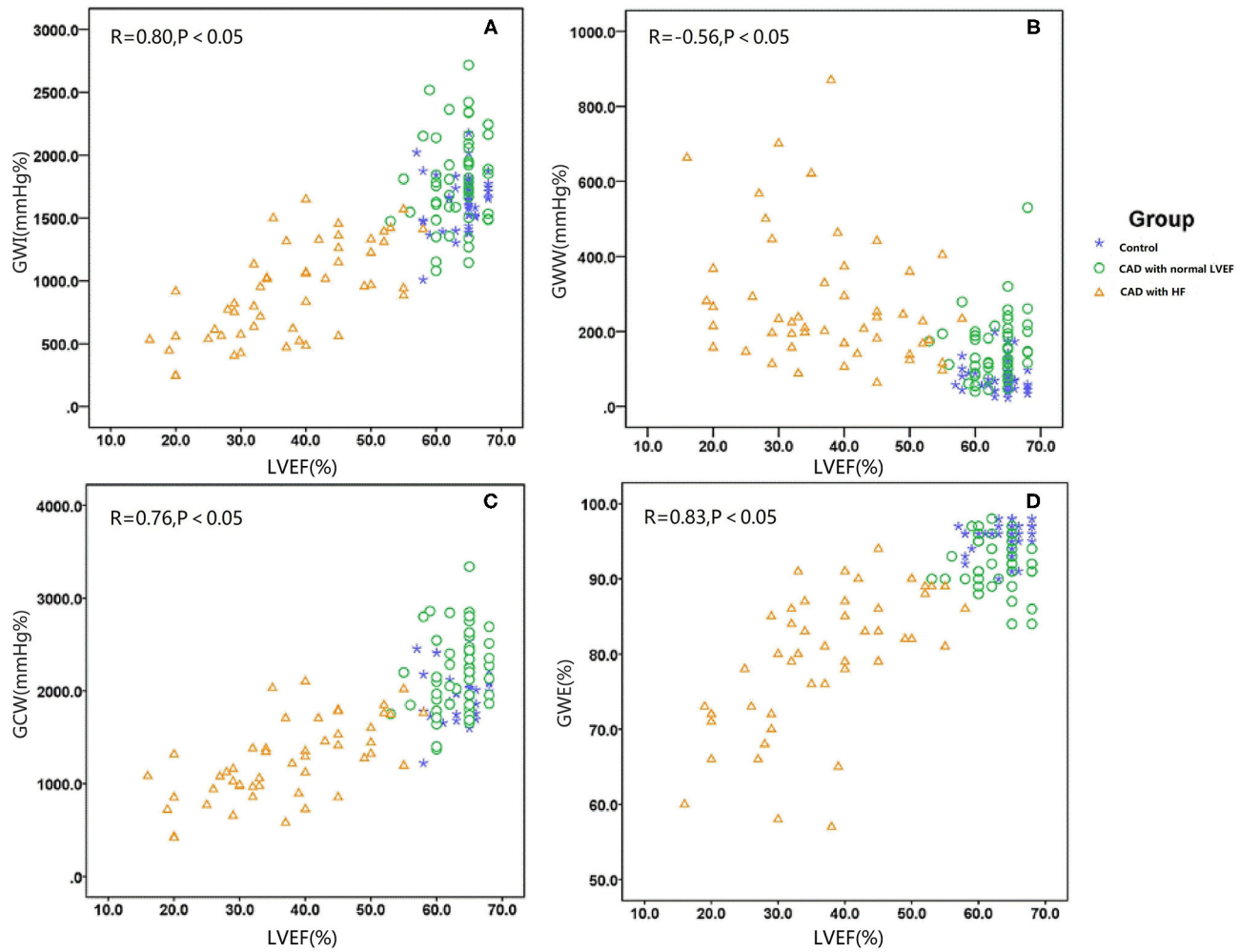

FIGURE 2 | Graph showing the median as well as 25th and 75th percentiles of the GWI **(A)**, GWW **(B)**, GCW **(C)**, and GWW **(D)**. $^{*}P < 0.05$, significantly different compared with the controls. GCW, global constructive work; GWE, global myocardial work efficiency; GWI, global work index; GWW, global waste work; HF, heart failure; HTN, hypertension.

mmHg%, P0.05; HTN: 183.4 ± 101.8 vs. 79.1 ± 40.3 mmHg%, $p < 0.05$). It also significantly increased in both HF subgroups (no HTN: 274.4 ± 175.9 vs. 79.1 ± 40.3 mmHg%, $p < 0.05$; HTN: 282.6 ± 174.3 vs. 79.1 ± 40.3 mmHg%; $p < 0.05$; **Figure 3B**).

GCW showed the same tendency as GWI in CAD patients. Compared with the control group, the GCW significantly increased in CAD patients with a normal LVEF and HTN (2,377.5 ± 427.8 vs. 1,964.5 ± 251.3 mmHg%, $p < 0.05$) but not in those with a normal LVEF and no HTN. In the HF group, GCW significantly decreased in all patients (no HTN: 1,275.1 ± 418.8 vs. 1,964.6 ± 251.3 mmHg%, $p < 0.05$; HTN: 1,176.2 ± 423.2 vs. 1,964.5 ± 251.3 mmHg%, $p < 0.05$; **Figure 3C**).

Compared with the control group, the GWE was decreased in the CAD subgroups (normal LVED but no HTN: 92.9 ± 3.2 vs. 95.2 ± 2.0%, $p < 0.05$; HTN: 91.6 ± 3.3 vs. 95.3 ± 2.0 %, $p < 0.05$) and HF subgroups (no HTN: 80.1 ± 9.7 vs. 95.3 ± 2.0%, $p < 0.05$; HTN: 78.4 ± 8.1 vs. 95.3 ± 2.0%, $p < 0.05$; **Figure 3D**).

DISCUSSION

In the current CAD study, MW appeared to be more predictive than LVEF and GLS for assessing LV function. Sub-endocardial fibers are susceptible to ischemia, so repetitive and intermittent minor ischemia may result in subtle forms of myocardial stunning. Minor ischemia may not necessarily result in reduced ventricular wall motion. In such cases, GLS is decreased, while LVEF and regional wall motions are normal (13, 14). As arterial blood pressure rises, the LV must spend more energy to eject the blood. This increase in afterload could reduce the absolute GLS, thus resulting in misinterpretation of the true contraction of the myocardium (2, 15). One study demonstrated that the MW in patients with a high SBP was significantly different from that in controls despite preservation of the strain and EF (4). MW derived from the PSL with consideration of the GLS and afterload enables the accurate assessment of the LV myocardial function.

TABLE 3 | Univariable and multivariable analyses of global work index.

Variable	Univariable analysis		Multivariable analysis	
	Coefficient	*p*-value	Coefficient	*p*-value
Sex	0.15	0.066		
Age	−0.149	0.069		
Body surface area	−0.01	0.901		
Body mass index	0.035	0.665		
SBP	**0.257**	**0.001**	0.283	<0.001
DBP	0.094	0.251	−0.116	0.025
IVSd (mm)	**−0.179**	**0.028**		
PWd (mm)	−0.155	0.059		
LVDd (mm)	**−0.674**	**<0.001**	−0.159	0.033
LVEF (%)	**0.801**	**<0.001**	0.671	<0.001
A wave (cm/s)	0.097	0.241		
E wave (cm/s)	**0.24**	**0.003**		
E/A ratio	0.062	0.447		
E/e′	**−0.317**	**<0.001**		

DBP, diastolic blood pressure; IVSd, diastolic interventricular septal thickness; LVDd, left ventricular diameter diastole; LVEF, left ventricular ejection fraction; PWd, diastolic posterior wall thickness; SBP, systolic blood pressure. p < 0.05 was considered significant.

TABLE 4 | Univariable and multivariable analyses of global waste work.

Variable	Univariable analysis		Multivariable analysis	
	Coefficient	*p*-value	Coefficient	*p*-value
Sex	−0.092	0.264		
Age	**0.403**	**<0.001**		
Body surface area	−0.041	0.612	−0.205	0.002
Body mass index	0.086	0.289		
SBP	**0.222**	**0.006**	0.247	<0.001
DBP	−0.018	0.824		
IVSd (mm)	**0.24**	**0.003**		
PWd (mm)	**0.287**	**<0.001**		
LVDd (mm)	**0.594**	**<0.001**	0.625	<0.001
LVEF (%)	**−0.556**	**<0.001**		
A wave (cm/s)	0.149	0.069		
E wave (cm/s)	−0.153	0.061		
E/A ratio	−0.186	**0.030**		
E/e′	0.441	**<0.001**		

DBP, diastolic blood pressure; IVSd, diastolic interventricular septal thickness; LVDd, left ventricular diameter diastole; LVEF, left ventricular ejection fraction; PWd, diastolic posterior wall thickness; SBP, systolic blood pressure. p < 0.05 was considered significant.

TABLE 5 | Univariable and multivariable analyses of global constructive work.

Variable	Univariable analysis		Multivariable analysis	
	Coefficient	*p*-value	Coefficient	*p*-value
Sex	0.133	0.104		
Age	−0.104	0.207		
Body surface area	−0.012	0.882		
Body mass index	0.059	0.466		
SBP	**0.315**	**<0.001**	0.345	<0.001
DBP	0.092	0.262	−0.144	0.008
IVSd (mm)	**−0.186**	**0.022**		
PWd (mm)	−0.129	0.116		
LVDd (mm)	**−0.623**	**<0.001**		
LVEF (%)	**0.765**	**<0.001**	**0.761**	**<0.001**
A wave (cm/s)	0.16	0.052		
E wave (cm/s)	**0.249**	**0.002**		
E/A ratio	0.034	0.708		
E/e′	**−0.254**	**0.003**		

DBP, diastolic blood pressure; IVSd, diastolic interventricular septal thickness; LVDd, left ventricular diameter diastole; LVEF, left ventricular ejection fraction; PWd, diastolic posterior wall thickness; SBP, systolic blood pressure. p < 0.05 was considered significant.

TABLE 6 | Univariable and multivariable analyses of global work efficiency.

Variable	Univariable analysis		Multivariable analysis	
	Coefficient	*p*-value	Coefficient	*p*-value
Sex	0.112	0.171		
Age	**−0.333**	**<0.001**		
Body surface area	0.021	0.793	0.168	0.003
Body mass index	−0.06	0.464	−0.129	0.024
SBP	−0.082	0.321	−0.095	0.026
DBP	0.047	0.568		
IVSd (mm)	**−0.234**	**0.004**		
PWd (mm)	**−0.248**	**0.002**		
LVDd (mm)	**−0.794**	**<0.001**	−0.366	<0.001
LVEF (%)	**0.831**	**<0.001**	0.547	<0.001
A wave (cm/s)	−0.111	0.174		
E wave (cm/s)	**0.256**	**0.002**		
E/A ratio	**0.204**	**0.022**	**−0.233**	**0.014**
E/e′	**−0.453**	**<0.001**		

DBP, diastolic blood pressure; IVSd, diastolic interventricular septal thickness; LVDd, left ventricular diameter diastole; LVEF, left ventricular ejection fraction; PWd, diastolic posterior wall thickness; SBP, systolic blood pressure. p < 0.05 was considered significant.

In our study, the GLS of patients with CAD was lower than that of controls, but the difference was not statistically significant. This lack of a difference may be related to the small sample size. However, MW detected differences between patients with CAD and controls. Furthermore, MW can indicate the subtle and accurate effects of ischemia on the myocardium by combining GLS and blood pressure information. Boe et al. investigated MW in patients with non-ST-segment acute coronary syndromes and reported that a regional GWI < 1,700 mmHg% was significantly superior to GLS or LVEF (16). Our results are consistent with this finding in that the MW indices derived from the GLS were more responsive to LV function than GLS.

MW indices were strongly correlated with LVEF in this study. These results are consistent with those of the Normal Reference Ranges for Echocardiography (NORRE) study, which demonstrated strong correlations between GWI and GLS and LVEF (17). In the current study, the GWI was decreased in CAD patients with HF because HF patients are

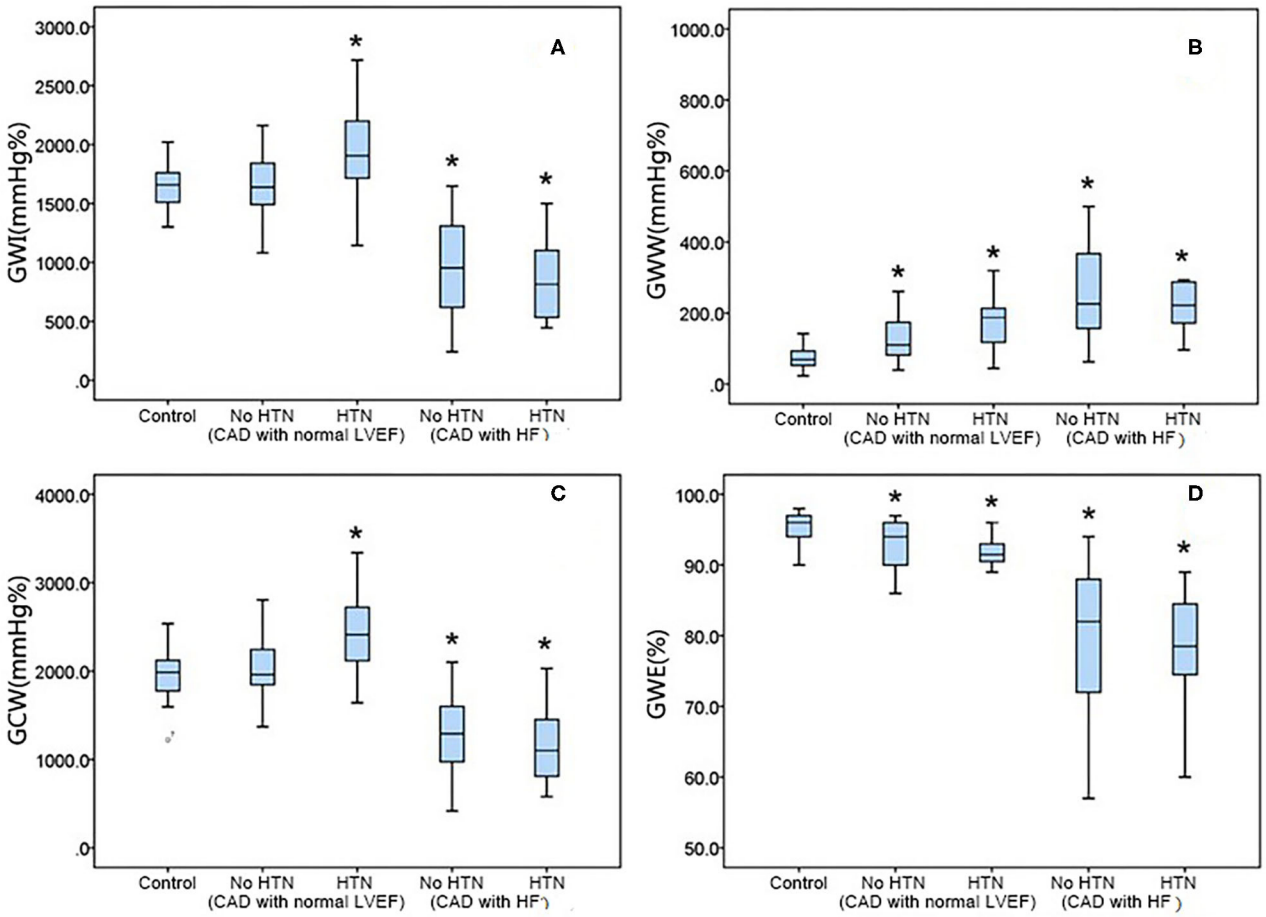

FIGURE 3 | Relationship of the myocardial work indices with the left ventricular ejection fraction. GCW, global constructive work; GWE, global myocardial work efficiency; GWI, global work index; GWW, global waste work; LVEF, left ventricular ejection fraction.

in a decompensated state of heart function due to myocardial damage. The GWI was significantly elevated in patients with HTN because the LV myocardium must compensate for the increased afterload in patients with HTN. Some studies reported that GWI was significantly increased in patients with high blood pressure, consistent with our study results (18, 19). Therefore, GCW can be used to estimate LV performance since it represents the work required for LV ejection. Another study indicated that GCW is significantly correlated with traditional parameters such as LVEF, E/e′, stroke volume, cardiac output, and cardiac index (17). GCW reflects the contractile and viable myocardium and is considered more useful than GLS (20). GWW, as the waste work of myocardium, was significantly elevated in the CAD-HTN subgroup and HF subgroup in our study. In patients with HTN, the increase in GWW may be due to resistance against the increasing afterload. In HF patients, dyssynchronous contractions and post-systolic shortening in the damaged myocardium may contribute to GWW (10). Dyssynchronization in wall motion increases GWW and reduces the ventricular ejection efficiency (21). GWW was reportedly alleviated in dyssynchronous ventricles along with increases in LVEF after cardiac resynchronization therapy (CRT) (3). Another study showed patients with higher GCW exhibit a favorable response to CRT (22). GWE reflects the efficiency of myocardial contraction. In our study, GWE was lower in the CAD subgroups than in controls, particularly in the HF subgroups. The GWI did not differ significantly between the CAD patients with a normal LVEF and HTN and the controls. GWE is considered a better indicator of myocardial impairment than GWI. A reduced GWE results from GCW reductions and GWW increases. GWE can reflect myocardial damage severity and LV function. A previous study reported a strong correlation between LVEF and GWE (8).

MW may provide additional information about dyssynchronous contractions, segmental MW, and myocardial contractility (23). GLS has become an important indicator of heart function and a prognostic factor in CAD patients (24, 25). In the early stages of CAD, MW derived from the combination of GLS and afterload is helpful in the evaluation of myocardial impairment and LV function. In this study, we found a strong correlation between MW and conventional echocardiographic parameters for assessing cardiac function. MW showed a particular pattern in CAD patients. Thus, MW is

of great value in evaluating heart function impairments in CAD patients, especially in patients in whom it cannot be identified by conventional echocardiography.

Limitations

Our study described various patterns of MW in CAD patients with different heart functions. However, the baseline patient ages varied among groups. No study to date demonstrated any effect of age on MW; however, this requires further investigation. Some CAD patients in this study had a history of diabetes and HTN, and it is unclear whether either condition affects MW. Poor image quality limits the assessment and application of MW. In this study, we used only one product (Vivid E95, GE Vingmed Ultrasound). Possible variations in speckle tracking strain findings among products have not been investigated. And finally, this was a small-sample single-center study; thus, larger studies are required to confirm our results.

CONCLUSION

MW manifested special patterns in the subgroups of CAD patients with different heart functions under different afterload conditions. Our findings suggest that MW enables an accurate and subtle assessment of ventricular function in CAD patients.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article will be made available by the authors upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Beijing Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HZ and YG contributed to the study planning and conduct, and the article writing. XW, CY, YL, and XM contributed to the patient enrolment process. ZP and RZ contributed to the data analysis and echocardiography examinations. YZ and FW contributed to the study design and take responsibility for the overall content as guarantors. 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.2021.727389/full#supplementary-material

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Interplay Between Gut Microbiota and Amino Acid Metabolism in Heart Failure

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Heart failure (HF) is a complex clinical syndrome of which the incidence is on the rise worldwide. Cardiometabolic disorders are associated with the deterioration of cardiac function and progression of HF. Recently, there has been renewed interest in gut microbiota (GM) and its metabolites in the cardiovascular disease. HF-caused hypoperfusion could increase intestinal permeability, and a "leaky" bowel leads to bacterial translocation and make its metabolites more easily enter the circulation. Considerable evidence shows that the composition of microbiota and amino acids (AAs) has been altered in HF patients, and AAs could serve as a diagnostic and prognostic biomarker in HF. The findings indicate that the gut–amino acid–HF axis may play a key role in the progression of HF. In this paper, we focus on the interrelationship between the AA metabolism and GM alterations during the development of heart failure. We also discuss the potential prognostic and therapeutic value of the gut–amino acid–HF axis in the cortex of HF.

Keywords: heart failure, gut microbiota, amino acids, metabolism, cardiometabolic disorders

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INTRODUCTION

Heart failure (HF) is a complex clinical syndrome and the end-stage of various cardiovascular diseases, which affects approximately 2% of adults worldwide (1). Although there have been great advances in the diagnosis and new treatments of HF, the overall prognosis for patients with HF is still not promising, and the hospital readmission and mortality rates of the patients are still high (2, 3). This makes it particularly important to understand and treat HF more precisely. Nowadays, with the advances of multiomic approaches (e.g., genomics, proteomics, transcriptomic, and metabolomics), novel therapeutic targets and strategies have been emerging to better characterize HF mechanistically as well as to investigate cutting-edge therapeutic strategies (4–7).

A growing number of research findings indicate that gut microbiota (GM) is strongly associated with human health and various diseases, including cardiovascular diseases (8–14). Recent researches have shown the regulating function of the gut microbiota and its metabolites in the manifestation and progression of HF (15, 16). A number of GM-associated metabolites, including short-chain fatty acids (SCFAs), bile acids (BAs), trimethylamine N-oxide (TMAO), amino acids (AAs) metabolites, and so on has been proven to take an active part in HF (16–21). These metabolites are the main pathways through which GM interacts with their hosts. Besides, the recent research have shown that the cardiac proteolysis attenuates in HF (22); thus, amino acids and its metabolites may have a strong impact on host through metabolic pathways. For example, the

tryptophan–kynurenine pathway and glycine-glyR α2-MAPK/ERK pathway have been indicated to obtain a cardioprotective effect (23, 24). However, with the emergence of other metabolite exploration in HF, there is basically no review mainly focused on the relationship among GM, AAs, and HF.

In this review, we reviewed the interrelationship between the long-term GM dysbiosis and AA perturbations in the development of HF. Moreover, we summarized the current researches regarding the alterations in the composition of intestinal microbiota in HF patients and the important pathogenic mechanism of AAs.

GUT MICROBIOTA ALTERATIONS IN HEART FAILURE

HF-caused hypoperfusion could increase intestinal permeability. The "leaky" bowel leads to bacterial translocation, making its metabolites more easily enter the circulation, such as endotoxin (LPS), the trigger of chronic inflammation (16, 25–28). However, except for the mechanism of a "leaky" gut, it is possible that the progression of HF could be regulated by alterations in the composition of GM and its metabolites (16).

Changes in the composition of GM in HF have been confirmed not only in the clinical study but also in experimental animal studies, which helped elucidate the role that microbiota played in the development of HF (14, 15, 29). **Table 1** summarizes the several cohort studies that have demonstrated the GM alterations in HF patients (**Table 1**) (15, 25, 27, 30–33).

The study of Sandek et al. might play a fundamental role in the exploration of the relationship between HF and microbiota (27, 30). They found that the mean density of microbiota within mucus was higher in CHF patients, and the mucosal biofilm was altered for a higher occurrence of strictly anaerobic *Eubacterium rectale* group and the strictly anaerobic *Fusobacterium prausnitzii*. The first study using 16s rRNA gene sequencing approach was reported by Luedde et al. in 2017 and revealed that HF patients had a decreased diversity of the microbiota in parallel with the downregulation of vital microbiota groups (31). More recently, Kummen et al. identified microbiota changes in 15 taxa, including depletion of taxa in the *Lachnospiraceae* family, which are known producers of butyrate (32). Another study showed that HFpEFs had a lower *Firmicutes*-to-*Bacteroidetes* ratio but not significantly and depleted bacteria that are short-chain fatty acid producers (33). Additionally, there has been evidence that *Bacteroides fragilis* and *Ruminococcus* had a positive effect on HF (41).

Nonetheless, current studies have a controversy on the shifting tendency of the specific microbiota and its function in HF (14). Further studies are required to explore the link between HF and microbiota and to investigate the exact effects of microbiota.

Abbreviations: HF, heart failure; GM, gut microbiota; SCFA, short-chain fatty acid; BA, bile acid; TMAO, trimethylamine N-oxide; AA, amino acid; IPA, indolepropionic acid; BCAA, branched-chain amino acid; KP, kynurenine pathway; 5-HT, serotonin; LV: left ventricular; DASH: Dietary Approaches to Stop Hypertension; FMT, fecal microbiota transplant.

BACTERIAL METABOLISM OF AMINO ACIDS

As an essential part of nutrients in the diet, AAs could affect the nutrition of the host by interacting with gut microbiota. In addition, AAs also play a vital role in regulating the diversity and abundance of AA-fermenting microbiota for their heterogeneity in turn (42–46).

The catabolism of protein in the diet is inseparable from the GM. GM promotes the proteolysis of protein through host- and bacteria-derived proteases and peptidases, which are used for incorporation of amino acids into structural and respective proteins. Besides, GM facilitates protein fermentation, the productions of which are SCFAs, gases (H_2, CO_2, CH_4, andH_2S), nitrogenous metabolites, amines, indoles, and so on. There are several ways of disposal of the AA production: (1) excretion by feces or breath, (2) utilization by microbiota, (3) detoxification by colonic epithelium, and (4) absorption by intestinal epithelium and entering circulation (42).

AA-fermenting microbiota are effectively distinct in the intestine for the preference of AA utilization. Preferred AA substrates of *Clostridium* genus bacteria are lysine or proline, and the *Peptostreptococcaceae* genus played a vital role in glutamate or tryptophan utilization (42, 47). Anaerobes ferment aromatic amino acids, including *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Peptostreptococcus* (43). Moreover, it is shown that *Clostridium sporogenes* using aromatic amino acids as substrates generated 12 compounds, one of which, indolepropionic acid (IPA), act as a major metabolite and could affect intestinal permeability and immunity (48). *Lactobacillus* could also use tryptophan to produce indole metabolites offering mucosal protection from inflammation (49).

Moreover, it is noteworthy that several species are the key driver of AA metabolism, including *Staphylococcus aureus*, *Megasphaera elsdenii*, *Bacteroides* spp. et al. (42, 47). In conclusion, AAs and microbiota have a strong interaction with each other, and microbiota contributes to host AA homeostasis.

DYSREGULATED AMINO ACID METABOLISM IN HEART FAILURE

As metabolomics is maturing, it is being utilized for early identification of organ function and dysfunction, and exploration of original disease pathways (7, 50). It is becoming clear that circulating metabolite profiles have a strong connection with HF severity and prognosis, which serve as promising novel biomarkers for identifying HF progression. Additionally, circulating amino acid profiles can directly reflect the host nutrition status, including food intake, absorption, tissue synthesis, and breakdown. Recent studies have provided evidence for microbial metabolism of AA in HF and investigated the value of AA for the identification and evaluation of HF.

Branched-chain amino acids (BCAAs), including valine, leucine, and isoleucine, are the major components in most mammals and account for nearly 35% of essential amino acids and 18% of the total amino acids (51, 52). One of the pivotal

TABLE 1 | Brief summary of studies investigating heart failure (HF) and alterations in microbiota and amino acids.

References	Sample groups	Methods	Key findings
Sandek et al. (27)	22 CHF patients and 22 controls	Biopsies of the sigmoid mucosa taken for fluorescence *in situ* hybridization (FISH)	Mean density of bacteria within mucus was higher in CHF patients; The most frequent strains were Bacteroides/Prevotella in HF patients and controls
Sandek et al. (30)	65 HF patients and 25 controls	Tested by FISH	CHF patients exhibited increased bacteria restricted to the juxtamucosal zone and a lower intestinal blood flow. The mucosal biofilm was altered in HF patients for higher occurrence of strictly anaerobic *Eubacterium rectal*e group and the strictly anaerobic *Fusobacterium prausnitzii*
Pasini et al. (25)	60 mild CHF patients, 30 moderate to severe CHF patients, and 20 controls	Microbiota in stool samples was measured after 48 h of incubation. Further proof by using bacterial metabolic tests	CHF patients had massive quantities of pathogenic bacteria and *Candida*, such as *Campylobacter*, *Shigella*, *Salmonella*, *Yersinia enterocolitica*, and *Candida* species
Luedde et al. (31)	20 HF patients and 20 controls	16s rRNA gene sequencing	HF patients had a lower diversity of the gut microbiota. There was a significant decrease in Coriobacteriaceae, Erysipelotrichaceae, and Ruminococcaceae observed on the family level in HFs. On the genus level, *Collinsella*, uncl. *Erysipelotrichaceae*, and uncl. Ruminococcaceae showed a significant decrease in HF
Kummen et al. (32)	Two cohorts. Discovery: 40 HFs; validation: 44 HFs; 266 controls	16s rRNA gene sequencing	HF patients had decreased microbial richness and identified changes in 15 taxa, including a depletion of taxa in the Lachnospiraceae family, which are known producers of butyrate
Beale et al. (33)	26 HFpEFs, 39 metropolitan controls and 28 regional controls	16s rRNA gene sequencing	There was a significant difference in α-diversity and β-diversity between both cohorts of controls and HFpEFs. HFpEFs had a lower Firmicutes-to-Bacteroidetes ratio but not significantly, and depleted bacteria that are short-chain fatty acid producers
Cheng et al. (34)	51 controls and 183 HFs; validation: 63 controls and 218 HFs with stage C	Untargeted metabolic analysis by LC-MS	A panel of metabolites, including histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4, has a diagnostic value similar to B-type natriuretic peptide (BNP). The prognostic value of the metabolite panel, which consisted of the asymmetric methylarginine/arginine ratio, butyrylcarnitine, spermidine, and the total amount of essential amino acids, was better than that of BNP
Wang et al. (35)	94 controls and 599 acute/decompensated HFs; validation: 391 HFs	Plasma was analyzed by UPLC	High-risk type 1 (leucine $\geq$145 μM and phenylalanine $\geq$88.9 μM), high-risk type 2 (leucine $<$81.2 μM) were associated with higher event rates. The prognostic value of types 1 and 2 remained significant after adjusting for age, BNP, and other risk factors in HF
Wang et al. (36)	890 HF outpatients to assess metabolic status, 387 patients to measure metabolic equivalents (MET).	Plasma samples measured by UPLC	HOP (plasma concentrations of histidine, ornithine, and phenylalanine) scores of $\geq$8.8 stratified patients at higher risk of composite events in a variety of HF populations. In multivariable analysis, HOP scores $\geq$8.8 remained a powerful event predictor, independent of other risk factors

(Continued)

TABLE 1 | Continued

References	Sample groups	Methods	Key findings
Chen et al. (37)	115 HFs and 37 controls	Plasma samples measured by UPLC	Phenylalanine ≥112 μM was associated with a lower accumulative survival rate and predicted death over 1 year independently
Lu et al. (24)	C57BL/6J mice and male SD rats	Cardiac hypertrophy and HF were induced by TAC surgery or Ang II	Glycine may be a novel cardioprotector against pressure overload-induced cardiac hypertrophy; the protection of glycine might be mediated by glyR α2 through the MAPK (JNK, ERK1/2, and p38) signaling pathways
Rozentryt et al. (38)	Placebo group:6; nutrition group:23	Intervention includes additional 600 kcal per day (proteins 20 g, carbohydrates 72 g, fat 26 g)	The feasibility of oral nutritional supplement in cachectic patients with heart failure and significant clinical benefit in terms of body size and body composition, laboratory parameters, and quality of life
Wu et al. (39)	Placebo group:12; active group:14	Intervention includes a combination of 8 g/day of L-alanyl-L-glutamine and 6.5 g/day of fish oil	The combined supplementation of L-alanyl-L-glutamine and PUFA did not improve exercise performance or muscle function but increased lean body mass and quality-of-life in patients with chronic stable HF
Pineda-Juárez et al. (40)	26 controls and 29 experimental group	Experimental group: the resistance exercise program and received 10 g/day BCAA supplementation, control group: the resistance exercise program.	Improvements in physical and functional capacities are attributed to resistance exercise program but not to the BCAA supplementation

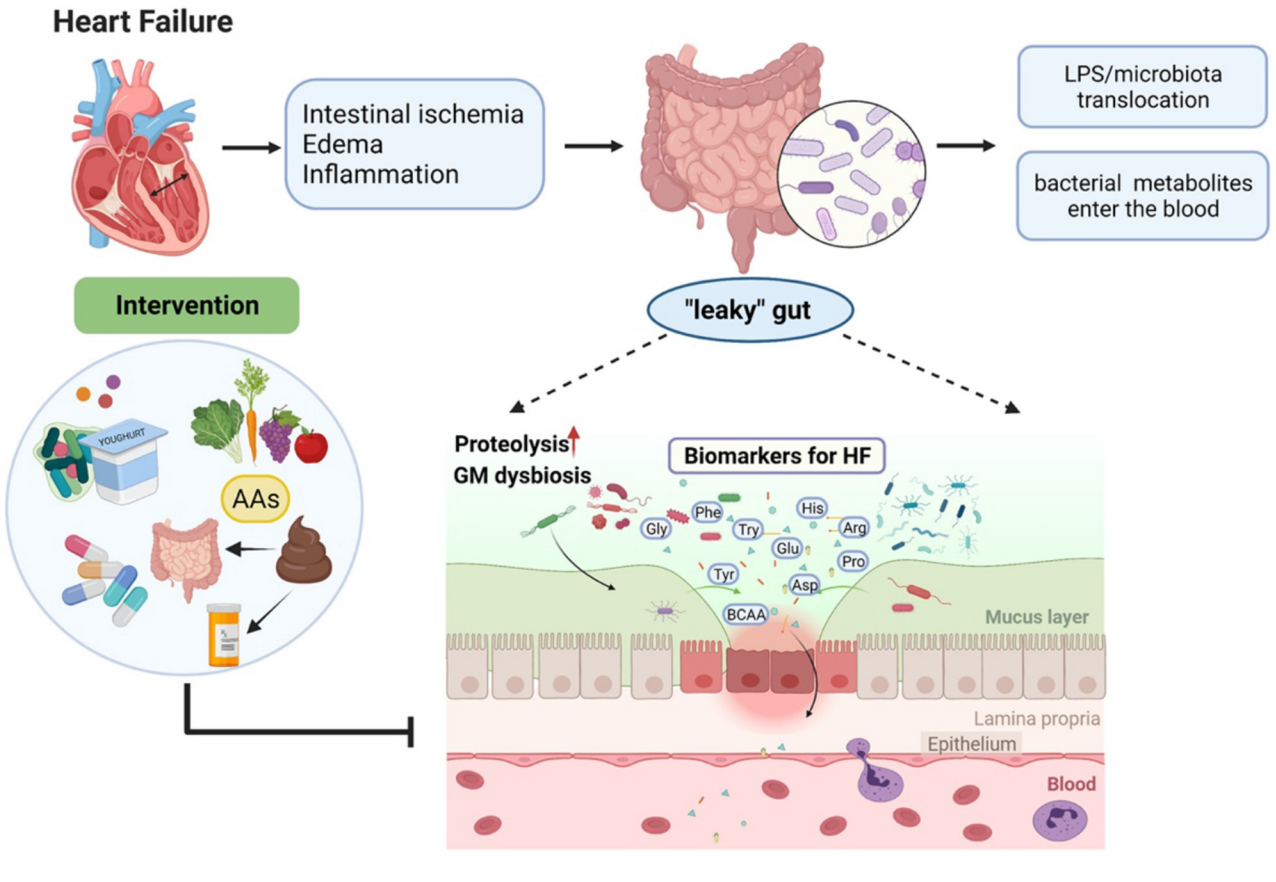

FIGURE 1 | Heart failure (HF)-caused gut microbiota dysbiosis leads to metabolite disorders and therapeutic interventions.

synthesis pathways of BCAA is in the microbiota, and uptake of BCAA from food is indispensable in humans. Different from not being synthesized in humans, BCAA catabolism not only occurs in the microbiota but also in humans and is mainly absorbed in the gut (52). More attention has been paid to the association between elevated circulating BCAA and HF (53–55). There are considerable evidence convincing the alterations of BCAA during HF, but these studies fail to explain the underlying mechanisms of how BCAA contributes to the progression of HF.

Large-scale trails, to date, have been carried out to demonstrate the relationship between circulating phenylalanine concentrations and HF (34, 35, 37, 56–59). Most of them suggest that increased phenylalanine could provide both diagnostic and prognostic value for HF. Consistent with such changings, there was a paralleling increasing trend in the serum tyrosine levels (35). Phenylalanine and tyrosine participates in the biopterin cycle; accumulation of them is a signal for tetrahydrobiopterin depletion, which often causes a problem with the production of NO and leading to cardiac dysfunction (34, 37). What is more, higher phenylalanine levels were correlated with higher C-reactive protein levels and higher pro-inflammation (37). It is an indication that HF patients with high phenylalanine levels have a more severe inflammation.

Tryptophan is one of the aromatic amino acids, and the kynurenine pathway (KP) is the key mechanism for tryptophan degradation (60–62). It has been shown that kynurenine has a strong relation to the pathophysiology of HF, such as inflammation, apoptosis, and oxidative stress (62). Increasing plasma levels of the kynurenine metabolites and the kynurenine:tryptophan ratio were associated with increased mortality in HF patients (23, 62). Furthermore, tryptophan is a pivotal substance for serotonin (5-HT) synthesis, and more than 90% of serotonin is synthesized from tryptophan in the intestine (61). Prior studies convincingly showed the considerable changes of 5-HT and 5-HT(2A)R in heart failure mice, and 5-HT2B receptor antagonists could inhibit right ventricular fibrosis through reducing collagen deposition (63–65). The newest study conducted by Cristina Razquin et al. examined the serotonin pathway and tryptophan-indole-3-propionic acid pathway of tryptophan metabolism, providing more evidence for understanding the relationship between tryptophan-related metabolites and HF (23).

In the pathophysiology of HF, failing hearts suffer from mitochondrial dysfunction, and their energy supplementation shifts from fatty acids to glucose utilization (34, 66). Histidine, arginine, and glutamine can be transferred into glutamate, offering energy and ornithine for cardiac tissues by participating into the Krebs cycle as alpha-ketoglutarate or the glutamate–ornithine–proline pathway (34). Previous studies further support the implicated mechanism of metabolic pathways in HF pathogenesis. It is almost certain that HF patients have a commonly decreasing trend in histidine, arginine, glutamine, and a reversely increase in ornithine (34). Furthermore, glutamate metabolism is ranked in the top 50 pathways in the enrichment overview for plasma in HF mice and patients (67).

As a nonessential amino acid, glycine has been repeatedly reported to have a hand in anti-inflammatory response, growth, and cytoprotection (68, 69). Recently, its beneficial effect in antihypertrophy and HF has been reported. Glycine antagonized left ventricular (LV) hypertrophy and cardiac fibrosis in HF mice (24). This paved a path for the generation of a novel treatment of HF.

In summary, a considerable number of previous studies confirmed that there commonly exists a disorder of AAs with a different trending in HF. Consistent with that, these results further supported the connection between HF and AAs.

AMINO ACID AS A DIAGNOSTIC AND PROGNOSTIC MARKER FOR HEART FAILURE

AAs have been shown to be a potential promising biomarker with significant diagnostic and prognostic values for HF. What we know about the values of AAs is largely based on the clinical studies that evaluate the possibility and stability of AAs as original biomarkers.

The study finished by Cheng et al. is one of the most influential researches to assess the metabolites and has identified a panel of four metabolites (histidine, phenylalanine, phosphatidylcholine diacyl C34:4, spermidine), which provided a similar diagnosis value and a better prognostic value than the conventional biomarker, BNP (34).

A recent study has classified patients at high risk for HF-related events into two groups: high-risk type 1 (leucine $\geq$145 μM and phenylalanine $\geq$88.9 μM), high-risk type 2 (leucine $<$81.2 μM), and other HF patients were placed into the low-risk group (35). Compared with the low-risk type, types of high-risk patients had a lower event-free survival rate, especially type 2 high-risk patients, which were characterized by severe malnutrition. This kind of novel simplified amino acid-based risk stratification offered a prognostic value for HF patients.

Wang et al. have assessed amino acid-based profile including histidine, ornithine, and phenylalanine (HOP score) (36). They have found a strong association between HOP score and cardiac function evaluated by the 6-min walking distance, which can compensate for the limitation of the NYHA Functional Classification System. A HOP score of $\geq$8.8 was associated with more risk factors for HF events.

In a study conducted by Chen et al., they measured the prognostic mortality value of phenylalanine in HF patients (37). Phenylalanine level has appeared to be positively related to the mortality of HF patients. In contrast to HF patients with phenylalanine $<$112 μM, patients with phenylalanine $>$112 μM had higher APACHE II and SOFA scores with higher mortality. Their analysis demonstrated that phenylalanine was an independent predictor of mortality and suggested setting 112 μM as a critical point for identifying different outcomes.

Up to now, there have been considerable clinical researches published to describe the significant value of AAs as diagnostic and prognostic biomarkers in HF. These results indicate that dysregulated AA is not only the result of HF but also a potential indicator for the progression of HF.

GUT–AMINO ACID–HEART FAILURE AXIS AS A POTENTIAL THERAPEUTIC TARGET FOR HEART FAILURE

A burgeoning number of relevant studies have demonstrated that there is a microbiota dysbiosis and amino acid alterations in HF; otherwise, there is a tight connection among gut microbiota, amino acids, and heart failure. What stands out is the promising values of the gut–amino acid–HF axis as a potential therapeutic target for HF.

Probiotics are live microorganisms, and prebiotics are nondigestible food products, which can change the microbiota composition and activity (14). Both of them are reported to be beneficial to the host and be cardioprotective. *Lactobacillus rhamnosus* GR-1 has been considered as a potential therapy for the attenuation of heart failure, so did *Lactobacillus plantarum 299v* and *Saccharomyces boulardii* (70, 71). However, antibiotics regulate the composition of microbiota through inhibiting specific types of negative bacteria (72). Vancomycin could improve the cardiac function in rats; polymyxin B had a similar function (70, 73). At the same time, it also increases the possibility of drug-resistant microbiota (74).

Dietary intervention for delaying the progression of HF mainly follows the guidelines from the American College of Cardiology/American Heart Association (75). The recommended Dietary Approaches to Stop Hypertension (DASH) eating plan has been assessed by several clinical trials and suggested its beneficial role for reducing HF incidence (76–79). It is also reported that several nutritional factors could change intestinal permeability, acting as a potential dietary intervention of HF (80).

Fecal microbiota transplant (FMT) is a budding way of transferring a positive microbiota into the unhealthy receiver. It has been proven to be effective in the treatment of *Clostridium difficile* infection, inflammatory bowel disease, and metabolic syndrome (81, 82). However, it also brings about a problem of virus transmission (83). Up to now, there are no related trials in HF.

Even though considerable studies have demonstrated that quite a few amino acids are useful for HF outcomes, there are some studies that evaluate how effective the amino acids are for HF treatment. There is one RCT study indicating that high-caloric protein-rich oral supplement has significant clinical benefits in terms of body composition and quality of life in CHF (38). Another RCT shows that supplementation of L-alanyl-L-glutamine and PUFA have the same effects in the CHF patients (39). However, BCAA supplementation has no improvements on physical and functional capacities in patients with HF (40). In one experimental study, combing β-alanine and histidine with exercise could elevate functional capacity and maximum strength in rats with CHF (84). Although it has been proven that glycine could reduce cardiac fibrosis in HF mice, its cardioprotective role remains to be validated in clinical trials.

CONCLUSIONS

Overall, current clinical studies have demonstrated the connection among gut microbiota, the circulating amino acids, and HF, the graphic abstract is shown in **Figure 1**. A number of AAs have been proven to serve as a diagnostic and prognostic biomarker in HF. Besides, several AAs are tested to show the positive effects of improving cardiac function in HF. Nevertheless, these results may not be adequate to elucidate the cause and effect between gut microbiota and HF. Further mechanism studies and clinical trials are needed to evaluate the gut–amino acid–HF axis as a potential therapeutic target for HF and to develop a deeper understanding of this axis in the future.

This review has pivotally presented the evidence of the gut–amino acid–HF axis as a whole and contributed in several ways for our understanding of HF. However, these findings and conclusions are limited by the quality and quantity of the relative research. Therefore, it is recommended that further research should be undertaken deeply in this axis.

AUTHOR CONTRIBUTIONS

GT, ZY, and YW designed and executed the review. GT, MG, XQ, BL, CW, HW, and JS drafted the manuscript, figure, and table. All authors contributed to the article and approved the submitted version.

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REVIEW
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Advances in Biomarkers for Detecting Early Cancer Treatment-Related Cardiac Dysfunction

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With the gradual prolongation of the overall survival of cancer patients, the cardiovascular toxicity associated with oncology drug therapy and radiotherapy has attracted increasing attention. At present, the main methods to identify early cancer treatment-related cardiac dysfunction (CTRCD) include imaging examination and blood biomarkers. In this review, we will summarize the research progress of subclinical CTRCD-related blood biomarkers in detail. At present, common tumor therapies that cause CTRCD include: (1) Chemotherapy—The CTRCD induced by chemotherapy drugs represented by anthracycline showed a dose-dependent characteristic and most of the myocardial damage is irreversible. (2) Targeted therapy—Cardiovascular injury caused by molecular-targeted therapy drugs such as trastuzumab can be partially or completely alleviated via timely intervention. (3) Immunotherapy—Patients developed severe left ventricular dysfunction who received immune checkpoint inhibitors have been reported. (4) Radiotherapy—CTRCD induced by radiotherapy has been shown to be significantly associated with cardiac radiation dose and radiation volume. Numerous reports have shown that elevated troponin and B-type natriuretic peptide after cancer treatment are significantly associated with heart failure and asymptomatic left ventricular dysfunction. In recent years, a few emerging subclinical CTRCD potential biomarkers have attracted attention. C-reactive protein and ST2 have been shown to be associated with CTRCD after chemotherapy and radiation. Galectin-3, myeloperoxidas, placental growth factor, growth differentiation factor 15 and microRNAs have potential value in predicting CTRCD. In this review, we will summarize CTRCD caused by various tumor therapies from the perspective of cardio-oncology, and focus on the latest research progress of subclinical CTRCD biomarkers.

Keywords: cancer treatment-related cardiac dysfunction, biomarkers, cardio-oncology, anthracyclines, HER2-targeted therapy, immune checkpoint inhibitors, radiotherapy

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INTRODUCTION

Improved early detection methods and the introduction of innovative cancer treatments have allowed a larger number of cancer patients to live longer. There were more than 16.9 million cancer survivors in the United States as of January 1, 2019, a figure that is projected to increase by 30% over the next decade to exceed 22.1 million (1). Long-term adverse events of cancer treatment

can affect longevity, and cancer treatment-related cardiovascular injuries are of particular interest. Early-stage breast cancer patients with underlying cardiovascular disease or who have survived for more than 5 years have a higher possibility of dying from cardiovascular disease than of dying from breast cancer (2). Common tumor treatment-related cardiac injuries include left ventricular dysfunction, heart failure (HF), angina, arrhythmia, acute coronary syndromes, thromboembolic ischemia, pericardial disease and myocardial fibrosis (3). Of these, the most serious cardiovascular injuries are cancer treatment-related cardiac dysfunction (CTRCD) and subsequent HF. Chemotherapy, molecular targeted therapy, immunotherapy and radiotherapy can all cause CTRCD. Chemotherapeutic agents such as anthracycline induce CTRCD with dose-dependent ultrastructural changes and irreversible damage, leading to severe HF and death. However, CTRCD induced by molecular targeted therapy drugs (represented by trastuzumab) is independent of drug dose, is not associated with any ultrastructural abnormalities, and can be partially or completely reversed with timely intervention (4). Regardless of whether the damage is reversible or not, the early recognition and prompt treatment of CTRCD are critical to protecting the cardiac function of cancer survivors. It is important to emphasize that not all types of cancer treatment-related cardiotoxicity contribute to heart failure. Fluoropyrimidines and vascular endothelial growth factor receptor targeting drugs often cause coronary spasm, resulting in clinical symptoms such as chest pain and angina on exertion or rest (5).

Experts from the American Society of Echocardiography and the European Association of Cardiovascular Imaging define CTRCD as a decrease in left-ventricular ejection fraction (LVEF) of more than 10%, confirmed by repeated cardiac images at 2–3-week intervals, to a value <53% (6). It should be noted that the diagnostic criteria for CTRCD caused by different solid tumors is the same. However, due to differences between the cancer treatment schemes of different solid tumors, and differences between treatment schemes for the same solid tumors at different stages, the characteristics, prevention and follow-up strategies for CTRCD are different. Echocardiography or radionuclide angiography are typically used to measure LVEF changes in order to diagnose CTRCD. However, the variability of echocardiography measurements, the high false negative rate of radionuclide angiography and the radiation involved in measurements cannot be ignored (7). Ejection fraction-preserved HF is very prevalent in cases of HF. Many other cases do not demonstrate a decline in resting LVEF in the early stages of CTRCD due to myocardial compensation (7). Under these conditions, changes in left-ventricular global longitudinal strain (GLS) appear earlier than CTRD, and GLS is a better predictor of CTRCD than LVEF (8). Over the past two decades, many studies have been performed on CTRCD-related serum biomarkers such as troponin (Tn) and B-type natriuretic peptide (BNP). These serum biomarkers have obvious value in the early identification, assessment and monitoring of CTRCD. In this review we will cover recent literature describing subclinical CTRCD biomarkers.

CANCER THERAPY AND CTRCD

Chemotherapy

Anthracyclines such as doxorubicin, epirubicin and daunorubicin are widely used as chemotherapy for breast cancer, leukemia, lymphoma and stomach cancer. Numerous studies have shown that anthracyclines can cause CTRCD. In a cohort of 2,625 patients treated with anthracyclines followed for 5.2 years, the overall incidence of CTRCD was approximately 9% and most cases occurred within 1 year of treatment (9). The occurrence of CTRCD is related to the cumulative dose of anthracycline (9). Studies have shown that the proportion of patients with adriamycin-related congestive HF is as high as 26% with a cumulative dose of 550 mg/m^2, and rises to 48% at 700 mg/m^2 (10). The mechanism through which anthracyclines induce CTRCD is closely related to oxidative stress, the anthracycline-Topoisomerase-II-DNA complex, microRNAs (miRNAs) expression and mitochondrial dysfunction (11–13).

Alkylating agents such as cyclophosphamide, ifosphamide and melphalan form the basis of chemotherapy for solid tumors, leukemia and lymphoma. In a retrospective study of autologous bone marrow transplantation with ifosfamide as part of a combination chemotherapy regimen, 17% of patients developed congestive HF (14). The cardiotoxicity of cyclophosphamide is closely related to the cumulative dose and dose based on body surface area. A dose of 180–200 mg/kg, or 1.5 g/m^2/d or higher, of cyclophosphamide is a risk factor for CTRCD (15).

The incidence of CTRCD due to taxane therapy was reported to be only 0.7%, which is significantly lower than other chemotherapy drugs (16). Taxanes affect the metabolism of anthracyclines *in vivo*, so when combined with high-dose anthracyclines, the incidence of CTRCD increases to 20% (16). However, the cardiotoxicity of taxane monotherapy was not found to be related to cumulative dose.

Targeted Therapy

Human epidermal growth factor receptor 2 (Her-2) is a transmembrane glycoprotein encoded by the proto-oncogene *ErBb2* that participates in the proliferation and differentiation of normal tissue cells (17). Her-2 is overexpressed in 15–25% of breast cancer patients, and its overexpression is associated with aggressive growth and a poor prognosis (18). Trastuzumab, a humanized monoclonal antibody against Her-2, is currently the most commonly used targeted drug for metastatic breast cancer and gastric cancer. Anti-Her-2-targeting drugs include pertuzumab, lapatinib and trastuzumab-DM1. Trastuzumab significantly improved the disease-free and overall survival of patients with positive Her-2 breast cancer (18). However, there is a significant risk of cardiotoxicity from trastuzumab-targeted therapy. Early-stage breast cancer patients treated with trastuzumab for 2 years saw a LVEF reduction of 7.2%, while the LVEF of patients treated without only decreased by 0.8% (19). Compared with chemotherapy alone, trastuzumab-combined treatment increased the risk of LVEF decline and congestive HF in patients with early-stage breast cancer 2.17- and 3.71-fold, respectively (20). Trastuzumab-related cardiac dysfunction mainly occurs during medication use, and the cardiac function

of most patients returns to normal after standard medical care (19). Trastuzumab-induced CTRCD is mainly due to its targeted inhibition of the neuregulin-1/ErbB pathway, which leads to myofibrillar injury and cardiac systolic dysfunction (21).

Immunotherapy

Immune checkpoint inhibitors (ICIs) are a primary focus of research on tumor immunotherapy. A total of seven ICIs have been approved for use by the FDA since 2011, including one cytotoxic T lymphocyte-associated protein 4 inhibitor (ipilimumab), three kinds of programmed cell death protein-1 (PD-1) inhibitors (pembrolizumab, nivolumab and cemiplimab), and three kinds of programmed death ligand-1 (PD-L1) inhibitors (atezolizumab, avelumab and durvalumab) (22). These drugs have been particularly beneficial in the treatment of melanoma, Hodgkin's lymphoma, non-small-cell lung cancer and liver cancer. However, the wide range of immune-related adverse events related to ICI use is a challenge. Immunotherapy-related cardiac events are relatively rare, but are often rapidly progressive and potentially fatal, which means that they require close monitoring. Up to now, immune myocarditis has been reported relatively more frequently. Most immunotherapy-induced left ventricular dysfunction is secondary to immune myocarditis. Cases of functional left ventricular dysfunction with no active inflammation, such as dilated cardiomyopathy with left ventricular damage and Takotsubo syndrome with acute left HF, have also been reported (23). In a multicenter study involving 964 patients receiving either one or two ICI treatments, the incidence of immune myocarditis was 1.14% (24) and 49% of patients with immunological myocarditis had LVEF <50% (24). A meta-analysis of 22 clinical trials using single ICIs in the treatment non-small cell lung cancer reported a 2% incidence rate of immune-related HF (25). The symptoms of some patients with immune-associated myocarditis or HF were reversed after timely high-dose corticosteroid treatment, and in some cases LVEF improved to baseline (23). Early recognition and timely treatment are therefore key to the effective management and treatment of immunologic-related cardiac events.

The mechanism behind CTRCD caused by ICIs is currently unclear. ICIs disrupt tumor immune escape and induce activated T cells to attack tumor cells. The same antigenic epitopes are present on both tumor cells and cardiomyocytes, suggesting that ICI activation of T cells against tumor cells also enhances the cross-reaction with cardiac antigens (23).

Radiotherapy

Radiation-induced heart disease (RIHD) is one of the most serious long-term complications of radiotherapy for thoracic tumors. The emergence of RIHD has led to a reexamination of the benefits and potential risks of radiation therapy. The incidence of HF after contemporary conformal radiotherapy is positively correlated with the mean radiation dose to the heart (26). The likelihood of HF approaches 18% over 20 years when the mean radiation dose to the heart exceeds 3.7 Gy, and is half that in patients who receive less or no radiotherapy (27). Patients are likely to benefit from emerging precision radiotherapy techniques such as intensity-modulated radiotherapy, image-guided radiotherapy and proton therapy, which are able to prevent high radiation doses to the heart. The average time from the end of radiotherapy to the onset of HF was 5.8 years, and more than half of HF cases were ejection fraction-preserved (26). It is therefore not reliable to predict radiation-related cardiac dysfunction based solely on LVEF changes. When radiotherapy is combined with anthracyclines or ICIs, the long-term incidence of cardiac dysfunction is significantly higher than with either treatment alone (28). Clinicians must be aware of the potential cardiotoxicity of combination therapy. Radiotherapy-induced CTRCD is related to endothelial cell injury and reactive oxygen species accumulation, which leads to oxidative stress and mitochondrial dysfunction (29). Myocardial tissue fibrosis is a common pathological outcome of RIHD, and can seriously interfere with left ventricular diastolic function and ejection fraction (30).

CLASSICAL BIOMARKERS

Troponin

The troponin complex is key to the regulation of skeletal muscle and cardiac muscle thin filament contraction. Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are expressed exclusively in the myocardium. cTn is released after cardiomyocyte necrosis, and is highly sensitive and specific for diagnosing a myocardial infarction. Increased circulating cTn can precede changes in echocardiographic indicators, and can therefore help in the early diagnosis of subclinical CTRCD (31). High-sensitivity cardiac troponin (hs-cTn) measurements can detect Tn concentrations 10- to 100-fold lower than the original detection method, thus further improving the accuracy and efficiency of myocardial injury diagnosis (32). Compared with hs-cTnT, hs-cTnI has superior early diagnosis potential and is not easily affected by circadian rhythm (33) (**Figure 1**).

Tn is one of the most popular biomarkers for the prediction of CTRCD. The elevation of Tn before the onset of cardiac symptoms in cancer patients is indicative subclinical functional and myocardial damage, and is closely related to all-cause mortality in cancer patients (34). cTn is a sensitive circulating biomarker for predicting left ventricular hypertrophy after anthracycline treatment. In a 3-year follow-up study, cTnI was significantly elevated during chemotherapy with anthracyclines (35). It reached its peak one month after chemotherapy (26 ng/L), gradually decreased to baseline (3 ng/L) over the proceeding 12 months, then remained at a low level (35). Recent studies have shown that the presence of hs-cTnT levels over 14 ng/L after anthracycline treatment for breast cancer represents a 2-fold increased risk of future CTRCD (36). In contrast, patients with a hs-cTnT level below 5 ng/L at the end of anthracycline treatment did not have CTRCD after 1-year (36).

Multiple studies have shown that Tn levels do not significantly change during trastuzumab monotherapy (36–38). Of 533 invasive breast cancer patients with positive HER-2 after neoadjuvant chemotherapy, only 31 had increased troponin levels during trastuzumab-targeted therapy (37). Patients with elevated cTnI and cTnT levels before trastuzumab had a 2.4-

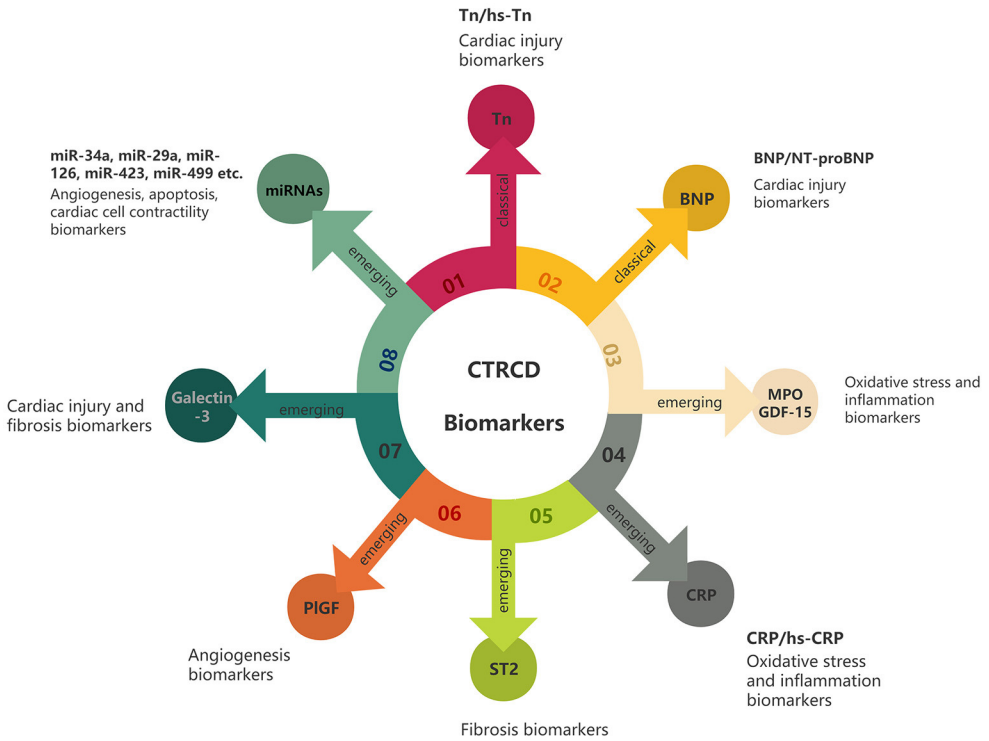

FIGURE 1 | CTRCD biomarkers. Classic biomarkers include Tn and BNP, which mainly reflect cardiac injury. Emerging biomarkers include MPO, GDF-15, CRP, ST2, PlGF, galectin-3 and miRNAs. They can reflect various aspects of the pathophysiology of the heart after cancer treatment, such as oxidative stress, inflammation and fibrosis.

to 4.5-fold increased risk of LVEF decline, although subsequent trastuzumab therapy did not impact this risk (37). The elevated baseline cTnI and cTnT levels were due to cardiac toxicity caused by anthracyclines received prior to the study. Another study of 42 breast cancer patients treated with trastuzumab failed to predict CTRCD using TnT, C-reactive protein (CRP) and BNP measurements (38). However, echocardiography with TVI and strain rate assessment was able to effectively detect preclinical changes in left ventricular systolic function (38). The poor ability of Tn to predict CTRCD efficacy during trastuzumab therapy may be because trastuzumab cardiotoxicity is weaker than that of anthracyclines, and trastuzumab-induced cardiotoxicity is primarily associated with myocardial infarction instead of myocardial injury (21). Interestingly, TnI helped to predict the reversibility of trastuzumab-induced cardiotoxicity. During trastuzumab treatment, all patients with CTRCD whose TnI level was less than 0.08 ng/mL recovered their normal cardiac function, while only 35% of CTRCD patients whose TnI level exceeded this threshold recovered (39).

Tn is the cornerstone of cardiotoxicity screening during ICI therapy. Patients with acute HF during ICI treatment had increased hs-TnT levels and a severely reduced LVEF (40). However, a cardiac MRI did not identify any notable features in these patients they were eventually diagnosed with lymphocytic myocarditis via endocardial biopsy (40). In a study of 35 patients with ICI-related myocarditis, 94% had increased TnT when clinical symptoms developed, and 51% had no significant LVEF abnormalities (41). An imaging examination should therefore not be used as the sole evaluation criteria for ICI-related cardiac events, and Tn is a potential biomarker for predicting ICI-related cardiac toxicity. The study also showed that when TnT was $\geq$1.5 ng/mL, the risk of cardiovascular death, cardiogenic shock and other major adverse cardiac events increased 4-fold (41). Elevated Tn represents a high-risk of a poor prognosis in patients with ICI-induced cardiotoxicity, although it is not a specific indicator of it. Increased Tn levels during ICI treatment should therefore be interpreted as a warning of an adverse cardiac event, and an immediate oncology–cardiological evaluation should be performed to guide further immunosuppressive therapy (42).

Changes in Tn in patients receiving thoracic radiotherapy has been of recent interest. In a cohort study of 87 patients treated with radiotherapy for breast cancer, lung cancer or mediastinal lymphoma, there was a downward trend in hs-cTnT from pre-radiotherapy to the completion of radiotherapy, especially in the subgroup that received an anthracycline and trastuzumab. This trend was thought to be related to cardiotoxicity caused by the previous treatment (43). However, approximately 15% of patients had an hs-TnT increase of over 30% during radiotherapy, and further studies are needed to explore the relationship between such an increase and the risk of RIHD. Of 58 patients with early left breast cancer who received radiotherapy, 21% had increased hs-cTnT (44). Increased hs-cTnT was positively correlated with

the whole heart and left ventricular radiation dose (44). It should be noted that none of these patients received chemotherapy, which rules out the possibility of undetected chemotherapy-induced cardiotoxicity and supports the hypothesis that changes in hs-TnT only represent radiation-induced cardiac injury. In contrast, D'Errico et al. measured TnI before and 5–22 months after radiotherapy for left breast cancer, and it did not exceed the defined positive threshold (45). Tn is inconsistently predictive of radiation-induced cardiotoxicity, so it may not be the best biomarker for diagnosing an acute cardiac injury after radiotherapy. In addition, due to the late onset of most RIHD, the effects of early fluctuations in Tn on the long-term development of heart disease are still yet to be determined by long-term follow-up studies.

Based on existing studies, cTn can identify early myocardial damage caused by chemotherapy or ICIs and predict CTRCD. However, Tn is inconsistently predictive of radiation-induced cardiotoxicity.

B-Type Natriuretic Peptide

BNP, a neuroendocrine hormone secreted by ventricular myocytes, responsively increases when the ventricular wall stretches due to the increased volume or load stress caused by HF, resulting in beneficial effects such as sodium regulation, diuresis and vasodilation (33). N-terminal proBNP (NT-proBNP), an amino-terminal fragment of BNP, is released into the circulation at an equal proportion to BNP, but has no biologic activity. Compared with BNP, NT-proBNP is stable *in vivo* and *in vitro* and is more popular in clinical practice. Negative BNP and NT-proBNP are used as exclusion criteria for acute and chronic HF, and both are widely used in the diagnosis and prognostic assessment of HF.

There have been numerous reports on the detection of subclinical CTRCD by BNP and NT-proBNP. NT-proBNP is closely related to long-term mortality in select cancer populations, and its serum level gradually increases with tumor progression (34). In a study of 71 breast cancer patients receiving non-high-dose anthracycline chemotherapy, serum NT-proBNP level measurements and echocardiography were performed before each cycle, within 24 h of the completion of chemotherapy, and at 3-, 6- and 12-month follow-ups. A sustained high level of NT-proBNP is associated with future left ventricular dysfunction, while no decrease in LVEF at 1-year follow-up was observed in patients with normal or transiently elevated NT-proBNP levels (46). NT-proBNP reflects reduced cardiac contractile reserve, which is a useful biomarker for detecting subclinical CTRCD. In addition, a cohort study of children with acute lymphoblastic leukemia who received doxorubicin chemotherapy reported that NT-proBNP (an indicator of increased left ventricular wall pressure) may be a more sensitive marker for the detection of subclinical CTRCD than cTnT (an indicator of cardiomyocyte death) (47). However, some researchers believe that the evidence that supports the use of BNP in the prediction of chemotherapy-induced cardiac dysfunction is insufficient, and further clinical studies are required (48).

NT-proBNP successfully predicted the risk of CTRCD in a prospective cohort study that included 323 breast cancer patients who received anthracycline or trastuzumab, with a maximum 3.7-year follow-up (36). Baseline levels of NT-proBNP are strongly associated with CTRCD development, with a 56% increased risk of CTRCD with each doubling of NT-proBNP over baseline values. However, due to the low incidence of CTRCD events in the trastuzumab group, no stratified analysis based on different treatment regimens was performed. Overall, routine screening NT-proBNP measurements are essential to monitoring for CTRCD, especially in those patients who received an anthracycline combined with trastuzumab. However, BNP has performed poorly in some studies. The incidence of cardiac dysfunction was very low in early-stage breast cancer patients who received no anthracyclines and anti-Her-2 therapy alone. In that treatment group the elevation of NT-proBNP was almost undetected (49). The ability of NT-proBNP to predict CTRCD following treatment with anti-Her-2 therapy alone was inferior to its predictive ability following previous exposure to anthracyclines. Some works have found that NT-proBNP was not associated with a reduced LVEF after trastuzumab treatment (38, 50, 51). Despite this, NT-proBNP is an important biomarker for the prediction of CTRCD and its negative predictive value may also be meaningful.

There are few studies that study the use of BNP or NT-proBNP in the prediction of ICI-related cardiac dysfunction. In a study of 30 patients with ICI-related cardiotoxicity, serum BNP levels were elevated in all 14 patients they were measured in (52). In another multicenter study of 35 patients with ICIs-associated myocarditis, 66% of patients had an elevated BNP, but the study did not further analyze the role of an elevated BNP in the development or clinical course of myocarditis (41). While BNP is a potential biomarker for predicting ICI-related cardiac dysfunction, further studies are required.

Elevated BNP levels after radiotherapy have been reported in multiple works, suggesting that it may be a potential biomarker for predicting RIHD. In one study, 25 patients with thoracic cancer received a mean cardiac radiation dose of $\geq$20 Gy, and elevated BNP levels were detected at the end of radiation therapy and at their first follow-up (1 to 2 months post-radiation) (53). Due to the high cardiac radiation dose used in the study, early changes in BNP may be related to cardiomyocyte inflammation, and radiation may have induced residual diastolic dysfunction. Significant increases in plasma BNP mostly occur at least 9 months after thoracic radiotherapy primarily in patients with radiation-induced myocardial damage (54). An elevated NT-proBNP level was better correlated with ventricular V3Gy, D1cm3/Dmean, and D0.5cm3/D50% than mean heart dose. This suggests that changes in NT-proBNP may be associated with damage caused by a local high dose of ventricular radiation. However, there was almost no difference in NT-proBNP levels before and 20 days after radiotherapy in 87 thoracic cancer patients (43). NT-proBNP therefore may not be effective at predicting acute RIHD. However, as most RIHD occurs late, long-term follow-up may be more useful for evaluating BNP levels after radiotherapy and their predictive value for RIHD.

Based on existing studies, BNP/NT-proBNP can effectively predict chemotherapy-induced cardiac dysfunction, especially

delayed HF. BNP/NT-proBNP also has potential in the prediction of targeted therapy or radiation-related cardiac dysfunction.

EMERGING BIOMARKERS

MPO

Myeloperoxidase (MPO), an enzyme primarily secreted by neutrophils, plays a vital role in the pathogenesis of atherosclerosis, congestive HF, hypertension and other cardiovascular diseases (55). Elevated MPO usually indicates a high risk of cardiovascular disease and a poor prognosis (55). MPO is an independent predictive factor of 1-year mortality in acute HF patients (56). The over-activation of MPO leads to inflammation and oxidative stress at the cellular level (57). Since oxidative stress plays a key role in the progression of CTRCD, MPO has recently emerged as the most promising biomarker for CTRCD.

Ky et al. first described the utility of MPO in predicting early CTRCD (58). The serum MPO levels of breast cancer patients treated with doxorubicin and paclitaxel followed by trastuzumab were significantly higher than baseline values 3 months after the start of cancer treatment, and gradually decreased over the next 15 months (58, 59). Patients with high MPO levels (422.6 pmol/L) 3 months into cancer treatment had a 36.1% chance of developing CTRCD by 15 months. In addition, MPO combined with TNI was of greater value in the prediction of CTRCD. Specifically, patients with elevated levels of either TnI or MPO had a 31.6 to 33.9% chance of developing CTRCD after 15 months, while patients with elevated levels of both had a 46.5% chance. Demissei et al. showed that high MPO levels prior to the start of anthracycline/trastuzumab therapy were closely associated with an increased risk of CTRCD (36). Each doubling of the baseline MPO level was associated with a 30% increased risk of CTRCD. MPO was more valuable at predicting CTRCD in patients who received a chemotherapy regimen that consisted of doxorubicin combined with trastuzumab compared with monotherapy. Moreover, Todorova et al. found that, of her Her-2-negative breast cancer patients who received doxorubicin chemotherapy and developed subclinical CTRCD had a significantly increased serum MPO level after the first cycle. In contrast, there was no obvious change in the MPO levels of patients with normal LVEF (60). MPO is therefore a potential biomarker of doxorubicin-related cardiotoxicity. The relationship between MPO and ICI-related cardiac dysfunction and RIHD is currently unclear and could be further explored by a cardio-oncologist in the future.

C-Reactive Protein

C-reactive protein (CRP) is an acute-phase protein that is secreted by the liver during inflammation, and whose expression is regulated by interleukin-6 and tumor necrosis factor-α. A high serum level of CRP (>10 mg/L) can predict the mortality of patients with acute decompensated HF one year after discharge (61). High-sensitivity CRP (hs-CRP) measured using high-sensitivity assays is an independent predictive factor of a poor prognosis for chronic congestive HF patients (62).

CRP/hs-CRP has recently emerged as an inexpensive, readily available and easily repeatable potential biomarker for the identification of early CTRCD. Onitilo et al. monitored cardiotoxicity during trastuzumab treatment in 54 patients with positive Her-2 breast cancer who had received standard chemotherapy (51). When the hs-CRP level was ≥3 mg/L, its sensitivity and specificity was 92.9 and 45.7%, respectively. The negative predictive value hs-CRP for patients with normal levels (hs-CRP <3 mg/L) was 94.1%. This suggests that most patients with normal hs-CRP levels can rule out the risk of future LVEF reduction, while patients with abnormal hs-CRP levels need to be strictly monitored during follow-up. The maximum hs-CRP value was detected at a median of 77.5 days before significant LVEF decline, and most subjects developed cardiotoxicity within 90 days of their peak hs-CRP level. Notably, the study evaluated both TnI and BNP, suggesting that neither were very potent biomarkers in their subjects. Similarly, Todorova et al. observed that the CRP levels of patients with decreased LVEFs after one cycle of doxorubicin chemotherapy were visibly higher than those who had no change in their LVEF (60). Considering that CRP is associated with surgery and infection, the negative predictive value of combined cTnI and CRP biomarkers is higher than either value alone. Other studies also found no clear association between CRP/hs-CRP and CTRCD induced by either chemotherapy or targeted therapy (38, 58).

CRP has recently been shown to have important predictive value in tumor immunotherapy. CRP is a prognostic biomarker for ICI treatment (63, 64). Metastatic renal cell carcinoma patients with high CRP levels (CRP ≥ 2.1 mg/dL) treated with nivolumab had a shorter progression-free survival and overall survival than those with low CRP levels (63). Similarly, elevated CRP levels in metastatic melanoma patients treated with ICIs suggests a poor prognosis (64). CRP can also predict tumor response to ICI treatment (65), and plays an important role in the prediction of immune-related adverse events. Abolhassani et al. analyzed 88 immune-related adverse events in 37 melanoma patients, which included cardiovascular adverse events, cutaneous adverse events and endocrine adverse events, and 93% of which were related to a 6.3-fold increase in CRP (66). An elevated CRP was observed in 42% of patients who had adverse events prior to the development of any clinical symptoms. There are few reports on the use of CRP for predicting ICIS-related cardiac adverse events, but existing studies suggest that CRP is a potential biomarker for these as well.

hs-CRP may also be a biomarker for radiation-induced cardiac dysfunction. Canada et al. found reported increased hs-CRP in 64% of 25 patients 1.8 years after the completion of thoracic radiotherapy (67). Abnormal hsCRP was significantly associated with decreased LVEF and reducede cardiac reserve. However, CRP was not very effective at predicting cardiac events during radiotherapy (68). Lipshultz et al. demonstrated found that hs-CRP level was higher in childhood cancer survivors than in normal controls regardless of exposure to cardiotoxic treatment (chemotherapy or radiotherapy) (69). Moreover, hs-CRP level was shown to be negatively correlated with left ventricular mass, wall thickness and dimension. Systemic inflammation may further impair cardiac function in childhood survivors, so

hs-CRP is expected to be an effective biomarker for monitoring the cardiovascular condition of cancer survivors.

CRP/hs-CRP demonstrated good CTRCD screening ability in the setting of chemotherapy, targeted therapy and immunotherapy. Of the emerging CTRCD biomarkers, CRP/hs-CRP has a relatively high predictive value and is expected to be widely used in clinical practice in the future.

ST2

ST2, a member of the interleukin 1 receptor family, is highly expressed when cardiac muscle cells are subjected to mechanical stress. The soluble receptor form of ST2 (sST2) is present in human circulation, and is an effective indicator of myocardial cell extension and fibrosis. sST2 levels are closely associated with sudden cardiac death and long-term mortality in HF patients (70, 71).

Few studies have addressed the predictive value of ST2 for CTRCD. Sawaya et al. followed 81 Her-2-positive breast cancer patients treated with anthracyclines followed by taxanes and trastuzumab or radiation for 15 months (50). ST2 levels were higher than normal serum reference values at baseline, but did not change significantly throughout the follow-up period (50, 72). Recent studies have suggested that there is a possible association between sST2 and radiation-induced cardiotoxicity. Zeng et al. assessed serum sST2 levels in 60 patients receiving chest radiotherapy (73). sST2 levels increased gradually over the course of radiotherapy, but no significant changes were observed in BNP or LVEF. It is worth noting that the ST2 change rate was positively correlated with heart V5, V10 and V20, and mean heart radiation doses. sST2 may therefore be useful in detecting acute radiological cardiotoxicity. Aula et al. grouped 63 breast cancer patients receiving radiotherapy via the severity of GLS deterioration, and correlated this with changes in sST2 levels (74). Patients with a GLS decline greater than 15% had a small but significant rise in sST2 at baseline, after radiotherapy, and three years after radiotherapy. Patients with a reduction in GLS of less than 15% had no significant fluctuation in sST2 level. sST2 may therefore be a potential biomarker for predicting radiation-related cardiotoxicity.

GDF-15

Growth differential factor 15 (GDF-15) is a member of the transforming growth factor beta superfamily that has cell protection, anti-apoptosis and anti-hypertensive properties. GDF-15 is upregulated in the setting of inflammation, oxidative stress, tissue hypoxia and cell injury (75). High serum GDF-15 levels in HF patients suggest a poor prognosis, and are predictive of 1- to 5-year mortality (76). Serum concentrations of GDF-15 were found to increase continuously during the 15-month follow-up of breast cancer patients treated with doxorubicin and trastuzumab, and increased GDF-15 levels were significantly associated with an increased risk of LVEF reduction (59). The GDF-15 levels of patients with mediastinal lymphoma or lung cancer who received thoracic radiotherapy were significantly higher after radiotherapy than before radiotherapy (1171ng/L increased to 1887ng/L) (43). However, early changes in GDF-15 were not observed in breast cancer patients, which may be related to the lower dose of radiation to the heart that is received during breast cancer radiotherapy. Moreover, changes in GDF-15 in this study were not associated with changes in LVEF during radiotherapy. Long-term follow-up studies may help define the predictive value of elevations in GDF-15 in the setting of radiation-related cardiotoxicity. It is worth noting that GDF-15 has been found to be upregulated during tumorigenesis and progression, so the value of GDF-15 in the prediction of CTRCD must be carefully considered (77).

PlGF

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor family. PlGF promotes the angiogenesis of ischemic heart and limb tissue by specifically binding with the Flt-1 receptor, and also participates in the pathologic processes of atherosclerosis and arthroinflammation (78, 79). It is worth noting that antagonistic PIGF/Flt-1 signaling can effectively inhibit tumor angiogenesis (79). PlGF is elevated in coronary heart disease and HF and is thought to have promising prognostic properties. High PlGF levels on admission can predict the long-term poor prognosis of acute decompensated HF (80). Putt et al. found that PlGF was consistently elevated and predictive of CTRCD in patients who received an anthracycline and trastuzumab (59). The potential predictive ability of PlGF may be related to the inhibitory effects of trastuzumab on angiogenesis (81). In addition, PlGF was acutely elevated in patients who received chest radiotherapy, and PIGF level was correlated with cardiac radiation dose (43). PlGF may therefore be a potential biomarker for predicting RIHD.

Galectin-3

Galectin-3, a member of the beta-galactoside-binding lectin family, is involved in the occurrence and development of cardiac fibrosis, HF and atherosclerosis (82). Galectin-3, secreted by macrophages, is key to heart remodeling (82). Numerous studies have shown that galectin-3 levels predict the risk of death in HF patients (83). Galectin-3 is upregulated in cancer cells, and promotes cancer progression and metastasis (84). Galectin-3 inhibitors can effectively block lung adenocarcinoma growth and metastasis, and increase the efficacy of PD-L1 ICIs (85). In animal studies, cardiac dysfunction induced by doxorubicin was accompanied by a massive accumulation of galectin-3, and cardiac function improved when galectin-3 was inhibited (86). Galectin-3 is expected to become a new therapeutic target for cancer and CTRCD. The expression of galectin-3 was upregulated in macrophages *in vitro* 24 h after radiation exposure (87). In contrast, Ky et al. monitored the galectin-3 serum concentrations in breast cancer patients during and after treatment with doxorubicin and trastuzumab and found no significant changes in galectin-3 levels throughout treatment (58). Moreover, galectin-3 could not effectively predict LVEF decline in their study. Altena et al. performed echocardiography and serum galectin-3 measurements in testicular cancer survivors who received cisplatin a median of 10 months and 6.9 years after chemotherapy (88). Cardiac abnormalities during long-term follow-up were more likely to manifest as diastolic dysfunction than systolic dysfunction. Galectin-3 levels were

markedly lower 6.9 years after chemotherapy than at baseline and 10 months, suggesting that the decreased diastolic function was not associated with cardiac fibrosis. The unsatisfactory predictive value of galectin-3 for CTRCD in humans may be related to the inability of echocardiography to accurately assess myocardial fibrosis. Cardiac magnetic resonance imaging may be a better imaging tool for assessing this outcome.

MicroRNAs

MicroRNAs (miRNAs) are highly conserved, single-stranded non-coding RNAs approximately 22 nucleotides in length that bind to messenger RNAs to silence gene expression. miRNAs participate in the regulation of approximately 30% of essential human gene expression, meaning that miRNAs regulate a large number of cell metabolic processes such as proliferation, apoptosis and metastasis (89). The combination of miRNAs and NT-probNP can accurately identify non-acute HF with retained ejection fraction (90). miRNAs are tissue-specific, relatively stable, easily stored at room temperature and can be accurately measured using quantitative real-time PCR and other techniques. miRNAs are therefore a promising biomarker for CTRCD.

miRNAs have recently been used to detect subclinical chemotherapy-related cardiotoxicity. Leger et al. observed a sharp increase in plasma miR-29b and miR-499 levels within 6 to 24 h of chemotherapy in children with an anthracycline chemotherapy-associated acute myocardial injury, and a significant correlation between elevated miRNAs and anthracycline dose (91). Lakhani et al. indicated that circulating levels of miR-34a, miR-29a, miR-126, miR-423 and miR-499 were upregulated in breast cancer patient 3 or 6 months after anthracycline treatment (31). There was a significant correlation between the upregulation of miRNAs and increased serum hs-cTn, a sign of myocardial injury. miRNAs can be used to effectively detect subclinical cardiac dysfunction caused by anthracyclines before LVEF decreased (31). Changes in miRNAs may be related to their involvement in regulating cardiac function. Not only does miR-34a activate the miR-34a-5p/SIRT1/p66SHC pathway to enhance cardiomyocyte apoptosis, it also increases the release rate of pro-inflammatory factors such as TNF-a and IL-6, thus playing an important role in the progression of anthracycline-induced cardiotoxicity (92). miR-29 is upregulated to inhibit the myocardial fibrosis response, which is an important aspect of cardiac remodeling after myocardial injury (93). miR-126 is expressed in endothelial cells and accelerates angiogenesis, and its upregulation after chemotherapy may be due to cellular stress and the anti-angiogenesis effects of chemotherapy drugs (94). The overexpression of miR-423 after chemotherapy directly targets O-GlcNAc transferase and induces apoptosis in cardiomyocytes, thus leading to HF (95). miRNAs are therefore promising new tools for detecting early chemotherapy-related cardiac dysfunction.

Recent studies suggest that increased levels of miR-130a during anthracycline plus trastuzumab treatment are an independent predictor of cancer treatment-related cardiotoxicity (96). Notably, Horie et al. proposed that miR-146a was significantly upregulated to inhibit ERBB4 expression after the application of doxorubicin. Although this inhibition is temporary, it blocks the activity of the NRG-1/ErbB signaling pathway and aggravates cardiomyocyte death. Combined treatment with trastuzumab, an ERBB2 inhibitor, aggravates the cardiotoxicity of the cancer treatment and further triggers HF (97). However, recent studies have shown that the administration of miR-146a-rich exosomes can not only alleviate doxorubicin/trastuzumab-induced oxidative stress in cardiomyocytes, but can also enhance the silencing effects of miR-146a on some target genes that encode the signaling mediators of the inflammation and cell death axis (98). miR-146a may therefore play a protective role in doxorubicin/trastuzumab-induced cardiotoxicity (98).

miRNAs have been found to be upregulated in animal models of autoimmune myocarditis, and correlated with the development of autoimmune myocarditis (99). miRNAs also play an important role in ICI-induced cardiac injury. Specifically, the PD-1 inhibitor promote a large amount of miR-34a aggregation in cardiomyocytes by regulating the miR-34a/KLF4 and miR-34a/Pnuts signaling pathways to induce myocardial inflammation and promote myocardial aging (100, 101). miRNAs are therefore potential therapeutic targets for ICI-induced cardiac injury.

Numerous studies have noted a close relation between miRNAs and RIHD. Hawkins et al. measured the circulating miRNA levels of 63 patients with NSCLC before radiotherapy and found that elevated miRNAs, such as miR-574, were correlated with a greater risk of radiation-related cardiotoxicity (102). In addition, the expression of miR-29a and miR-150 decreased with an increased radiation dose during chest radiotherapy (103). Decreased miR-29a during a myocardial infarction can enhance the cardiac fibrosis response (104). The changes in miR-29a during chest radiotherapy may be related to the pre-fibrotic state after radiation, making miR-29a is a potential biomarker for predicting radiation-related cardiotoxicity. A large number of animal studies have found that miR-21 is the most significantly upregulated miRNA in irradiated cardiomyocytes, and that its expression nearly doubles after radiation (105). The expression of miR-21 can inhibit apoptosis and promote cell proliferation, which is beneficial to the early resistance of cardiomyocytes to radiation injury. It is important to note that the expression of miR-1 in myocardial cells was significantly decreased after radiation, especially in the left ventricle (105). miR-34a was upregulated in irradiated cardiomyocytes and promoted cell senescence through the miR-34a/sirtuin 1 signaling pathway (106). miRNAs are important cardiovascular regulatory factors, and miRNA profiling before, during and after radiotherapy may provide more predictive and prognostic information about radiation-related cardiotoxicity.

In sum, miRNAs are a new tool for detecting early CTRCD. miRNAs profiles can provide a great deal of information about CTRCD, especially in those who have received chemotherapy and radiotherapy.

Other Emerging Biomarkers

There are many other clinical biomarkers that may be useful in the prediction of CTRCD. Glycogen phosphorylase BB

TABLE 1 | Researches on emerging biomarkers for CTRCD.

Reference	Biomarkers	Treatment	Patient population	Positive results
Lakhani et al. 2021 (31)	MiR-34a, MiR-29a, MiR-126, MiR-423, MiR-499	Doxorubicin	17 breast cancer patients with triple negative status	Circulating miRNAs were upregulated at 3 or 6 months after treatment and effectively detected CTRCD before LVEF decreased
Demissei et al. 2020 (36)	MPO, Thrombomodulin, thrombin-antithrombin complex,nucleosomes, CRP	Doxorubicin,cyclophosphamide, paclitaxel and/or trastuzumab	323 breast cancer patients	Each doubling of baseline MPO level was associated with a 30% increased risk of CTRCD
Demissei et al. 2019 (43)	PlGF and GDF-15	Radiotherapy	87 breast cancer, lung cancer, or mediastinal lymphoma patients	PlGF and GDF-15 were significantly increased during radiotherapy in lung cancer/lymphoma patients
Onitilo et al. 2012 (51)	hs-CRP	Trastuzumab	54 Her-2+ Breast Cancer patients	Normal hs-CRP levels may be associated with low future risk for decreased LVEF
Ky et al. 2014 (58)	CRP, GDF-15, MPO, PlGF, galectin-3 and soluble fms-like tyrosine kinase receptor(sFlt)-1	Doxorubicin, Cyclophosphamide, Paclitaxel, Trastuzumab	78 Her-2+ Breast Cancer patients	Levels of CRP, GDF-15, MPO, PlGF and sFIT-1 were significantly higher than baseline three months after the start of treatment, and elevated MPO levels were associated with an increased risk of CTRCD
Putt et al. 2015 (59)	MPO, PlGF and GDF-15	Doxorubicin, Cyclophosphamide, Paclitaxel, Trastuzumab	78 Her-2+ Breast Cancer patients	Increased levels of MPO, PlGF and GDF-15 were associated with an increased risk of CTRCD
Todorova et al. 2020 (60)	MPO, Thrombomodulin, thrombin-antithrombin complex,nucleosomes, CRP	Doxorubicin, Cyclophosphamide	51 early Her-2- breast cancer patients	The levels of MPO, CRP and thrombin-antithrombin complex were significantly increased in patients with subclinical CTRCD before and after the first cycle of chemotherapy, while there were no significant changes in patients with normal LVEF
Canada et al. 2020 (67)	hs-CRP	Radiotherapy	25 breast or lung cancer patients	hs-CRP was elevated in 64% of patients 1.8 years after radiotherapy, and the elevated hsCRP level was significantly correlated with decreased LVEF and smaller cardiac reserve.
Zeng et al. 2020 (73)	sST2	Radiotherapy	60 thoracic malignancy cancer patients	Serum sST-2 levels were elevated over time during radiotherapy. Heart V5, V10, V20 and mean heart dose were independently and positively associated with the elevated ST-2 change rate.
Aula et al. 2020 (74)	sST2	Radiotherapy	53 breast cancer patients	Patients with a GLS decline greater than 15% showed a small but significant increase in sST2 at baseline, after radiotherapy, and three years after radiotherapy, while patients with a GLS decline less than 15% showed no significant fluctuation in sST2 levels.
Frères et al. 2018 (94)	sST2, miR-126, miR-199a, miR-423, miR-34a	Cyclophosphamide, epirubicin and paclitaxel±trastuzumab or lapatinib	45 breast cancer patients	The levels of sST2 and miRNAs were significantly increased
Feng et al. 2021 (96)	miR-130a	Epirubicin/cyclophosphamide, docetaxel plus trastuzumab	72 Her-2+ breast cancer patients	Elevated miR-130a was an independent risk predictor for CTRCD

(Continued)

TABLE 1 | Continued

Reference	Biomarkers	Treatment	Patient population	Positive results
Hawkins et al. 2019 (102)	14 circulating miRNAs species	Radiotherapy	63 non-small cell lung cancer patients	Elevated levels of miRNAs, represented by miR-574, suggest an increased risk of radiation-associated cardiotoxicity
Dinh et al. 2016 (103)	circulating miRNAs	Radiotherapy	5 non-small cell lung cancer patients	The expression of miR-29a and miR-150 decreased with the increase of radiation dose
Horacek et al. 2013 (108)	GPBB, myoglobin and heart-type fatty acid binding protein	Anthracyclines	24 acute leukemia patients	A significant increase in GPBB levels after chemotherapy continued until 6 months after chemotherapy and the increased GPBB was significantly associated with left ventricular diastolic dysfunction
Finkelman et al. 2017 (110)	arginine, citrulline, ornithine, asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and N-monomethylarginine (MMA)	Doxorubicin ± trastuzumab	170 breast cancer patients	Changes in serum levels of arginine-nitric Oxide Metabolites were associated with an increased risk of CTRCD at up to 5.4 years of follow-up
Yu et al. 2018 (111)	40 distinct chemokines, 9 matrix metalloproteinases and 33 potential markers of cardiovascular diseases	Doxorubicin	27 breast cancer patients	Patients with abnormal immune response proteins were more likely to develop CTRCD
Chalubinska-Fendler et al. 2019 (113)	lipopolysaccharide-binding protein, fatty acid binding protein, CRP	Radiotherapy	129 breast cancer patients	Lipopolysaccharide Binding Protein could predict diastolic dysfunction 3 years after radiotherapy

(GPBB), one of the subtypes of glycogen phosphorylase, is mainly distributed in the heart and brain. GPBB is the key enzyme in glycogenolysis, supplying energy to cardiomyocytes during myocardial ischemia (107). GPBB is released into the plasma via the cell membrane, which has increased permeability during myocardial ischemia. Horacek et al. observed that GPBB significantly elevated six months after high-dose anthracycline chemotherapy, and was associated with left ventricular diastolic dysfunction (108). In contrast, the levels of other biomarkers (such as myoglobin and heart-type fatty acid-binding protein) did not significantly fluctuate. GPBB may therefore be an emerging biomarker for predicting CTRCD whose potential requires further confirmation in large prospective studies.

Anthracycline-induced CTRCD is closely associated with oxidative stress and endothelial dysfunction. The arginine–nitric oxide metabolic pathway plays an important role in these two pathophysiological processes. Arginine–nitric oxide metabolites such as asymmetric dimethylarginine and N-monomethylarginine can inhibit the activity of endothelial nitric oxide synthase and cause DNA damage, myocardial cell apoptosis and endothelial cell dysfunction (109). In a follow-up study of 170 breast cancer patients treated with doxorubicin and/or trastuzumab, the serum concentrations of dimethylargeinine and N-monomethylargeinine were significantly increased soon after chemotherapy (110). Changes in the serum levels of arginine–nitric oxide metabolites were correlated with an increased risk of CTRCD over 5.4 years of follow-up. As inflammation and immune response are essential to the early stages of doxorubicin-induced cardiotoxicity, the high expression of immune response proteins such as chemokines and matrix metalloproteinases may be related to the sensitivity of individual cardiomyocytes to doxorubicin (111). Doxorubicin-induced CTRCD is more likely to occur in patients with abnormal immune response proteins, so immune response proteins could be predictive biomarkers of CTRCD. Moreover, as carriers of some proteins and genetic materials, exosomes can aid in the early diagnosis of cardiotoxicity induced by doxorubicin (112). It is noteworthy that the serum lipopolysaccharide-binding protein could be used to predict diastolic dysfunction 3 years after radiotherapy, and is a promising predictor of radiation-related cardiac dysfunction (113) (**Table 1**).

Biomarkers and Management of CTRCD

Prevention strategies for CTRCD currently include: (1) Correction of pre-existing cardiovascular risk factors; (2) Direct reduction of cardiotoxicity: through a reduced cumulative dose of chemotherapy drugs, use of anthracycline liposomes and altered administration regimens; (3) Cardioprotectants such as dexrazoxane, which has been approved by the FDA for the cardiac protection of metastatic breast cancer patients receiving

TABLE 2 | Predictive value of biomarkers for cancer treatments.

	Chemotherapy	Targeted therapy	Immunotherapy	Radiotherapy
Tn/hs-Tn	+++	+	+++	++
BNP/NT-pro BNP	+++	++	+	++
MPO	++	++	-	-
CRP/hs-CRP	++	++	++	+
ST2	-	-	-	++
GDF-15	+	+	-	+
PlGF	+	+	-	+
Galectin-3	+	-	-	-
miRNAs	++	+	+	++
GPBB	+	-	-	-
Arginine-nitric oxide metabolites	+	+	-	-
Immune response proteins	+	-	-	-
Lipopolysaccharide-binding protein	-	-	-	+

(–) Indicates that there is currently no research report on the relationship between biomarkers and cancer treatments. (+) Indicates that biomarkers have been reported to be associated with cancer treatment. (++) Represents a large number of studies reported an association between biomarkers and cancer treatment. (+ + +) Represents that biomarkers are recognized as predictors of cancer treatment.

high doses of doxorubicin. (4) Cardiovascular support drugs such as angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, selected beta blockers and statins have been shown to prevent CTRCD (114). Tn provides important guidance during the development of a CTRCD preventive strategy. Early use of enalapril in patients with elevated Tn after high-dose chemotherapy can effectively prevent cardiotoxicity and improve CTRCD prognosis (115). The prophylactic use of enalapril even after the development of troponin abnormalities can effectively reduce the incidence of left ventricular dysfunction, with no significant difference between the two strategies (116). Beta-blockers can help patients who have already developed trastuzumab-induced cardiac dysfunction continue to complete targeted therapy (117). An angiotensin-receptor-neprilysin inhibitor that significantly improves cardiovascular outcomes was a breakthrough in the treatment of HF, and is expected to be used in the treatment of CTRCD in the future. ESMO consensus recommendations clearly state that asymptomatic patients receiving cardiotoxic anti-cancer therapy should seek the help of cardio-oncologists immediately after the detection of an elevated Tn, and it is recommended that patients be evaluated for LVEF and GLS and cardiac protective therapy started after the exclusion of ischemic heart disease (114). Patients with elevated blood markers without significant cardiac dysfunction can continue anti-cancer therapy under close monitoring. However, clinicians still rely on biomarkers to guide the management of CTRCD rather than hierarchical management. The normal threshold of NT-proBNP for diagnosing heart failure varies between different age groups, and should be of particular interest to clinicians who wish to guide the management of CTRCD.

CONCLUSION AND PROSPECT

In this review, we emphasize the vital role of serum biomarkers in the detection of subclinical CTRCD. We summarized the progress of research on two classical biomarkers (Tn and BNP) and several emerging biomarkers (MPO, CRP, ST2, GDF-15, PlGF, Galectin-3, miRNAs and GPBB) in the prediction of early CTRCD. Classical biomarkers have good sensitivity and specificity, low cost, and provide quantifiable and reproducible data. A large number of studies have confirmed that classical markers can predict the risk of CTRCD and guide further treatment. Emerging biomarkers are involved in many biological processes, and their addition may further enhance the utility of biomarkers in the management of CTRCD. For example, CRP and ST2 are associated with inflammation, galectin-3 is associated with cell proliferation, MPO and GDF-15 are associated with oxidative stress, PlGF is associated with angiogenesis and miRNAs are associated with apoptosis. However, disadvantages of emerging biomarkers are their high cost and low popularity (**Table 2**).

In addition to blood biomarkers, cardiac imaging has been widely used in the detection of subclinical CTRCD. Echocardiography, currently the most commonly used imaging tool for detecting CTRCD in clinical practice, is non-invasive, highly repeatable, simple and safe. LVEF is the most widely used cardiac function indicator in echocardiography, and can independently predict the short-term and long-term mortality of CTRCD. However, due to the early compensatory response of LVEF after heart injury, subclinical CTRCD cannot be accurately detected by LVEF. Strain-echocardiography is a new tool for evaluating myocardial deformation. GLS, as assessed by speckle-tracking echocardiography, can accurately measure early myocardial changes caused by cancer treatment. A 10–15% reduction in GLS is the most effective predictor of early CTRCD (3). A large number of studies have shown that GLS can effectively detect CTRCD before LVEF changes. The randomized controlled trial by Thavendiranathan et al. showed that compared with LVEF, preventive cardioprotective therapy based on GLS decline can significantly reduce the incidence of CTRCD (118). GLS is increasingly being used by clinicians to monitor the cardiac function of patients undergoing cancer treatment at baseline, during treatment, and during follow-up. However, the detection of GLS in elderly patients, obese patients and patients with valvular heart disease and coronary artery disease is limited (119). Although a few of the studies mentioned above have shown that GLS can predict CTRCD before blood markers change, the competition between GLS and blood markers for predictive ability requires further exploration. A combination of blood markers and imaging techniques may be the best choice for predicting CTRCD. The sensitivity of hs-TnI combined with longitudinal strain measurements was significantly higher than that of simple biomarker measurements (74% increased to 87%),

with a negative predictive value of 91% (50). NT-proBNP and GLS measurements also help to improve the detection rate of early CTRCD, and this mode can successfully detect CTRCD before LVEF declines and clinical symptoms appear (120). It is necessary to note that during the COVID-19 pandemic, patients without symptoms associated with CTRCD can be monitored over extended imaging intervals (121). In order to reduce the risk of exposure, cardiac toxicity can be monitored mainly using routine blood markers. CTRCD was further evaluated by imaging when blood markers were abnormal.

Most clinical studies on the prediction of early CTRCD focused on the traditional biomarkers Tn and BNP, with few studies on emerging markers. Future studies can be improved in the following ways: (1) a longer follow-up time will provide valuable data for improve the predictive power of biomarkers for late cardiotoxicity; (2) increasing the sample size will improve study accuracy; (3) the direction of cardiotoxicity caused by targeted therapy or ICIs should be further explored. Genetic testing, human stem cell-derived cardiomyocytes and artificial intelligence have all been used to predict subclinical CTRCD (122).

In conclusion, the early diagnosis, treatment and prognostic assessment of CTRCD in the era of precision medicine all require close cooperation between oncologists and cardiologists. Emerging biomarkers have strong potential to predict subclinical CTRCD and provide direction for future cardio-oncology.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception, development, and critical appraisal of this manuscript. All authors contributed to the article and approved the submitted version.

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Association of Systemic Immune-Inflammation Index With Short-Term Mortality of Congestive Heart Failure: A Retrospective Cohort Study

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Purpose: The present study aimed to clarify the potential predictive significance of Systemic immune-inflammation index (SII) in assessing the poor prognosis of critically ill patients with congestive heart failure (CHF).

Methods: Detailed clinical data were extracted from the Multiparameter Intelligent Monitoring in Intensive Care III database after gaining access and building the local platform. The 30- and 90-day and hospital all-cause mortalities of the patient was the primary outcome, and the readmission rate and the occurrence of major cardiovascular adverse events (MACEs) were the secondary outcomes. the Cox proportional hazard model and Logistic regression analysis were selected to reveal the relationship between SII level and the research outcome. Further, the propensity score matching (PSM) analysis was performed to improve the reliability of results by reducing the imbalance across groups.

Results: There were a total of 4,606 subjects who passed the screening process and entered the subsequent analysis. Multivariate regression analysis showed that after adjusting for possible confounders, including age, heart rate, and albumin, etc., the high level of SII was independently associated with 30- and 90-day and hospital mortalities (tertile 3 vs. tertile 1: HR, 95% CIs: 1.23, 1.04-1.45; 1.21, 1.06-1.39; 1.26, 1.05-1.50) and the incidence of MACEs (tertile 3 vs. tertile 1: OR, 95% CI: 1.39, 1.12-1.73) in critically ill patients with CHF, but no significant correlation was found between SII and the readmission rate. Consistently, patients with high SII level still presented a significantly higher short-term mortality than patients with low SII in the PSM subset.

Conclusion: In critically ill patients with CHF, high level of SII could effectively predict high 30- and 90-day and hospital mortalities, as well as the high risk of occurrence of MACEs.

Keywords: congestive heart failure, systemic immune-inflammation index, mortality, biomarker, MIMIC-III database

INTRODUCTION

Congestive heart failure (CHF) is defined as a pathological condition in which the cardiac output is insufficient to maintain the perfusion and metabolic needs of various tissues and organs, mainly characterized by the congestion of pulmonary circulation, accompanied by the corresponding clinical manifestations such as shortness of breath and decreased activity tolerance (1). CHF is the severe or terminal stage of most primary cardiovascular diseases and has increasingly become a major public problem threatening human health. In the United States and Europe, more than 1 million heart failure patients are hospitalized each year, and as the population ages, this number is expected to increase by more than 50% in the next 15 years (2). In addition, although some progress has been made in the treatment in recent years, the prognosis of CHF patients is still poor, with 5-year mortality rate as high as 40-50% (3). Therefore, accurate assessment and stratification of prognosis are critical to the clinical management of CHF, and the development of certain prognostic-related biomarkers is also pressing, which can help clinicians to identify high-risk patients early and take more aggressive treatment measures (4).

Although the specific pathogenesis of heart failure remains unclear, abnormal immune activation and chronic inflammation play an important role. The damage, repair and remodeling of myocardium are the important link in the occurrence and development of heart failure, of which immune/inflammatory cells (neutrophils, lymphocytes, etc.) and the inflammatory factors (tumor necrosis factor, interleukin-6, etc.) released by them are involved (5). Inflammatory factors can induce cardiomyocyte hypertrophy, apoptosis and fibrosis, and ultimately lead to adverse cardiac remodeling and progressive left ventricular dysfunction (6). Inflammatory factors have been considered to be biomarkers for poor prognosis of heart failure, and anti-inflammatory therapy is also expected to become a new target for the treatment of heart failure (7).

Systemic immune-inflammation index (SII) is a composite inflammatory indicator that combines three significant immune cells, including neutrophil, lymphocyte, and platelet, and is considered to be an excellent indicator of local immune response and systemic inflammation (8). Neutrophils, platelets and the cytokines they produce are mainly related to non-specific immune responses, while lymphocytes are considered to be mainly related to specific immune pathways. Compared with the absolute count of single immune cells, SII has a better representation in reflecting the inflammation status of the body, with better stability. Until now, SII has been confirmed to be closely correlated with the poor prognosis of a variety of cardiovascular diseases, including coronary artery disease (9), aortic stenosis (10), infective endocarditis (11), etc., showing good application prospects, but no studies have shown that SII is correlated with the prognosis of patients with CHF. In view of this, the present study attempted to elucidate the independent association between SII and the occurrence of adverse events such as short-term mortality in critically ill patients with CHF, so as to provide reference for the prevention and treatment of heart failure.

Abbreviations: CHF, Congestive heart failure; SII, Systemic immune-inflammation index; MIMIC-III, Medical Information Mart for Intensive Care III; ICD-9, The ninth revision of the International Classification of Diseases; ICU, Intensive care unit; MACEs, Major adverse cardiac events; SOFA, The sequential organ failure assessment; SAPSII, The simplified acute physiology score II; SD, Standard deviation; GAM, Generalized additive model; K-M curve, Kaplan-Meier curve; BUN, Blood urea nitrogen; cTnT, Troponin T; NT-proBNP, N-terminal probrain natriuretic peptide; VIF, Variance inflation factor; PSM, Propensity score matching; VHD, Valvular heart disease; AMI, Acute myocardial infarction; TNF-α, Tumor necrosis factor α; IL-6, Interleukin 6; MPV, Mean platelet volume; FDR, False discovery rate.

MATERIALS AND METHODS

Data Sources

All data used in the research and analysis of this study are derived from MIMIC-III (Medical Information Mart for Intensive Care III) database, a free and open-access large-scale critical care medicine database developed and run by the Massachusetts Institute of Technology for researchers all over the world. Another advantage of this database, in addition to including the detailed clinical data such as demographic data, vital signs, laboratory tests, and various treatment information, is that it provides accurate death information of all subjects, including time of death in the hospital or within 90 days after discharge, which makes it possible for the clinicians to carry out the prognostic related research. Besides, since this database has been approved by the local Ethics Committee as a whole and the individual identifying information of the research subjects has been deleted, this study no longer needs additional ethical approval.

Population Selection and Exclusion

The subjects of this study are all from the MIMIC- III database (12), and patients who meet all the following requirements are included in the subsequent analysis: (1) Patients diagnosed with CHF based on the ninth revision of the International Classification of Diseases (ICD-9) code (code 428.0); (2) Adult patients 18 years of age and older; (3) First admission to intensive care units (ICU). Patients who met one of the following criteria were excluded from the study: (1) The length of ICU stay was shorter than 24 h; (2) Absence of SII results during hospitalization; (3) Survival time was <0 (the time of death of some organ donors may be earlier than the admission).

Study Outcomes

The short-term mortality, including the 30- and 90-day and hospital all-cause mortalities of critically ill patients with CHF, were selected as the primary study outcome, while the secondary study outcome events were defined as the patients' readmission rate and the occurrence of major adverse cardiac events (MACEs), which is a composite outcome event, including all-cause death, readmission for acute heart failure, the use of mechanical circulatory support and the implementation of heart transplantation (13).

Data Extraction and Integration

Through the PostgreSQL software (version 9.6, https://www.postgresql.org/), we extracted detailed clinical data of the research subjects from the MIMIC-III database, including demographic data (age, gender, race, etc.), comorbidities (hypertension, diabetes mellitus, atrial fibrillation, etc.), vital signs (heart rate, blood pressure, respiratory rate, etc.), severity of illness scores [the sequential organ failure assessment (SOFA) and the simplified acute physiology score II (SAPSII) scores], laboratory tests (blood routine, electrolytes, etc.) and intervention measures (dialysis, mechanical ventilation, etc.). And SII was calculated as follows: SII = platelet × neutrophil/lymphocyte (14).

After separately extracting the required tables from the MIMIC-III database, the Stata software (version 16, https://www.stata.com/) was used to process and merge these original table and generate a complete table that can be used for subsequent analysis. Use the "winsorize" function to identify and process outliers, and fill in missing values through multiple compensation methods.

Statistical Analyses

The continuous variables were presented in the form of mean ± standard deviation (SD) or median (interquartile range). If continuous variables satisfy both normal distribution and homogeneity of variance, *t*-test was used for analysis, while the Mann-Whitney U test was conducted if the normal distribution or homogeneity of variance were not satisfied. Categorical variables are expressed in the form of the number of cases (percentage), with the chi-square test (or Fisher's exact method) for analysis.

We constructed a generalized additive model (GAM) to determine the non-linear relationship between SII and 30- and 90-day all-cause mortalities in critically ill patients with CHF. In addition, we also visually show the relationship between SII and patients' survival through the Kaplan-Meier (K-M) curve, and use the Log-rank test for hypothesis testing.

In order to relieve the interference of possible confounding factors on the results, we completed univariate and multivariate regression analysis to further clarify the relationship between SII and outcome variables. In the crude model, no variables were adjusted. In model I, the age, gender, and race variables were adjusted, while model II further adjusted other 12 variables on the basis of these variables of model II, including heart rate, blood urea nitrogen (BUN), albumin, troponin T (cTnT), N-terminal probrain natriuretic peptide (NT-proBNP), urine output of first day, cardiac index, the type of first ICU admission, the use of mechanical ventilation and vasopressor, pneumonia, and liver diseases. The selection of confounding factors follows the following principles (15): (1) A certain factor has an influence of more than 10% on the research variable; (2) Some factors may have a significant impact on the outcome variable based on past experience. Besides, we used the variance inflation factor (VIF) to test the multicollinearity between variables with 5 as the threshold, and the variables with a high degree of collinearity, serum chloride, were deleted to avoid over-fitting of the model. The Cox regression analysis was used to determine the relationship between SII and short-term mortalities in critically ill patients with CHF, while Logistic regression analysis was used to analyze the association between SII and readmission rates or MACEs.

To further enhance the credibility of our analysis, we performed the subgroup analysis and propensity score matching (PSM) analysis. First, By the subgroup analysis, determine whether the correlation between the SII and the high 30-day all-cause mortality in critically ill patients with CHF was consistent across various subgroups stratified mainly by comorbidities, and reflect the stability of SII as a prognostic marker. The optimal cut-off value of SII for the short-term mortality was determined by the X-tile (version 3.6.1, Yale University School of Medicine) software, and then the entire study population was divided into two groups, namely the high SII group and the low SII group. In this study, all variables with uneven distribution between the two groups of patients were included in the PSM

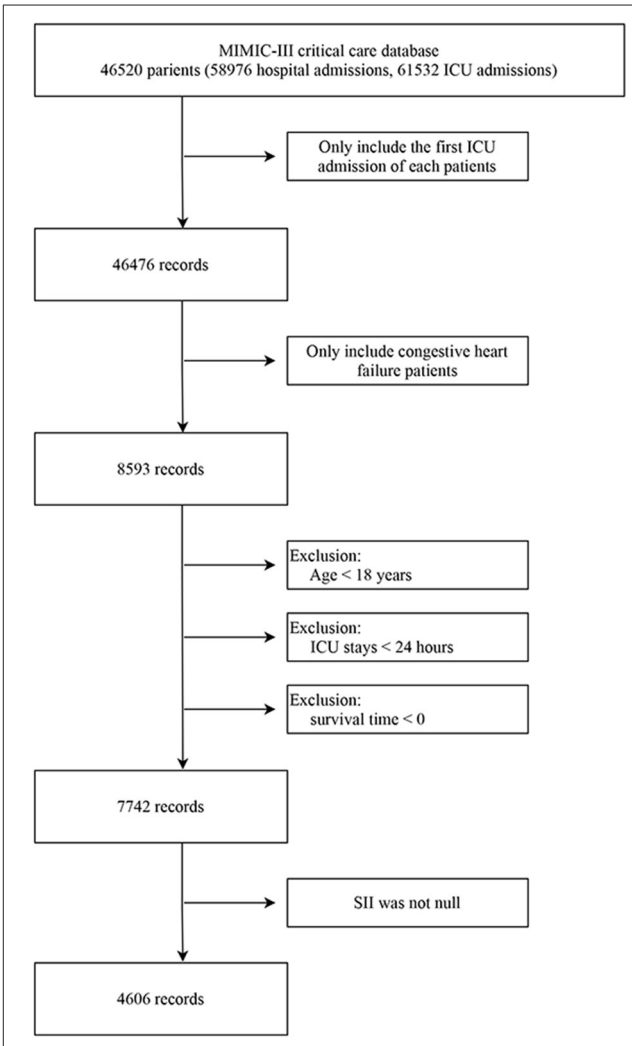

FIGURE 1 | Workflow of exclusion and inclusion as utilized to select the final 4,606 patients. ICU, intensive care unit. SII, systemic immune-inflammation index.

TABLE 1 | The baseline clinical characteristics of critically ill patients with CHF.

Parameter	All (n = 4,606)	Survivors (n = 3,226)	Non-survivors (n = 1,380)	P-value
Demographics				
Age, years	74.91 (64.06-83.07)	72.70 (61.97-81.65)	78.94 (69.40-84.82)	<0.001
Male, *n* (%)	2436 (52.89%)	1690 (52.39%)	746 (54.06%)	0.298
Ethnicity, *n* (%)				<0.001
White	3349 (72.71%)	2361 (73.19%)	988 (71.59%)	
Black	332 (7.21%)	254 (7.87%)	78 (5.65%)	
Others	925 (20.08%)	611 (18.94%)	314 (22.75%)	
Vital signs				
HR, beats/minute	85.02 (74.53-96.54)	84.33 (74.33-95.77)	86.67 (75.24-98.65)	<0.001
RR, times/minute	19.42 (16.87-22.38)	19.17 (16.76-21.91)	20.03 (17.24-23.45)	<0.001
SBP, mmHg	113.64 (104.46-125.96)	114.89 (105.50-126.98)	111.04 (101.60-123.48)	<0.001
DBP, mmHg	56.93 (50.87-63.71)	57.85 (51.64-64.34)	55.10 (49.46-61.64)	<0.001
Temperature, °C	36.76 (36.37-37.21)	36.80 (36.42-37.22)	36.65 (36.26-37.13)	<0.001
SpO2, %	97.23 (95.74-98.46)	97.25 (95.81-98.49)	97.19 (95.52-98.40)	0.018
Weight, kg	77.75 (64.70-93.60)	79.80 (65.60-96.00)	73.80 (62.00-88.00)	<0.001
Therapies, n (%)				
ACEI	1266 (27.49%)	1012 (31.37%)	254 (18.41%)	<0.001
ARB	183 (3.97%)	151 (4.68%)	32 (2.32%)	<0.001
β-blocker	2827 (61.38%)	2074 (64.29%)	753 (54.57%)	<0.001
Digoxin	475 (10.31%)	303 (9.39%)	172 (12.46%)	0.002
Furosemide	3190 (69.26%)	2275 (70.52%)	915 (66.30%)	0.004
Statins	1451 (31.50%)	1121 (34.75%)	330 (23.91%)	<0.001
Dialysis	478 (10.38%)	278 (8.62%)	200 (14.49%)	<0.001
Vasopressor	1234 (26.79%)	727 (22.54%)	507 (36.74%)	<0.001
Ventilation	1879 (40.79%)	1133 (35.12%)	746 (54.06%)	<0.001
Assisted circulation	282 (6.12%)	186 (5.77%)	96 (6.96%)	0.123
Laboratory events				
Hemoglobin, g/dl	10.90 (9.70-12.30)	11.10 (9.80-12.50)	10.70 (9.40-11.90)	<0.001
Creatinine, mg/dl	1.20 (0.90-1.80)	1.20 (0.90-1.70)	1.30 (0.90-2.10)	<0.001
BUN, mg/dl	27.00 (18.00-43.00)	25.00 (17.00-40.00)	33.00 (21.00-51.00)	<0.001
Glucose, mg/dl	135.33 (115.00-166.12)	134.23 (115.47-164.03)	137.71 (114.36-170.52)	0.111
Sodium, mmol/L	139.00 (136.00-141.00)	139.00 (136.00-141.00)	138.00 (135.00-141.00)	0.023
Potassium, mmol/L	4.20 (3.80-4.60)	4.10 (3.70-4.60)	4.20 (3.80-4.70)	0.002
Chloride, mmol/L	103.00 (99.00-107.00)	103.00 (99.25-107.00)	103.00 (99.00-107.00)	0.035
Bicarbonate, mmol/L	24.00 (21.00-28.00)	25.00 (22.00-28.00)	24.00 (20.00-27.00)	<0.001
PT, second	14.30 (13.20-16.80)	14.20 (13.10-16.50)	14.80 (13.40-17.90)	<0.001
APTT, second	31.80 (27.30-40.40)	31.30 (27.10-40.20)	32.70 (27.87-41.10)	0.001
Lactate, mmol/L	1.82 (1.30-2.90)	1.80 (1.20-2.83)	2.00 (1.30-3.00)	<0.001
Albumin, g/dl	3.00 (2.56-3.40)	3.00 (2.60-3.42)	2.89 (2.45-3.24)	<0.001
Bilirubin, mg/dl	1.40 (0.60-3.05)	1.40 (0.60-3.00)	1.41 (0.60-3.10)	0.339
ALT, IU/L	60.00 (22.00-298.33)	69.00 (23.92-327.15)	45.00 (19.00-207.75)	<0.001
NT-proBNP, ng/ml	10.98 (4.67-18.83)	10.70 (4.26-18.34)	11.91 (5.67-19.96)	<0.001
cTnT, ng/ml	1.34 (0.10-2.87)	1.46 (0.11-2.94)	1.12 (0.09-2.58)	<0.001
CI, L/min/m^2	2.76 (2.13-3.47)	2.75 (2.13-3.48)	2.78 (2.14-3.45)	0.626
Urine output, L	1.54 (0.89-2.44)	1.73 (1.02-2.65)	1.14 (0.67-1.87)	<0.001
SII ×10	174.76 (92.24-344.55)	163.11 (88.62-309.02)	213.97 (105.80-430.34)	<0.001
Hospitalization type				<0.001
Emergency, *n* (%)	4062 (88.19%)	2804 (86.92%)	1258 (91.16%)	
Elective, *n* (%)	367 (7.97%)	300 (9.30%)	67 (4.86%)	

(Continued)

TABLE 1 | Continued

Parameter	All (n = 4,606)	Survivors (n = 3,226)	Non-survivors (n = 1,380)	P-value
First ICU admission				<0.001
CCU, *n* (%)	1236 (26.83%)	898 (27.84%)	338 (24.49%)	
MICU, *n* (%)	2005 (43.53%)	1308 (40.55%)	697 (50.51%)	
Comorbidities, *n* (%)				
Hypertension	937 (20.34%)	655 (20.30%)	282 (20.43%)	0.919
Hyperlipemia	1260 (27.36%)	975 (30.22%)	285 (20.65%)	<0.001
Diabetes mellitus	1657 (35.97%)	1218 (37.76%)	439 (31.81%)	<0.001
Atrial fibrillation	2077 (45.09%)	1361 (42.19%)	716 (51.88%)	<0.001
AMI	346 (7.51%)	241 (7.47%)	105 (7.61%)	0.871
VHD	463 (10.05%)	289 (8.96%)	174 (12.61%)	<0.001
Peripheral vascular	577 (12.53%)	397 (12.31%)	180 (13.04%)	0.489
Pulmonary circulation	292 (6.34%)	183 (5.67%)	109 (7.90%)	0.005
Pneumonia	1350 (29.31%)	858 (26.60%)	492 (35.65%)	<0.001
COPD	245 (5.32%)	156 (4.84%)	89 (6.45%)	0.025
Liver diseases	232 (5.04%)	138 (4.28%)	94 (6.81%)	<0.001
Renal failure	1120 (24.32%)	757 (23.47%)	363 (26.30%)	0.040
Stroke	250 (5.43%)	148 (4.59%)	102 (7.39%)	<0.001
Hypothyroidism	555 (12.05%)	392 (12.15%)	163 (11.81%)	0.746
Malignancy	253 (5.49%)	128 (3.97%)	125 (9.06%)	<0.001
Depression	359 (7.79%)	276 (8.56%)	83 (6.01%)	0.003
Scores				
SOFA	5.00 (3.00-7.00)	4.00 (3.00-6.00)	6.00 (4.00-8.00)	<0.001
SAPSII	40.00 (32.00-49.00)	38.00 (30.00-46.00)	47.00 (39.00-56.00)	<0.001
Length of ICU stay, h	94.00 (50.00-187.00)	88.00 (48.00-166.75)	122.00 (62.00-240.25)	<0.001

HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO2, percutaneous oxygen saturation; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BUN, blood urea nitrogen; PT, prothrombin time; APTT, activated partial thromboplastin time; ALT, alanine transaminase; cTnT, troponin T; NT-proBNP, N-terminal pro brain natriuretic peptide; CI, cardiac index; SII, Systemic immune-inflammation index; CHF, congestive heart failure; AMI, acute myocardial infarction; VHD, valvular heart disease; COPD, chronic obstructive pulmonary disease. CCU, cardiac care unit; MICU, medical intensive care unit.

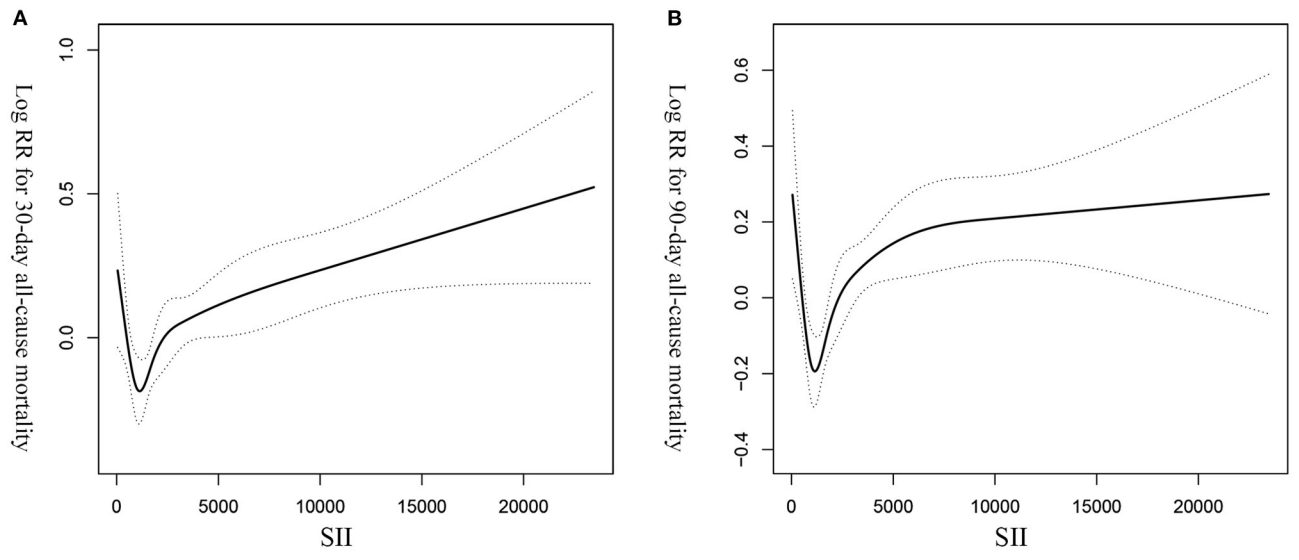

FIGURE 2 | The non-linear curve fitting of the association between SII and 30- **(A)** and 90- **(B)** day all-cause mortalities in critically ill patients with CHF after adjusting for age, gender, race, and other potential variables. SII, systemic immune-inflammation index. CHF, congestive heart failure.

model as covariates, and the corresponding propensity score was calculated by Logistic regression. Then the two groups of individuals with similar propensity scores were matched by 1:1 using nearest neighbor matching method with a caliper width of 0.05, and the histogram of the propensity score distribution was draw, which was carried out through the Stata software. Then, K-M curves were depicted and Cox regression analysis was conducted in the matching cohort after PSM, which further validated SII as an independent risk factor for poor prognosis in the critically ill patients with CHF.

The statistical analysis above was conducted by EmpowerStats software (version 2.20, http://www.empowerstats.com/cn/, X&Y solutions, Inc, Boston, MA) and R software (version 3.4.3). $p < 0.05$ (two-sided) was considered statistically significant.

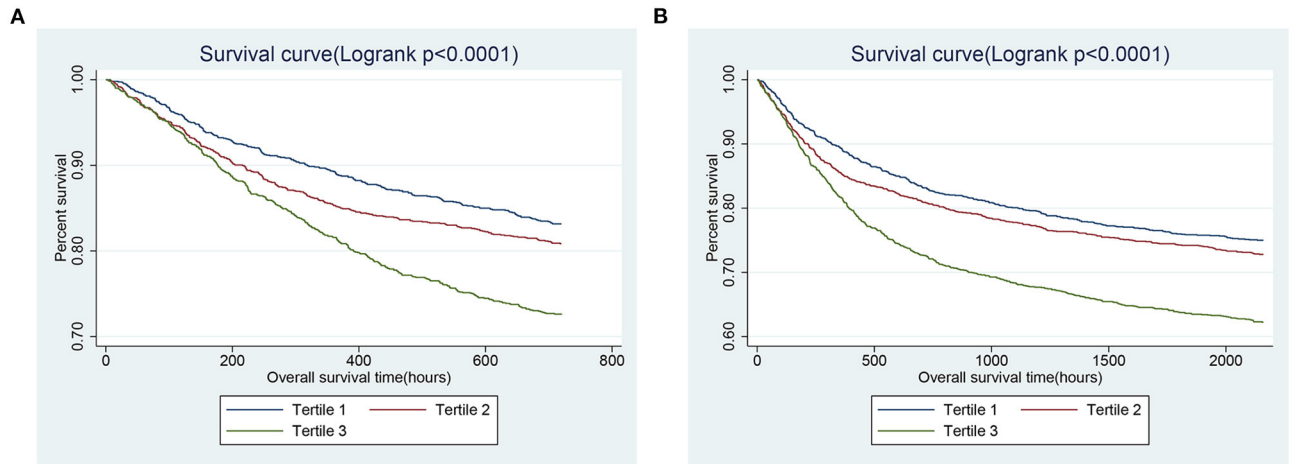

FIGURE 3 | Kaplan-Meier survival curves showing the 30- **(A)** and 90- **(B)** day all-cause mortalities stratified by SII tertile in critically ill patients with CHF. SII, systemic immune-inflammation index. CHF, congestive heart failure.

TABLE 2 | The univariate and multivariate Cox regression analysis exploring the association of SII tertile with short-term mortality of critically ill patients with CHF.

	Crude		Model I		Model II	
	HR (95%CIs)	**FDR**	**HR (95%CIs)**	**FDR**	**HR (95%CIs)**	**FDR**
30-day all-cause mortality						
SII (tertile)						
<1144.28	1 (ref)		1 (ref)		1 (ref)	
≥1144.28, <2730.11	1.17 (0.99, 1.38)	0.101	1.14 (0.97, 1.35)	0.134	1.05 (0.89, 1.25)	0.524
≥2730.11	1.73 (1.48, 2.03)	0.003	1.63 (1.40, 1.91)	0.003	1.23 (1.04, 1.45)	0.022
P for trend	<0.001		<0.001		<0.001	
90-day all-cause mortality						
SII (tertile)						
<1144.28	1 (ref)		1 (ref)		1 (ref)	
≥1144.28, <2730.11	1.12 (0.97, 1.28)	0.171	1.10 (0.96, 1.27)	0.202	1.02 (0.88, 1.17)	0.820
≥2730.11	1.65 (1.45, 1.88)	0.003	1.57 (1.38, 1.79)	0.003	1.21 (1.06, 1.39)	0.010
P for trend	<0.001		<0.001		<0.001	
Hospital all-cause mortality						
SII (tertile)						
<1144.28	1 (ref)		1 (ref)		1 (ref)	
≥1144.28, <2730.11	1.27 (1.06, 1.52)	0.016	1.21 (1.01, 1.45)	0.042	1.17 (0.97, 1.40)	0.096
≥2730.11	1.61 (1.36, 1.91)	0.003	1.45 (1.22, 1.72)	0.003	1.26 (1.05, 1.50)	0.017
P for trend	<0.001		<0.001		<0.001	

Crude model was adjusted for none.

Model I was adjusted for age, gender, and race.

Model II was adjusted for age, gender, race, heart rate, blood urea nitrogen, albumin, troponin T, NT-proBNP, urine output of first day, cardiac index, the type of first ICU admission, the use of mechanical ventilation and vasopressor, pneumonia, and liver diseases.

SII, Systemic immune-inflammation index; CHF, congestive heart failure; HR, hazard ratio; CI, confidence interval. FDR, false discovery rate.

TABLE 3 | The univariate and multivariate Logistic regression analysis exploring the association of SII tertile with readmission and MACEs of critically ill patients with CHF.

	Crude		Model I		Model II	
	OR (95%CIs)	**FDR**	**OR (95%CIs)**	**FDR**	**OR (95%CIs)**	**FDR**
Readmission						
SII (tertile)						
<1144.28	1 (ref)		1 (ref)		1 (ref)	
≥1144.28, <2730.11	0.91 (0.77, 1.07)	0.581	0.94 (0.80, 1.11)	0.581	0.94 (0.80, 1.11)	0.581
≥2730.11	0.88 (0.75, 1.04)	0.581	0.93 (0.78, 1.10)	0.581	0.97 (0.81, 1.15)	0.703
P for trend	<0.001		<0.001		<0.001	
MACEs						
SII (tertile)						
<1144.28	1 (ref)		1 (ref)		1 (ref)	
≥1144.28, <2730.11	1.17 (1.02, 1.35)	0.036	1.17 (1.02, 1.35)	0.036	0.92 (0.74, 1.13)	0.417
≥2730.11	2.01 (1.73, 2.32)	0.003	2.02 (1.74, 2.35)	0.003	1.39 (1.12, 1.73)	0.006
P for trend	<0.001		<0.001		<0.001	

Crude model was adjusted for none.
Model I was adjusted for age, gender, and race.
Model II was adjusted for age, gender, race, heart rate, blood urea nitrogen, albumin, troponin T, NT-proBNP, urine output of first day, cardiac index, the type of first ICU admission, the use of mechanical ventilation and vasopressor, pneumonia, and liver diseases.
SII, Systemic immune-inflammation index; CHF, congestive heart failure; OR, odds ratio; CI, confidence interval. FDR, false discovery rate.

Besides, in the regression analysis, we corrected *p*-value with false discovery rate (FDR) to avoid the false positives with multiple comparisons and used the false discovery rate (FDR) of 0.05 as cutoff.

RESULTS

Clinical Characteristics of Study Subjects

The study subjects were screened according to the process mentioned above, and 4,606 subjects were finally included (**Figure 1**). The demographic data, vital signs, comorbidities, treatment, scores and laboratory examinations and other relevant information between survivors and non-survivors' cohorts were shown in **Table 1** in detail. Besides, the clinical characteristics of critical ill patients with CHF across three group divided according to SII levels were presented in **Supplementary Table 1**. Overall, the median age of the study subjects included was 74.91 years old, mainly male patients, reaching 2,436, accounting for 52.89%. And the majority of the subjects were whites, accounting for 72.71%, blacks only 7.21%, and other races including Asians accounted for 20.08%. The duration of follow-up was 90 days, of which 3,226 survived and 1,380 died due to various reasons before the end of the study. SII (213.97 vs. 163.11, $p < 0.001$) was significantly higher in the non-survivor cohort. Compared with the survivor group, subjects in the non-survivor cohort were older (median age: 78.94 vs. 72.70 years old, $p < 0.001$), with more unstable vital signs, higher incidence of comorbidities such as valvular heart disease (VHD), atrial fibrillation, and renal failure, as well as higher SOFA and SAPSII scores.

SII Distinguishing a Poor Short-Term Prognosis

GAM analysis was used to identify the non-linear correlation between SII and the short-term all-cause mortality of patients, which showed that there was a U-shaped curve relationship between SII and 30-day (**Figure 2A**) and 90-day (**Figure 2B**) all-cause mortalities in critically ill patients with CHF, indicating that higher or lower SII may be associated with increased mortality. The K-M survival curve (**Figure 3**) also showed that compared with the low SII group, patients with high SII level had a lower overall survival rate and shorter survival time, with a statistically significant *p*-value.

In order to verify the independent relationship between SII and the poor short-term mortality of CHF patients, we performed the univariate and multivariate Cox regression analysis (**Table 2**), with SII stratified by tertiles. In crude model, the third tertile of SII increased significantly the risk of 30- (HR, 95% CI: 1.73, 1.48-2.03) and 90-day (HR, 95% CI: 1.65, 1.45-1.88) and hospital (HR, 95% CI: 1.61, 1.36-1.91) all-cause mortalities compared with the first tertile. In multivariate model I, after adjusting for age, sex, and race, the third tertile of SII group also suffered from the higher risk of 30- (HR, 95% CI: 1.63, 1.40-1.91) and 90-day (HR, 95% CI: 1.57, 1.38-1.79) and hospital (HR, 95% CI: 1.45, 1.22-1.72) all-cause mortalities. In model II, in addition to adjusting for the variables in model I and other possible confounders such as pneumonia, the use of dialysis, and BUN level, high levels of SII were still strongly associated with poor 30- (HR, 95% CI: 1.23, 1.04-1.45) and 90-day (HR, 95% CI: 1.21, 1.06-1.39) and hospital (HR, 95% CI: 1.26, 1.05-1.50) all-cause mortalities.

Association Between SII and Readmission and MACEs

For MACEs, a similar trend was observed. In model II (**Table 3**), patients with an SII ≥ 2730.11 were still at higher incidence of MACEs (OR, 95% CI: 1.39, 1.12-1.73). However, in the multivariate regression

TABLE 4 | Subgroup analysis of the relationship between SII and 30-day all-cause mortality.

	N	SII			*P* for interaction
		<1144.28	≥1144.28, <2730.11	≥2730.11	
Hypertension					0.178
No	3,669	1.0 (ref)	1.09 (0.90, 1.31)	1.70 (1.44, 2.02)	
Yes	937	1.0 (ref)	1.57 (1.07, 2.31)	1.88 (1.28, 2.76)	
Diabetes					0.384
No	2,949	1.0 (ref)	1.16 (0.95, 1.42)	1.62 (1.34, 1.95)	
Yes	1,657	1.0 (ref)	1.22 (0.90, 1.65)	2.01 (1.52, 2.67)	
Hyperlipemia					0.006
No	3,346	1.0 (ref)	1.13 (0.94, 1.36)	1.51 (1.27, 1.80)	
Yes	1,260	1.0 (ref)	1.40 (0.96, 2.05)	2.77 (1.94, 3.96)	
AMI					0.016
No	4,260	1.0 (ref)	1.09 (0.92, 1.30)	1.71 (1.46, 2.01)	
Yes	346	1.0 (ref)	2.63 (1.30, 5.30)	2.31 (1.14, 4.69)	
VHD					0.602
No	4143	1.0 (ref)	1.15 (0.96, 1.38)	1.76 (1.49, 2.07)	
Yes	463	1.0 (ref)	1.23 (0.76, 2.00)	1.50 (0.94, 2.41)	
PCD					0.931
No	4,314	1.0 (ref)	1.17 (0.98, 1.39)	1.72 (1.46, 2.02)	
Yes	292	1.0 (ref)	1.15 (0.61, 2.19)	1.86 (1.05, 3.32)	
Atrial fibrillation					0.437
No	2,529	1.0 (ref)	1.04 (0.82, 1.31)	1.59 (1.28, 1.98)	
Yes	2,077	1.0 (ref)	1.29 (1.02, 1.64)	1.84 (1.47, 2.31)	
Pneumonia					0.061
No	3,256	1.0 (ref)	1.18 (0.96, 1.44)	1.89 (1.56, 2.29)	
Yes	1,350	1.0 (ref)	1.09 (0.82, 1.45)	1.33 (1.03, 1.74)	
COPD					0.962
No	4,361	1.0 (ref)	1.16 (0.98, 1.38)	1.72 (1.47, 2.02)	
Yes	245	1.0 (ref)	1.32 (0.54, 3.20)	1.86 (0.83, 4.17)	
Liver diseases					0.065
No	4,374	1.0 (ref)	1.26 (1.06, 1.50)	1.85 (1.57, 2.18)	
Yes	232	1.0 (ref)	0.62 (0.34, 1.13)	1.24 (0.70, 2.21)	
Renal failure					0.396
No	3,486	1.0 (ref)	1.10 (0.91, 1.33)	1.72 (1.44, 2.05)	
Yes	1,120	1.0 (ref)	1.39 (1.00, 1.94)	1.79 (1.30, 2.48)	
Hypothyroidism					0.628
No	4,051	1.0 (ref)	1.13 (0.95, 1.35)	1.71 (1.45, 2.01)	
Yes	555	1.0 (ref)	1.46 (0.90, 2.38)	1.96 (1.23, 3.12)	
Stroke					0.618
No	4,356	1.0 (ref)	1.14 (0.96, 1.36)	1.69 (1.44, 1.99)	
Yes	250	1.0 (ref)	1.45 (0.79, 2.65)	2.24 (1.26, 3.97)	
Depression					0.216
No	4,247	1.0 (ref)	1.17 (0.98, 1.39)	1.79 (1.52, 2.10)	
Yes	359	1.0 (ref)	1.11 (0.55, 2.25)	1.02 (0.51, 2.03)	
Malignancy					0.879
No	4,353	1.0 (ref)	1.18 (0.99, 1.40)	1.72 (1.46, 2.02)	
Yes	253	1.0 (ref)	1.02 (0.57, 1.83)	1.57 (0.94, 2.63)	

(Continued)

TABLE 4 | Continued

	N	SII			*P* for interaction
		<1144.28	≥1144.28, <2730.11	≥2730.11	
SOFA					0.172
<5	2,149	1.0 (ref)	1.24 (0.91, 1.70)	2.21 (1.66, 2.96)	
≥5	2,457	1.0 (ref)	1.20 (0.99, 1.46)	1.58 (1.31, 1.90)	
SAPSII					0.255
<40	2,181	1.0 (ref)	1.22 (0.87, 1.71)	1.99 (1.43, 2.75)	
≥40	2,425	1.0 (ref)	1.13 (0.93, 1.37)	1.42 (1.19, 1.69)	

SII, Systemic immune-inflammation index; AMI, acute myocardial infarction; VHD, valvular heart disease; PCD, peripheral vascular diseases; COPD, chronic obstructive pulmonary disease.

analysis, there was no obvious independent correlation between SII and readmission rate in the critically ill patients with CHF (tertile 3 vs. tertile 1: OR, 95% CI: 0.97, 0.81-1.15).

Subgroup Analyses

The subgroup analysis was conducted to reveal the correlation between SII and 30-day mortality across comorbidities and different parameters, and the results were shown in **Table 4**. First, the results of the study showed that in all subgroups, the increase in SII level was closely related to the increase in the 30-day all-cause mortality of critically ill patients with CHF. Besides, most of the stratification factors have not been found to have a significant impact on the relationship between the SII and 30-day all-cause mortality (interaction p-value > 0.05), except for hyperlipemia ($p = 0.006$) and acute myocardial infarction (AMI, $p = 0.016$).

Prognostic Value of SII After PSM

Determine the optimal cut-off value of SII for 90-day all-cause mortality of critically ill patients with CHF through X-tile software, which is 5533.4. Subjects were divided into two groups according to the cut-off value, and their clinical characteristics were summarized in **Table 5**, most of which were unevenly distributed across the two groups. In order to effectively balance these confounding factors and improve the credibility of our results, we conducted a PSM analysis with 1:1 matching. After PSM, 602 pairs of research objects were generated and the difference of almost all variables were balanced between the two groups, with a good matching performance (**Figure 4**). At the same time, after PSM, compared with the low SII level group, subjects in the high SII level group still underwent higher 30- (24.9 vs. 33.2%, $p = 0.002$) and 90-day (35.0 vs. 42.0%, $p = 0.013$) and hospital (22.4 vs. 27.7%, $p = 0.033$) all-cause mortalities, with shorter survival time (**Table 5**; **Figure 5**). Besides, after adjusting for these covariable in the model II, the increased SII was also associated with increased risk of short-term all-cause mortality in critically ill patients with CHF after PSM and the results were shown in **Figure 6** in the form of a forest map.

DISCUSSION

CHF is widespread all over the world, with gradually increasing incidence with age, and has become one of the diseases with the highest rates of hospitalizations and deaths in the world, bringing about enormous medical expenditure and social burden, especially in developing countries (16). In addition to exploring promising treatments, the development of novel prognostic markers for risk stratification of patients is also of great value for improving the prognosis of patients. As we all know, as a commonly used indicator of heart failure, NT-proBNP can make a good performance in prognostic evaluation. However, the clinical applications of NT-proBNP for risk assessment has some drawbacks and problems. First, compared to the real value, the detection of NT-proBNP tend to be underestimated because of the short half-life. Moreover, the test of NT-proBNP may be affected by various factors such as age, gender, and kits (17). Therefore, the search for more possible biomarkers has gradually attracted the attention of researchers and clinicians.

In the present study, we extracted the clinical data of 4,606 critically ill patients who diagnosed with CHF between 2001 and 2012 in MIMIC-III database, and analyzed the relationship between SII and short-term prognosis by univariate and multivariate regression analyses for the first time. And the results have verified that SII could be an independent biomarker for poor short-term prognosis of CHF. There was a non-linear relationship between SII and 30- and 90-day mortalities. And the higher SII level, the higher risk of 30- and 90-day and hospital mortalities, as well as the higher incidence of MACEs. The correlation remained significant after adjusting for possible confounders, stratifying according to comorbidities, and PSM matching, respectively.

Inflammation, a widely accepted hallmark of heart failure, has been proven to play an indispensable role in the pathogenesis of heart failure (18). The increase and activation of inflammation-related cytokines not only present a reflection of the activation of inflammation *in vivo*, but have also been identified as being associated with poor prognosis in

TABLE 5 | The clinical characteristics in critically ill patients with CHF before and after PSM.

Parameter	Before PSM			After PSM		
	<5533.4	≥5533.4	*P* value	<5533.4	≥5533.4	*P*-value
N	3,995	611		602	602	602
Demographics						
Age, years	74.4 (63.4-82.9)	76.9 (68.2-83.7)	<0.001	77.7 (66.1-84.2)	76.9 (68.1-83.7)	0.984
Male, n (%)	2154 (53.9%)	282 (46.2%)	<0.001	279 (46.3%)	279 (46.3%)	1.000
Ethnicity, *n* (%)		0.001	0.001			0.526
White	2879 (72.1%)	470 (76.9%)		463 (76.9%)	461 (76.6%)	
Black	309 (7.7%)	23 (3.8%)		30 (5.0%)	23 (3.8%)	
Others	807 (20.2%)	118 (19.3%)		109 (18.1%)	118 (19.6%)	
Vital signs						
HR, beats/minute	84.5 (74.1-96.0)	88.2 (77.0-99.6)	<0.001	87.5 (77.3-98.8)	87.9 (76.8-99.2)	0.840
RR, times/minute	19.3 (16.8-22.3)	20.3 (17.6-23.2)	<0.001	20.2 (17.3-23.5)	20.2 (17.6-23.1)	0.876
SBP, mmHg	113.8 (104.6-126.0)	113.8 (104.6-126.0)	0.145	113.9 (103.4-125.6)	112.1 (103.5-125.7)	0.964
DBP, mmHg	57.1 (51.1-63.9)	55.7 (50.2-62.5)	0.009	55.3 (49.1-61.9)	55.6 (50.2-62.5)	0.219
Temperature, °C	36.8 (36.4-37.2)	36.7 (36.3-37.1)	0.010	36.8 (36.4-37.2)	36.7 (36.3-37.1)	0.254
SpO2, %	97.3 (95.8-98.5)	97.0 (95.5-98.4)	0.157	97.3 (95.6-98.5)	97.0 (95.5-98.4)	0.474
Weight, kg	78.4 (65.0-94.3)	74.4 (61.0-90.0)	<0.001	73.0 (61.0-88.5)	74.4 (61.0-90.0)	0.560
Therapies, *n* (%)						
ACEI	1117 (28.0%)	149 (24.4%)	0.065	153 (25.4%)	147 (24.4%)	0.689
ARB	158 (4.0%)	25 (4.1%)	0.872	26 (4.3%)	25 (4.2%)	0.886
β-blocker	2454 (61.4%)	373 (61.0%)	0.858	373 (62.0%)	367 (61.0%)	0.722
Digoxin	411 (10.3%)	64 (10.5%)	0.888	85 (14.1%)	63 (10.5%)	0.053
Furosemide	2766 (69.2%)	424 (69.4%)	0.937	412 (68.4%)	416 (69.1%)	0.804
Statins	1274 (31.9%)	177 (29.0%)	0.148	158 (26.2%)	174 (28.9%)	0.302
Dialysis	426 (10.7%)	52 (8.5%)	0.104	86 (14.3%)	51 (8.5%)	0.001
Vasopressor	1042 (26.1%)	192 (31.4%)	0.008	171 (28.4%)	187 (31.1%)	0.313
Ventilation	1561 (39.1%)	318 (52.0%)	<0.001	306 (50.8%)	310 (51.5%)	0.818
Assisted circulation	259 (6.5%)	23 (3.8%)	0.009	21 (3.5%)	23 (3.8%)	0.759
Scores						
SOFA	5.0 (3.0-7.0)	5.0 (3.0-7.0)	0.556	5.0 (3.0-7.0)	5.0 (3.0-7.0)	0.405
SAPSII	40.0 (32.0-49.0)	45.0 (36.0-53.5)	<0.001	44.0 (35.0-53.0)	45.0 (36.0-53.0)	0.440
Laboratory events						
Hemoglobin, g/dl	11.0 (9.7-12.4)	10.7 (9.5-12.1)	0.008	10.7 (9.5-11.9)	10.7 (9.5-12.1)	0.290
Creatinine, mg/dl	1.2 (0.9-1.8)	1.2 (0.9-2.1)	0.250	1.3 (0.9-2.0)	1.2 (0.9-2.1)	0.160
BUN, mg/dl	26.0 (18.0-42.0)	30.0 (19.0-49.0)	<0.001	31.0 (20.0-49.0)	30.0 (19.0-48.0)	0.613
Glucose, mg/dl	134.0 (114.4-163.6)	145.5 (119.7-176.8)	<0.001	141.2 (117.1-180.3)	145.5 (119.6-176.2)	0.610
Sodium, mmol/L	139.0 (136.0-141.0)	138.0 (135.0-141.0)	<0.001	138.0 (135.0-141.0)	138.0 (135.0-141.0)	0.487
Potassium, mmol/L	4.2 (3.8-4.6)	4.2 (3.7-4.7)	0.575	4.2 (3.8-4.7)	4.2 (3.7-4.7)	0.634
Chloride, mmol/L	103.0 (100.0-107.0)	102.0 (98.0-107.0)	<0.001	103.0 (99.0-106.0)	102.0 (98.0-107.0)	0.377
Bicarbonate, mmol/L	25.0 (21.0-28.0)	24.0 (20.0-27.0)	<0.001	24.0 (20.0-27.0)	24.0 (20.0-27.0)	0.878
PT, second	14.3 (13.2-16.8)	14.4 (13.2-17.1)	0.245	14.4 (13.2-17.3)	14.4 (13.2-17.2)	0.846
APTT, second	31.8 (27.3-41.1)	31.6 (27.3-38.6)	0.329	31.3 (27.3-40.2)	31.7 (27.3-38.7)	0.920
Lactate, mmol/L	1.9 (1.3-2.9)	1.8 (1.3-2.8)	0.654	1.8 (1.3-3.0)	1.8 (1.3-2.8)	0.654
Albumin, g/dl	3.0 (2.6-3.4)	2.9 (2.5-3.3)	<0.001	2.8 (2.4-3.3)	2.9 (2.5-3.3)	0.255
Bilirubin, mg/dl	1.5 (0.6-3.1)	0.9 (0.4-2.5)	<0.001	1.1 (0.5-2.4)	0.9 (0.4-2.6)	0.282
ALT, IU/L	62.0 (22.0-309.0)	50.0 (21.0-242.7)	0.034	51.0 (21.0-225.8)	50.0 (21.0-248.5)	0.637
NT-proBNP, ng/ml	10.9 (4.6-18.6)	12.1 (5.1-19.8)	0.019			
cTnT, ng/mL	1.4 (0.1-2.9)	1.1 (0.1-2.5)	0.016	1.1 (0.1-2.8)	1.1 (0.1-2.5)	0.768
CI, L/min/m^2	2.8 (2.1-3.5)	2.8 (2.2-3.5)	0.309	2.8 (2.1-3.4)	2.8 (2.2-3.5)	0.938
Urine output, L	1.6 (0.9-2.5)	1.4 (0.8-2.1)	<0.001	1.4 (0.7-2.2)	1.4 (0.8-2.1)	0.522
Hospitalization type			0.002			0.238
Emergency, *n* (%)	3498 (87.6%)	564 (92.3%)		538 (89.4%)	555 (92.2%)	

(Continued)

TABLE 5 | Continued

Parameter	Before PSM			After PSM		
	<5533.4	≥5533.4	*P* value	<5533.4	≥5533.4	*P*-value
Elective, *n* (%)	338 (8.5%)	29 (4.7%)		39 (6.5%)	29 (4.8%)	
First ICU admission			<0.001			0.004
CCU, *n* (%)	1098 (27.5%)	138 (22.6%)		143 (23.8%)	138 (22.9%)	
Length of ICU stay, h	93.0 (50.0-184.0)	109.0 (54.0-204.0)	0.003	118.5 (59.0-228.8)	109.0 (53.0-201.8)	0.128
Comorbidities, *n* (%)						
Atrial fibrillation	1783 (44.6%)	294 (48.1%)	0.107	281 (46.7%)	290 (48.2%)	0.603
AMI	301 (7.5%)	45 (7.4%)	0.882	39 (6.5%)	44 (7.3%)	0.570
VHD	402 (10.1%)	61 (10.0%)	0.952	88 (14.6%)	61 (10.1%)	0.018
Peripheral vascular	511 (12.8%)	66 (10.8%)	0.167	88 (14.6%)	64 (10.6%)	0.037
Pulmonary circulation	239 (6.0%)	53 (8.7%)	0.011	45 (7.5%)	52 (8.6%)	0.459
Pneumonia	1108 (27.7%)	242 (39.6%)	<0.001	235 (39.0%)	237 (39.4%)	0.906
COPD	188 (4.7%)	57 (9.3%)	<0.001	48 (8.0%)	53 (8.8%)	0.603
Liver diseases	214 (5.4%)	18 (2.9%)	0.011	20 (3.3%)	18 (3.0%)	0.742
Renal failure	982 (24.6%)	138 (22.6%)	0.284	162 (26.9%)	136 (22.6%)	0.083
Stroke	227 (5.7%)	23 (3.8%)	0.051	38 (6.3%)	23 (3.8%)	0.052
Hypothyroidism	461 (11.5%)	94 (15.4%)	0.007	97 (16.1%)	92 (15.3%)	0.692
Malignancy	209 (5.2%)	44 (7.2%)	0.047	31 (5.1%)	43 (7.1%)	0.150
Depression	303 (7.6%)	56 (9.2%)	0.175	50 (8.3%)	56 (9.3%)	0.542
Hypertension	830 (20.8%)	107 (17.5%)	0.062	126 (20.9%)	106 (17.6%)	0.144
Hyperlipemia	1120 (28.0%)	140 (22.9%)	0.008	133 (22.1%)	138 (22.9%)	0.730
Diabetes mellitus	1449 (36.3%)	208 (34.0%)	0.285	205 (34.1%)	205 (34.1%)	1.000
30-day mortality	769 (19.2%)	204 (33.4%)	<0.001	150 (24.9%)	200 (33.2%)	0.002
90-day mortality	1120 (28.0%)	260 (42.6%)	<0.001	211 (35.0%)	253 (42.0%)	0.013
Hospital-day mortality	659 (16.5%)	172 (28.2%)	<0.001	135 (22.4%)	167 (27.7%)	0.033

PSM, propensity score matching; HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; SpO2, percutaneous oxygen saturation; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BUN, blood urea nitrogen; PT, prothrombin time; APTT, activated partial thromboplastin time; ALT, alanine transaminase; cTnT, troponin T; NT-proBNP, N-terminal pro brain natriuretic peptide; CI, cardiac index; SII, Systemic immune-inflammation index; CHF, congestive heart failure; AMI, acute myocardial infarction; VHD, valvular heart disease; COPD, chronic obstructive pulmonary disease. CCU, cardiac care unit.

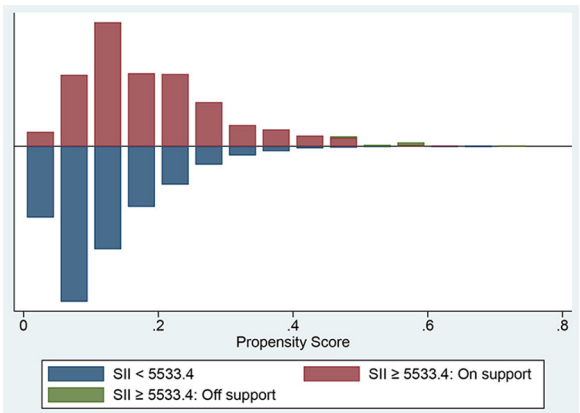

FIGURE 4 | The histogram presenting the range of propensity scores and the corresponding number of matches in high (red) and low (blue) SII groups. SII, systemic immune-inflammation index.

heart failure. Tumor necrosis factor α (TNF-α) was the first cytokine found to be significantly elevated in the serum of patients with heart failure, which could directly induce myocardial apoptosis and necrosis, bringing about ventricular adverse remodeling (19). The elevated levels of TNF-α often indicated impaired cardiac systolic function and poor long-term survival (20). In addition, interleukin 6 (IL-6), another classic cytokine mainly derived from monocytes, could be significantly increased in patients with left ventricular systolic dysfunction without clinical symptoms, and may be a sensitive indicator for the early diagnosis of heart failure (21). IL-6 was also positively correlated with the severity of heart failure, the concentration of which was negatively correlated with left ventricular ejection fraction and overall survival (22). Although these inflammatory molecules had good performance in the prognostic evaluation of patients, the expensive detection cost limited their clinical application. As a novel biomarker, SII can systematically and comprehensively reflect the status of inflammation *in vivo*. Blood routine is a routine examination for almost all hospitalized patients. It is simple, fast, and inexpensive. Compared with NT-proBNP, cytokines and other prognostic indicators, SII can effectively screen high-risk patients, guide the formulation of individualized treatment plans and improve the prognosis of patients without additional cost, with a broad application prospect, especially in underdeveloped areas.

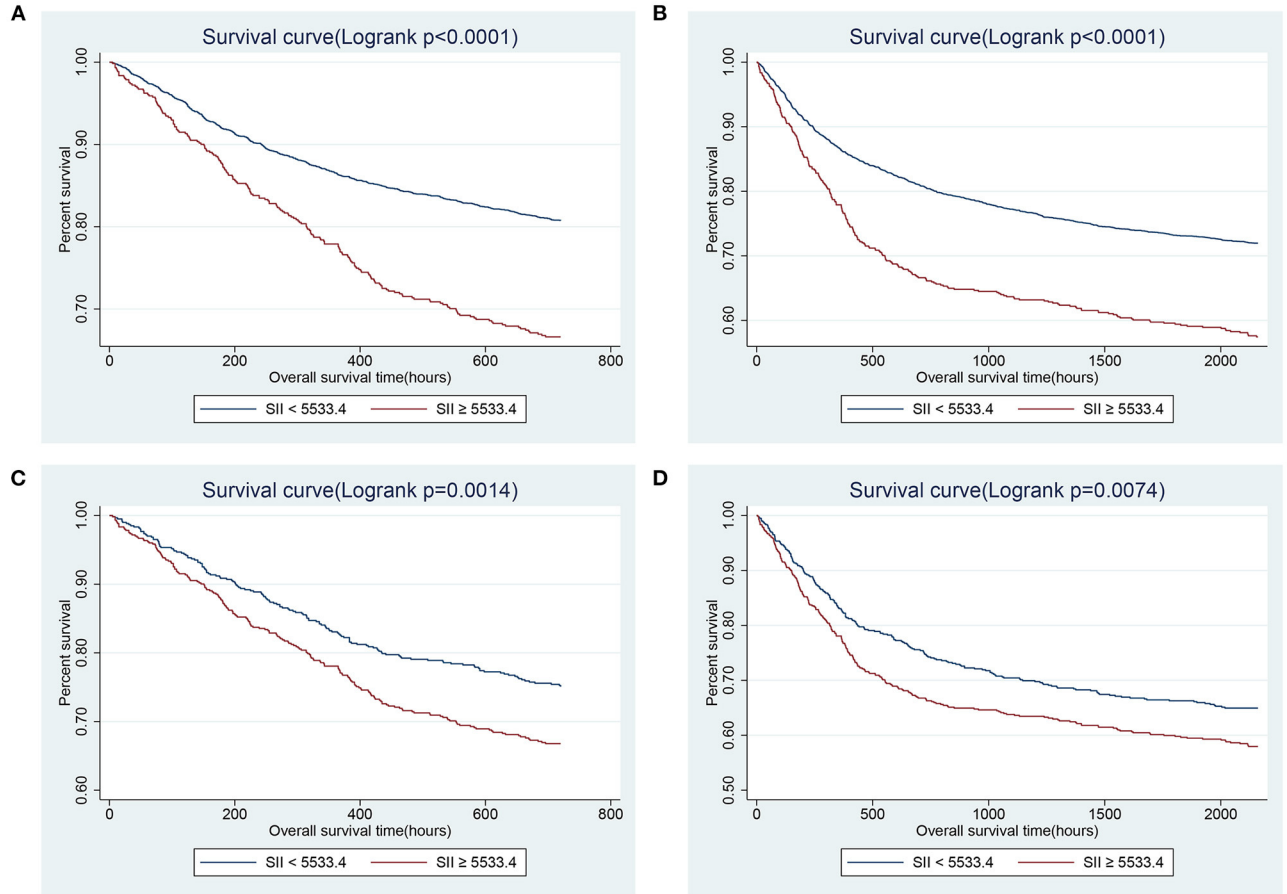

FIGURE 5 | Kaplan-Meier survival curves showing the 30- **(A)** and 90- **(B)** day all-cause mortalities stratified by cut-off value of SII in critically ill patients with CHF before PSM. **(C,D)** presented the correlation between SII and 30- and 90-day mortalities after PSM, separately. SII, systemic immune-inflammation index. CHF, congestive heart failure. PSM, propensity score matching.

The mechanisms of the relationship between SII and adverse prognosis of CHF remains unclear, and possible explanations are as follows. Recently, it has been revealed that circulating immune cells and their subtypes had a vital indicative effect on the prognosis of cardiovascular diseases (23). SII was a prognostic indicator that integrates three circulating immune cells, including neutrophils, lymphocytes, and platelets, the increase of which indicates either a relative increase in platelets and neutrophils counts or a relative decrease in lymphocytes. Neutrophils are the main component of white blood cells, accounting for 60-70% of the total, and play an important role in the non-specific immune system. In the inflammatory response, neutrophils react rapidly, with strong chemotaxis and phagocytosis, and participate in the progression of various cardiovascular diseases, including heart failure (24). In addition, neutrophils could contribute to oxidative stress and endothelial dysfunction by releasing a large amount of myeloperoxidase, NADPH oxidase, and so on (25). According to a community-based study (26), absolute neutrophils can effectively predict the increased incidence of acute decompensated heart failure after AMI, and it was also an independent risk factor for death (27). Lymphocytes mainly consist of two subtype including T and B cells, which were often associated with the specific immunity. Several previous studies have reported that lymphocyte counts in patients with heart failure were lower than in the normal population and lymphopenia served as an independent predictor of poor survival of patients with chronic and advanced heart failure (28, 29). Lymphopenia was considered to be an important feature of systemic inflammation, which may be caused by the following mechanisms. First, the circulating lymphocytes were attracted into the cardiac tissue, leading to its redistribution. Then, lymphopenia was related to the activation of renin-angiotensin-aldosterone hormone and the adrenergic nervous system, which could exert pro-apoptotic effects on lymphocytes (28). Platelets were differentiated from the mononuclear-phagocyte system and were the key mediator linking the two pathological processes of inflammation and thrombosis (30). On the one hand, platelets were the main effector molecules for hemostasis after cardiovascular injury. On the other hand, the activation of platelets plays a major role in pathogenic thrombosis and participates in the pathogenesis of many groups of cardiovascular diseases, including coronary heart

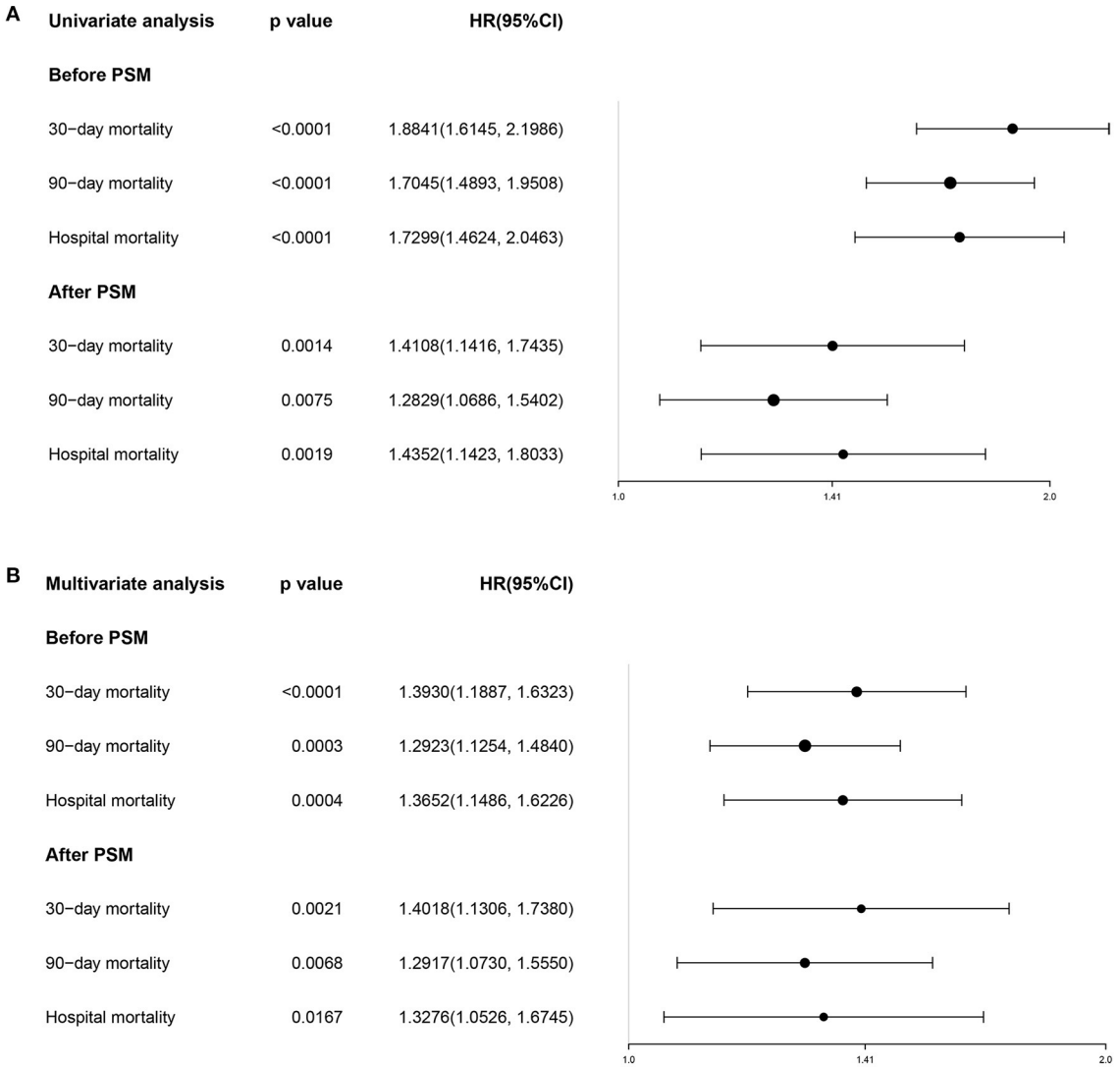

FIGURE 6 | The forest plot showing the univariate **(A)** and multivariate **(B)** Cox regression for effects of SII on short-term mortality in the critically ill patients with CHF before and after PSM. SII, systemic immune-inflammation index. CHF, congestive heart failure. PSM, propensity score matching.

disease (31). Moreover, Platelets interacted with leukocytes and their subtypes (neutrophil, lymphocytes, etc.) and endothelial cells and activated them, inducing monocyte adhesion and transport, releasing inflammatory factors such as TNF-α and IL-1, which together promoted local inflammation and fibrosis in heart failure (31). The study by Kandis et al. (32) demonstrated that mean platelet volume (MPV), indicating the activation of platelets, increased significantly in decompensated heart failure patients. In addition, MPV at admission was independently associated with the hospital and 6-month mortalities.

Besides, the subgroup analysis was also conducted, and the results demonstrated that the association was stable and consistent between the high level of SII and the poor 30-day mortality across CHF patients with different comorbidities or severity scores. We also noted that, among the critically ill patients with CHF, patients complicated with AMI and hyperlipidemia had a higher risk of 30-day mortality, and this risk was higher for higher SII, which implied that SII may be more valuable for the prognostic evaluation of CHF patients with AMI or hyperlipidemia. Similarly, the previous study has also shown that in patients with coronary heart disease, the high level of SII indicated a high risk of cardiac death in the future, as well as the high incidence of non-fatal stroke and heart failure (9). Although there was no research on the correlation between SII and hyperlipidemia, the interconnection of hyperlipidemia and inflammation involved in the pathogenesis of cardiovascular disease has been recognized (33).

As a retrospective cohort study, the existence of confounding factors cannot be ignored, and it was difficult to intuitively and accurately judge the correlation between SII level and the prognosis of CHF patients. Another highlight of this study was

the use of PSM analysis to reduce the imbalance of confounding factors across different groups. After PSM, the most baseline characteristics were well-balanced between these two groups, except for the use of dialysis, the type of first ICU admission, and the peripheral vascular diseases. More importantly, the conclusion that high levels of SII were independently related to high short-term mortality remained stand before and after PSM, which improved the reliability of SII as a prognostic marker of CHF.

But at the same time, there were certain limitations in this study. First, the present study was conducted at a single center and did not validate the prognostic value of SII in a validation cohort. Second, the clinical data were collected retrospectively from databases and it was difficult to ensure that variables were evenly distributed across groups, although multiple regression models were conducted to adjust for confounders and PSM analysis was used to minimize inter-group differences in baseline characteristics. Third, some significant variables may be omissive due to the lack of data. Last, this study was unable to determine the underlying mechanism of the association between high SII and poor prognosis of CHF patients, and further experiments are necessary.

CONCLUSION

In summary, the high level of SII is closely related to the poor short-term prognosis in critically ill patients with CHF, including 30- and 90-day and hospital all-cause mortalities, as well as the occurrence of MACEs, and is expected to be a simple and effective prognostic evaluation indicator.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at: https://mimic.mit.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Boards of Massachusetts Institute of Technology (Cambridge, MA, USA) and Beth Israel Deaconess Medical Center (Boston, MA, USA). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZY and LZ conceived and designed the study. YT and XZ collected and analyzed the clinical data. YT drafted the manuscript. YF and QC participated in the implementation of statistical methods in this study and put forward constructive suggestions. ZL and HL reviewed the study and participated in the interpretation of the results. All authors gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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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.2021.753133/full#supplementary-material

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Validity of the ESC Risk Assessment in Idiopathic Pulmonary Arterial Hypertension in China

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Background: The 2015 European pulmonary hypertension (PH) guidelines recommend a risk stratification strategy for pulmonary arterial hypertension (PAH). We aimed to investigate the validation and potential prognostic information in Chinese patients.

Methods: The risk assessment variables proposed by the PH guidelines were performed by using the WHO function class, 6-min walking distance, brain natriuretic peptide or its N-terminal fragment, right arterial pressure, cardiac index, mixed venous saturation, right atrium area, pericardial effusion, peak oxygen consumption, and ventilatory equivalents for carbon dioxide. An abbreviated version also was applied.

Results: A total of 392 patients with idiopathic PAH (IPAH) were enrolled between 2009 and 2018. After a median interval of 13 months, re-evaluation assessments were available for 386 subjects. The PAH guidelines risk tool may effectively discriminate three risk groups and mortality ($p < 0.001$) both at the baseline and re-evaluation. Meanwhile, its simplified risk version was valid for baseline and accurately predicted the risk of death in all the risk groups ($p < 0.001$). At the time of re-evaluation, the percentage of low-risk group has an increase, but a greater proportion achieved the high-risk group and a lesser proportion maintained in the intermediate-risk group.

Conclusion: The 2015 European PH guidelines and its simplified version risk stratification assessment present an effective discrimination of different risk groups and accurate mortality estimates in Chinese patients with IPAH. Changes of risk proportion at re-evaluation implicated that natural treatment decisions may not be consistently with goal-oriented treatment strategy.

Keywords: pulmonary arterial hypertension, idiopathic pulmonary arterial hypertension, risk assessment, guideline, prognosis

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INTRODUCTION

The assessment of the prognosis of patients has been considered as an important section in patients with pulmonary arterial hypertension (PAH); different baseline and follow-up variables have been utilized individually or combined to predict outcome. Up to date, the 2015 European Society of Cardiology (ESC)/European Respiratory Society (ERS) pulmonary hypertension (PH) guidelines proceedings summarized risk stratification strategy advances (1), each focusing on

different countries or registries, including the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL) studies (2, 3), the Swedish PAH Registry (4), the Comparative, Prospective Registry of Newly Initiated Therapies for Pulmonary Hypertension (COMPERA) Registry (5), and the French PH Network (FPHN) (6). The updated analysis of risk stratification recommended a flexible and comprehensive approach by using the clinical features such as right ventricular function, hemodynamic parameters, biomarkers, and exercise. Based on the cutoff values gathered from the 2015 ESC/ERS guidelines, three risk categories were defined as a low-, intermediate-, or high-risk group (1).

The accuracy of this risk assessment strategy has been validated by the COMPERA Registry and mortality rate was significantly different between the three risk strata in baseline and follow-up (5). However, the COMPERA study used an abbreviated version risk analysis including six variables such as the WHO function class (FC), 6-min walk distance (6MWD), brain natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP), right arterial pressure (RAP), cardiac index (CI), and mixed venous oxygen saturation (S_VO_2), not capturing disease progression, syncope, echocardiography, and cardiopulmonary exercise testing (CPET) data. These findings confirm and extend previous study by Kylhammar et al. (4), who used the same subset of parameters [plus right atrial area and the presence/absence of pericardial effusion (PE)]. Although simplified variables could discriminate the risk groups, the most reliable dataset from echocardiography and CPET needed to determine (1).

TABLE 1 | Variables and cutoff values from the risk assessment from the ESC/ERS 2015 guidelines*.

	Low risk	Intermediate risk	High risk
WHO FC	I, II	III	IV
6MWD, meter	>440	165–440	<165
BNP, ng/L	<50	50–300	>300
NT-proBNP, ng/L	<300	300–1,400	>1,400
Hemodynamics			
RAP, mmHg	<8	8–14	>14
CI, L/min/m^2	≥2.5	2.0–2.4	≤2.0
S_VO_2, %	>65	60–65	<60
Imaging (echocardiography)			
RA area, cm^2	<18	18–26	>26
Pericardial effusion	No	No or minimal	Yes
Cardio-pulmonary exercise testing			
Peak VO_2, mL/min/kg	>15 (>65% pred.)	11–15 (35–65% pred.)	<11 (<35% pred.)
VE/VCO_2 slope	<36	36–44.9	≥45

Simplified version included the WHO FC, 6MWD, NT-proBNP, BNP, RAP, CI, and S_VO_2%. BNP, brain natriuretic peptide; CI, cardiac index; ESC, European Society of Cardiology; ERS, European Respiratory Society; 6MWD, 6-minute walk distance; NT-proBNP, N-terminal pro-BNP; RA, right atrium; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; VE/VCO_2, ventilatory equivalent for carbon dioxide; VO_2, oxygen consumption; FC, function class.

Patients with PAH usually show a typical pattern with low peak oxygen uptake [peak oxygen consumption (VO_2)], providing prognostic information and therapeutic decision-making (1, 7). Echocardiography remained an important determinant as right ventricular function was key prognostic variables (8, 9). Similarly, the risk assessment of the French PH Network proposed by the European PH guidelines in patients with idiopathic PAH (IPAH) was available to work at baseline and follow-up (6). In fact, the risk stratification tool itself has a level of evidence C; also, the cutoff points are derived from several studies (1). However, it is unknown to validate the efficiency of this instrument in a real-world cohort in specific treatment era, especially in China.

The principle aim of this study was to apply the risk assessment from the 2015 ESC/ERS guidelines to a newly diagnosis cohort of patients with IPAH in China. We attempted to test the discrimination of the risk instrument presented in guidelines and to explore the potential prognostic changes at follow-up.

MATERIALS AND METHODS

Study Patients

All the newly diagnosed patients with IPAH (≥18 years of age at diagnosis) were retrospectively reviewed in the Shanghai Pulmonary Hospital between January 2009 and September 2018. IPAH at baseline was set by right heart catheterization (RHC) according to standard criteria: a mean pulmonary artery pressure (mPAP) ≥25 mm Hg and pulmonary vascular resistance (PVR) >3 Wood units at rest in the presence of a normal pulmonary artery wedge pressure (PAWP) ≤15 mm Hg (1). Patients were excluded if they have definite causes for PAH such as connective tissue disease and congenital heart disease, those with portopulmonary hypertension, chronic pulmonary thromboembolism, and pulmonary hypertension due to left heart diseases and lung diseases and/or hypoxemia. Major endpoint was defined as all-cause mortality and no patients received lung or heart–lung transplantation. This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Shanghai Pulmonary Hospital Ethics Committee (K19-054). Written informed consent was obtained from all the participants.

Risk Stratification

Risk assessment was performed according to the 2015 ESC/ERS PH guidelines and patients were categorized as "low risk," "intermediate risk," or "high risk" in **Table 1** (5). An abbreviated version of this guideline risk stratification strategy used the WHO FC, 6MWD, BNP or NT-proBNP, RAP, CI, and S_VO_2. The cutoff values proposed in the guidelines were graded as 1, 2, and 3 (1 = low risk, 2 = intermediate risk, and 3 = high risk). When the baseline 6-MWD did not detect, it was considered as a grade 3 (4). For each patient, the sum of all the grades was divided by the number of available variables. The mean grade was rounded to the next integer to define the risk group. For the follow-up risk stratification,

TABLE 2 | Characteristics of patients with IPAH in baseline risk stratification.

	N	Low risk	Intermediate risk	High risk	All
Subjects, *n* (%)		96 (25)	267 (68)	29 (7)	392
Age, years		35 ± 14	42 ± 16	39 ± 16	40 ± 16
Female, *n* (%)		70 (73)	180 (67)	14 (48)	264(67)
BMI, kg/m^2		23 ± 6	22 ± 4	23 ± 3	23 ± 4
WHO FC, *n* (%)					
Class I–II		69 (72)	63 (24)	0 (0)	132 (34)
Class III		27 (28)	188 (70)	19 (66)	234 (60)
Class IV		0 (0)	16 (6)	10 (35)	26 (7)
6MWD, meters	392	436 ± 97	364 ± 100	280 ± 95	379 ± 107
BNP, ng/L	164	46 (24, 94)	262 (149, 438)	661 (306, 880)	211 (65, 426)
NT-proBNP, ng/L	250	162 (40, 267)	1096 (542, 1892)	2428 (1949, 3771)	748 (255, 1679)
Hemodynamics					
RAP, mmHg	389	4 (2, 7)	6 (4, 10)	15 (12, 17)	6 (3, 10)
mPAP, mmHg	392	53 (45, 63)	58 (50, 69)	63 (56, 80)	58 (48, 68)
PAWP, mmHg	392	7 (6, 10)	8 (5, 10)	10 (7, 11)	8 (5, 10)
CI, L/min/m^2	388	3.2 (2.8, 3.7)	2.2 (1.9, 2.7)	1.6 (1.5, 1.8)	2.4 (1.9, 3.0)
PVR, Wood units	392	9 (7, 12)	15 (11, 18)	20 (16, 26)	14 (9, 18)
S_VO_2, %	388	72 (68, 76)	60 (56, 65)	46 (42, 52)	62 (56, 69)
Echocardiographic variables					
RA area, cm^2	235	16 (13, 20)	23 (18, 33)	38 (29, 47)	22 (16, 30)
No PE, *n* (%)		90 (94)	163 (61)	7 (24)	260 (66)
Minimal PE, *n* (%)		1 (1)	73 (27)	15 (52)	89 (25)
PE, *n* (%)		0 (0)	5 (2)	3 (10)	8 (2)
Cardiopulmonary exercise testing					
Peak VO_2, mL/min/kg	100	18 ± 4	12 ± 3	9 ± 2	14 ± 4
VE/VCO_2 slope	100	36 ± 7	62 ± 36	79 ± 22	56 ± 33
Initial therapies (within 3 months after diagnosis), *n* (%)					
No specific/CCB therapy		13 (14)	20 (8)	0 (0)	33 (8)
Monotherapy		68 (71)	169 (63)	21 (72)	258 (66)
Combination therapy		15 (16)	78 (29)	8 (28)	101 (26)

Values are expressed as mean ± SD, medians (interquartile range), or n (%), unless otherwise stated.
BMI, body mass index; BNP, brain natriuretic peptide; CCB, calcium channel blocker; CI, cardiac index; mPAP, mean pulmonary arterial pressure; 6MWD, 6-minute walk distance; NT-proBNP, N-terminal pro-BNP; PAWP, pulmonary artery wedge pressure; PE, pericardial effusion; PVR, pulmonary vascular resistance; RA, right atrium; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; VE/VCO_2, ventilatory equivalents for carbon dioxide; VO_2, oxygen consumption; FC, function class; IPAH, idiopathic pulmonary arterial hypertension.

we chose the visit that included follow-up hemodynamics after the baseline risk assessment at least 3 months. If no hemodynamic follow-up was available, we selected the follow-up visit that contained most of the data such as echocardiography or CPET. Variables listed in the guidelines that are not captured both at the baseline and follow-up are disease progression and syncope.

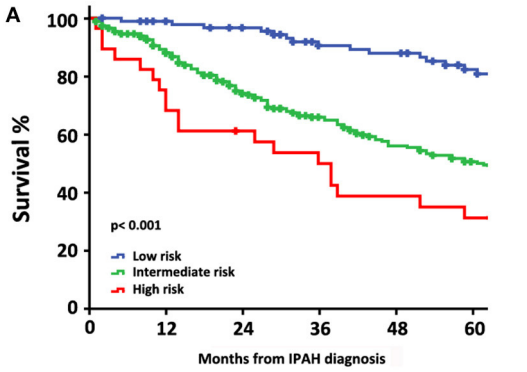
Statistical Analysis

Continuous variables are expressed as mean ± SD or medians with corresponding 25th and 75th percentiles [interquartile range (IQR)]. Categorical variables are expressed as numbers and percentages. When data were not normally distributed, a non-parametric test was used. Changes between baseline and re-evaluation were assessed by using the chi-squared test where appropriate. Survival analyses were performed by using the Kaplan–Meier method, truncated at 5 years, and were compared by using the log-rank test. Survival time was calculated from the date of diagnostic RHC to the date of final follow-up and re-evaluation to final follow-up. Survival was compared for patients who were remained in the low-, intermediate-, or high-risk group, respectively, improved to the low- or intermediate-risk group, or worsened to the intermediate- or high-risk group. The univariate and multivariate Cox proportional hazards regression analysis was performed to assess the risk of death by using the respective low-risk group as reference. Patients were censored at termination December 31, 2018. A p-value <0.05 was considered as statistically significant. All the analyses were performed by using the Statistical Package for the Social Sciences (SPSS) version 14.0 statistical software package (SPSS, Chicago, Illinois, USA).

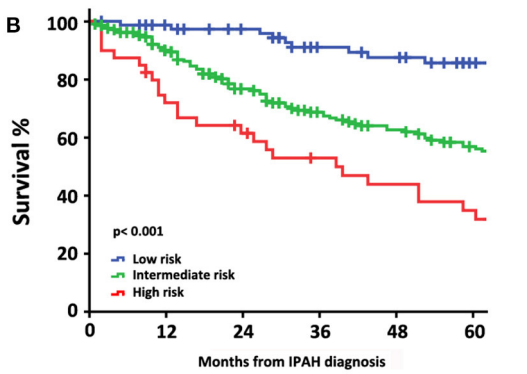
RESULTS

Study Patients

The baseline data reviewed total newly 392 patients with IPAH who fulfilled the criteria including 11 variables of interest for



Months after enrolment	Patients at risk, n			Survival, %		
	Low risk	Intermediate risk	High risk	Low risk	Intermediate risk	High risk
0	96	267	29	100	100	100
12	95	219	21	99	88	69
24	90	168	19	98	75	62
36	73	138	15	91	67	51
48	70	110	12	88	57	40
60	58	96	10	83	52	33



Months after enrolment	Patients at risk, n			Survival, %		
	Low risk	Intermediate risk	High risk	Low risk	Intermediate risk	High risk
0	76	207	39	100	100	100
12	75	170	28	99	90	72
24	70	130	23	97	76	61
36	56	104	19	91	68	52
48	49	88	15	87	62	43
60	46	74	12	85	56	34

FIGURE 1 | The survival estimates in patients with idiopathic pulmonary arterial hypertension at baseline according to **(A)** the 2015 European Society of Cardiology/European Respiratory Society (ESC/ERS) risk stratification strategy; **(B)** a simplified version.

this study (**Table 1**). Out of the 10 variables at baseline, at least 4 variables were available in all the 392 patients, at least 10 in 59 (15%) patients and at least 8 in 177 (45%) patients. All the patients underwent the RHC examination. The characteristics of these patients in baseline were shown in **Table 2**. At the time of diagnosis, most patients (67%) were women and mean age was 40 years old. A total of 260 (67%) patients were in the WHO FC III or IV, whereas 34% patients were in the WHO FC I-II. All cause death survival for the overall study patients ($n = 392$) was shown in **Supplementary Figure 1**. For the initial treatment, 258 (66%) patients received monotherapy, 101 (26%) patients received combination therapy, and 33 (8%) patients received no specific/calcium channel blocker (CCB) therapy. At baseline, 186 (48%) patients used phospodiesterase 5 inhibitor (PDE5i) treatment in monotherapy group and 68 (17%) patients used PDE5i plus endothelin receptor antagonist (ERA) (**Supplementary Table 1**).

Risk Assessment at Baseline and Mortality

At the time of diagnosis, 96 (25%) of patients were in the low-risk group, 267 (68%) of patients were in the intermediate-risk group, and 29 (7%) of patients were in the high-risk group, respectively (**Table 2**). After the diagnosis of IPAH within 5 years, 141 (36%) patients had died, 19 (20%) patients were in the low-risk group, 104 (39%) patients were in the intermediate-risk group, and 18 (62%) patients were in the high-risk group. In the low-risk group, the survival rate at 1-, 2-, 3-, 4-, and 5-year was 99, 98, 91, 88, and 83%, respectively. The corresponding survival was 88, 75, 67, 57, and 52% in the intermediate-risk group, respectively, and 69, 62, 51, 40, and 33% in the high-risk group, respectively ($p < 0.001$ for all the group comparisons). The predictive values of each variable at baseline are shown in **Figure 1A**.

Similarly, by using simplified version, all the six variables were available in 322 patients, 76 (24%) of patients were in the low-risk group, 207 (64%) of patients were in the intermediate-risk group, and 39 (12%) of patients were in the high-risk group, respectively (**Supplementary Table 2**). Accordingly, the survival differences in the three risk categories were still statistical significant ($p <$ 0.001 for all the group comparisons; **Figure 1B**). The predictive values of each variable in those patients at baseline are shown in **Figure 2**.

Risk Assessment at Re-evaluation and Mortality

At the end of follow-up, among the 106 patients with missing re-evaluation information, 24 (6%) patients died, 57 (15%) patients were below two variables, and 25 (6%) patients lost to follow-up for other specified reasons (**Supplementary Figure 2**). For re-evaluation assessment, out of the 10 variables, at least 2 variables were available in 286 patients, at least 8 variables were available in 11 (4%) patients, and at least 4 variables were available in 210 (73%) patients, respectively. Median interval between diagnosis and re-evaluation was 13 [5, 33] months. At the time of re-evaluation, 85 (30%) patients were in the low-risk group, 159 (56%) patients were in the intermediate-risk group, and 42 (15%) patients were in the high-risk group, respectively. There were increased 5% patients attaining high-risk group and decreased 12% patients with intermediate-risk group, although the percentage of low-risk group has an increased 8% ($p < 0.001$, **Figure 3**). Only 46 (16%) of these re-evaluation patients were available for hemodynamic data; however, 186 (65%) of these re-evaluation patients were available for right area, 253 (88%) of these re-evaluation patients were available for PE, and 88 (31) of these re-evaluation patients were available for CPET.

The characteristics of these patients in re-evaluation were shown in **Table 3**. At re-evaluation, 147 (51%) patients received monotherapy, 126 (44%) patients received combination therapy,

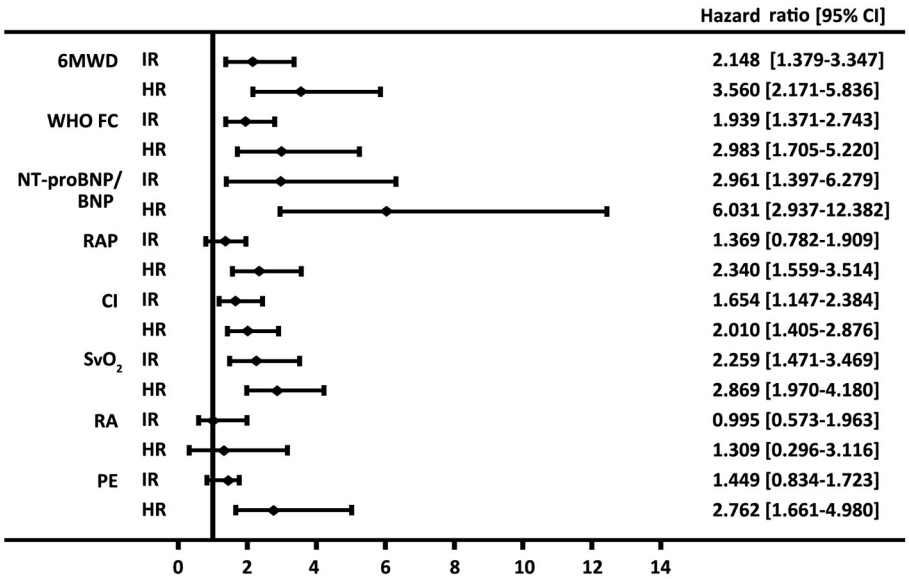


FIGURE 2 | Forest plot based on the prognostic values of 6-min walk distance (6MWD), the WHO function class (FC), brain natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP), right arterial pressure (RAP), cardiac index (CI) and mixed venous oxygen saturation (S_VO_2), right atrium (RA) area, and pericardial effusion (PE) in the intermediate-risk (IR) and high-risk (HR) groups. Values for the variables were obtained from baseline. The reference value is from the respective low-risk group.

and 13 (4%) patients received no specific/CCB therapy. Within combination therapy group, 102 (36%) patients used PDE5i plus. Compared with those variables at baseline, there was significant improvement in 6MWD, CI, PVR, S_VO_2, and proportion of combination therapy (**Supplementary Table 3**). After re-evaluation of these patients within 5 years, 36 (13%) patients had died, 4 (8%) patients were in the low-risk group, 16 (10%) patients were in the intermediate-risk group, 7 (17%) patients were in the high-risk group, and 9 (3%) patients in censor. In the low-risk group, the survival rate at 1-, 2-, 3-, 4-, and 5-year was 94, 91, 89, 82, and 76%, respectively. The corresponding survival was 75, 66, 54, 46, and 38% in the intermediate-risk group, respectively, and 53, 28, 25, 21, and 18% in the high-risk group, respectively ($p < 0.001$ for all the group comparisons; **Figure 4**). The predictive values of each variable from the multivariate Cox proportional hazards regression analysis at re-evaluation are shown in **Supplementary Figure 3**. The WHO FC, 6MWD, NT-proBNP/BNP, and SVO_2 were independent predictors. From baseline to re-evaluation, the changes in the risk assessment were associated with a shift in the mortality risk ($p < 0.001$ for all the group comparisons; **Figure 5**).

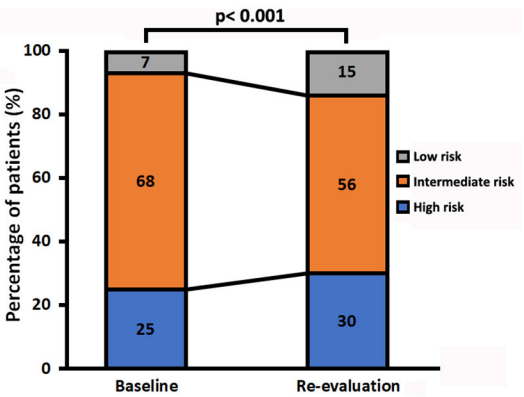


FIGURE 3 | Change in the three risk groups in patients with idiopathic pulmonary arterial hypertension from baseline to re-evaluation.

DISCUSSION

There was much evidence to support that the multiparametric approach stratified the patients with PAH in different risk groups for mortality. According to the risk status, different strategies can be utilized to guide therapeutic decisions (8). However, the validation of these comprehensive risk assessments for Chinese patients with IPAH is unclear. Among the above three registries, IPAH was a major etiology of PAH, such as 77% in the FPHN, 67% in the COMPERA Registry, and 51% in the Swedish PAH Registry, which indicated that IPAH was a special type and provided available strategy (4–6). The main findings of this study can be demonstrated as follows: (1) the 2015 European PH guidelines risk stratification effectively discriminated a low, intermediate, and high risk at baseline and re-evaluation assessments; (2) accurately predicted the risk of death in patients with IPAH; (3) its simplified version risk strategy was valid for baseline; and (4) the percentage of the low-risk group has an increase at re-evaluation, but a greater proportion of patients achieved the high-risk group and a lesser proportion maintained in the intermediate-risk group. Despite of

TABLE 3 | Characteristics of patients with IPAH in re-evaluation risk stratification.

	N	Low risk	Intermediate risk	High risk	All
Subjects, *n* (%)		85 (30)	159 (56)	42 (15)	286
WHO FC, *n* (%)					
Class I–II		48 (56)	28 (18)	0 (0)	76 (27)
Class III		8 (9)	94 (59)	20 (48)	122 (43)
Class IV		0 (0)	6 (4)	19 (45)	25 (9)
6MWD, meters	135	472 ± 69	370 ± 106	204 ± 154	396 ± 120
BNP, ng/L	43	25 (13, 42)	297 (150, 453)	726 (385, 874)	184 (64, 453)
NT-proBNP, ng/L	224	74 (40, 142)	1,160 (541, 2,403)	1,691 (1,444, 2,844)	806 (146, 2,326)
Hemodynamics					
RAP, mmHg	46	5 (3, 7)	10 (6, 12)	12 (5, 14)	6 (4, 11)
mPAP, mmHg	46	43 (34, 52)	64 (57, 70)	78 (68, 84)	57 (40, 65)
PAWP, mmHg	46	9 (7, 11)	10 (6, 11)	10 (8, 14)	10 (7, 11)
CI, L/min/m^2	46	3.5 (3.2, 4.6)	2.3 (1.9, 2.6)	1.9 (1.6, 2.0)	2.6 (2.2, 3.4)
PVR, Wood units	46	5 (4, 8)	15 (12, 20)	21 (17, 22)	10 (5, 15)
S_VO_2, %	46	74 (71, 78)	60 (52, 63)	50 (41, 58)	65 (58, 74)
Echocardiographic variables					
RA area, cm^2	186	15 (12, 18)	22 (18, 29)	45 (37, 52)	23 (17, 34)
No PE, *n* (%)		74 (87)	91 (57)	19 (45)	170 (67)
Minimal PE, *n* (%)		2 (2)	45 (28)	12 (29)	73 (29)
PE, *n* (%)		0 (0)	3 (2)	7 (17)	10 (4)
Cardiopulmonary exercise testing					
Peak VO_2, mL/min/kg	88	17 ± 3	12 ± 3	9 ± 1	13 ± 4
VE/VCO_2 slope	88	35 ± 5	56 ± 25	81 ± 48	52 ± 28
Therapies (within 3 months after re-evaluation), *n* (%)					
No specific/CCB therapy		5 (6)	7 (4)	1 (2)	13 (4)
Monotherapy		54 (64)	77 (48)	16 (38)	147 (51)
Combination therapy		26 (31)	75 (47)	25 (60)	126 (44)

Values are expressed as mean ± SD, medians (interquartile range), or n (%), unless otherwise stated.
BMI, body mass index; BNP, brain natriuretic peptide; CCB, calcium channel blocker; CI, cardiac index; mPAP, mean pulmonary arterial pressure; 6MWD, 6-minute walk distance; NT-proBNP: N-terminal pro-BNP; PAWP, pulmonary artery wedge pressure; PE, pericardial effusion; PVR, pulmonary vascular resistance; RA, right atrium; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; VE/VCO_2, ventilatory equivalents for carbon dioxide; VO_2, oxygen consumption; FC, function class.

the methodical risk assessments that are applicable for Chinese patients with IPAH, actual treatment seems not consistent with this goal-oriented treatment strategy.

A comprehensive assessment is used, since no single variable provides sufficient diagnostic and prognostic information. As we known, the 2015 ESC/ERS guidelines recommended 13 variables and the REVEAL risk score consisted of 19 variables (1, 2). The number of variables seems possible to discriminate risk groups accurately, but not all the variables may be done in PH centers. Except for the progression and syncope of the symptom, the variables selected in this study included all the RHC parameters, BNP or NT-proBNP, and 6WMD at baseline. Meanwhile, right atrium (RA) area was available in 235 (60%) patients, PE was available in 357 (91%) patients, and CPET was available in 100 (26%) patients (**Tables 2**, **3**). It was significantly discrimination of different risk groups, i.e., 25% patients were in the low-risk group, 68% patients were in the intermediate-risk group, and 7% patients were in the high-risk group, respectively. If we used the simplified version of risk criteria, the proportion of the high-risk group was increased to 12% (**Supplementary Table 2**). Our results were closed to previous findings by the Swedish PH Registry, which the proportion of low-, intermediate-, and high-risk patients, respectively, was 23, 67, and 10% (530 patients with PAH and 49% patients with IPAH) (4). However, in the COMPERA IPAH subgroup study, the proportion of the high-risk group increased to 19% (5). The reasons for differences are partly attributed to different variables used for risk assessment or severity of different parameters. For example, 6MWD was 299 ± 123 m in the COMPERA IPAH subgroup, but 369 ± 107 m in this study. Our previous study has been reported that 6-min walk test values in Chinese patients with IPAH were significantly higher than those recorded in foreign registries (3, 10–12). Hence, it is necessary to discuss the feasibility of statistical risk calculation method. Given that echocardiography and CPET were not available for all the studies, most reliable indicators needed to further determine.

Regardless of whether regular follow-up, the three risk groups had significantly different long-term survival at baseline and in re-evaluation. It suggested that 13 variables of the 2015 ESC/ERS guidelines were relatively stable to discriminate risk stratification.

However, we still found that an increased proportion of the high-risk group and a lesser proportion of patients with the intermediate-risk group, although the percentage of the low-risk group has an increase (**Figure 3**). The changes in risk category reflected that the patients in the low-risk group may be benefit from initial treatment, but those in the intermediate- and high-risk groups seemed not be sufficient. In this study, primary combinations of PAH-targeted drugs were observed in 26% of all the patients and in 28% of the high-risk patients, which implicated that combination treatment goal was not achieved in majority of our patients. Ample evidence was proposed to use of initial monotherapy or combination therapies in patients with naïve PAH (13–19). Initial combination therapy could improve exercise capacity and prognosis compared with initial monotherapy (14, 17). It is intelligible that the treatment goals are not always realistic and physicians may modify the therapeutic strategies with advanced disease or severe comorbidities. Certainly, the improved survival rates may be attributable to success of specific treatment and the increasing economic burden for patients cannot be ignored (10, 19). Even at the time of re-evaluation, over 50% patients are still in monotherapy or no specific therapies condition after all.

Of note, the parameters of echocardiography and CPET were used for risk stratification in this study including RA area, PE, peak VO_2, and ventilatory equivalents for carbon dioxide (VE/VCO_2) slope proposed by the 2015 PH guidelines. Presence of PE is common and thought to be an important indicator for right heart failure in patients with PAH (20, 21). Fenstad et al. reported that even modest degrees of pericardial fluid were associated with a significant increase in mortality in patients with PAH (22). In this study, we also found the degree of severe PE in the high-risk group that may be overestimated (**Table 3**, **Figure 3**). A preserved RA function is crucial to maintain sufficient right heart function, partly since the change of RA size alters the motion of the tricuspid annulus (23). Accordingly, impaired right ventricle systolic function and RA dilation (RA area > 18 cm^2) were associated with worse long-term survival in patients with IPAH (24, 25). Grapsa et al. have reported that clinical deterioration was better associated with RA rather than RV remodeling in patients with PAH (26). There was no difference of RA area between baseline and re-evaluation (median was 22 cm^2 at baseline and 23 cm^2 in re-evaluation). Our data suggested that the parameters of PE and RA area were useful information for the risk stratification strategy.

Cardiopulmonary exercise testing may provide suggestive information in patients with PAH both at the circulation impairment and ventilatory inefficiency (27). Lower peak VO_2 and higher VE/VCO_2 slope were considered to establish the severity of exercise capacities or to assess outcomes (28–30). Wensel et al. have reported that average peak VO_2 and VE/VCO_2 slope during exercise were 11.2 ± 0.5 ml/min/kg and 54 ± 2 (2002) (31) and 13 ± 5 ml/min/kg and 54 ± 18 (2013) (30), respectively; also, patients with peak $VO_2 \leq 10.4$ ml/min/kg had poor survival. Our data showed that peak VO_2 was 14 ± 4 ml/min/kg and VE/VCO_2 slope was 54 ± 2 at baseline. Reference to the criteria of the 2015 ESC PH guidelines, the value of peak VO_2 was arrived to the intermediate-risk group and VE/VCO_2 slope was arrived to the high-risk group in this study. However, the two variables of CPET were all removed from the Cox multivariate model equation (at last step). This does not mean that CPET *per se* is not relevant, but that exercise function might not be superior to resting hemodynamics or echocardiography in this study. Although CPET is not widely utilized in patients with PAH, an increasing recognition of potential values should be emphasized (32). A total of 26% (100/392) patients of this study have CPET values, but further studies need still more valuable information to evaluate comprehensive score system for risk.

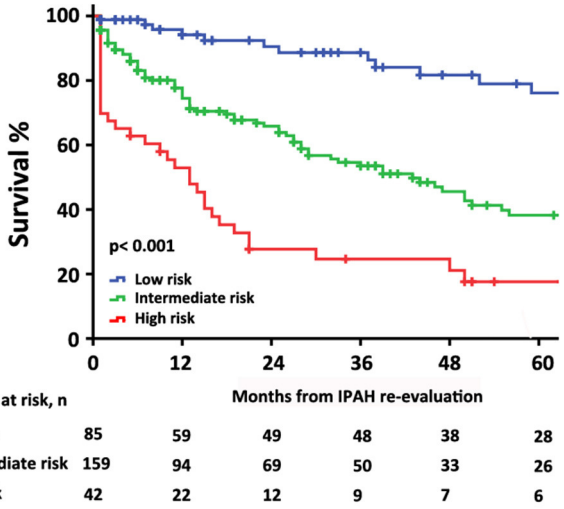
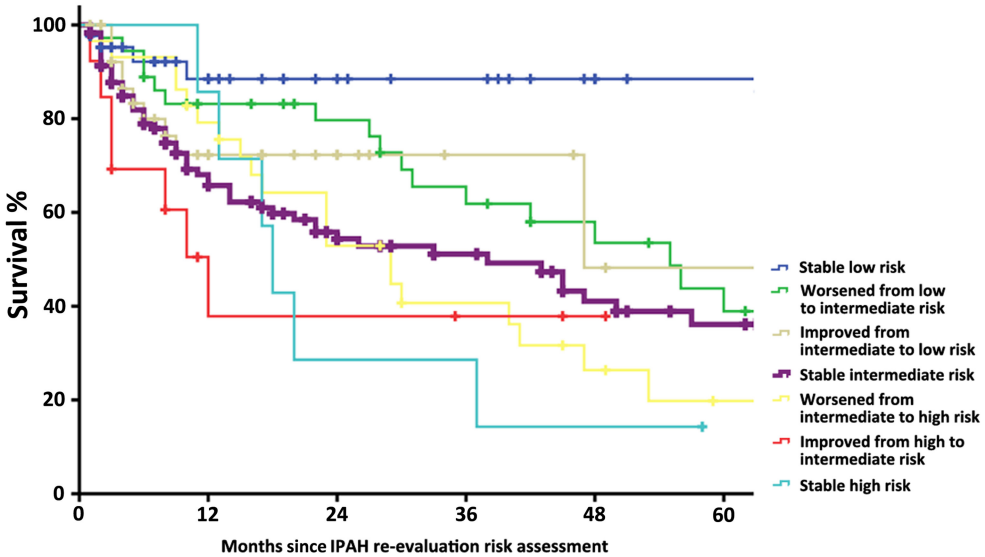


FIGURE 4 | Survival estimates in patients with idiopathic pulmonary arterial hypertension at re-evaluation according to the 2015 ESC/ERS risk stratification strategy.

Study Limitations

The major strengths of this study were the availability of complete data for invasive hemodynamics and non-invasive echocardiography and CPET variables at diagnosis in patients with IPAH. There are several limitations in this study. First, this is a retrospective study in a single center and the sample size was not large enough to provide sufficient numbers of the patients in three risk stratifications. Second, the follow-up assessments were not standardized and the proportion of RHC testing was lower at re-evaluation. However, we selected the follow-up visit that contained most of the data such as echocardiography or CPET. So, 88% patients at re-evaluation assessments had values of PE, 65% patients at re-evaluation assessments had values of RA area, and ∼30% patients at re-evaluation assessments had values of CPET. Additionally, despite of median interval between diagnosis and re-evaluation was 13 months, ∼15% of that was over 24 months, which may be biased toward the time-effect test. Finally, this study does not include prognostic variables, such as age, sex, comorbidities, disease progression, and syncope, and the individual risk is further modified by these factors. Further studies should



Patients at risk, n							
Months after enrolment	Stable low risk	Worsened from low to intermediate risk	Improved from intermediate to low risk	Stable intermediate risk	Worsened from intermediate to high risk	Improved from high to intermediate risk	Stable high risk
0	43	36	41	117	29	13	7
12	30	30	19	58	23	6	6
24	25	24	11	39	15	4	2
36	25	18	7	31	11	4	2
48	25	13	4	20	6	4	1
60	23	9	3	14	4	4	1

Survival, %							
Months after enrolment	Stable low risk	Worsened from low to intermediate risk	Improved from intermediate to low risk	Stable intermediate risk	Worsened from intermediate to high risk	Improved from high to intermediate risk	Stable high risk
0	100	100	100	100	100	100	100
12	97	83	80	66	79	84	71
24	95	80	76	54	53	69	57
36	92	62	72	51	45	61	29
48	92	58	48	43	40	51	29
60	89	44	48	36	26	40	14

FIGURE 5 | The survival estimates in patients with idiopathic pulmonary arterial hypertension according to the 2015 ESC/ERS risk category from baseline to re-evaluation. This figure was based on $n = 286$ patients.

organize more prospective studies or explore exiting registries in China.

CONCLUSION

In conclusion, the present data show that the 2015 ESC/ERS PH guidelines and its simplified version risk stratification strategy may effectively discriminate different risk groups at baseline and re-evaluation. Meanwhile, this study validated an accurate prediction of mortality. Non-invasive echocardiography assessment might help to identify predictive usefulness of risk categorization strategies. The parameters of CPET seems to be less sensitive to the risk level designation, but need to be clarified in future and prospective studies. Changes of risk proportion at re-evaluation implicated that natural treatment decisions may not behave consistently with goal-oriented treatment strategy, but patients with IPAH may benefit from initial therapy.

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 K19-054. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RZ and LW contributed to the study design, study conduct, supervision, scientific overview, data analysis, editing of the manuscript, and also directly involved in the recruitment and care of the patients. S-GG and W-HW contributed to enrolment of the patient, data analysis, scientific interpretation, drafting, and editing the original manuscript. CL, Q-HZ, RJ, C-JL, H-LQ, and J-ML contributed to recruitment of participants, data collection, curation, and formal analysis. All authors have reviewed the manuscript, approved the final version for submission, participated in the design of this study, patient enrolment, and meet criteria for authorship.

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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.2021.745578/full#supplementary-material

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Genetically Determined Inflammatory Biomarkers and the Risk of Heart Failure: A Mendelian Randomization Study

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Background: Positive associations between inflammatory biomarkers and the risk of heart failure (HF) have been reported in conventional observational studies. However, the causal effects of inflammatory biomarkers on HF have not been fully elucidated. We conducted a Mendelian randomization (MR) study to examine the possible etiological roles of inflammatory biomarkers in HF.

Methods: Summary statistical data for the associations between single nucleotide polymorphisms (SNPs) and C-reactive protein (CRP), fibrinogen, and components of the interleukin-1 (IL-1)-interleukin-6 (IL-6) inflammatory signaling pathway, namely, interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1ra), IL-6, and soluble IL-6 receptor (sIL-6r), were obtained from genome-wide association studies (GWASs) for individuals of European descent. The GWAS dataset of 977,323 participants of European ancestry, which included 47,309 HF cases and 930,014 controls, was collected to identify genetic variants underlying HF. A two-sample Mendelian randomization framework was implemented to examine the causality of the association between these inflammatory biomarkers and HF.

Results: Our MR analyses found that genetically determined CRP and fibrinogen were not causally associated with HF risk (odds ratio [OR] = 0.93, 95% confidence interval [CI] = 0.84–1.02, $p = 0.15$; OR = 0.94, 95% CI = 0.55–1.58, $p = 0.80$, respectively). These findings remained consistent using different Mendelian randomization methods and in sensitivity analyses. For the IL-1-IL-6 pathway, causal estimates for IL-6 (OR = 0.86, 95% CI 0.81–0.91, $p < 0.001$), but not for IL-1β, IL-1ra, or sIL-6r, were significant. However, the association between genetically determined IL-6 and HF risk became non-significant after excluding SNPs with potential pleiotropy (OR = 0.89, 95% CI = 0.77–1.03, $p = 0.12$).

Conclusion: Our study did not identify convincing evidence to support that CRP and fibrinogen, together with their upstream IL-1-IL-6 signaling pathway, were causally associated with HF risk.

Keywords: heart failure, Mendelian randomization, C-reactive protein, fibrinogen, interleukin

INTRODUCTION

Heart failure, a debilitating condition in which the heart fails to respond to increased cardiac output to meet peripheral demands, is a worldwide health burden (1). Currently, ∼1–2% of adult populations in developed countries are victims of heart failure (2). Given the prolonged life expectancy of the general population and the vulnerability of the elderly to cardiac dysfunction (3), the prevalence of HF is predicted to have a two-fold increase by 2060 (4). Therefore, exploring underlying pathophysiological mechanisms to identify therapeutic targets for improving HF prognosis is a clinically unmet need.

Inflammation has been implicated in the pathogenesis of heart failure (HF). Previously, observational studies have reported that C-reactive protein (CRP), a representative biomarker of systemic inflammation, can predict the development and prognosis of HF (5–7). Another inflammatory marker, fibrinogen, is a vital determinant of blood viscosity and platelet aggregation, and has also been suggested to be associated with HF risk (8, 9). However, residual confounding and reverse causality may remain alternative explanations for the strong association between CRP and fibrinogen with heart failure because of the inherent limitation of conventional observational studies (10).

"Upstream" proinflammatory cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6), are major initiators for the production of "downstream" biomarkers, such as CRP and fibrinogen, from the liver (11, 12). Established associations between the classic IL-1-IL6-CRP signaling and adverse cardiac remodeling have led to investigations targeting this pathway to reduce inflammation and alleviate cardiac dysfunction (13). However, contradictory results from HF therapy based on anti-inflammation suggested otherwise (14, 15). High serum levels of IL-1 receptor antagonist (IL-1ra) and soluble IL-6 receptor (sIL-6r) have been reported to mimic the effects of endogenous inhibition of IL-1 and IL-6 signaling, respectively (16). However, investigation of these proximal inflammatory mediators is difficult given that their concentrations are subjected to constant fluctuations in the bloodstream.

Mendelian randomization is a form of analysis that uses genetic variants as instrumental variables (IVs) to generate more reliable causal estimates of long-exposure effects of risk factors on disease outcomes (17). This approach takes advantage of the naturally occurring random allocation of alleles at conception (18). Therefore, the level of a specific exposure will usually be independent of other exposures and unaffected by disease status (19). Thus, Mendelian randomization (MR) is capable of overcoming the limitations of residual confounding and reverse causation in observational studies. Here, we perform a two-sample MR analysis to test the hypothesis that genetically determined CRP and fibrinogen and their upstream inflammatory biomarkers, IL-1 and IL-6, are associated with HF risk in a European population.

METHODS

Study Design and Data Source

Mendelian randomization is built upon three main assumptions (**Figure 1**) (20). First, genetic variants selected as instrumental variables should be robustly associated with the risk factor. Second, no association should exist between genetic variants and confounders. Third, genetic variants should affect the risk of outcome through the risk factor and not *via* other pathways.

Circulating CRP-associated variants were collected from the largest genome-wide association study (GWAS) aimed at identifying variants in relation to CRP concentration (involving 204,402 individuals from 88 population-based cohort studies) (21). Genetic variants for fibrinogen were identified from a GWAS meta-analysis, which enrolled >90,000 individuals from 28 studies (22). Genetic instruments for IL-1β were obtained from a GWAS of the Northern Finland Birth Cohort (23), and those for IL-1ra were obtained from a GWAS meta-analysis of 11 cohorts (24). Genetic variants for IL-6 were collected from a GWAS of the SardiNIA project (25), and those for sIL-6r were collected from a collaborative meta-analysis of human genetic and biomarker data (26) (the analytical procedure is presented in **Figure 2**).

Following the identification of the genetic variants, summary statistical data on the association of genetic variants with heart failure were extracted from the published GWAS performed by the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium on 47,309 cases and 930,014 controls (27). HF cases from 26 cohorts of the HERMES Consortium were identified based on the clinical diagnosis of HF of any etiology with no specific inclusion criteria for left ventricular (LV) ejection fraction (**Supplementary Table S1**). Details of participant selection can be found elsewhere (27).

The datasets used in our study included individuals of European ancestry to reduce selection bias and to improve the robustness of the analysis. All the data used in this study were derived from GWAS for which ethical approval and patient consent were previously obtained. The study protocols were in accordance with the guidelines of the Helsinki Declaration and approved by the ethics committee of all participating sites.

Genetic Variant Selection

Single-nucleotide polymorphisms that reached genome-wide significance ($p < 5 * 10^{-8}$) were selected as instrumental variables. These SNPs were further linkage disequilibrium (LD)-pruned (distance threshold = 10,000 kb, $r^2 < 0.001$) to ensure independence among the genetic variants (28). If the selected SNPs were not collected in the GWAS of HF, proxy SNPs in the linkage disequilibrium ($r^2 > 0.8$) were chosen for substitution. Subsequently, palindromic SNPs were removed to ensure that the effects of the SNPs on the exposure corresponded to the same allele as their effects on HF. Accordingly, for CRP and fibrinogen, 41 and 19 SNPs, respectively, were included in the primary analysis of the association with HF. One SNP for IL-1β (rs6917603), two SNPs for IL-1ra (rs4251961, rs6759676), two SNPs for IL-6 (rs4129267, rs643434), and one SNP for

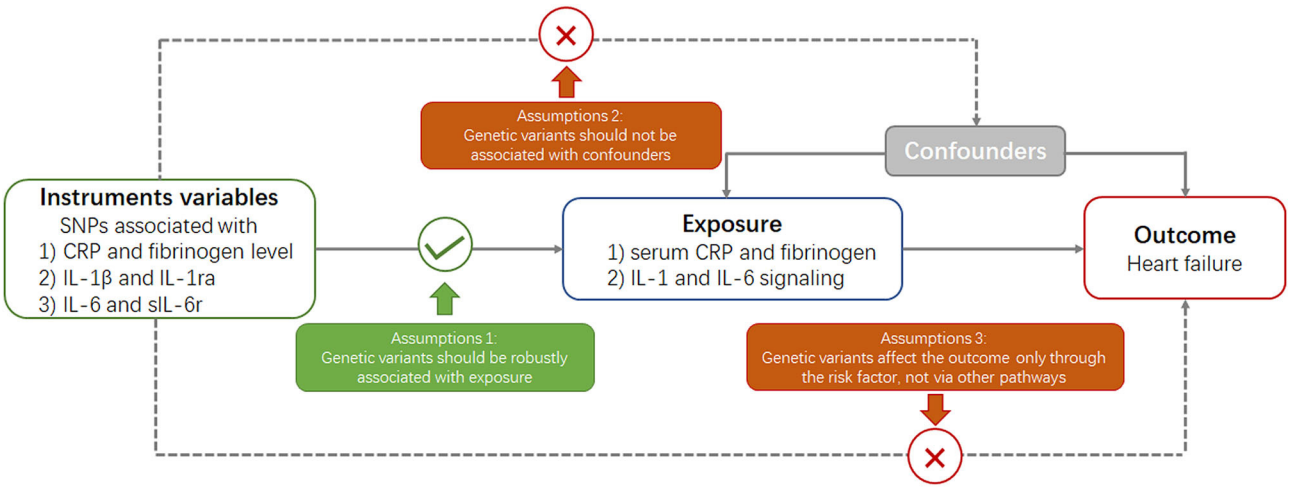

FIGURE 1 | Key assumptions underlying Mendelian randomization study design. CRP, C-reactive protein; SNP, single nucleotide polymorphism.

sIL-6r (rs2228145) were also enrolled. The phenotypic variance explained by the selected SNPs was ∼6.5% for CRP variation, 3.7% for fibrinogen variation, 1% for IL-1β, 2% for IL-1ra, 0.6% for IL-6, and 4% for sIL-6r.

Statistical Analysis

We first harmonized the summary exposure and outcome data based on a previously described method (**Supplementary Table S2**) (29). Then, for CRP- and fibrinogen-associated instruments, four different methods of two-sample MR, namely, inverse-variance weighted, weighted median, MR-Egger, and MR-PRESSO, were implemented to calculate estimates and address possible heterogeneity and horizontal pleiotropy across the causal estimates (30). We used a predefined decision tree to select the best statistical estimation from the four methods as previously described (**Supplementary Figure S1**) (18). For biomarkers of IL-1β, IL-1ra, IL-6, and sIL-6r, the Wald ratio method was used to calculate estimates by dividing the beta coefficient for the SNP-outcome association with the beta coefficient for the SNP-biomarker effect. The inverse-variance weighted method with fixed effects was used to combine the Wald estimates for two SNPs.

To test the reliability of causal effect estimates, several sensitivity analyses were performed. First, a leave-one-out analysis was further conducted by removing a single variant from the analysis each time to determine whether the influence of a single SNP disproportionately affected the association. An additional sensitivity analysis was performed to address horizontal pleiotropic bias by excluding any SNPs significantly associated with potential confounders, such as body mass index (31), type 2 diabetes (32), systolic blood pressure (33), diastolic blood pressure (33), high-density lipoprotein cholesterol (34), low-density lipoprotein cholesterol (34), triglyceride (34), and smoking (35).

All results are presented as odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of the outcomes with per predicted increase in CRP and fibrinogen concentrations (36). A two-sided $P < 0.05$ was defined as statistically significant. All the analyses were performed with the TwoSampleMR and MR-PRESSO packages with R version 4.0.2.

RESULTS

Causal Effect of CRP and Fibrinogen on Heart Failure

The causal effect estimates of genetically determined CRP and fibrinogen on the risk of HF are presented in **Figures 3**, **4**, respectively. Significant heterogeneity was detected by the Cochran heterogeneity test among SNPs of CRP ($Q = 108.8$; $p < 0.001$) and fibrinogen ($Q = 38.2$; $p = 0.004$). The MR-Egger method showed significant directional pleiotropy for the association of CRP with HF [odds (intercept), 0.0076; $p = 0.025$] but not for fibrinogen [odds (intercept), 0.0074; $p = 0.229$]. Thus, based on a predefined decision tree (**Supplementary Figure S1**), no significant causal effects between CRP (MR Egger; OR = 0.93, 95% CI = 0.84–1.02, $p = 0.15$) and fibrinogen (weighted median; OR = 0.94, 95% CI = 0.55–1.58, $p = 0.80$) and HF risk were observed (**Table 1**). The lack of causal association persisted using all of the MR methods employed here. Leave-one-out sensitivity analyses did not reveal any significant change (**Supplementary Figure S2**). After excluding SNPs with potential pleiotropy, 13 SNPs for CRP and 11 SNPs for fibrinogen remained for analysis, and the results confirmed that neither genetically predicted CRP levels (weighted median; OR = 1.01, 95% CI = 0.93–1.09, $p = 0.83$) nor fibrinogen concentration (IVW, OR = 1.18, 95% CI = 0.77–1.81, $p = 0.46$) was associated with HF risk (**Supplementary Table S3**; **Supplementary Figure S3**).

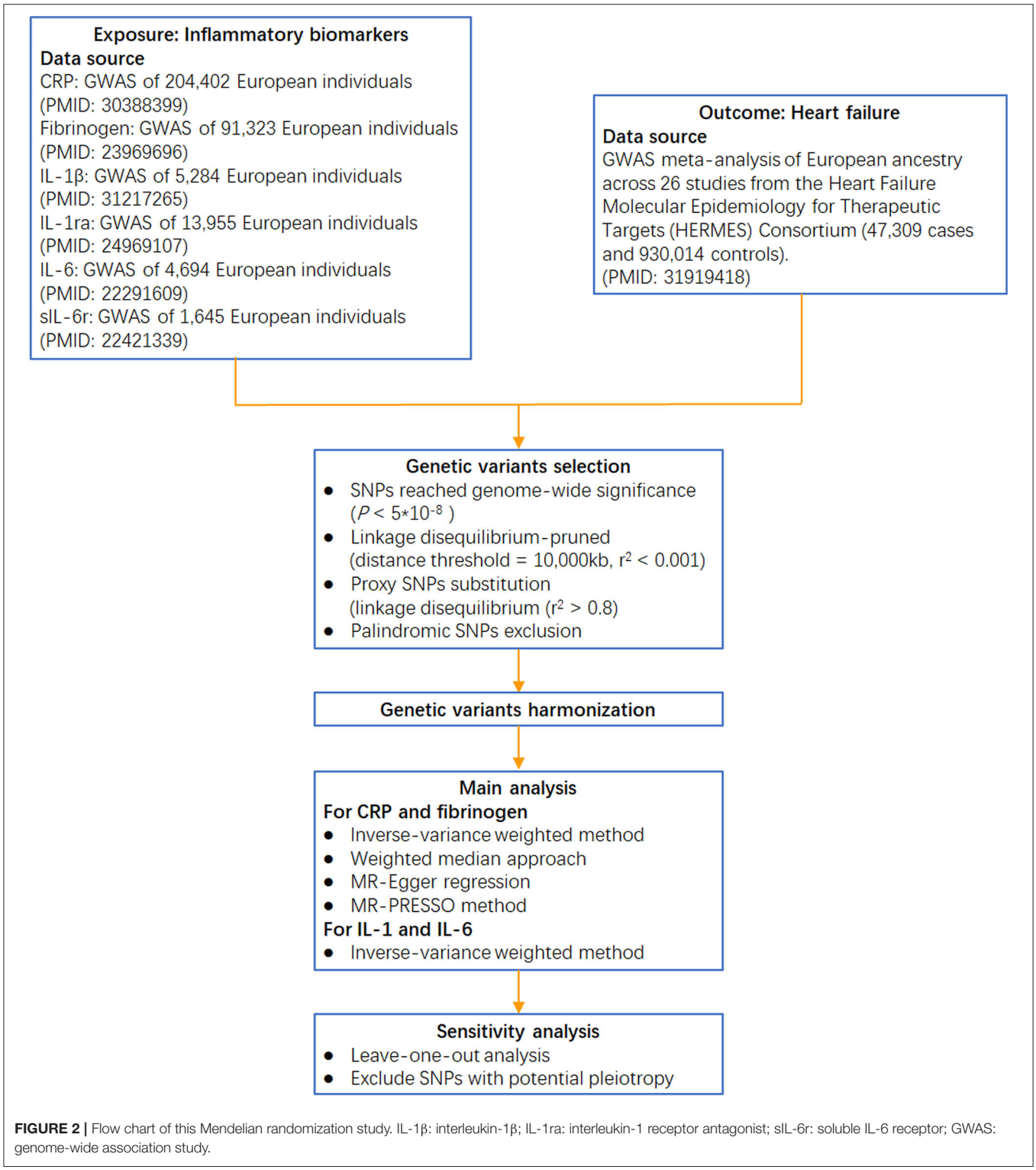

FIGURE 2 | Flow chart of this Mendelian randomization study. IL-1β: interleukin-1β; IL-1ra: interleukin-1 receptor antagonist; sIL-6r: soluble IL-6 receptor; GWAS: genome-wide association study.

Causal Association Between the IL-1-IL-6 Pathway and Heart Failure

The results of the causal association between IL-1 and IL-6 signaling and HF are presented in **Table 2**. For IL-1 signaling, one SNP (rs6917603) was selected for IL-1β, and two SNPs (rs4251961 and rs6759676) were selected for IL-1ra. For IL-6 signaling, two SNPs were selected for IL-6 (rs4129267, rs643434), and one SNP was selected for sIL-6r (rs2228145). Primary MR analyses revealed an inverse causal effect of IL-6 on HF risk (OR = 0.86, 95% CI 0.81–0.91, $p < 0.001$), whereas no significant

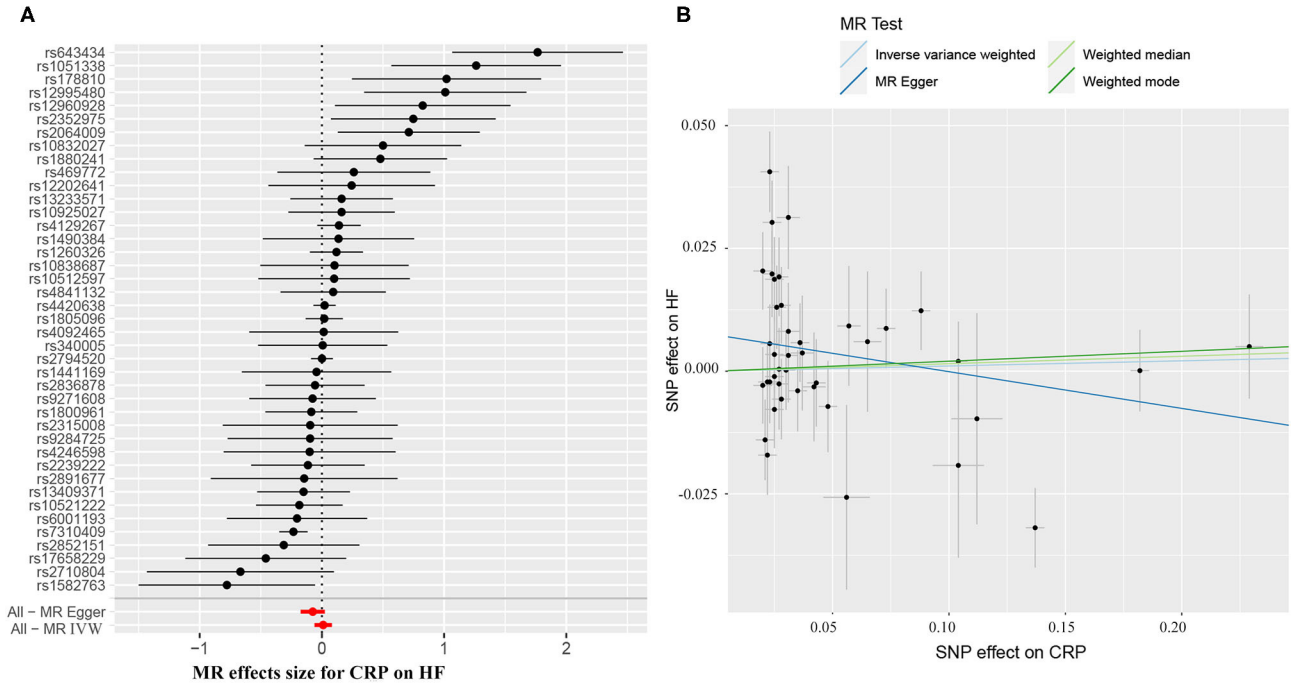

FIGURE 3 | (A) Forest plot and **(B)** scatter plot of the potential effects of CRP-associated SNPs on heart failure (HF). IVW, inverse variance weighted. MR, Mendelian randomization.

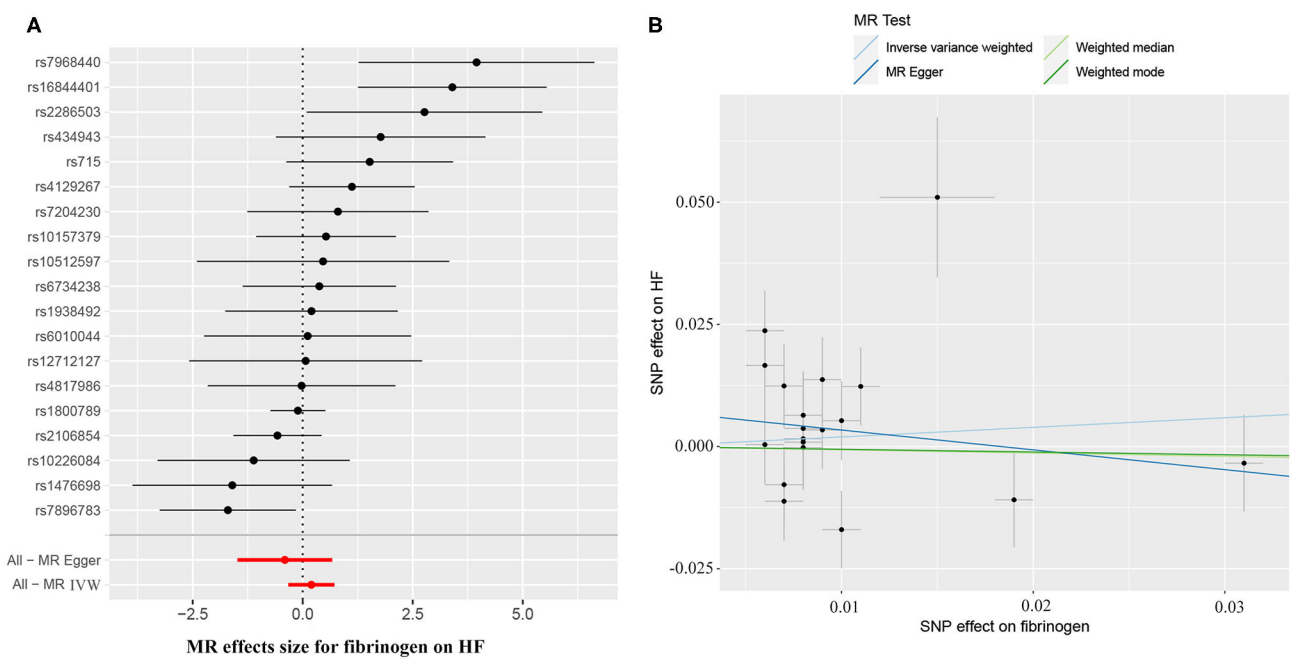

FIGURE 4 | (A) Forest plot and **(B)** scatter plot of the potential effects of fibrinogen-associated SNPs on HF.

association between genetically determined IL-1β (OR = 1.04, 95% CI 0.86–1.25, $p = 0.70$), IL-1ra (OR = 0.95, 95% CI 0.83–1.10, $p = 0.51$), and sIL-6r (OR = 0.96, 95% CI 0.91–1.01, $p = 0.14$) with HF development was observed (**Table 2**). However, strong pleiotropic associations were noted between rs4251961 and rs643434 and cardiometabolic traits. After excluding the

TABLE 1 | Mendelian randomization (MR) estimates of CRP and fibrinogen with heart failure.

Phenotype and methods	IVs (SNPs)	OR (95% CI)	*P*-value	*Q*-statistics	*Ph*
CRP					
IVW	41	1.01 (0.94–1.09)	0.77	108.8	<0.001
Weighted median	41	1.02 (0.95–1.08)	0.64		
MR-Egger	41	0.93 (0.84–1.02)	0.15		
MR-PRESSO	37	1.03 (0.98–1.10)	0.21		
Fibrinogen					
IVW	19	1.21 (0.72–2.06)	0.46	38.2	0.004
Weighted median	19	0.94 (0.55–1.58)	0.80		
MR-Egger	19	0.67 (0.23–1.96)	0.15		
MR-PRESSO	19	1.05 (0.62–1.77)	0.85		

CRP, C-reactive protein; IV, instrumental variable; OR, odds ratio; CI, confidence interval; Ph, P for heterogeneity.

TABLE 2 | Association between IL-1 and IL-6 signaling with heart failure risk estimated in MR analysis.

Biomarker	IVs (SNPs)	OR (95% CI)	*P*-value
IL-1β	1	1.04 (0.86–1.25)	0.70
IL-1ra	2	0.95 (0.83–1.10)	0.51
Exclude rs4251961	1	1.00 (0.82–1.24)	0.95
IL-6	2	0.86 (0.81–0.91)	<0.001
Exclude rs643434	1	0.89 (0.77–1.03)	0.12
sIL-6r	1	0.96 (0.91–1.01)	0.14

IL-1β, interleukin-1β; IL-1ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; sIL-6r, soluble IL-6 receptor.

SNPs with pleiotropy, one SNP remained for IL-1ra and one for IL-6, and no significant causal effect of the IL-1-IL-6 pathway components on HF risk was observed (**Table 2**).

DISCUSSION

In our MR analyses of a European population, our findings did not support an important etiological role of CRP and fibrinogen in HF development. Various sensitivity analyses supported our initial findings. In our study investigating IL-1 and IL-6 signaling, there was some evidence of an association between genetically determined IL-6 and HF risk but not for IL-1β, IL-1ra, or sIL-6r. However, this association became non-significant after excluding SNPs with pleiotropy.

Inflammation has been considered to contribute to the pathogenesis and progression of HF through various mechanistic pathways, such as cardiomyocyte apoptosis, cardiac fibrosis, and endothelial dysfunction (14). Activation of systemic inflammation has been widely reported in patients with HF as elevated levels of various markers, such as CRP and fibrinogen (37, 38). Evidence from observational cohort studies has shown that increased CRP and fibrinogen are independent risk factors for HF, suggesting that these inflammation biomarkers may play etiological roles in HF. However, given that the hemodynamic stress of HF itself can induce a state of sterile inflammation (39), whether the elevation of specific biomarkers of inflammation is a reflection of their involvement in disease pathogenesis or an epiphenomenon of HF remains unsolved. Meanwhile, the inherent limitations of conventional observational studies, such as residual confounding and reverse causation, limit the ability to ascertain causal inferences. Random control trials (RCTs) are the most powerful method to demonstrate the etiology hypothesis observed in epidemiological studies (40). However, it is difficult to implement RCTs because of rigorous research designs and expensive costs. In recent years, MR research has been acknowledged as the best alternative to RCTs given that it is a very reliable method that uses genetic variants inherited randomly from parents to infer a causal relationship between risk factors and diseases (40, 41). Previous MR studies have indicated that CRP was unlikely to be a causal factor for ischemic cardiovascular disease (42), and there was only very weak evidence of a causal effect of fibrinogen on coronary heart disease (CHD) (43). In our MR study, we analyzed the correlation between inflammatory biomarkers and HF risk with the aid of large-scale GWAS datasets. Our findings revealed that genetically elevated CRP and fibrinogen were not significantly associated with HF, suggesting that these inflammatory biomarkers may function as bystanders instead of causative factors in HF.

The "upstream" proinflammatory biomarkers IL-1 and IL-6 act as important initiators of inflammation and can trigger a cascade of inflammatory mediators, such as CRP and fibrinogen (44). Although evidence from preclinical and clinical studies suggested that the IL-1-IL-6 signaling pathway led to impaired systolic and diastolic cardiac function (45), clinical trials choosing these pathways as anti-inflammatory therapeutic targets have reported controversial results. Administration of anakinra, an IL-1 receptor antagonist, yielded beneficial effects on aerobic capacity improvement but did not reduce the length of hospital stay or rehospitalization in HF (14, 46). An RCT found a significant reduction in IL-6 and CRP concentrations in 267 patients treated with colchicine compared with those treated with placebo (47). However, there was no improvement in cardiac function or measures of LV remodeling in the colchicine group (47). Recent results from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) provided evidence that targeting IL-1β with canakinumab significantly reduced major cardiovascular event rates, including HF hospitalizations (48), and that the magnitude of risk reduction was correlated with the magnitude of IL-6 reduction (44). Thus, canakinumab exhibited minimal improvement in patients who did not achieve substantial reductions in IL-6. Our MR analyses initially found a significant causal effect for IL-6 on HF but not for IL-1β, IL-1ra, or sIL-6r. The inverse association between genetically predicted levels of circulating IL-6 and HF was consistent with previous MR studies that focused on CHD (49, 50), indicating that IL-6 inhibition may be associated with lower risk of HF. However, after excluding an SNP with pleiotropy (rs643434), the association became non-significant. Nevertheless, the MR

analysis likely reflects lifelong exposure to risk factors, which may explain the different effects between endogenous inhibition of IL-6 and exogenous blockade of IL-6 signaling. Therefore, it is possible that the administration of an IL-6 inhibitor under a specific condition may reduce HF risk.

A major strength of this study was the design of MR analysis based on the largest GWAS meta-analysis on HF, which can prevent reverse causation and the influence of potential confounders. We also conducted additional analyses excluding SNPs with potential pleiotropy, which can minimize the bias in causal effect estimates. In addition, we performed a comprehensive evaluation of causal inferences of inflammatory biomarkers in the IL-1-IL-6-CRP pathway, which may provide a better understanding of the role of this pathway in the pathogenesis of HF.

Our study had several limitations. First, we restricted the study population to European ancestry to reduce bias from population stratification. This restriction reduced the transferability to individuals with other genetic backgrounds. Second, because of the unavailability of individual data, we could not conduct analyses stratified by subtypes and severity of HF. Since observational studies suggested that a stronger association of inflammatory markers may exist in the context of HF with preserved EF (HFpEF), the etiological role of inflammation in specific subphenotypes requires future research. Third, we only investigated the causal relationship between certain inflammatory biomarkers and HF from a genetic point of view. Therefore, our results should be treated with caution, since the causal effect of SNP exposure on SNP outcome can be modified by compensatory processes during development, and the etiological roles of other inflammatory factors in HF need further exploration.

CONCLUSION

Our MR analysis did not identify convincing evidence to support the causal relationship between inflammatory biomarkers, such as CRP and fibrinogen, or their upstream IL-1-IL-6 pathway with HF. Additional human and animal studies are needed to confirm our MR results further.

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

XL and SP designed this study and conducted the main analysis. BG, SC, GZ, YW, CG, JX, XL, XZ, and SL reviewed and edited 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.2021.734400/full#supplementary-material

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The Diagnostic and Prognostic Value of Plasma Galectin 3 in HFrEF Related to the Etiology of Heart Failure

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Aim: The aim of present study is to evaluate the diagnostic and prognostic value of plasma galectin 3 (Gal-3) for HF originating from different causes.

Methods: We investigated the plasma levels and expression of Gal-3 in cardiac tissues in two transgenic (TG) strains of mice with cardiomyocyte-restricted overexpression of either β2- adrenergic receptor (β2- AR TG) or Mammalian sterile 20-like kinase 1 (Mst1-TG) in the present study. Additionally, 166 patients suffering from heart failure with reduced ejection fraction (HFrEF) in two hospitals within the Shaanxi province were examined in this study. All these patients were treated according to the Chinese HF guidelines of 2014; subsequently, they were followed up for 50 months, and we analyzed the prediction value of baseline Gal-3 to endpoints in these patients.

Results: Gal-3 was localized in the cytoplasm and nucleus of cardiomyocytes, often formed aggregates in Mst1-TG mice. Extracellular Gal-3 staining was uncommon in Mst1-TG hearts. However, in β2-AR TG mice, although Gal-3 was also expressed in myocardial cells, it was more highly expressed in interstitial cells (e.g., fibroblasts and macrophages). Plasma Gal-3 was comparable between nTG and Mst1-TG mice. However, plasma Gal-3 was higher in β2-AR TG mice than in nTG mice. In the cohort of HFrEF patients, the median plasma Gal-3 concentration was 158.42 pg/mL. All participants were divided into two groups according to Gal-3 levels. Patients with Gal-3 concentrations above the median were older, and had lower plasma hemoglobin, but higher plasma creatinine, tissue inhibitor of metalloproteinases 1 (TIMP-1), left ventricular end systolic diameter (LVESD), left ventricular end-systolic volumes (LVESV) and end-diastolic, as well as left ventricular end-diastolic volumes (LVEDV). Spearman correlation analysis revealed that Gal-3 was positively correlated with TIMP-1 ($r = 0.396$, $P < 0.001$), LVESV ($r = 0.181$, $P = 0.020$) and LVEDV ($r = 0.190$, $P = 0.015$). The 50-month clinical follow-up revealed 43 deaths, 97 unplanned re-hospitalizations, and 111 composite endpoint events. Cox analysis demonstrated that although Gal-3 did not provide any prognostic value in either total-HF subjects or coronary-heart-disease (CHD) patients, it did provide prognostic value in non-CHD patients.

Conclusion: Although plasma Gal-3 is associated with TIMP-1 and echocardiographic parameters, the diagnostic and prognostic value of Gal-3 in HFrEF is determined by the etiology of HF.

Keywords: heart failure, galectin-3, prognosis, risk factor, diagnostic, etiology

HIGHLIGHT

The diagnosis and prognostic value of Galectin-3 in HFrEF.

INTRODUCTION

Heart failure (HF) is a disease of high morbidity and mortality regardless of therapies (1, 2). Hence, there is an increasing demand for early diagnosis, better prognostic evaluation and management of HF. Thus, as indicators of pathological processes and responses to therapeutic interventions, circulating blood biomarkers have been increasingly studied due to their non-invasive determinations that tend to be sufficiently sensitive and accurate. Due to different etiologies and underlying pathophysiological processes, HF was a heterogeneous disease. Although there are many different available biomarkers [e.g., NT-proBNP (3), GDF-15 (4)], the diagnosis and prognostic values of these biomarkers were discordant.

Galectin 3 (Gal-3) is a soluble β-galactoside-binding protein, expressed in epithelial and inflammatory cells in several organs, located both intracellularly and extracellularly (5, 6), involved in cellular functions related to cell adhesion (7, 8), proliferation (9) and differentiation (10–12), the Gal-3 is considered as a biomarker of cardiac fibrosis and remodeling (5, 13, 14). In the myocardium, Gal-3 is primarily expressed in fibroblasts and macrophages that play an important role in the formation of myocardial fibrosis through the activation of fibroblasts (15), and have been linked to fibrosis in a spectrum of medical conditions, including HF. Although many previous studies have demonstrated elevated plasma concentrations of Gal-3 in both acute and chronic HF, the prognostic value of Gal-3 in predicting re-hospitalization and mortality has not yet been determined. Zhao et al. (16) and we (17) found that plasma and cardiac levels of Gal-3 were different across distinct HF caused by different etiologies in experimental animals. Therefore, it seems reasonable to hypothesize that the diagnostic and prognostic value of plasma Gal-3 is related to and affected by the etiology. The researchers of this study, therefore, evaluated the diagnosis and prognostic value of plasma Gal-3 within HF patients caused by different causes.

Abbreviations: Gal-3, galectin 3; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CHD, coronary heart disease; MLHFQ, the Minnesota Living with Heart Failure Questionnaire; SBP, systolic blood pressure; DBP, diastolic blood pressure; CR, creatinine; BUN, urea nitrogen; HB, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TIMP-1, tissue inhibitor of metalloproteinases 1; BMI, Body mass index; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic dimension; LVEDD, left ventricular end-diastolic dimension; LVESV, left ventricular end-systolic volumes; LVEDV, left ventricular end-diastolic volumes; FS, left ventricular fractional shortening; NYHA, New York Heart Association; ARNI, Sacubitril/Valsartan; MRA, mineralocorticoid receptors antagonist; HR, hazard ratio; CI, confidence intervals; nTG, non-transgenic mice; β2-AR TG, β2-adrenergic receptor transgenic; Mst1-TG, Mammalian sterile 20-like kinase 1 transgenic.

METHODS

This study was conducted in mouse models and HF patients.

Animals

Two transgenic (TG) strains of mice with cardiomyocyte-restricted overexpression of either β2- adrenergic receptor (β2-AR TG) or Mammalian sterile 20-like kinase 1 (Mst1-TG) were used in the present study. Our previous works have characterized cardiomyopathic phenotypes of both models (16, 18–20). These two strains of mice were both from the C57Bl/6 genetic background and female mice were excluded. Age-matched non-transgenic (nTG) littermates were used as controls. Mice were housed in standard conditions with food and water provided *ad libitum*. All experimental procedures were approved by a local animal Ethics Committee in compliance with both the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition) and the ARRIVE guidelines.

Subjects

This protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Shaanxi 710061, China, approval number: 2013-133) and was in accordance with the Helsinki Declaration's guidelines. Informed consent was obtained from all participants. The cohort study consisted of chronic HF patients, aging between 18 and 80 years, who were diagnosed with heart failure with reduced ejection fraction (HFrEF) in the Department of Cardiovascular Medicine at Xunyi Hospital and Jingyang Hospital from May 2014 to May 2015 by the physicians who were blinded to the study. According to China HF guidelines of 2014, diagnosis of HFrEF was based on: ① typical symptoms and signs, ② clinical history, ③ NT-proBNP ≥125 pg/mL, ④ left ventricual ejection fractions <40%. Patients were excluded from the present study if they had acute HF, active neoplasia, acute myocardial infarction, acute or chronic liver disease (alanine aminotransferase level >5 times the upper normal limit), acute stroke, serious kidney disease, chronic consumption disease, thyroid dysfunction, fibrotic pathologies (e.g., pulmonary fibrosis, collagenases), valvular heart disease and/or cancer. A total of 166 HFrHF patients were recruited to satisfy these exclusion criteria. All participants were divided into two groups according to the media level of Gal-3. Then, based on their clinical features, as well as their past medical history and/or the result of coronary angiography, patients from each group

were further divided into two subgroups, namely, the group of those with coronary-heart-disease (CHD) group and the group of those without CHD group. Patients were then followed up for a period of 50 months and were evaluated for the development of major adverse cardiovascular events (MACEs).

Plasma and Histological Gal-3 Analysis in Mice

Blood was collected in heparin-containing vials when mice were killed, centrifuged at 4°C (3,000 rpm in 20 min), and stored at −80°C. Plasma Gal-3 levels were detected by a mice Galectin-3 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN, USA) in twin duplicates wells, following protocols provided by the manufacturer. The detection range of the plasma Gal-3 was 8.19–2,000 pg/mL, and the limit of detection was 6 pg/mL.

Paraffin-embedded LV sections (6 μm) were prepared and used for Gal-3 immunofluorescent staining within mice. For Gal-3 immunofluorescent staining, after dewaxed, heat-induced antigen retrieval and permeabilization were carried out (with 10 mM of Na-citrate buffer containing 0.05% Tween 20; pH 6.0; 95°C for 25 min) followed by blocking with DAKO Protein Block (X0909, Agilent, 1 h at room temperature). Sections were incubated with primary goat anti-mouse Gal-3 (1:100, AF1197, R&D Systems) overnight at 4°C, then they were incubated with the secondary antibody, Alexa Fluor 594 donkey anti-goat IgG (1:200, A11058, Invitrogen by Thermo Fisher Scientific). The cardiomyocyte boundary was revealed by wheat-germ-agglutinin FITC staining (1:80, FL-1021, Vector Labs, 1 h at room temperature). Images were acquired with an Olympus BX61 fluorescent microscope.

Clinical Measurements

Investigators and a trained interviewer collected all of the clinical data. At baseline, all medical visits to the patients were conducted and patients' information was collected, including demographic data, past medical history, history of cardiovascular diseases, the Minnesota Living with Heart Failure Questionnaire (MLHFQ), New York Heart Association (NYHA) functional class, smoking behavior, and alcohol abuse. Smoking was defined as smoking cigarettes within one month of the indexed hospital admission. Hypertension was defined as a cuff blood pressure $\geq$140/90 mmHg and/or the current use of antihypertensive medications. Subjects were also questioned about their past histories of diabetes mellitus and their current use of anti-diabetic drugs. Diagnosis of diabetes mellitus (DM) was confirmed if plasma fasting glucose was $\geq$7.0 mM (or if the 2-h postprandial glucose was $>$11.1 mM) or if there was current use of anti-diabetic medication. Anthropometric measurements, such as body weight (kg) and height (m), were taken during the first visit. Body mass index (BMI) was calculated as weight divided by height squared.

Analysis of Patients' Blood Parameters

For measurements of hemoglobin (HB), creatinine (CR), urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), blood was collected from each patient after gaining the consent. These blood parameters were measured at the Central Clinical Laboratory of the First Affiliated Hospital of Xi'an Jiaotong University. HB was measured with automated cell counters via standard techniques by HST201 (Sysmex, Japan). CR, BUN, ALT and AST were examined by Beckman automatic biochemical analyzer AU5431 (America). After overnight fasting, between 6–7 a.m., blood from the median cubital vein was drawn into ethylenediaminetetraacetic acid (EDTA)-containing tubes, centrifuged at 4°C (3,000 rpm in 10 min) within 2 h after collection and stored at −80°C for later analyses (i.e., to detect Gal-3 and TIMP-1 levels).

Plasma Gal-3 levels and TIMP-1 were detected by a Human Gal-3 Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN, USA) and a human TIMP-1 Quantikine ELISA Kit (Abbkine, Inc., China) in duplicates wells following protocols provided by the manufacturer, respectively. The mean plasma Gal-3 and TIMP-1 were calculated as the final level. The detection range of the plasma Gal-3 was 0–4,000 pg/mL. The calibration range of serum TIMP-1 was 31.25–2,000 ng/mL, and the limit of detection was 16 ng/mL.

Echocardiographs and Electrocardiograms

At baseline, echocardiographs were performed with a Phillips iE33 system by a single trained operator blinded to the Gal-3 plasma concentration of each subject. All Echocardiographs data were analyzed by a single operator to limit inter-observer variability. The following standard parameters were collected: left ventricular end-systolic and end-diastolic volumes (LVESV and LVEDV), left ventricular end-systolic and end-diastolic dimensions (LVESD and LVEDD), and left ventricular fraction shortness (LVFS). The left ventricular ejection fraction (LVEF) was calculated by the Simpson biplane model.

The 12-leads electrocardiographic were made up of three standard limb leads (I, II, and III), augmented limb leads (aVR, aVL and aVF), and six precordial leads (V1, V2, V3, V4, V5, and V6). The QT interval was best measured between the beginning of the Q wave until the end of the T wave in lead II.

Treatments and Evaluations of Patient Outcomes

All HF patients were actively followed up at times of 1, 3, 6, and 50 months after the initial treatments. The complete follow-up information was gained for all 166 patients (100%). Information was obtained by face-to-face interviews or telephone conversations. Information regarding secondary cardiovascular events and treatments was collected, since the start of the treatment in the present study. Cardiovascular events were defined as MACEs as either the main cause of death, re-hospitalization because of HF, or composite endpoint events. All patients received β-blockers, as well as angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), according to the China HF guidelines of 2014, unless there were contraindications to these drugs. Mineralocorticoid-receptor antagonists (MRA), diuretics, and digoxin were prescribed to patients who had corresponding indications according to the China HF guidelines of 2014.

Statistical Analysis

Analyses were performed using SPSS version 13.0. Normally distributed values were presented as mean ± standard deviation (SD), and differences between groups were determined using Student's *t*-tests. Variables with a skewed normal distribution were presented as medians (inter-quartile range), and between-group differences for these variables were determined using Rank-Sum tests. Categorical variables were presented as percentages, and differences between groups were tested using Chi-squared tests. Owing to the skewed distribution of Gal-3, Spearman correlation analysis was used to analyze the relationship between plasma Gal-3 levels and clinical characteristics. MACE-rate estimates were generated via the Kaplan-Meier method. Cox regression modeling was used to assess the relative importance of baseline risk factors to the resulting endpoints. Hazard ratios (HR) with 95% confidence interval (CI), were presented to show the risk of an event when a given factor was present. Significance was defined at the 5% level using a two-tailed statistical test.

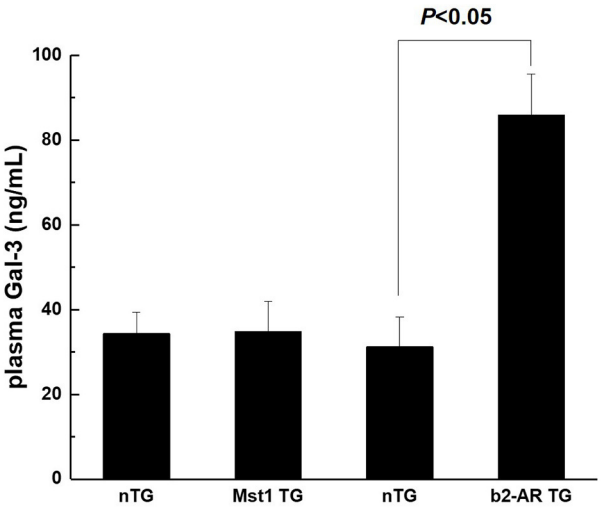

FIGURE 1 | Plasma concentration of Gal-3 from nTG, Mst1-TG, and β2-AR-TG hearts in mice. Gal-3, galectin-3; nTG: non-transgenic mice; b2-AR TG, β2- adrenergic receptor transgenic; Mst1-TG, Mammalian sterile 20-like kinase 1 transgenic.

RESULTS

Myocardial and Serum Gal-3 Expression Levels in Cardiomyopathic Mice

Our previous studies showed that myocardial Gal-3 concentrations were higher in both Mst1-TG mice and β2-AR-TG mice compared to that in nTG mice (16, 19, 21). In keeping with our previous findings, the plasma Gal-3 concentration in β2-AR-TG mice was significantly elevated vs. nTG mice, whereas Mst1-TG mice showed no change in plasma Gal-3 concentration compared with that of respective nTG group (**Figure 1**). By immunohistochemistry, Gal-3 was localized in the cytoplasm and nucleus of cardiomyocytes, and often formed aggregates in Mst1-TG mice. Extracellular Gal-3 staining was uncommon within Mst1-TG mice. However, in β2-AR TG mice, although certain number of cardiomyocytes were positively stained by Gal-3, Gal-3 was more often expressed in interstitial cells (e.g., fibroblasts and macrophages) (**Figure 2**).

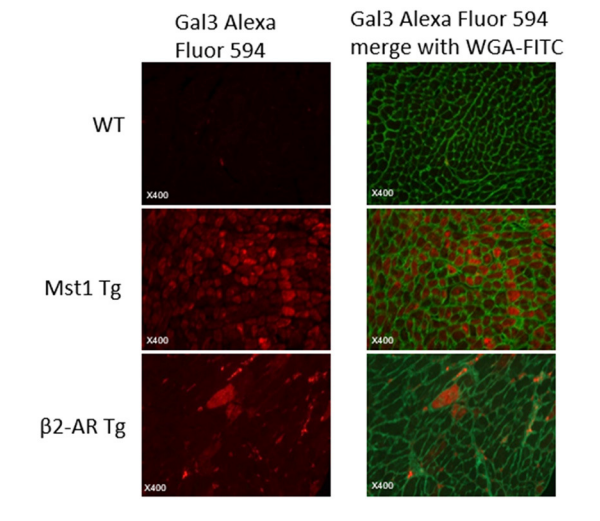

FIGURE 2 | Immunofluorescent staining of LV sections from wild-type, Mst1-Tg, and β2-AR-Tg hearts in mice. Gal-3 staining: red fluorescence. Merged images also show cell boundaries in green (i.e., wheat-germ agglutinin-FITC staining).

Baseline Characteristics of HF Patients

The participants of this cohort study included 105 (63.2%) males and 61 (36.7%) females. Plasma Gal-3 concentrations were between 23.88–1157.63 pg/mL, and the median Gal-3 concentration was 158.42 pg/mL. All participants were divided into two groups according to the cut-off level of 158.42 pg/ml (below median Gal-3 and above median Gal-3). Next, the clinical data were compared between these two groups. As shown in **Table 1**, there were no statistical differences between two groups in terms of gender, hypertension history, DM history, treatments, and MACEs. However, patients with Gal-3 plasma concentrations above the median were older ($P = 0.043$). **Table 1** also demonstrates that patients with increased plasma Gal-3 concentration had lower HB ($P = 0.002$) but higher plasma CR ($P = 0.011$), TIMP-1 ($P = 0.002$), LVESD ($P = 0.036$), LVESV ($P = 0.043$), and LVEDV ($P = 0.036$).

Relationships Between Plasma Gal-3 Levels and Clinical Characteristics

Figure 3 illustrates the associations between both Gal-3 and echocardiographic variables and the relationship between Gal-3 and TIMP-1. The results of spearman correlation analysis showed

TABLE 1 | Baseline characteristics of all HFrEF patients.

	Below median Gal-3 ($n = 83$)	Above median Gal-3 ($n = 83$)
Age (years)	60.6 ± 9.2	63.6 ± 9.3*
Gender (M/F)	55/28	50/33
Hypertension (%)	29.3	39.0
Diabetes mellitus (%)	7.2	7.2
Smoking (%)	57.8	47.0
Alcohol consumption (%)	41.5	41.0
Coronary heart disease (%)	34.9	48.2
HF history (years)	4.0 (2.0, 6.0)	4.0 (3.0, 7.0)
MLHFQ	25.0 (14.0, 34.0)	29.0 (16.0, 38)
SBP (mmHg)	122 ± 19	122 ± 23
DBP (mmHg)	77 ± 12	78 ± 11
CR (umol/L)	78.0 ± 12.1	84.3 ± 18.5*
BUN (mmol/L)	6.6 ± 1.5	7.1 ± 2.2
HB (g/L)	151.3 ± 21.7	141.9 ± 15.7*
AST (U/L)	24.6 ± 8.3	24.1 ± 9.4
ALT (U/L)	21.5 ± 10.6	20.9 ± 11.6
TIMP-1 (ng/mL)	113.3 ± 89.7	160.1 ± 103.7*
QT interval (ms)	419 ± 49	422 ± 50
BMI (Kg/m^2)	22.9 ± 3.3	23.4 ± 3.9
Heart Rate (bpm)	78.5 ± 14.9	78.4 ± 18.3
LVEF (%)	35.3 ± 7.1	35.5 ± 7.7
LVESD (mm)	56.4 ± 8.6	57.3 ± 10.0*
LVEDD (mm)	68.9 ± 8.4	70.2 ± 9.4
LVESV (ml)	135 ± 55	156 ± 71*
LVEDV (ml)	199 ± 73	225 ± 85*
FS (%)	17.9 ± 4.1	17.8 ± 4.8
NYHA functional class		
I	20.5	10.8
II	53.0	60.2
III	22.9	26.5
IV	3.6	2.4
β-blocker (%)	79.5	81.9
ACEI/ARB (%)	90.4	89.2
MRA (%)	73.5	79.5
Digoxin (%)	19.3	27.7
Diuretics (%)	55.4	59.0
Death rate (%)	20.3	31.3
Re-hospitalization rate (%)	52.4	65.4
Composite-endpoint event (%)	61.4	72.3

*Data are mean (SD) or n (%) unless otherwise stated. F, female; M, male; MLHFQ, the Minnesota Living with Heart Failure Questionnaire; SBP, systolic blood pressure; DBP, diastolic blood pressure; CR, creatinine; BUN, urea nitrogen; HB, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TIMP-1, tissue inhibitor of metalloproteinases 1; BMI, body mass index; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic dimension; LVEDD, left ventricular end-diastolic dimension; LVESV, left ventricular end-systolic volumes; LVEDV, left ventricular end-diastolic volumes; FS, left ventricular fractional shortening; NYHA, New York Heart Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockers; MRA, mineralocorticoid receptors antagonist; *P < 0.05 vs. below median Gal-3 group.*

that, Gal-3 was positively correlated with TIMP-1 ($r = 0.396$, $P < 0.001$), LVESV ($r = 0.181$, $P = 0.020$), and LVEDV ($r = 0.190$, $P = 0.015$).

Prognostic Value of Plasma Gal-3 Levels in all HFrEF Patients

During a 50-month follow-up, there were 43 deaths, 97 unplanned re-hospitalizations, and 111 composite endpoint events including death and unplanned re-hospitalizations. COX regression analysis and Kaplan-Meier analysis were performed. Following univariate Cox analysis, Gal-3 did not provide any prognostic value when all HF subjects were analyzed together (**Figure 4**; **Table 2**).

The Comparison of Baseline Characteristics of HFrHF Patients With or Without CHD

Furthermore, we performed stratified analysis, respectively, in accordance with the two subgroups: the group with CHD and the other one without CHD. Gal-3 concentration was higher in subjects with CHD than without CHD (219.30 ± 20.73 pg/mL vs. 164.21 ± 8.85 pg/mL). Within HFrEF with CHD, there were no statistical differences between two groups in terms of age, gender, hypertension history, DM history, BUN, AST, ALT, QT intervals, LVEDD, LVESD, LVEDV, LVESV, treatments, and MACEs. However, Within HFrEF with CHD and Gal-3 plasma concentrations above the median patients had lower HB but higher plasma CR and TIMP-1. Whereas, within HFrEF patients with CHD, there were no statistical differences between two groups in terms of age, gender, hypertension history, DM history, BUN, AST, ALT, QT intervals, LVEDD, LVESD, and treatments. However, within HFrEF without CHD group, patients whose Gal-3 plasma concentrations above the median had lower HB but longer HF history, higher MLHFQ and death rate, larger LVEDV and LVESV (**Table 3**).

Prognostic Value of Plasma Gal-3 Levels in HFrHF Patients With or Without CHD

By COX regression analysis and Kaplan-Meier analysis, the researchers of this study found that Gal-3 did not provide any prognostic value in participants with CHD. In contrast, as shown in **Figure 5** and **Table 4**, respectively, Gal-3 did predict prognoses in HF subjects without CHD.

DISCUSSION

Our present study revealed four primary findings. First, Gal-3 levels in plasma were different between two mouse models of cardiomyopathy (i.e., Mst1-TG mice and β2-AR TG mice). Second, the expression of Gal-3 in myocardial tissue was different between these two mouse models. Third, plasma concentrations of Gal-3 were associated with TIMP-1 and echocardiographic parameters within HFrEF patients. Finally, although plasma concentrations of Gal-3 did not predict prognoses in all HFrEF participants, it had predictive power of prognoses in HFrEF without CHD subjects.

Gal-3 is primarily expressed in macrophages and fibroblasts, and is involved in myocardial fibrosis through activation of fibroblasts (6, 22). In the present study, we found that Gal-3 was also expressed in cardiomyocytes; moreover, the

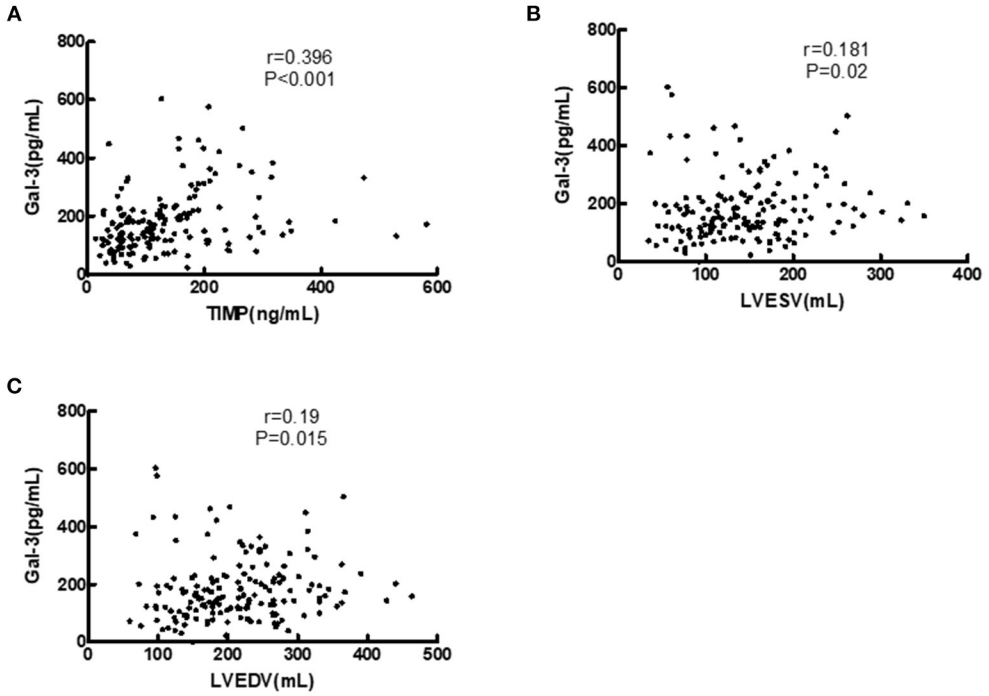

FIGURE 3 | Correlations of serum Gal-3 levels with TIMP-1, LVEDV, and LVESV in HF patients. **(A)** Correlation of Gal-3 with TIMP-1. **(B)** Correlation of Gal-3 with LVESV. **(C)** Correlation of Gal-3 with LVEDV. Gal-3, galectin-3; LVESV, left ventricular end systolic volume; LVEDV, left ventricular end diastolic volume.

expression of Gal-3 was different between two mouse cardiopathy models. In Mst1-TG mice, Gal-3 was primarily expressed in cardiomyocytes, while it was mainly expressed in myocardial interstitial cells in β2-AR TG mice. This phenomenon has also been confirmed in our previous studies. We found that Gal-3 expression was confined to the infarcted area and was localized to both non-cardiomyocytes and cardiomyocytes in infartion-reperfusion (I/R) mice model, importantly, plasma levels of Gal-3 were also transiently elevated at three-days post-infarction. However, plasma Gal-3 was not elevated, despite of the increased Gal-3 mRNA and the protein levels in myocardial tissue within TAC mice (17). As we know, macrophages were regarded as the major cell type expressing Gal-3, in addition to cardiomyocytes and fibroblasts. The expression of Gal-3 in myocardial tissue was affected by inflammation (23, 24), β-adrenaline receptor pathway (25) and the Hippo pathway (22). We (18) found that in different cardiomyopathy models (Mst1-TG mice, β2-AR-TG mice, the I/R model and mice treated with isoproterenol), the density of macrophages within myocardial tissue was different between each other. Meanwhile, the increase of plasma Gal-3 was positively correlated with the increase of macrophages in these cardiomyopathy models. These results indicated that the expression of Gal-3 in myocardial tissue and plasma Gal-3 level depended on the etiology of HF. Although only two cardiomyopathy mice models were used in this study and there were not healthy participants as control to analysis the diagnostic value of Gal-3, to match with mice studies, these results could partially respond to the diagnostic value of plasma Gal-3 in HFrEF.

Myocardial fibrosis is an important pathophysiological mechanism involved in the development and progression of chronic heart failure (CHF) (26–28). Collagen synthesis by myocardial fibroblasts is activated in CHF and is affected by many determinants [e.g., Gal-3 (29, 30) and TIMP-1 (31–33)]. Some studies had disclosed that Gal-3 could stimulate myocardial fibrosis through various mechanisms (6, 10, 15, 30, 34) and Gal-3 deficiency ameliorates fibrosis and remodeling in dilated cardiomyopathy animal models (35–37). It has been demonstrated that TIMP-1 did contribute to ventricular remodeling and myocardial fibrosis in experimental HF models (33, 38). Since Gal-3 has been linked to myocardial fibrosis, it is plausible that elevated plasma concentrations of Gal-3 may also be linked to TIMP-1. In the present study, plasma Gal-3 was positively correlated with LVEDV and LVESV in chronic HFrEF patients. Few prior studies have systematically evaluated the relationship between echocardiographic measures and blood concentrations of Gal-3. The DEAL-HF trial performed serial echocardiographic measures in 240 HF patients with NYHA Class-III and -IV symptoms and found a positive association between increased plasma concentrations of Gal-3 and changes in LVEDV, whilst there was no correlation between baseline LVEDV and Gal-3 levels (39). These previous results are different from those of our present study. This discrepancy may be related to the different research subjects in each study. All subjects in the DEAL-HF trial were

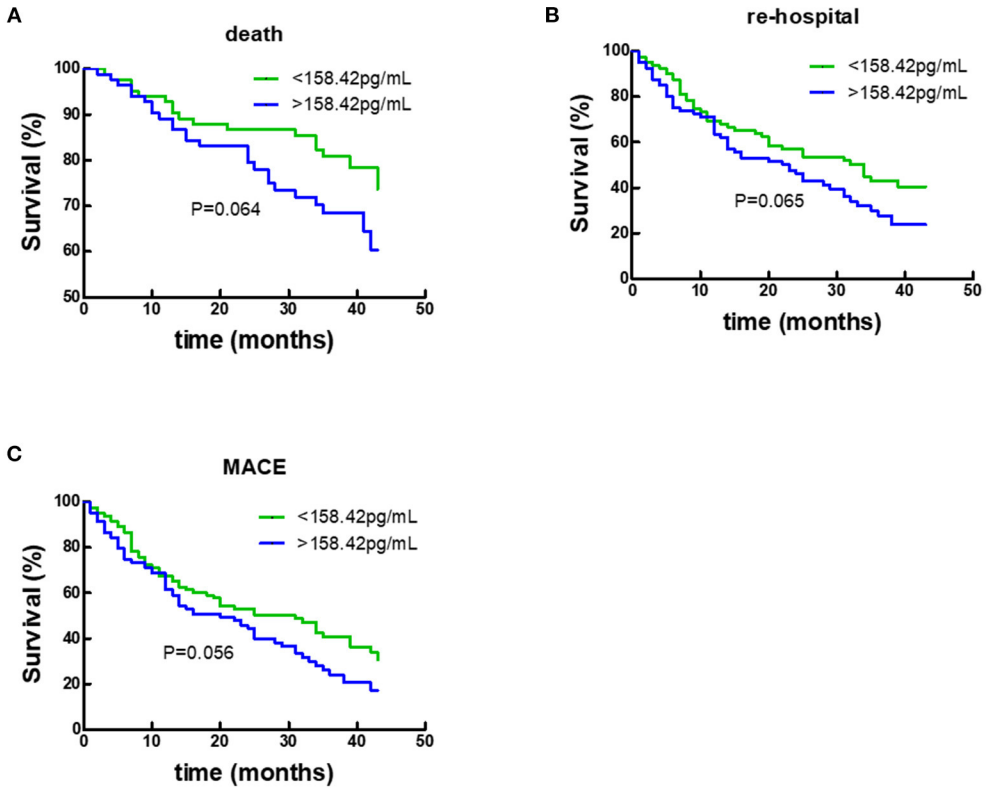

FIGURE 4 | Kaplan–Meier survival curves according to baseline plasma Gal-3 levels in all HF subjects. **(A)** Death rates according to higher of lower baseline plasma Gal-3 levels. **(B)** Re-hospitalization rate according to higher of lower baseline plasma Gal-3 levels. **(C)** Composite-endpoint event rates according to higher of lower baseline plasma Gal-3 levels. Gal-3, Galectin-3.

TABLE 2 | Predictive value of baseline plasma Gal-3 to long-term outcomes in all HFrEF patients.

	HR (95% CI)	*P*
Death	1.769 (0.957, 3.268)	0.064
Re-hospitalization	1.454 (0.968, 2.184)	0.065
Composite-endpoint event	1.433 (0.983, 2.088)	0.056

HR, hazard ratio; CI, confidence intervals.

patients with NYHA Class-III and -IV symptoms, whereas all patients in our present study exhibited NYHA class I–IV symptoms. Therefore, future studies should further investigate the roles and mechanisms of Gal-3 in myocardial fibrosis and HF.

Although there have been many studies investigating the relationship between blood levels of Gal-3 and mortality in HF patients (40–42), the predictive value of Gal-3 for the prognosis of HF remains to be illusive (43). Recently, the PARADIGM-HF trial revealed that baseline and eight-month changes in serum Gal-3 levels did not predict outcomes in HFrEF patients (44). However, based on the results of animal experiments, it is speculated that the predictive value of Gal-3 in the prognosis of HF may be related to the etiology of HF (16–18) and the specific therapies used to treat HF (25). In the present study, we found that plasma Gal-3 did not predict the mortality in all HF subjects, while it did correlate with mortality in HF without CHD subjects. As some researchers proposed, due to different etiologies and underlying pathophysiological processes, HF was a heterogeneous disease. There were some results about the comparison of inflammation between CHD and non-CHD, and inflammation was less robust in non-ischaemic HF animal models than in ischaemic HF models. Plasma Gal-3 level may represent more serious damage of cardiomyocytes. To clarify the value of Gal-3 in HF, it may require preclinical investigations in more animal models of HF in addition to clinical studies.

Limitations

We examined Gal-3 expression in mice and assessed the predictive value of blood Gal-3 on clinical endpoints in HFrEF patients. However, our present study had several limitations. First, only two cardiomyopathy mice models were used in this study, and we did not co-stain Gal-3 with macrophage or monocyte or fibroblast. Second, although 166 HFrEF patients were included, there were not healthy participants as control group to analysis the

TABLE 3 | Baseline characteristics of HFrEF with or without CHD patients.

	HFrEF with CHD ($n = 69$)		HFrEF without CHD ($n = 97$)	
	Below median Gal-3 ($n = 29$)	Above median Gal-3 ($n = 40$)	Below median Gal-3 ($n = 54$)	Above median Gal-3 ($n = 43$)
Age (years)	63.8 ± 9.7	65.7 ± 9.3	58.9 ± 8.4	61.7 ± 9.1
Gender (M/F)	20/9	27/13	35/19	23/20
Hypertension (%)	50.0	38.5	18.5	18.6
Diabetes Mellitus (%)	10.3	12.5	5.6	2.3
Smoking (%)	62.1	52.5	55.6	41.9
Alcohol consumption (%)	37.9	40.0	43.4	41.9
HF history (years)	4 (2.8)	3 (2.7)	3 (1.25, 5)	5 (3, 7.75)†
MLHFQ	26 (10.41)	26 (11.37)	26 (16.34)	31 (23.39)†
SBP (mmHg)	126 ± 21	129 ± 22	120 ± 18	115 ± 22
DBP (mmHg)	78 ± 11	80 ± 10	77 ± 12	75 ± 11
CR (umol/L)	79.7 ± 14.0	88.8 ± 20.3*	77.1 ± 10.9	80.1 ± 15.7
BUN (mmol/L)	6.5 ± 1.4	7.2 ± 2.5	6.7 ± 1.5	7.1 ± 1.9
HB (g/L)	154.3 ± 21.6	144.3 ± 15.7*	149.6 ± 21.8	139.6 ± 15.7†
AST (U/L)	22.3 ± 7.6	24.5 ± 9.7	26.1 ± 8.6	23.3 ± 9.2
ALT (U/L)	20.2 ± 11.7	22.5 ± 12.6	22.3 ± 9.9	20.5 ± 9.8
TIMP-1 (ng/mL)	127.6 ± 115.9	183.3 ± 104.9*	105.6 ± 72.1	138.6 ± 99.0
QT interval (ms)	427 ± 48	407 ± 47	416 ± 48	429 ± 51
BMI (Kg/m2)	23.2 ± 3.6	24.3 ± 3.4	22.7 ± 3.2	22.5 ± 3.9
Heart Rate (bpm)	77.9 ± 13.2	78.6 ± 20.2	80.7 ± 25.2	76.2 ± 17.2
LVEF (%)	36.2 ± 7.3	38.2 ± 6.7	34.9 ± 7.0	33.0 ± 7.8
LVESD (mm)	55.0 ± 8.9	53.6 ± 9.3	57.2 ± 8.3	60.7 ± 9.6
LVEDD (mm)	67.5 ± 8.5	67.3 ± 8.5	69.7 ± 8.3	72.8 ± 9.6
LVESV (ml)	127 ± 57	137 ± 64	140 ± 54	172 ± 73†
LVEDV (ml)	190 ± 78	204 ± 93	204 ± 70.	244 ± 85†
FS (%)	18.0 ± 4.6	19.2 ± 5.1	17.7 ± 3.8	16.5 ± 4.1
NYHA functional class				
I	31.0	17.5	14.8	4.7
II	55.2	55.0	51.9	65.1
III	10.3	25.0	29.6	27.9
IV	3.4	2.5	3.7	2.3
β-blocker (%)	72.4	80.0	83.3	83.7
ACEI/ARB (%)	82.8	90.0	94.4	88.4
MRA (%)	72.4	82.5	74.1	73.7
Digoxin (%)	24.1	32.5	16.7	23.3
Diuretics (%)	48.3	52.5	59.3	65.1
Death rate (%)	20.7	22.5	20.4	39.5†
Re-hospitalization rate (%)	58.6	64.1	49.1	66.7
Composite-endpoint event (%)	65.5	70.0	59.3	74.4

*Data are mean (SD) or n (%) unless otherwise stated. F, female; M, male; MLHFQ, the Minnesota Living with Heart Failure Questionnaire; SBP, systolic blood pressure; DBP, diastolic blood pressure; CR, creatinine; BUN, urea nitrogen; HB, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TIMP-1, tissue inhibitor of metalloproteinases 1; BMI, body mass index; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic dimension; LVEDD, left ventricular end-diastolic dimension; LVESV, left ventricular end-systolic volumes; LVEDV, left ventricular end-diastolic volumes; FS, left ventricular fractional shortening; NYHA, New York Heart Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blockers; MRA, mineralocorticoid receptors antagonist; *$P < 0.05$ vs. below median Gal-3 patients with CHD; †$P < 0.05$ vs. below median Gal-3 patients without CHD.*

diagnosis value of Gal-3, which leads to a mismatch with mice studies. Third, the sample size that was small, needs to be enlarged in the future. Finally, we only assessed baseline Gal-3 concentrations in the present study, whereas broad assessment such as concentrations after treatments could be performed.

CONCLUSIONS

Although plasma concentrations of Gal-3 were associated with TIMP-1 and echocardiographic parameters, the diagnostic and prognostic value of plasma Gal-3 in HFrEF were decided by the etiology of HF.

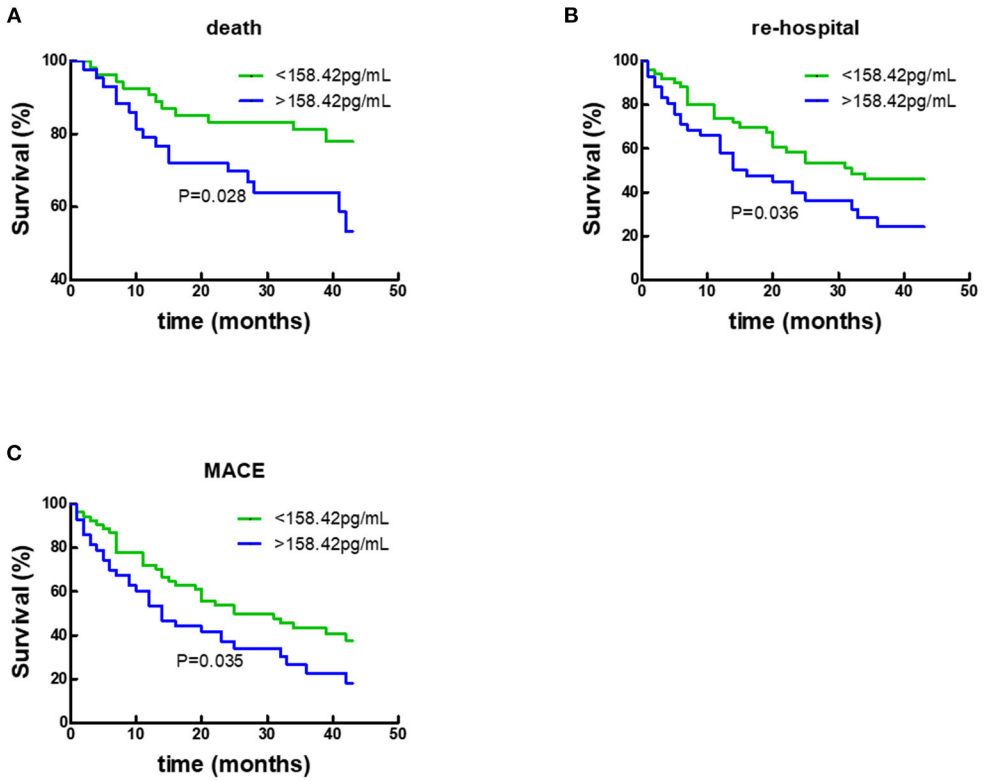

FIGURE 5 | Kaplan–Meier survival curves according to baseline plasma Gal-3 levels in non-CHD subjects. **(A)** Death rates according to higher of lower baseline plasma Gal-3 levels. **(B)** Re-hospitalization rates according to higher of lower baseline plasma Gal-3 levels. **(C)** Composite-endpoint event rates according to higher of lower baseline plasma Gal-3 levels. Gal-3, Galectin-3.

TABLE 4 | Predictive value of baseline plasma Gal-3 to long-term outcomes in HFrEF with or without CHD patients.

	HFrEF without CHD patients		HFrEF with CHD patients	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Death	2.292 (1.071, 4.905)	0.028	1.899 (0.664, 5.435)	0.232
Re-hospitalization	1.756 (1.021, 3.018)	0.036	1.473 (0.799, 2.716)	0.215
Composite-endpoint event	1.673 (1.022, 2.740)	0.035	1.545 (0.858, 2.780)	0.147

HFrEF, heart failure with reduced ejection fraction; CHD, coronary heart disease; HR, hazard ratio; CI, confidence intervals.

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 First Affiliated Hospital of Xi'an Jiaotong University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Xi'an Jiaotong University.

AUTHOR CONTRIBUTIONS

QL, X-JD, LB, and A-QM designed the study. QL, S-PC, YW, TL, Y-BX, JL, and XH followed up with the included patients. QL and Y-DS performed the experiments. QL and R-CZ collected and analyzed the data. QL prepared the manuscript. All authors read and approved the final manuscript.

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α-Linolenic Acid and Risk of Heart Failure: A Meta-Analysis

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Background: The α-linolenic acid is a plant origin n-3 fatty acid that may reduce the risk of cardiovascular disease. However, the effect of α-linolenic acid (ALA) on the risk of heart failure (HF) remains unclear. In this meta-analysis, we aimed to determine the role of ALA in the risk of incident HF.

Methods: Electronic databases were searched for studies up to August 10, 2021. Studies were included for meta-analysis if the adjusted risk of HF in different dietary intake or circulating levels of ALA was reported. We used the random-effects model to calculate the estimated hazard ratios (HRs) and 95% CI for higher ALA.

Results: A total of 6 studies (7 cohorts) comprising 135,270 participants were included for meta-analysis. After a median follow-up duration of 10 years, 5,905 cases of HF were recorded. No significant heterogeneity was observed among all the included studies. Random-effects model analyses showed that there was no significant association between ALA and the risk of incident HF, either assessed as quintiles (highest quintile vs. lowest quintile: HR = 0.95, 95% CI = 0.86–1.06) or per 1 *SD* increment (HR = 0.99, 95% CI = 0.95–1.01). Furthermore, we did not observe any association between ALA and the risk of HF in subgroup analyses performed according to age, sex, follow-up duration, and measuring method of ALA.

Conclusions: We found no association between ALA and the risk of incident HF, suggesting that ALA might not be effective in the prevention of HF.

Keywords: α-linolenic acid, fatty acid, heart failure, risk, meta-analysis

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INTRODUCTION

Heart failure has become a growing global public health burden, with high rates of re-hospitalisation and mortality. Heart failure (HF) incidence increases dramatically with a growing ageing population, it was estimated that nowadays, there were more than 37.7 million HF patients worldwide (1). Although there have been significant improvements in the management of HF, the morbidity and mortality in HF patients were still high, which underscores the importance of the identification of novel risk factors and treatment modalities of HF (2–4).

Long-chain (LC) omega-3 polyunsaturated fatty acids (n-3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may play a protective role in the incidence and progression of HF, (5–7) through multiple mechanisms including anti-inflammation effects, improving cardiomyocytes' energy metabolism endothelial function (8).

However, considering the sustainable supply and safety of the marine sources, plant-based n-3 PUFA is gaining much attention recently and is considered as an important alternative to n-3 PUFA sources (9). α-linolenic acid (ALA), a plant origin essential n-3 fatty acid, can be converted to EPA and DHA. Therefore, it is important to explore whether ALA has similar effects on the risk of HF. However, although previous studies showed that dietary patterns that included ALA-rich foods may reduce the risk of cardiovascular disease (10, 11), the association between ALA and HF was unclear. The Physicians' Health Study has found that dietary ALA intake level showed an inverse trend towards an association with HF, (12) while other studies did not find a similar association (13–15). To address the knowledge gap, we performed a pooled analysis of observational studies to generate data on the associations between ALA and the risk of incident HF.

METHODS

Databases Search Strategy and Inclusion Criteria of Studies

We performed the meta-analysis following the recommendations of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group (16). Electronic databases, including PubMed, Google scholar, Cochran Library, and EMBASE, were searched for studies from inception until 31 July 2021, using a combined text and medical subheadings (MeSH) search strategies, with multiple terms related to "Omega-3 PUFA" or "α-linolenic acid" and "heart failure". We were restricted to human studies. Reference lists of the related articles and included studies were manually reviewed to identify potentially missed pieces of research. The detailed strategy used for searching PubMed is presented in **Supplementary File 1**. When searching other databases, the strategies were similar.

We included observational studies for meta-analysis if they met the following criteria: (1) observational studies (including cohort studies, nest case-control studies, and *post hoc* analyses of randomised controlled trials) with adult participants (aged ≥18 years); (2) dietary intakes or circulating levels of ALA were detected; (3) the risk of HF associated with different levels of ALA were reported.

We excluded studies if: (1) they were cross-sectional studies; (2) the risk of HF was unadjusted for confounders; (3) the follow-up duration was <1 year; (4) duplicated articles were reported from the same cohort dataset. If more than one report used data from the same participants' cohort, only the latest published article was included.

Data Extraction and Quality Assessment of the Included Studies

Two investigators (J.W. and M.Q.) independently conducted article searches and screened the retrieved articles. Full copies of potentially suitable studies were reviewed. Key information of the included studies, such as country, cohort characteristics, methods for measurement of ALA, study sample, sex proportion, mean/median age, HF cases, and follow-up duration was recorded. If the essential message was not reported, we contacted the principal authors for clarification.

The Newcastle–Ottawa Quality Assessment Scale for observational cohort studies was used to determine the study quality (17), which is based on: the selection (3 items with 1 point each), comparability (1 item with up to 2 points), and exposure/outcome (3 items with 1 point each), with the total score from 0 to 9. Included studies were classified as good quality (≥7 points), fair quality (4–6 points), or poor (<4 points), respectively (18, 19).

Data Synthesis

The outcome evaluated in this analysis was the risk of incident HF associated with different levels of dietary intake or circulating ALA. We extracted outcome data which adjusted for the maximal number of potential confounders for analysis. In the included studies, the associations of the HF risk and ALA levels were reported in different ways, such as per quartiles or quintiles of ALA levels, or per 1 *SD* increment in the continuous trait. Therefore, we used two methods to transfer the data into consistent comparisons. We compared the hazard ratio (HR) of HF risk in participants with the highest quintile of ALA with those with the lowest quintile and calculated the HR as per 1 *SD* increment in ALA. If effect measures were not reported as per quintile or per 1 *SD* change in the included studies, we converted the data following the previously published methods (20). Briefly, we assumed that the exposure variable (ALA) was with normal distribution and its association with the interesting outcome (risk of HF) was log-linear. The expected difference in means of the highest vs. lowest tertile is 2.18 *SD*s, and 2.54 *SD*s for the highest vs. lowest quartile, 2.8 *SD*s for the highest vs. lowest quintile, respectively (20). Therefore, HR reported as top vs. bottom quintile comparison can be converted to per 1 *SD* change by using a division conversion factor of 2.8 to the log HR. Accordingly, HRs for comparisons of extreme tertiles and quartiles are divided by 2.18 and 2.54, respectively. Conversely, HRs and 95% CIs reported as comparisons of quartiles or tertiles can be converted to comparisons of quintiles, by using a multiplication conversion factor of 2.8/2.54 or 2.8/2.18 to the log HRs.

The inverse variance approach was used to calculate the log HRs and corresponding SEs with the random-effects models (21). The I^2 statistics were used to test heterogeneity. An I^2 value > 50% was regarded as with significant heterogeneity among the included studies (2). Subgroup analyses of the primary outcome were conducted according to sex, age, follow-up period, and measure of ALA. We evaluated the publication bias by inspecting the funnel plots, as well as by using Egger's test and Begg's test. Finally, we performed the sensitivity analyses by changing the random-effects model into the fixed-effects model in the pooled analysis. We also recalculated the HRs by excluding one study at a time to determine the effect of every study on the pooled risk.

All analyses were performed using Stata 15.0 (StataCorp LP, College Station, TX, USA) and RevMan 5.3 (The Cochrane Collaboration, Copenhagen, Denmark). All *P*-values are two-tailed, and a $P < 0.05$ is considered as with statistical significance.

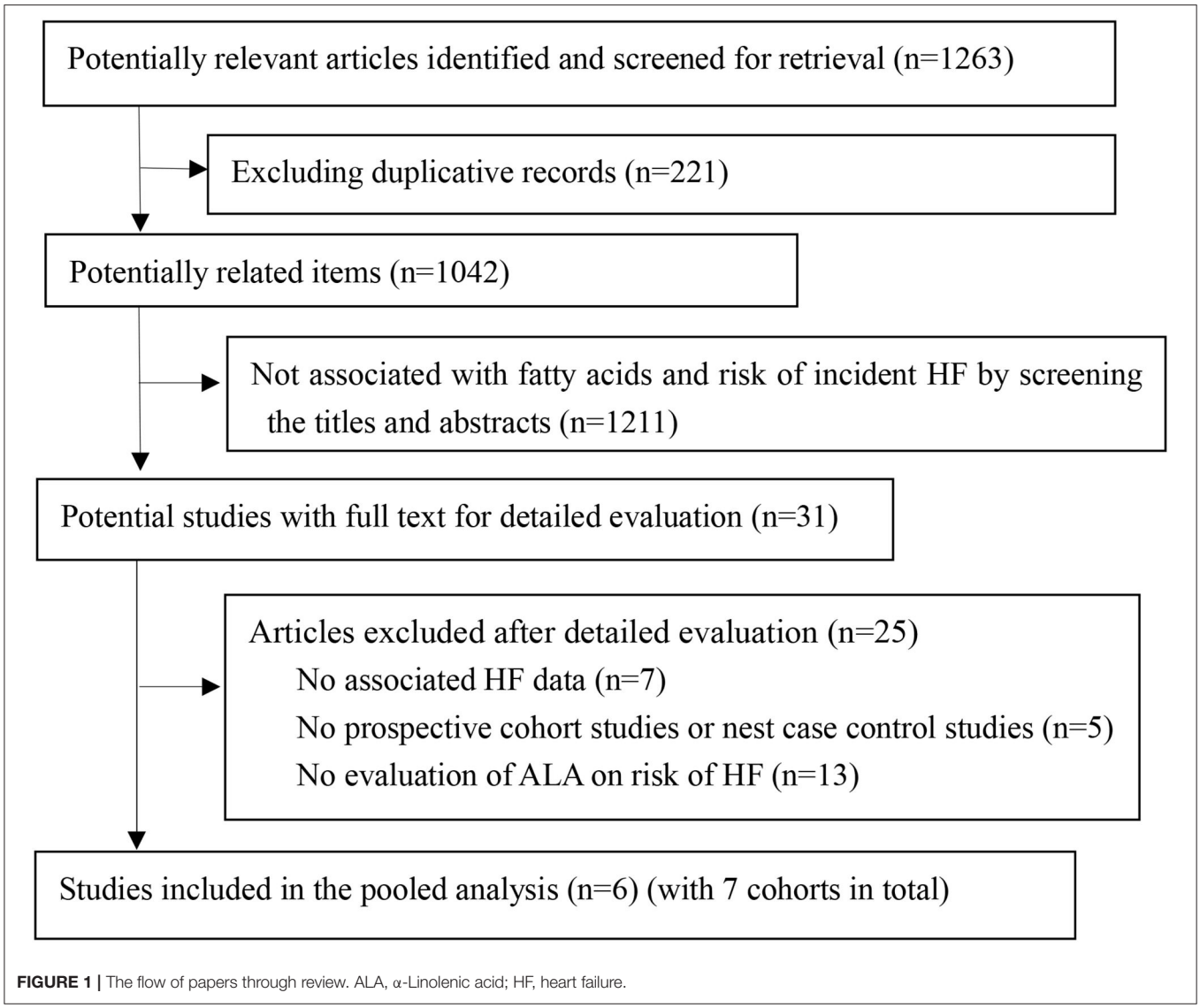

FIGURE 1 | The flow of papers through review. ALA, α-Linolenic acid; HF, heart failure.

RESULTS

Studies Characteristics and Quality

We retrieved 1,263 article items from the searched electronic databases. After removing duplication records, two authors independently screened the titles and abstracts. Thirty-one articles were fully evaluated, and 6 studies were included in the pooled analysis finally (**Figure 1**). In the included studies, two of them reported dietary intake of ALA (14, 22), 3 reported circulating ALA level (12, 15, 23), and only one study reported both dietary and circulating ALA level (13). The 6 studies (7 cohorts) included 135,270 participants for analysis, with their main characteristics resented in **Table 1**. One study only enrolled men, two studies only enrolled women, while all other studies enrolled both genders. After the median follow-up duration of 10 years (ranged from 8.4 to 14.3 years), 5,905 cases of HF were recorded. The adjusted confounders for HF risk in the pooled studies are presented in **Supplementary File 5**. Only one study was graded as fair, and all other 5 studies were considered as of good quality according to our predefined quality assessment criteria (**Supplementary File 3**).

ALA and Risk of Incident HF

No statistical heterogeneity observed among all the included studies for the association between ALA and incident HF (I^2 = 0%, P = 0.77). Random-effects model analyses showed that there was no significant association between ALA and risk of incident HF, either assessed as quintiles (**Figure 2**, highest quintile vs. lowest quintile: HR = 0.95, 95% CI = 0.86–1.06, P = 0.36) or per 1 *SD* increment (**Figure 3**, HR = 0.99, 95% CI = 0.95–1.01, P = 0.36). We did not find any evidence of publication bias by the funnel plot (**Supplementary Files 4**, **5**), Egger's or Begg's tests (both $P > 0.1$).

TABLE 1 | Characteristics of studies on ALA and risk of HF.

Study	Country/Region	Cohort characteristics	Measure of ALA (method)	Sample size (%women)	HF case (n)	Age (y), average (range or SD)	Follow-up (y)
Djousse et al. (23)	United States	Community residents	Serum (GC)	2003 (61.2%)	655	77.7 (4.4)	9.7
Wilk et al. (12)	United States	Healthy male physicians	Plasma Phospholipid (GC)	1576 (0%)	786	58.7 (NA)	8.4
Lemaitre et al. (13)	United States	Community residents	Dietary (FFQ)	4432 (NA)	1072	≥65 y (NA)	11.9
Lemaitre et al. (13)	United States	Community residents	Plasma Phospholipid (GC)	2957 (63.2%)	686	72.1 (5.2)	10.4
Belin et al. (14)	United States	Postmenopausal women	Dietary (FFQ)	84493 (100%)	1858	63.3 (50-79)	10.0
Levitan et al. (22)	Sweden	Swedish women	Dietary (FFQ)	36234 (100%)	651	61.6 (48-83)	9.0
Yamagishi et al. (15)	United States	Community residents	Plasma Phospholipid (GLC)	3575 (53.4%)	197	53.7 (5.6)	14.3

ALA, α-linolenic acid; ARIC, Atherosclerosis Risk in Communities; FFQ, food frequency questionnaire; GC, gas chromatography; GLC, gas-liquid chromatogram; HF, heart failure; NA, not available.

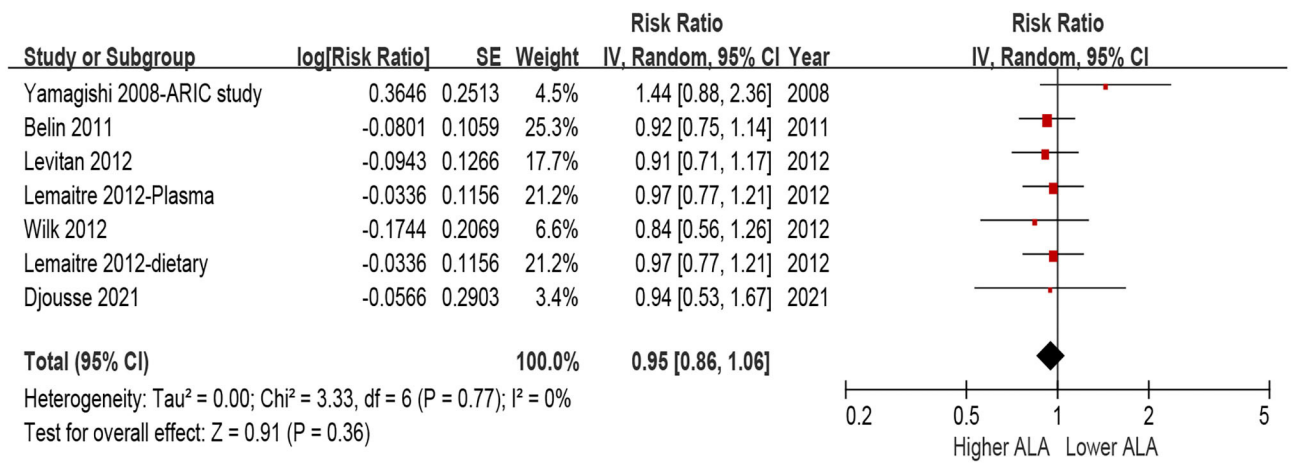

FIGURE 2 | Forest plot for risk of HF associated with ALA level by quintiles. ALA, α-Linolenic acid; CI, confidence interval; HF, heart failure.

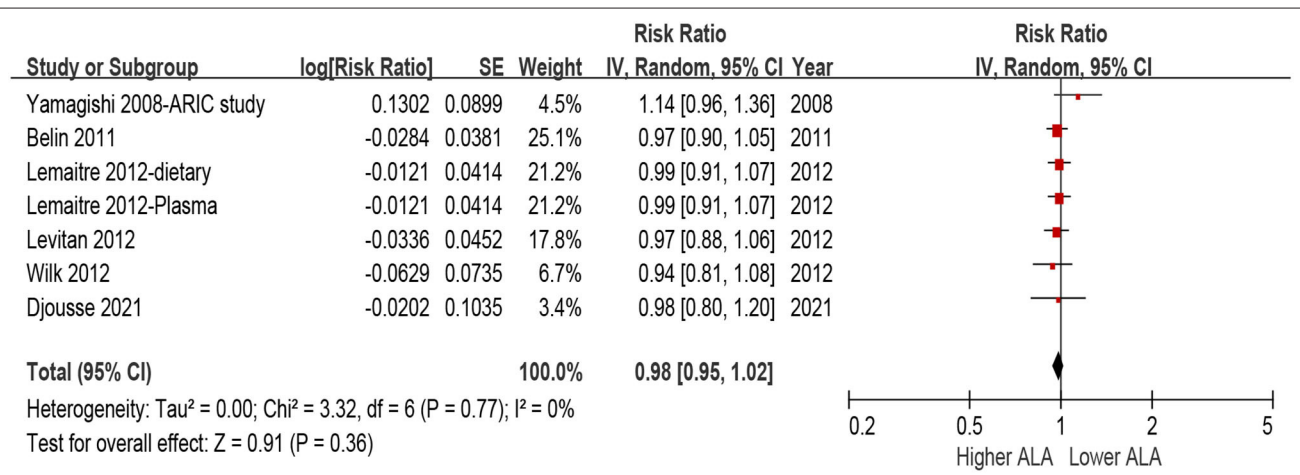

FIGURE 3 | Forest plot for risk of HF associated with ALA level by per 1 SD increment. ALA, α-Linolenic acid; CI, confidence interval; HF, heart failure.

Sensitivity Analyses and Subgroup Analyses

We conducted several sensitivity analyses to confirm the HF outcome was consistent by using the fixed-effects models instead of random-effects models for meta-analysis or recalculating the estimated HRs and 95% CI by excluding one study at each time. Furthermore, in subgroups performed according to age, sex, follow-up duration, and measuring method of ALA, we did not observe any significant association between ALA levels and the risk of incident HF. Furthermore, no significant heterogeneity was observed among all subgroup comparisons (all $I^2 = 0\%$, $P \geq 0.48$, **Table 2**).

DISCUSSION

To the best of our knowledge, this is the first pooled analysis evaluating the association between ALA and the risk of incident HF. We did not find any association between ALA (neither detected by dietary intake nor circulating level) and the risk of HF. The null result was further supported by no heterogeneity observed among multiple subgroup comparisons.

Basic research on ALA on cardiac function had yielded inconsistent results. In a mice/rat model with doxorubicin-mediated cardiotoxicity, the prophylactic administration of the ALA was cardioprotective by decreasing inflammation, apoptosis, and mitochondrial dysfunction (24, 25). Enrichment of ALA in rodent diet reduced oxidative stress and inflammation, resulted in less left ventricle dilation, and decreased myocardial fibrosis during myocardial infarction (26, 27). In diabetic or obese mice, dietary ALA counters cardioprotective dysfunction mainly through anti-inflammatory effects (28, 29). ALA may also confer beneficial anti-platelet and anti-inflammatory effects, and interaction with gut microbiota, which is significantly associated with the risk HF (20, 30). However, these effects were not observed in a rat model under pressure overload conditions (31).

In humans, evidence suggests that increasing ALA is associated with favourable cardiometabolic status and lower inflammation status (32, 33), which may play a protective role in cardiac function. Data from NHANES found that ALA and DHA can enhance the negative association between maximal oxygen uptake and C-reactive protein, suggesting that the anti-inflammatory response to maximal oxygen uptake capacity is related to levels of ALA and (34). However, in a randomised clinical trial, it was also found that increasing plasmatic ALA had no impact on plasma inflammatory biomarkers (35). Taking these studies with our results together indicates that humans may not benefit from ALA intake in the view of prevention of HF.

In contrast, marine n-3 PUFA had been shown to be protective for HF (12, 13, 36). In humans, ALA can be bioconverted into EPA and DHA, however, the rate is not sufficiently. The rate of bioconversion from ALA to DHA is about 0.05–0.5%, and to EPA is 0.2–10% (9). The limited bioconversion rate may limit the potential cardioprotective effects. Furthermore, this conversion rate from ALA to EPA/DHA was influenced by sex and genetic factors (especially for variants of desaturase and elongase enzymes), as well as dietary intake level of EPA/DHA. In a Danish cohort with 53,909 participants followed for a median of 13.4 years, an inverse association between dietary ALA intake and the risk of CVD was observed in individuals with a low intake of marine n-3 PUFA, while not in those with a higher intake of marine n-3 PUFA. These findings suggested that ALA may be associated with a lower risk of CVD only in those individuals with not adequate intake of marine n-3 PUFA (37). However, whether the relationship between ALA and the risk of incident HF was similar remains unexplored.

Our study has several major strengths. First, most of the included studies were adjusted for multiple confounders, including traditional cardiovascular risk factors and energy intake. This may mitigate the effect of potential confounders influencing the results. Second, the associations were consistently determined by category (highest quintile *vs.* lowest quintile)

TABLE 2 | Subgroup analyses of the association between ALA and risk of HF.

	Studies (N)	Quintile 5 vs. Quintile 1		Per 1 SD increment	
		HR (95%CI)	*P* value*/I^2	HR (95%CI)	*P* value*/I^2
Average age (years)					
<65	4	0.94 [0.81, 1.10]	0.85/0%	0.99 [0.93, 1.04]	0.85/0%
≥65	3	0.97 [0.83, 1.13]		0.98 [0.93, 1.03]	
Sex					
Female	2	0.92 [0.78, 1.08]	0.69/0%	0.97 [0.92, 1.03]	0.68/0%
Male	1	0.84 [0.56, 1.26]		0.94 [0.81, 1.08]	
Follow-up duration (years)					
<10 years	3	0.90 [0.74, 1.09]	0.48/0%	0.96 [0.90, 1.03]	0.48/0%
≥10 years	4	0.98 [0.86, 1.10]		0.99 [0.95, 1.04]	
Measure of exposure					
Dietary	3	0.92 [0.81, 1.04]	0.51/0%	0.98 [0.94, 1.02]	0.68/0%
Circulating	4	0.99 [0.83, 1.18]		1.00 [0.94, 1.06]	

**For heterogeneity among subgroups. ALA, α-linolenic acid; CI, confidence intervals; HF, Heart failure; HR, hazard ratios; SD, standard deviation.*

or continuous (per 1-*SD*) increment of ALA. Furthermore, no heterogeneity was observed among the included studies ($I^2 = 0\%$) and pooled results were also consistent among comprehensive subgroup analyses. However, some limitations of the current study should be discussed. First, most of the included studies were from the United States, and only one study was from Sweden. The association between ALA and incident HF was still unclear in other populations, especially those with different dietary patterns. Second, we performed this meta-analysis based on the study level. No individual participant data were available, so residual bias cannot be totally avoided. Third, the cardio-protective effects of ALA may be modified by different intakes of LC n-3 PUFAs (37). However, only two studies adjusted the level of DHA and EPA in the analysis. Finally, only one measurement of ALA at baseline was detected in most of the included studies, and the change of ALA overtime was not accounted for. However, the Physicians' Health Study showed that the use of single baseline level ALA, or mean level of between baseline and long-term follow-up measures (up to 15 years), yielded similar associations (both null) on their relationship with HF (38).

CONCLUSIONS

We did not find any association between ALA (measured either from dietary questionnaires or with a circulating biomarker) and the risk of incident HF. These results suggest that plant origin ALA cannot be regarded as a substitute for LC n-3 fatty acids in the viewpoint for the prevention of HF. Further studies with other populations (e.g., Asians, Africans) are needed to determine whether a high intake of ALA can prevent HF.

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

JWu, MQ, LS, D-lL, JWe, and YH: research idea and study design. JWu, MQ, and LS: data acquisition. JWu and MQ: data analysis and interpretation. JWu and LS: statistical analysis. JWu and YH: supervision and mentorship. All authors contributed important intellectual content during manuscript drafting or revision and accept accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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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.2021.788452/full#supplementary-material

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

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
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E/E′ Is a New Independent Predictor of Recovered Ejection Fraction in Patients With Systolic Heart Failure Undergoing Ablation for Atrial Fibrillation

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Aims: Catheter ablation should be considered in patients with atrial fibrillation (AF) and with heart failure (HF) with reduced ejection fraction (EF; HFrEF) to improve survival and reduce heart failure hospitalization. Careful patient selection for AF ablation is key to achieving similar outcome benefits. However, limited data exist regarding predictors of recovered ejection fraction. We aimed to evaluate the predictors of recovered ejection fraction in consecutive patients with HF undergoing AF ablation.

Methods and Results: A total of 156 patients [67.3% men, median age 63 (11)] with AF and HF underwent initial catheter ablation between September 2017 and October 2019 in the First Affiliated Hospital of Dalian Medical University. Overall, the percentage of recovered ejection fractions was 72.3%. Recovered EFs were associated with a 39% reduction in all-cause hospitalization compared to non-recovered EFs at the 1-year follow-up [23.8 vs. 62.8 (odds ratio) OR 2.09 (1.40–3.12), $P < 0.001$]. Univariate analysis for recovered EFs showed that diabetes ($P = 0.083$), prevalent HF ($P = 0.014$), prevalent AF ($P = 0.051$), LVEF ($P = 0.022$), and E/E′ ($P = 0.001$) were associated with EF improvement. Multivariate analysis showed that the only independent predictor of EF recovery was E/E′ [OR 1.13 (1.03–1.24); $P = 0.011$]. A receiver operating characteristic analysis determined that the suitable cut-off value for E/E′ was 15 (sensitivity 38.7%, specificity 89.2%, the area under curve 0.704).

Conclusions: Ejection fraction (EF) recovery occurred in 72.3% of patients, associated with a 39% reduction in all-cause hospitalization compared to the non-recovered EFs in our cohort. The only independent predictor of recovered EF was E/E′ < 15 in our series.

Keywords: atrial fibrillation, E/E′, recovered ejection fraction, heart failure, catheter ablation

INTRODUCTION

The atrial fibrillation epidemic has been closely linked to a concomitant rise in heart failure (HF) morbidity and mortality (1). The estimated incidence of HF among patients with atrial fibrillation (AF) is 1.58 to 4.4 per 100 person-years. HF and AF often coexist in clinical practice (2). When present in combination, AF and HF portend a worse prognosis than either condition alone.

Recent randomized controlled trials (RCTs) reported clinical improvements in mortality, HF hospitalizations, left ventricular ejection fraction (LVEF), and quality of life in patients with HFrEF (HF with reduced ejection fraction) who had AF ablation (3–5). The Catheter Ablation vs. Medical Rate Control in AF and Systolic Dysfunction (CAMERA-MRI) and Pulmonary-Vein Isolation for AF Patients with HF (PABACHF) trials reported that 58–76% of patients had normalization of EF after AF ablation compared with patients receiving another medical therapy (6, 7). CASTLE-AF was the largest RCT to compare the hard endpoints between ablation and medical therapy in patients with AF and HF (8). Catheter Ablation versus Standard Conventional Therapy in Patients with Left Ventricular Dysfunction and Atrial Fibrillation (CASTLE-AF) revealed a benefit of mortality and HF hospitalizations in AF ablation patients. However, only a small number of selected patients underwent AF ablation in these trials, the largest one having only 179 patients randomized to the AF ablation group. The AF Management in Congestive HF with Ablation (AMICA) trial was a large RCT to compare the absolute increase in LVEF from baseline at 1 year between ablation and the best medical therapy in patients with persistent AF and HF (9). The AMICA trial did not reveal any benefit of AF ablation in patients with AF and advanced HF. These controversial results raised the issue that stratification for AF in HF patients remains challenging in clinical practice. Patients with HF and AF benefit the most from catheter ablation should be fully evaluated.

In this study, we carried out a retrospective study to evaluate predictors of LVEF recurrence after ablation for AF in systolic HF patients.

METHODS

Patient Selection

The protocol was reviewed and approved by the ethics committee of the First Affiliated Hospital of Dalian Medical University. Patients presenting with a documented episode of symptomatic AF and systolic HF (LVEF $< 50\%$) were eligible for enrollment in the study. Potential clinical predictors were analyzed, including age, sex, type of AF, CHA2DS2-VASc score, complications, echocardiogram characteristics, and health history.

Atrial fibrillation (AF) classification was defined as:

Paroxysmal AF: Self-terminating, in most cases within 48 h. Some AF paroxysms may continue for up to 7 days. AF episodes that are cardioverted within 7 days should be considered paroxysmal.

Persistent AF: AF that lasts longer than 7 days, including episodes terminated by cardioversion, either with drugs or by direct current cardioversion, after 7 days or more.

Catheter Ablation Strategy

The indications for AF ablation procedures and periprocedural anticoagulation were in accordance with the current guidelines. Preprocedural left atrial CT was performed to evaluate the anatomy of the pulmonary veins. Immediately before the ablation procedure, the presence of a left atrial appendage thrombus was excluded with transesophageal echocardiography. In patients undergoing catheter ablation, circumferential PV isolation was mandatory as the primary method. Additional ablation techniques, including the creation of linear lesions, ablation of complex fractionated atrial electrograms, or combinations thereof, were left to the investigator's discretion for a secondary

TABLE 1 | Characteristics of patients at baseline.

	***n* = 156**
Age, mean (SD), years	63.7 (11.0)
Sex, no. (%)	
Male	67.3%
Female	32.7%
Type of AF, no. (%)	
Paroxysmal	30.8%
Persistent	65.4%
Long-standing	4.8%
Duration of AF, mean (SD), years	3.3 (5.0)
Hypertension, no. (%)	73 (46.8)
Post MI, no (%)	16 (10.3)
Dilated cardiomyopathy, no. (%)	5 (3.2)
Hypertrophic cardiomyopathy, no. (%)	1 (0.6)
Ischaemic cardiomyopathy, no. (%)	11 (7.1)
Stroke, transient ischaemic attack, peripheral embolism, no. (%)	16 (10.9)
Diabetes, no. (%)	28 (17.9)
Coronary heart disease, no. (%)	21 (13.5)
Valvular heart disease, no. (%)	16 (10.3)
CHA_2DS_2-VASc score, mean (SD)	2.37 (1.45)
MYHA class, no. (%)	
I	28 (17.9)
II	52 (33.3)
III	62 (39.7)
IV	14 (9.0)
LVEF, mean (SD), %	37.9 (7.8)
NT-proBNP, mean (SD), pg/ml	640 (934.8)
TnI, mean (SD), ng/ml	0.06 (0.17)
D-dimer, mean (SD), mg/l	0.61 (1.26)
Oral anticoagulation (%)	152 (97.4)
β blocker used (%)	66 (42.3)
ARNI used (%)	71 (45.2)
ACEI/ARB used (%)	67 (42.9)

AF, atrial fibrillation; LVEF, left ventricular ejection fraction; MI, myocardial infarction.

ablative approach. The endpoint of the ablation strategy was complications of the PVI and/or additional lesions. Mapping was performed with the Carto 3D cardiac mapping system (CARTO, Biosense Webster, Inc., US).

Recovered Ejection Fraction

A working definition of RecEF that is consistent with the majority of studies in the literature includes the following: (1) Documentation of a decreased LVEF < 40% at baseline; > 10% absolute improvement in LVEF and the second measurement of LVEF > 40% (10). (2) Documentation of a decreased LVEF 40–50% at baseline and the second measurement of LVEF > 50%. (3) LVEF measurements were obtained under sinus rhythm (SR) or AF at baseline (30.8% under SR, 69.2% under AF). After ablation, a second measurement was obtained at sinus rhythm (78.2% under SR, 21.8% under AF).

Follow-Up and Echocardiograms

For the whole 1-years follow-up (FU), all patients were monitored in the out-patient department of our institution. Oral anticoagulation was uninterrupted during the follow-up. A designated follow-up clinic completed the follow-ups. Patients had follow-ups in the postoperative months at 1, 3, 6, and 12 *via* clinic visit. ECG or 24-h Holter and echocardiograms were obtained at the clinic visit. Echocardiograms were centrally assessed at our echocardiography laboratory. LVEF assessment was originally intended to use contrast echocardiography at sinus rhythm. All patients underwent standardized, 2D non-contract transthoracic echocardiographic imaging. LVEF was determined according to the Simpson rule from left ventricular end-diastolic and end-systolic volumes in apical 5- and 2-chamber views. All causes of death and hospitalization were obtained at 12 months.

Statistics

Baseline characteristics were summarized as means and *SD*s for continuous variables or frequency numbers and percentages for categorical variables. Differences between the two groups were estimated with 2-sample *t*-tests for continuous variables or chi-square analyses for categorical variables. Logistics proportional hazards models were used to adjust for differences in baseline characteristics or pertinent covariates on outcomes. We estimated univariable and multivariable models, hazard ratios (HRs), and their relative 95% CIs were derived. Covariates selected for multivariable models were based on significant variables in the univariable analyses and entered into models stepwise. The multivariate analysis and logistic regression were conducted to evaluate the predictors. A two-tailed $P < 0.05$ was considered significant. All statistical analyses were performed using IBM SPSS Statistics software version 24 (SPSS Inc., IBM, Somers, New York, USA).

RESULTS

Characteristics of Enrolled Patients

During the 3 years, 156 patients with AF and systolic HF (LVEF < 50%) were enrolled in the study. The median age was 64 years (53–69 years), and 33% were females. The average LVEF was 37.9%. Baseline characteristics for the study population are shown in **Table 1**.

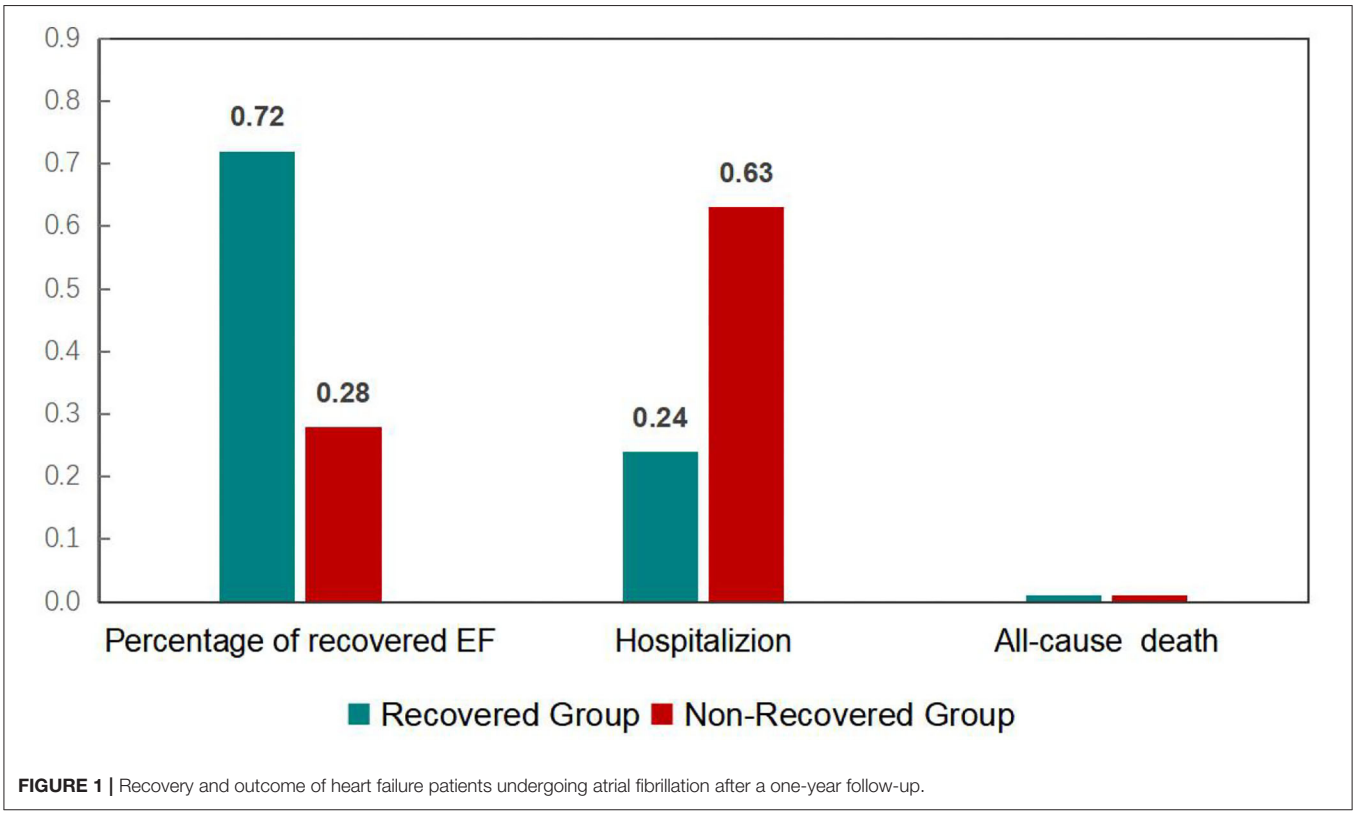

FIGURE 1 | Recovery and outcome of heart failure patients undergoing atrial fibrillation after a one-year follow-up.

Recovery and Outcome of Heart Failure After Atrial Fibrillation Ablation

Left ventricular ejection fraction (LVEF) recovery occurred 1.5 ± 0.3 month after ablation was performed. The recovered and ablation outcomes are shown in **Figure 1** and **Table 2**. The overall number of patients of LVEF recovered was 113 (72%). The mean LVEF was improved from 38% to 57% in the recovered group ($P < 0.001$), and the mean LVEF was not significant in the non-recovered group (from 0.36 to 0.38). After the 1-year follow-up, 25 (24%) hospitalizations occurred in the recovered group, and 27 (63%) hospitalizations occurred in the non-recovered group. The difference was significant [(odds ratio) OR 2.09 (1.40–3.12), $P < 0.001$]. No death occurred during the follow-up.

Predictors of LVEF Recovered

We analyzed patients' baseline characteristics and procedural characteristics to evaluate the predictors of LVEF recovery (**Tables 1**, **2**). Univariate analysis for recovered LVEF showed that diabetes ($P = 0.083$), prevalent HF ($P = 0.014$), prevalent AF ($P = 0.051$), LVEF ($P = 0.022$), and E/E′ ($P = 0.001$) were associated with LVEF improvement. Multivariate analysis

TABLE 3 | Multivariate analysis of predictors of recovered ejection fractions.

Examined parameters	OR (95% CI)	*P*
Diabetes	0.23 (0.04–1.42)	0.115
Prevalent HF	0.35 (0.07–1.78)	0.205
Prevalent AF	0.45 (0.16–1.22)	0.115
LVEF	0.98 (0.92–1.05)	0.564
E/E′	1.13 (1.03–1.24)	0.011

AF, atrial fibrillation; HF, heart failure; prevalent AF, existing AF and new-onset HF; prevalent HF, existing HF and new-onset AF.

TABLE 2 | Univariate analysis of predictors of recovered ejection fractions.

	Recovered group ($n = 113$)	Non-recovered group ($n = 43$)	OR (95%CI)	*P*
Age, mean (SD), years	62.8 (11.2)	65.9 (10.1)		0.175
Sex, no. (%)				
Male	77 (68.1)	28 (65.1)		0.356
Female	36 (31.9)	15 (34.9)		
Type of AF, no. (%)				
Paroxysmal	36 (31.9)	13 (30.2)		0.124
Persistent	77 (68.1)	30 (69.8)		
Duration of AF, mean (SD), years	3.1 (4.6)	3.9 (5.9)		0.427
Hypertension, no. (%)	53 (46.9)	20 (46.5)		0.965
Diabetes, no. (%)	24 (21.2)	4 (9.3)	0.38 (0.12–1.17)	0.083
Coronary disease, no. (%)	14 (12.4)	7 (16.3)		0.525
Valvular disease, no. (%)	10 (8.8)	6 (14.0)		0.348
CHA2DS2-VASc score, mean (SD)	2.4 (1.5)	2.3 (1.3)		0.914
LVEF, mean (SD), %	38.57 (8.0)	36.1 (6.8)	0.90 (0.91–1.00)	0.022
LAD, mean (SD), mm	43.0 (5,0)	43.9 (4.2)		0.278
LVD, mean (SD), mm	52.5 (6.8)	54.3 (6.8)		0.148
E/E′, mean (SD)	10.8 (4.2)	16.6 (9.5)	1.17 (1.08–1.26)	0.001
HR_{max}, mean (SD), beats/min	140.5 (40.0)	140.8 (46.8)		0.972
HR_{min}, mean (SD), beats/min	55.7 (11.5)	54.4 (14.8)		0.623
HR_{mean}, mean (SD), beats/min	85.8 (16.9)	86.0 (20.7)		0.970
Ablation Strategy, no. (%)				
PVI only	53 (46.9)	20 (46.5)		0.913
PVI+line	48 (42.5)	18 (41.9)		0.827
PVI+CAFE	5 (4.4)	3 (7.0)		0.114
PVI+Line+CAFE	7 (6.2)	2 (4.6)		NS
Recurrence of AF, no. (%)	42 (37.2)	22 (51.2)		0.112
Prevalent AF, no. (%)	62 (54.9)	16 (37.2)	0.49 (0.24–1.00)	0.051
Prevalent HF, no. (%)	6 (5.3)	8 (18.6)	0.24 (0.08–0.76)	0.014
Concomitant AF and HF, no. (%)	45 (39.8)	18 (41.9)		0.817
All cause hospitalization, no. (%)	25 (23.8)	27 (62.8)	2.09 (1.40–3.42)	<0.001
All cause death, no. (%)	0 (0)	0 (0)	–	NS

AF, atrial fibrillation; HF, heart failure; PVI, pulmonary vein isolation; CAFE, complex fractionated atrial electrograms; HR, heart rate; LVD, left ventricular diameter; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; prevalent AF, existing AF and new-onset HF; prevalent HF, existing HF and new-onset AF; NS, no significant.

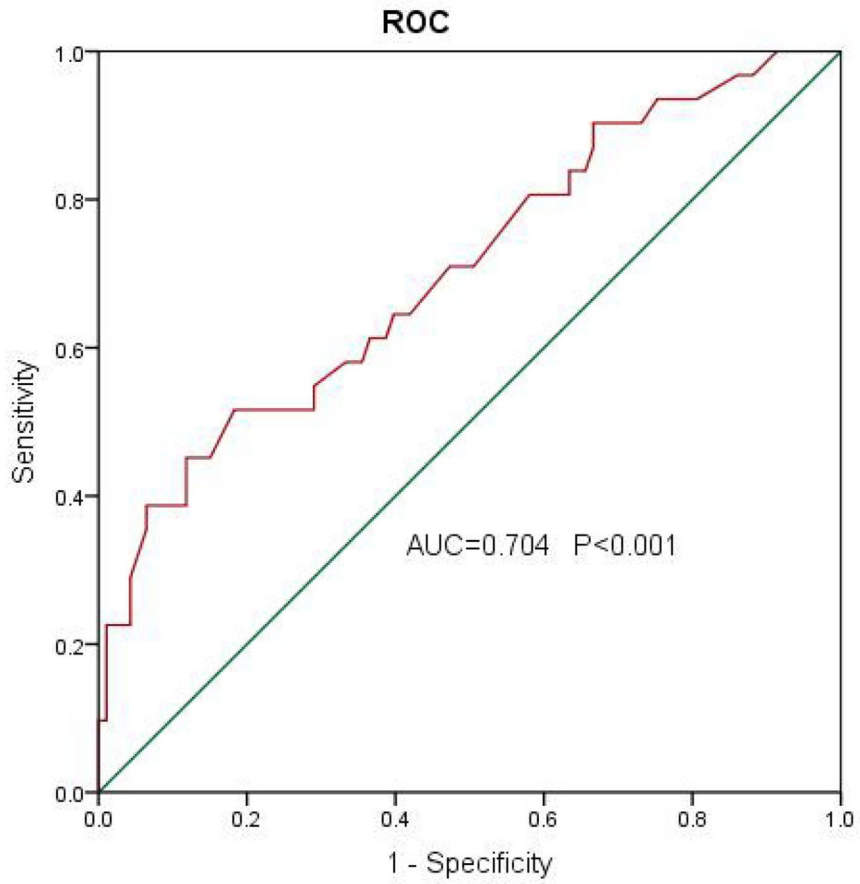

FIGURE 2 | A receiver operating characteristic analysis revealed a moderate accuracy of predicting the improvement of left ventricular ejection fraction (LVEF) by E/E′ with a cut-off of 15 (sensitivity: 38.7%, specificity: 89.2%, area under the curve: 0.704).

showed that the only independent predictor of LVEF recovery was E/E′ [OR 1.13 (1.03–1.24); $P = 0.011$] (**Table 3**). A receiver operating characteristic analysis revealed a moderate accuracy of predicting the improvement of LVEF by E/E′ with a cut-off of 15 (sensitivity: 38.7%, specificity: 89.2%, area under the curve: 0.704) (**Figure 2**).

DISCUSSION

Main Findings

Our main finding is that the only independent predictor of recovered LVEF is E/E′ < 15. In agreement with the literature, diabetes-prevalent HF, prevalent AF, and basic LVEF were significant predictors at the univariate level. Still, they were not independent predictors according to the multivariate analysis in our study. Therefore, this study may suggest a new predictor for LVEF recovered in systolic HF patients who receive AF ablation.

The Outcome of Atrial Fibrillation Ablation in Systolic Heart Failure Patients

Since the early landmark study on AF ablation in congestive HF by the Bordeaux group, several observational and randomized studies have provided substantial evidence that AF ablation in patients with HFrEF results in high rates of successful sinus rhythm maintenance along with significant clinical and LV functional improvements (5). In the PABACHF trial (6), 76% of patients in the AF ablation group significantly improved LVEF vs. 25% in the atrioventricular nodal ablation/biventricular pacing group. In the CAMERA-MRI study (7), where only patients with persistent AF and idiopathic cardiomyopathy were included, 58% of patients had normalization of LVEF after AF ablation, compared with 9% of patients receiving medical rate control only. In our cohort of 156 consecutive cases, 72% of patients had recovered LVEF after AF ablation.

The most recently published larger prospective RCTs have evaluated the role and efficacy of AF ablation in selected patients with HFrEF. AF ablation was associated with a greater reduction in all-cause mortality and HF hospitalizations. In our study, recovered LVEF was associated with a 39% reduction in HF hospitalizations [23 vs. 63%; 2.09 (1.40–3.12), $P < 0.001$] compared to non-recovered LVEF. No deaths occurred in the study due to the small sample size and short follow-up. However, considering all RCTs together, it remains unanswered which AF ablation strategy is optimal and recommended to achieve

the reported favorable outcomes. We compared the ablation strategy between the groups, and there was no difference in AF ablation for LVEF recovery. Advanced structural remodeling or non-convertible AF in HF patients following PVI (11) and additional ablation are often needed. Whether empirical linear lesions (12), targeted non-PV trigger ablation (13), ablation of complex fractionated atrial electrograms (CFAE) (14), or substrate (low voltage) modification (15) ensure superior success is a controversial matter of opinion, and comparison of the different ablation strategies in patients with HF lacks evidence.

Predictors of Recovered LVEF

It should be considered in selected AF patients with HFrEF to improve survival and reduce HF hospitalization (IIa) following the 2020 ESC guidelines (16). We are aware that not every patient will benefit from an approach to ablation-based rhythm control. Thus, careful patient selection for AF ablation is key to achieving similar outcome benefits and should currently be geared to HF populations. Few studies have identified important factors that may be independent predictors of recovered LVEF after AF ablation (3). Ukita et al. (17) found that left ventricular end-diastolic dimension <53 mm might be an independent predictor of LVEF improvement after catheter ablation of persistent AF in HFrEF patients. In an academic review performed by Richter et al. (3), clinical guidance was proposed for the choice of treatment for AF in patients with HF, including ages <65, idiopathic cardiomyopathy, and LV diameter <55 mm. Studies suggest that a higher ventricular rate may be associated with an increased risk of HF in AF patients. However, in one study (18), among 50 patients with AF and HF who underwent AF ablation, only 15 (22%) had heart rate >100 beats per minute at baseline. However, LVEF normalized in 36 patients (72%) at 6 months post-ablation, suggesting that HF may develop in patients with no tachycardia.

We found that E/E′ is a new independent predictor of recovered ejection fraction in patients with systolic HF undergoing ablation for AF. Scarce data are available regarding the role and predictive value of E/E′ on the recovered ejection fraction. Our results suggested that E/E′ < 15 predicted an LVEF increase after AF ablation in patients with HF. E/E′ is the ratio between early mitral inflow velocity and mitral annular early diastolic velocity, which has become central in the guidelines for diastolic evaluation. The use of E/E' is generally the most feasible and among the most reproducible method for estimation of filling pressure. Several prominent validation studies have confirmed the correlation of this ratio with filling pressure, and the prediction of normal and abnormal filling pressure is most reliable when the ratio is <8 or >15 (19). Diastolic dysfunction is often associated with irreversible ventricular remodeling and poor outcomes (20). Higher E/E′ predicted more myocardial fibrosis (21), in addition to poor outcome after AF in HF. Patients with impaired diastolic and systolic functions suggest that cardiac reserve function is lost. Even if arrhythmias such as AF are corrected, cardiac function may not be restored. Concomitant systolic and diastolic dysfunction in patients with HF and AF may be a specialized form of cardiomyopathy and needs to be further assessed.

LIMITATIONS

The major limitation of our registry is that it represents a single-center experience and the population size was underpowered. The follow-up TTE was performed at various times, which might have underestimated the improvement in LVEF. The "true" predictors of LVEF recovery were unclear. Further factors, such as genetics, atrial cardiomyopathy, tachycardiomyopathy, need to be studied. Our research is a retrospective study, and bias exists, such as patient selection, ablation strategy, etc.

CONCLUSIONS

Left ventricle ejection fraction (LVEF) recovery occurred in 72.3% of patients, which was associated with a 39% reduction in all-cause hospitalization compared to the non-recovered LVEFs in our cohort. The only independent predictor of recovered LVEF was E/E′ < 15 in our series.

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 authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of First Affiliated Hospital of Dalian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YX, LG, and YaY contributed to conception and design of the study. MY, RZ, and HT organized the database. XG and YiY performed the statistical analysis. RZ and YS wrote the first draft of the manuscript. XX, XYu, YD, and XYi wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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The Value of IGF-1 and IGFBP-1 in Patients With Heart Failure With Reduced, Mid-range, and Preserved Ejection Fraction

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Background: Previous studies have reported inconsistent results regarding the implications of deranged insulin-like growth factor 1 (IGF-1)/insulin-like growth factor-binding protein 1 (IGFBP-1) axis in patients with heart failure (HF). This study evaluates the roles of IGF1/IGFBP-1 axis in patients with HF with reduced ejection fraction (HFrEF), mid-range ejection fraction (HFmrEF), or preserved ejection fraction (HFpEF).

Methods: Consecutive patients with HFrEF, HFmrEF, and HFpEF who underwent comprehensive cardiac assessment were included. The primary endpoint was the composite endpoint of all-cause death and HF rehospitalization at one year.

Results: A total of 151 patients with HF (HFrEF: $n = 51$; HFmrEF: $n = 30$; HFpEF: $n = 70$) and 50 control subjects were included. The concentrations of IGFBP-1 ($p < 0.001$) and IGFBP-1/IGF-1 ratio ($p < 0.001$) were significantly lower in patients with HF compared to controls and can readily distinguish patients with and without HF (IGFBP-1: areas under the curve (AUC): 0.725, $p < 0.001$; IGFBP-1/IGF-1 ratio: AUC:0.755, $p < 0.001$; respectively). The concentrations of IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio were similar among HFpEF, HFmrEF, and HFrEF patients. IGFBP-1 and IGFBP-1/IGF-1 ratio positively correlated with N-terminal probrain natriuretic peptide (NT-proBNP) levels ($r = 0.255$, $p = 0.002$; $r = 0.224$, $p = 0.007$, respectively). IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio did not predict the primary endpoint at 1 year for the whole patients with HF and HF subtypes on both univariable and multivariable Cox regression.

Conclusion: The concentrations of plasma IGFBP-1 and IGFBP-1/IGF-1 ratio can distinguish patients with and without HF. In HF, IGFBP-1 and IGFBP-1/IGF-1 ratio positively correlated with NT-proBNP levels.

Keywords: IGF-1, IGFBP 1, heart failure, HFrEF—heart failure with reduced ejection fraction, HFpEF—heart failure with preserved ejection fraction, HFmrEF—heart failure with mid-range ejection fraction

INTRODUCTION

Heart failure (HF) is the final common pathway of many cardiovascular diseases and can be classified into reduced ejection fraction (HFrEF), mid-range ejection fraction (HFmrEF), or preserved ejection fraction (HFpEF) based on the 2016 European Society of Cardiology (ESC) guideline for HF (1). Risk stratification should be based on a multimodality approach but can differ between HF subtypes (2–5). Although survival for patients with HFrEF has been improved substantially due to advances in drug and device-based therapies, the use of medications to improve prognosis in patients with HFmrEF and HFpEF is less well defined. Patients with HFmrEF and HFpEF constitute more than one-half of the HF cohort, but the risk stratification for both subtypes remains difficult (6–9). Nevertheless symptomatic HFmrEF and HFpEF show a poorer prognosis compared to their HFrEF counterparts (10). Better understanding the pathophysiology of the three HF subtypes will provide additional insights for guiding medical therapies (11).

Circulating biomarkers reflect the pathophysiological state of HF and are of potential value for its diagnosis and prognosis (12, 13). The peptic hormone-insulin-like growth factor 1(IGF-1) regulates proliferation, differentiation, metabolism, and cell survival in various tissues. Over recent years, an increasing number of studies have reported the link of IGF-with to all-cause mortality and cardiovascular diseases, such as HF, atrial fibrillation, and stroke (14–17). By upregulating the IGF1-PI3K-Akt pathway, IGF-1 tends to show cardioprotective effects (18), improves cardiomyopathy (19), and modulates the cellular processes implicated in short-term ventricular remodeling of the infarcted myocardium (20). IGF-binding proteins bind to IGF-1, thereby regulating its activity. Among these IGF-binding proteins, in particular, IGF-binding protein 1 (IGFBP-1) has numerous actions, including peripheral binding and potent inhibition of IGF-1 (21). A previous study (22) has investigated the ability of IGF-1/IGFBP-1 to distinguish between HF subtypes, and found that IGF-1 levels were different between HFpEF and HFrEF, and have prognostic roles. In this study, we investigated the plasma concentrations of IGF-1 and IGFBP-1 in patients with HF and compared their levels between HF subtypes, their correlations with N-terminal prohormone brain natriuretic peptide (NT-proBNP), and their prognostic values.

METHODS

Study Population

This study enrolled consecutive patients from October 2018 to January 2020. The inclusion criteria were: (1) HF symptoms or signs; (2) NT-proBNP >125 ng/ml; and (3) patients were divided into left ventricular ejection fraction (LVEF) < 40% (HFrEF); LVEF ≥ 40% and < 50% (HFmrEF) and LVEF ≥ 50% (HFpEF) groups. The control group enrolled patients referred for elective angiography or treatment of uncontrolled hypertension with NT-proBNP ≤ 125 ng/l. Exclusion criteria included acute myocardial infarction, myocarditis, moderate-to-severe valvular heart disease, severe systemic inflammatory disease, or severe renal or hepatic disease. The study was approved by the local ethics committee of the Second Hospital of Tianjin Medical University and conformed to the Declaration of Helsinki.

Clinical, Biochemical, and Echocardiographic Data

Baseline data with regard to demographic and clinical variables involving age, gender, hospital stay, smoking history, comorbidities (such as hypertension, diabetes, coronary revascularization history, and atrial fibrillation), blood pressure, heart rate, biochemical results (in particular, NT-proBNP), and discharge medication were collected.

Blood samples were drawn at rest and collected with ethylenediamine tetraacetic acid (EDTA) anticoagulant tubes to analyze routine laboratory parameters. The blood tubes were centrifuged at 3,000 *g* at room temperature for 10 min and plasma was separated from cellular compartments and stored at −80°C for later analysis of IGF-1 and IGFBP-1. An ELISA was performed to measure the concentration of IGF-1 and IGFBP1 using IGF-1 and IGFBP1 assay kit (Cusabio, China).

Echocardiography was performed using a standard ultrasound system (PHILIPS iE33). LVEF was measured based on modified biplane Simpson's method. Measurement of left atrial anterior and posterior diameter (LAD), interventricular septum thickness (IVS), left ventricular end-diastolic diameter, and left ventricular end-systolic diameter were from parasternal long-axis view.

Follow-Up and Outcomes

All the patients with HF continued the standardized treatment for HF after discharge. Patients were followed up by clinical visits or telephone calls for 12 months. The primary endpoint was the composite endpoint of all-cause death and HF rehospitalization at 1 year. The follow-up time was calculated from discharge to all-cause death, first readmission, or termination of the study.

Statistics

Baseline continuous variables were reported as mean ± SD or median and interquartile range, which is based on a continuous distribution of data: Student's *t*-test or ANOVA is used for normal distribution, and the Mann–Whitney test or the Kruskal–Wallis test is used for abnormal distribution. Categorical variables are expressed as numbers and percentages and compared using the Chi-square test or Fisher's exact test.

Concentrations of IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio were compared in HFrEF, HFmrEF, HFpEF, and controls. The correlation was performed between levels of IGF-1, IGFBP-1, IGFBP-1/ IGF-1 ratio, and NT-proBNP using Pearson's *r*. The diagnostic value of IGF-1, IGFBP-1, and IGFBP-1/ IGF-1 ratio to identify HF were investigated and compared *via* the areas under the curve (AUCs) of receiver operating characteristics (ROC) curves. The Cox proportional hazard model was also performed to investigate the prognostic value of IGF-1 concentration, IGFBP-1 concentration, and IGFBP-1/IGF-1 ratio. Log-rank tests for the Kaplan–Meier survival curves were performed according to different HF subtypes. All data were analyzed using SPSS statistical software (SPSS 25.0) R programming version 4.1.1. A p-value < 0.05 was considered statistically significant.

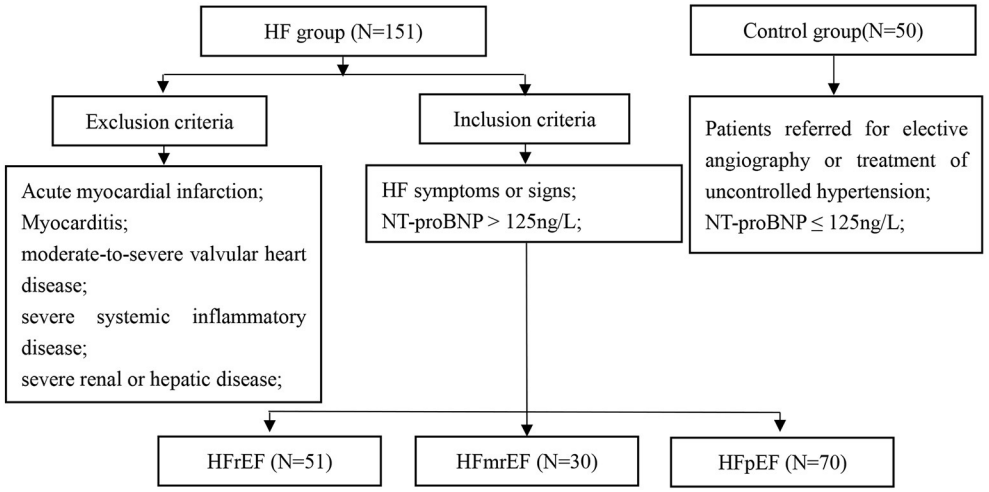

FIGURE 1 | Study flowchart. HF, heart failure; HFrEF, HF with reduced ejection fraction; HFmrEF, HF with mid-range ejection fraction; HFpEF, HF with preserved ejection fraction; NT-proBNP, N-terminal pro-B natriuretic peptide.

RESULTS

Baseline Characteristics and Biomarkers of Patients With HF and Controls

A total of 163 patients with HF were enrolled. Of these, 12 patients were lost to follow-up, and therefore 151 consecutive patients with HF (mean age 68.9 ± 11.4 years; 59.6% men) were included in the final analysis. In total, 50 subjects without HF were included as controls (**Figure 1**). The baseline characteristics of the study cohort are shown in **Table 1**. Compared to controls, patients with HF had a higher male frequency (59.6 vs. 42.0%, $p = 0.030$), had a longer hospital stay, had higher rates of atrial fibrillation, prior myocardial infarction, stroke, prior coronary revascularization, and more likely to use digoxin, diuretics, and cardioprotective medicine at discharge, such as beta-blocker, spironolactone, angiotensin system antagonist [angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), and angiotensin receptor-neprilysin inhibitors (ARNI)]. In addition, patients with HF had a larger atrium ($p < 0.001$) and ventricle ($p < 0.001$), and lower LVEF ($p < 0.001$) compared to controls.

Heart failure had a higher creatinine level ($p < 0.001$) and a lower hemoglobin level ($p = 0.036$) compared with control. NT-proBNP levels were higher in HF than in control subjects without HF ($p < 0.001$). The levels of IGF-1 in patients with HF and controls were, median (IQR), 50.9 (37.4, 72.6) ng/ml, and 50.0 (34.8, 67.3) ng/ml, respectively, but no difference was found between the two groups ($p = 0.392$). In contrast, the levels of IGFBP-1 ($p < 0.001$) and IGFBP-1/IGF-1 ratio ($p < 0.001$) were significantly lower in patients with HF compared with controls.

The diagnostic performance for HF diagnosis was analyzed by ROC analysis for IGF-1, IGFBP-1, and the IGFBP-1/IGF-1 ratio in the patients with HF and controls (**Figure 2**). IGFBP-1 and IGFBP-1/IGF-1 ratios have moderate values for distinguishing between patients with HF and non-HF (AUC = 0.725 and 0.755, respectively). IGF-1 was not useful for this classification. The predictive abilities of NT-proBNP were superior to those of IGFBP-1 and IGFBP-1/IGF-1 ratio (AUC for NT-proBNP, 0.981).

Baseline Characteristics and Biomarkers in HFrEF, HFmrEF, and HFpEF

Of the 151 patients with HF, 51 had HFrEF, 30 had HFmrEF, and 70 had HFpEF. Their baseline characteristics are summarized in **Table 2**. As compared with HFpEF and HFmrEF, patients with HFrEF were more commonly men, had lower systolic blood pressure, tended to have a prior myocardial infarction, and were more likely to be prescribed with angiotensin system antagonist (ACEI, ARB, and ARNI), diuretic, spironolactone, and digoxin. Compared to HFrEF and HFmrEF, patients with HFpEF were less likely to have an ischemic etiology of HF ($p = 0031$). Otherwise, according to echocardiography, patients with HFrEF had more enormous left atrium and left ventricle and thinner IVS than patients with HFpEF and HFmrEF. In contrast, the percent of calcium channel blockers was higher in the HFpEF group than in HFrEF and HFmrEF group. There was no difference in the primary endpoint among the three groups (HFrEF 58.5% vs. HFmrEF 43.3% vs. HFpEF 51.4%, ($p = 0.395$), however, HFrEF was a trend to a higher risk of all-cause death at 12 months compared with HFmrEF and HfpEF ($p = 0.069$).

The levels of IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio were similar among patients with HFpEF, HFmrEF, HFrEF (**Table 2** and **Figures 3A–C**). The difference of these biomarkers between HFrEF, HFmrEF, and HFpEF remained insignificant after adjustment for gender, age, and NT-proBNP. There was a progressive increase in NT-proBNP levels from HFpEF to HFmrEF to HFrEF, with patients with HFrEF having the highest levels ($p = 0.014$, **Table 2**). IGFBP-1 levels and IGFBP-1/IGF-1 ratio were positively correlated with NT-proBNP levels

TABLE 1 | Baseline characteristics of study participants.

	HF ($n = 151$)	Control ($n = 50$)	*P* value
Demographics and vital signs			
Age (years)	71.0 (61.8, 77.0)	66.0 (60.3, 72.0)	0.048
Male	90 (59.6%)	21 (42.0%)	0.030
Hospital stay (days)	7.5 (5.7, 11)	4 (3, 5.7)	<0.001
Systolic BP (mmHg)	130.5 (114.0, 150.0)	141.5 (126.5, 149.0)	0.025
Diastolic BP (mmHg)	80.0 (69.7, 89.2)	81.0 (72.2, 86.0)	0.872
Heart rate (bpm)	80.0 (69.7, 82.0)	70.5 (63.0, 80.5)	0.002
Medical history			
Atrial fibrillation	56 (37.1%)	3 (6%)	<0.001
Prior MI	52 (34.4%)	3 (6.0%)	<0.001
Hypertension	108 (71.7%)	38 (76.0%)	0.689
Diabetes mellitus	62 (41.3%)	13 (26.0%)	0.052
Stroke	44 (29.1%)	5 (10.0%)	0.006
COPD	8 (5.3%)	0 (0.0%)	0.214
Coronary revascularization history	45 (29.8%)	6 (12.0%)	0.012
Smoking history	67 (44.4%)	18 (36.0%)	0.299
Echocardiographic parameters			
LVEF, %	48.0 (38.0, 59.2)	64.00 (60.0, 67.7)	<0.001
LAD (mm)	44.5 (40.3, 50.0)	38.2 (35.0, 40.6)	<0.001
LVEDD (mm)	51.9 (47.8, 58.8)	47.9 (44.1, 50.5)	<0.001
IVS (mm)	9.35 (8.4, 11.0)	9.00 (8.40, 9.60)	<0.001
Discharge medications			
ARB or ACEI or ARNI	97 (64.2%)	22 (44.9%)	0.017
Digoxin	18 (11.9%)	0 (0.0%)	0.011
Beta blocker	96 (63.6%)	22 (44.9%)	0.021
Calcium channel blocker	48 (31.8%)	20 (40.8%)	0.246
Spironolactone	87 (57.6%)	3 (6.1%)	<0.001
Diuretics	89 (59.3%)	5 (10.2%)	<0.001
Biomarkers			
Creatinine (umol/L)	88.8 (68.7, 126.4)	73.3 (60.3, 87.6)	<0.001
Hemoglobin (g/L)	130 (108, 144)	136 (128, 143)	0.036
Troponin I (ng/ml)	0.02 (0.01, 0.10)	0.02 (0.01, 0.03)	0.521
CK-MB (U/L)	14.0 (10.0, 22.0)	11.2 (9.5, 19.2)	0.181
D-dimer (mg/L)	797.9 (439.1, 1,208.1)	328.3 (233.2, 510.6)	<0.001
Total cholesterol (mmol/L)	4.1 (3.3, 5.3)	4.6 (3.7, 5.1)	0.241
Triglyceride (mmol/L)	1.1 (0.8, 1.6)	1.7 (1.1, 2.4)	<0.001
NT-proBNP (ng/L)	3,334.5 (1,855.7, 8,112.2)	86.7 (38.9, 210.0)	<0.001
IGF-1 (ng/ml)	50.9 (37.4, 72.6)	50.1 (34.8, 67.3)	0.392
IGFBP-1 (ng/ml)	60.3 (5.7, 461.4)	439.7 (404.2, 523.2)	<0.001
IGFBP-1/IGF-1	1.36 (0.1, 8.7)	8.5 (6.3, 13.1)	<0.001

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor-neprilysin inhibitors; BP, blood presure; COPD, chronic obstructive pulmonary disease; HF, heart failure; IGF-1, insulin-like growth factor 1; IGFBP-1, IGF binding protein 1; IVS, interventricular septum thickness; LAD,left atrial anterior and posterior diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MI, myocardial infaction; NT-proBNP, N-terminal prohormone brain natriuretic peptide.

($r = 0.255$, $p = 0.002$; $r = 0.224$, $p = 0.007$, respectively; **Figures 3D–F**).

The primary endpoint occurred in 79 (52.3%) patients, of whom 16 (10.6%) died and 63 (41.7%) were rehospitalized for HF. There was no difference in the primary endpoint among the three groups (HFrEF 58.5% vs. HFmrEF 43.3% vs. HFpEF 51.4%, $p = 0.540$, **Figure 4**). As shown in **Figure 5**, with the aggravation of cardiac function (New York Heart Association, NYHA), the incidence of the primary endpoint was significantly increased ($p = 0.012$). Multivariable Cox regression showed that IGF-1 levels, IGFBP-1 levels, and IGFBP-1/IGF-1 ratio were not predictive of prognosis after adjusting for age, gender, atrial fibrillation, and NT-proBNP in patients with HF (**Figure 6**).

DISCUSSION

This is, to the best of our knowledge, the first study comparing the concentrations of IGF-1 between various HF subtypes. The

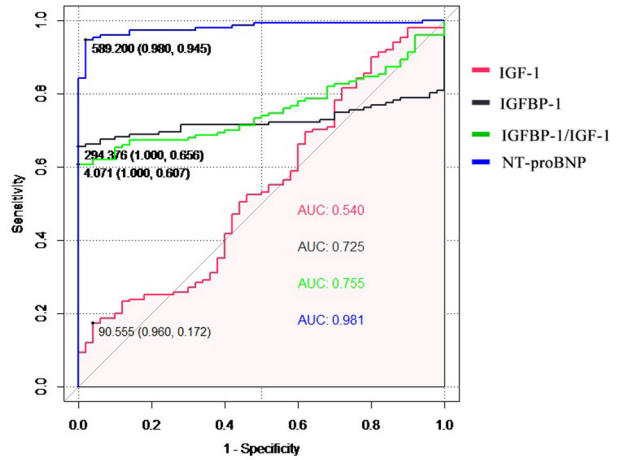

FIGURE 2 | The receiver operating characteristic (ROC) curve of the diagnostic ability for heart failure of IGF-1, IGFBP-1, IGFBP-1/IGF-1 ratio, and NT-proBNP. IGF-1, insulin-like growth factor 1; IGFBP-1, IGF binding protein 1; NT-proBNP, N-terminal pro-B natriuretic peptide.

main results are that (1) IGFBP-1 and IGFBP-1/IGF-1 ratio were significantly lower in patients with HF compared to those without HF, (2) in HF, IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio did not differ among HFpEF, HFmrEF, and HFrEF, and (3) IGFBP-1 and IGFBP-1/IGF-1 ratio positively correlated with NT-proBNP.

There is an increasing body of evidence that IGF-1 can have protective roles in the heart. Patients with HF are more likely to have a lower concentration of IGF-1. As supported by previous studies, ACEIs have been shown to improve survival in patients with HFrEF (23) and are recommended for the treatment of every patient with HFrEF according to guidelines (1), and can regulate IGF-1 levels (24, 25). Nevertheless, studies on the relationship between IGF-1concentrations and cardiovascular disease vary significantly, reporting to be reduced, normal, and even increased. On the one hand, lower IGF-1 levels seemed to be harmful and associated with diastolic dysfunction and even HFpEF (26). On the other hand, as shown by Faxen et al. (26) compared with the control group, IGF-1 levels were higher in HFpEF but lower in HFrEF. The normal range of IGF-1 in patients with HF was also ever reported (27). Our patients were enrolled consecutively in the hospital, and all samples were obtained during the acute phase. However, IGF-1 could neither identify patients with HF from controls nor distinguish HF subtypes. We supposed that IGF-1 might have a protective role in the process of modulating heart activity, and as a possibility, the divergent results may be due to the susceptibility of IGF-1 to baseline environments, such as age, race, and acute period and unrecognized differences in lifestyle factors modulating IGF-1 levels.

Insulin-like growth factor-binding proteins are widely expressed in most tissues, and are endocrine and autocrine/paracrine regulators of IGF activity, which is essential for this crucial physiological system. IGF-1 activity is regulated by IGFBPs. However, IGFBPs function their biological roles not only by binding to IGF but also play roles independent of the IGF system (28, 29). IGFBP-1 binds IGF1 and IGF2 with equal affinity, inhibiting or enhancing IGF actions (30, 31). Previous studies reported diverse conclusions about the prognostic role of IGFBP-1. One study showed that IGFBP-1 was associated with long-term all-cause and cancer mortality but not cardiovascular events (32). Other studies indicated that IGFBP-1 is a long-term predictor of HF in survivors of a first acute myocardial infarction (33) and predicts adverse clinical outcomes during outpatient follow-up of patients with chronic HF (34). In contrast, consistent with our results, Faxen et al. reported IGFBP-1 was similar in HFpEF and HFrEF phenotypes and revealed no associations with outcomes (22).

In this study, lower IGFBP-1 concentration and IGFBP-1/IGF-1 ratio values showed a correlation from controls to patients with HF, while neither IGF-1 nor IGFBP-1 have value in distinguishing HF subtypes or predicting prognosis. In addition, the correlation between levels of IGFBP-1 and IGFBP-1/IGF-1 ratio and NT-proBNP, a well-recognized prognostic marker and indicator of elevated ventricular filling pressures among patients regardless of ejection fraction (35, 36), indicated that IGFBP-1 and IGFBP1/IGF-1 may serve as a supplementary to better estimate prognosis of HF, despite their negative role in this study.

Heart failure with reduced ejection fraction, HFmrEF, and HFpEF sharing common clinical features constitute different entities with distinct pathogenetic backgrounds. Efforts are made to find biomarkers identifying subjects with different HF entities. Recent years have emerged studies reporting several biomarkers which can discriminate HFpEF from HFrEF. High-Density Lipoprotein Particle Subfractions can distinguish between HFpEF and HFrEF (37). A study investigating inflammation mediated by the tumor necrosis factor-alpha (TNFa) axis in patients with HF indicated that there was a significant difference in TNF receptor-2 (TNFR2) between patients with HFrEF and HFpEF (38). An ensemble of the male-specific transcriptomic panel with NT-proBNP has been estimated to be able to differentiate between HFpEF and HFrEF (39). Otherwise, some biomarkers cannot distinguish HF subtypes but have an indicative value. For instance, cystatin C was higher in HFpEF than HFrEF but not significantly (40). Higher levels of adiponectin were associated with the adverse outcome only in HFrEF, not HFpEF (41). Growth differentiation factor 15 is similarly elevated and has an independent prognostic utility in both HFrEF and HFpEF (42), without differentiating value.

Several limitations of this study must be acknowledged. First, the definitive determination of cause and effect relationships was not clear due to the retrospective observational nature of the present study. As reported by previous studies, the activity of IGF-1 and IGFBP-1 are regulated by insulin, while it is a pity that concentrations of insulin were not measured at baseline. Second, single-center experience with a limited sample size affects its wide application. Multi-center research and long-term follow-up would allow us to better understand the mechanism of levels IGF-1, IGFBP-1, and IGFBP-1/IGF-1 ratio in identifying HF subtypes and predicting clinical outcomes. Third, we enrolled control groups referred for elective angiography or treatment of uncontrolled hypertension, which may not be

TABLE 2 | Baseline characteristics of heart failure patients.

	HFrEF (*N* = 51)	HFmrEF (*N* = 30)	HFpEF (*N* = 70)	*P* value
Demographics and vital signs				
Age (years)	68.4 ± 11.2	66.3 ± 11.9	70.3 ± 11.1	0.255
Male	41 (80.4%)	19 (63.3%)	30 (42.9%)	<0.001
Hospital stay (days)	9.6 ± 5.7	7.7 ± 3.8	9.2 ± 5.8	0.316
Systolic BP (mmHg)	123.8 ± 26.6	139.7 ± 28.7	137.9 ± 27.1	0.008
Diastolic BP (mmHg)	80.5 ± 16.8	87.3 ± 19.4	77.2 ± 15.0	0.022
Heart rate (bpm)	85.4 ± 20.8	81.6 ± 14.1	78.2 ± 20.2	0.135
NYHA I	2 (3.9%)	6 (20.0%)	5 (7.1%)	0.305
NYHA II	13 (25.5%)	8 (26.7%)	18 (25.7%)	
NYHA III	25 (49.0%)	13 (43.3%)	32 (45.7%)	
NYHA IV	11 (21.6%)	3 (10.0%)	5 (21.4%)	
Medical history				
Ischemic etiology	34 (66.7%)	22 (73.3%)	34 (48.6%)	0.031
Atrial fibrillation	14 (27.5%)	9 (30.0%)	33 (47.1%)	0.119
Prior MI	29 (56.9%)	9 (30.0%)	14 (20.0%)	<0.001
Hypertension	31 (60.8%)	22 (73.3%)	55 (78.6%)	0.193
Diabetes mellitus	25 (49.0%)	16 (53.3%)	21 (30.4%)	0.041
Stroke	15 (29.4%)	5 (16.7%)	24 (34.3%)	0.206
COPD	2 (3.9%)	3 (10.0%)	3 (4.3%)	0.437
Coronary revascularization history	18 (35.2%)	5 (16.7%)	22 (31.4%)	0.192
Smoking history	26 (51.0%)	13 (43.3%)	28 (40.0%)	0.483
Echocardiographic parameters				
LVEF, %	30.0 (24.0, 37.7)	41.0 (41.00, 47.00)	60.0 (55.2, 63.7)	<0.001
LAD (mm)	48.7 ± 9.0	43.4 ± 6.8	45.2 ± 7.0	0.008
LVEDD (mm)	59.3 ± 11.1	54.2 ± 7.8	48.3 ± 8.4	<0.001
IVS (mm)	8.7 ± 2.1	10.0 ± 1.9	10.4 ± 2.3	0.001
Discharge medications				
ARB or ACEI or ARNI	43 (84.3%)	18 (60.0%)	36 (51.4%)	0.001
Digoxin	13 (25.5%)	1 (3.3%)	4 (5.7%)	0.001
Beta blocker	36 (70.6%)	19 (63.3%)	41 (58.6%)	0.398
Calcium channel blocker	8 (15.7%)	8 (26.7%)	32 (45.7%)	0.002
Spironolactone	41 (80.4%)	17 (56.7%)	29 (41.4%)	<0.001
Diuretic	41 (80.4%)	16 (53.3%)	32 (46.4%)	0.001
Biomarkers				
Creatinine (umol/L)	99.7 (79.7, 123.9)	79.1 (62.8, 121.5)	88.5 (65.5, 147.8)	0.206
Hemoglobin (g/L)	130.0 (117.0, 146.5)	130.5 (113.0, 147.0)	128.0 (103.0, 141.0)	0.448
Troponin I (ng/ml)	0.05 (0.01, 0.11)	0.05 (0.01, 0.65)	0.01 (0.00, 0.06)	0.087
CK-MB (U/L)	14.0 (11.5, 23.5)	17.3 (11.0, 28.1)	13.9 (9.0, 23.6)	0.304
D-dimer (mg/L)	879.5 (539.38, 1,554.1)	591.2 (392.4, 1,039.6)	619.1 (397.6, 2,473.6)	0.167
Total cholesterol (mmol/L)	3.7 (2.9, 4.6)	4.4 (3.5, 5.6)	4.5 (3.7, 5.6)	0.008
Triglyceride (mmol/L)	0.9 (0.6, 1.5)	1.2 (0.8, 1.6)	1.3 (0.9, 1.8)	0.235
NT-proBNP (ng/L)	5,981.5 (2,349.7, 11,501.0)	3,550.0 (2,495.0, 7,813.0)	2,488.0 (1,633.7, 5,216.0)	0.014
IGF-1 (ng/ml)	49.4 (36.0, 73.5)	50.3 (33.6, 88.7)	51.3 (39.2, 62.4)	0.979
IGFBP-1 (ng/ml)	103.3 (6.1, 615.8)	133.3 (13.2, 497.9)	50.9 (4.7, 487.3)	0.456
IGFBP-1/IGF-1	2.3 (0.1, 9.7)	1.5 (0.3, 12.3)	1.2 (0.1, 8.7)	0.617
Outcomes				
Primary endpoint, n (%)	30 (58.5%)	13 (43.3%)	36 (51.4%)	0.395
Heart failure hospitalization, n (%)	21 (41.2%)	11 (36.7%)	29 (41.4%)	0.890
All cause death, n (%)	9 (17.6%)	2 (6.7%)	7 (10.0%)	0.269

HFrEF, heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction; NYHA, New York Heart Association. Other abbreviations are as ***Table 1****.*

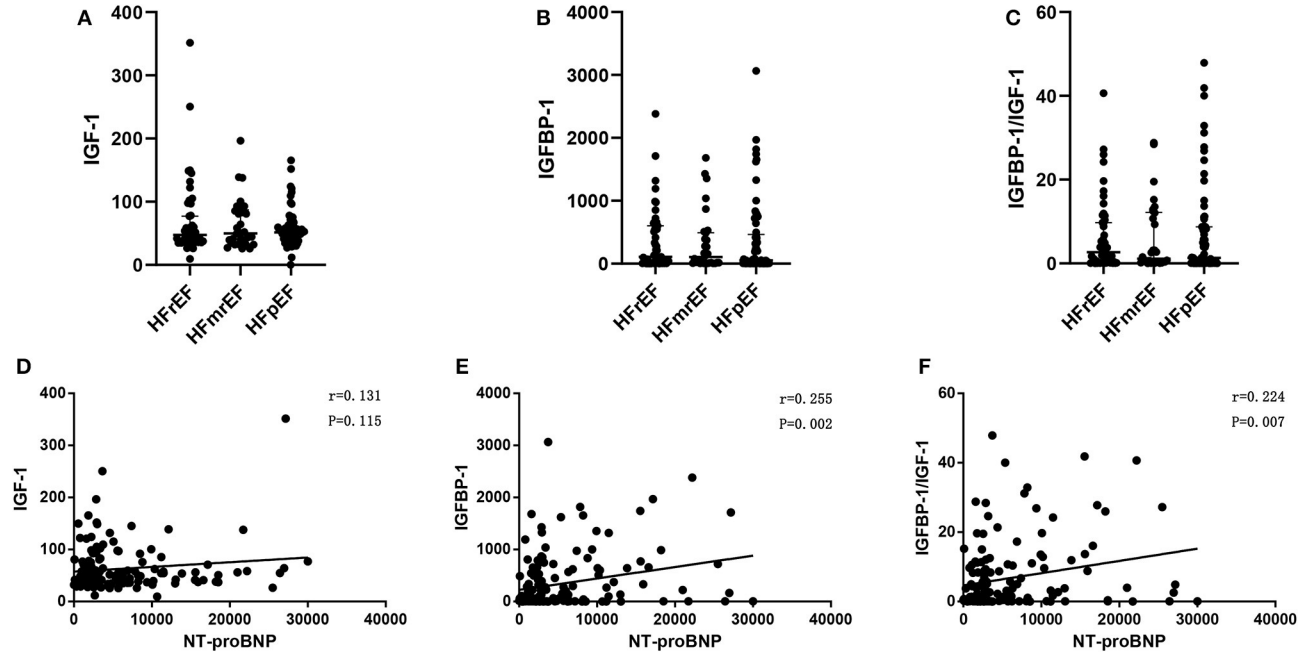

FIGURE 3 | IGF-1 **(A)**, IGFBP-1 **(B)**, and IGFBP-1/IGF-1 ratio **(C)** in HFrEF, HFmrEF,and HFpEF; Pearson's correlation between IGF-1 and NT-proBNP **(D)**, IGFBP-1 and NT-proBNP **(E)**, and IGFBP-1/IGF-1 ratio and NT-proBNP **(F)**, in patients with heart failure. Abbreviations as **Figures 1**, **2**.

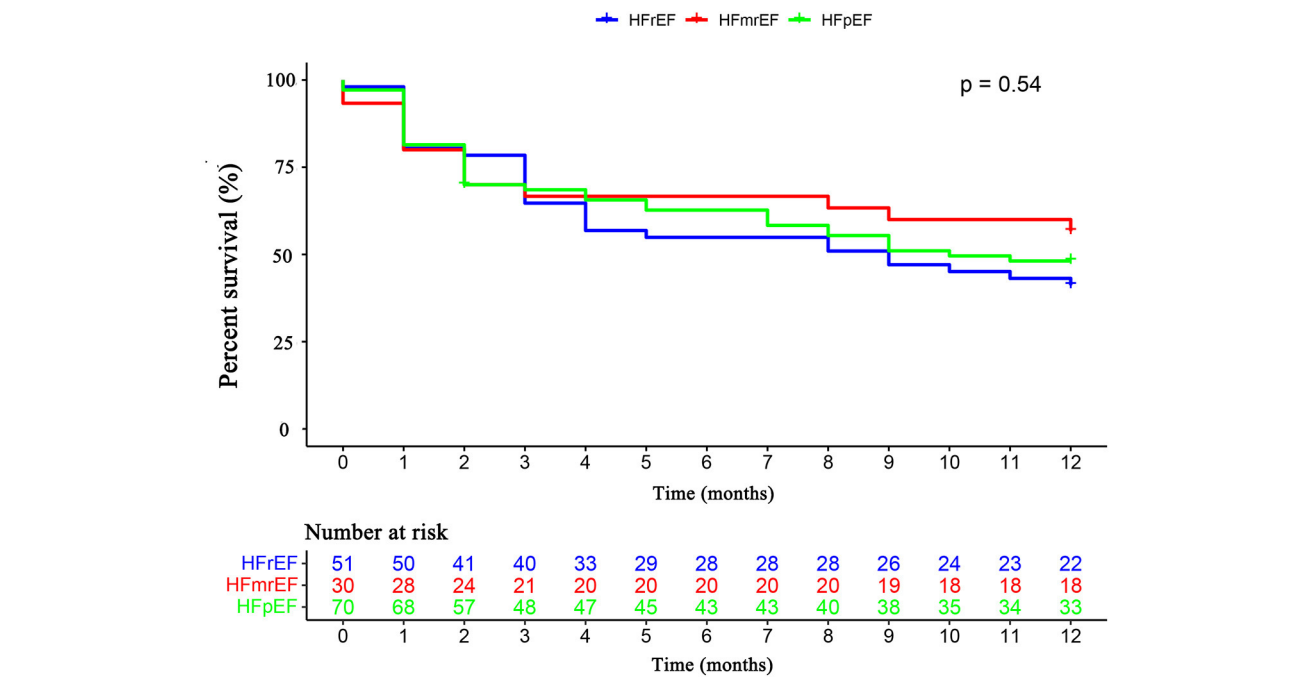

FIGURE 4 | The Kaplan–Meier survival curves according to HFrEF, HFmrEF, and HFpEF. Abbreviations as for **Figure 1**.

representative of the general population. However, this would better reflect real-world clinical scenarios where a diagnosis of HF would be important for guiding management. Finally, metabolic abnormalities especially diabetes mellitus would influence the levels of IGF-1/IGFBP-1. There was a borderline significant difference between the frequency of diabetes in the HF and control groups. Further studies are required to expand on the sample size to allow us to conduct further analyses,

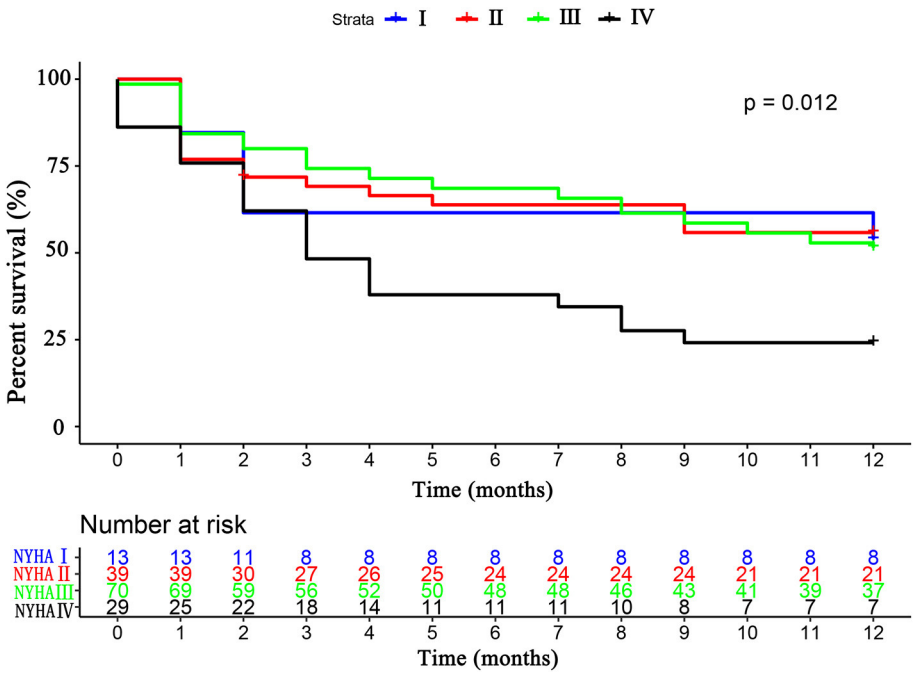

FIGURE 5 | The Kaplan–Meier survival curves according to the NYHA cardiac classification. NYHA, New York Heart Association.

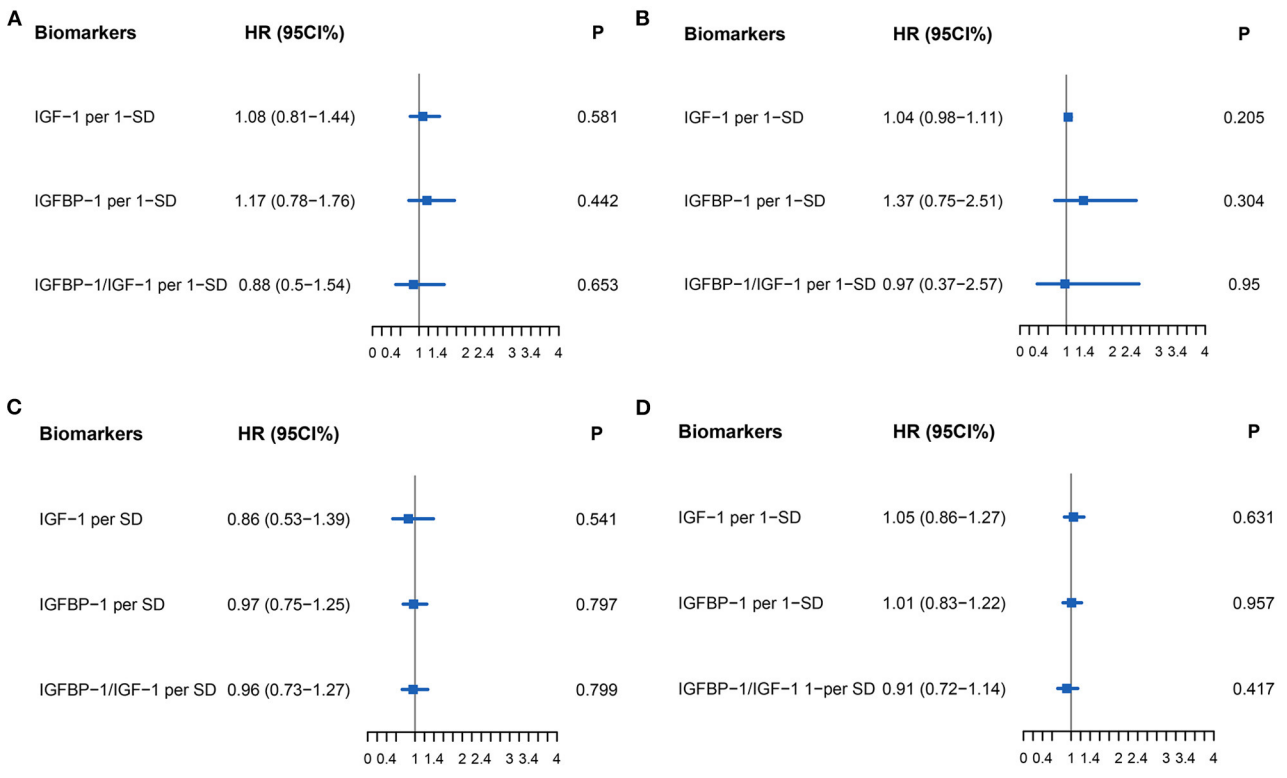

FIGURE 6 | Forest plot of biomarkers IGF-1 (Per 1-SD), IGFBP-1 (Per 1-SD), and IGFBP-1/IGF-1 (Per-SD) for Cox multivariable adjustment of age, gender, atrial fibrillation, and NT-proBNP for the prediction of the primary endpoint (all-cause death and rehospitalization for heart failure at one-year follow-up) in patients with heart failure. **(A)** for HFrEF, **(B)** for HFmrEF, **(C)** for HFpEF, **(D)** for all patients with HF. Abbreviations as for **Figures 1**, **2**.

such as propensity score matching for diabetes status and HbA1c levels.

CONCLUSION

The concentrations of plasma IGFBP-1 and IGFBP-1/IGF-1 ratio can distinguish patients with and without HF. In HF, IGFBP-1 and IGFBP-1/IGF-1 ratio positively correlated with NT-proBNP levels.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Ethics Committee of the Second Hospital of Tianjin Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TL and GL put forward conception and study design. SG and MG researched data, tested biomarkers, wrote the manuscript, and contributed to statistical analysis. GT, SG, and TL edited and contributed to the manuscript, data interpretation, and discussion. All authors have read and approved the final version of the manuscript.

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Heart-Rate Recovery at 1 Min After Exercise Predicts Response to Balloon Pulmonary Angioplasty in Patients With Inoperable Chronic Thromboembolic Pulmonary Hypertension

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Background: Dysfunction of autonomic nervous system plays an important role in the development of pulmonary hypertension. The present study aimed to investigate the interaction between balloon pulmonary angioplasty (BPA) and cardiac autonomic function by using heart-rate recovery at 1 min (HRR1) after exercise as a surrogate marker.

Methods and Results: We retrospectively enrolled 89 consecutive patients with inoperable chronic thromboembolic pulmonary hypertension who underwent BPA from May, 2018 to Jan, 2021. According to hemodynamics at follow-up, patients were categorized as BPA responders if they met one or both of the following criteria: (1) mean pulmonary arterial pressure $\leq$ 30 mmHg and (2) a reduction of pulmonary vascular resistance $\geq$ 30%. Compared with baseline, HRR1 tended to increase within 7 days after the first BPA session, and this improvement persisted at follow-up. HRR1 at baseline and at follow-up were associated with well-validated markers of CTEPH severity, including N-terminal pro-brain natriuretic peptide, mean pulmonary arterial pressure and pulmonary vascular resistance. Furthermore, the change of HRR1 from baseline to follow-up was also associated with the change of those variables. After adjustment for confounders, baseline HRR1 was still a strong independent predictor of BPA outcome. Receiver operator characteristic curve analysis showed that the cutoff value for HRR1 in predicting BPA outcome was 19 beats.

Conclusions: BPA could significantly improve HRR1, suggesting the alleviation of sympathovagal imbalance. Easily available and non-invasive HRR1 seems to be a useful tool in predicting outcome of BPA and dynamically monitoring the efficacy of BPA.

Keywords: chronic thromboembolic pulmonary hypertension, balloon pulmonary angioplasty, heart-rate recovery at 1 min, cardiac autonomic function, prognosis

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INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is featured by organized chronic thrombi in proximal or distal pulmonary arteries and small-vessel remodeling in non-occluded areas, which increases pulmonary arterial pressure and results in right-sided heart failure (1). The prognosis of CTEPH is poor, with a 5-year survival rate of 10% for patients with a mean pulmonary arterial pressure (mPAP) > 50 mmHg (2).

Currently, pulmonary endarterectomy is the first therapeutic option for CTEPH (3). However, as many as 40% of patients are ineligible for surgical intervention due to reasons like severe comorbidities, distal lesions, and other patient specific factors (4, 5). Over the past decade, refined balloon pulmonary angioplasty (BPA) is emerging as an alternative option for inoperable CTEPH. Increasing evidences suggest that BPA could significantly improve hemodynamics, exercise tolerance and pulmonary function (6, 7).

Sympathetic, parasympathetic, and sensory nerve fibers innervate the pulmonary vasculature (8). In general, sympathetic nerve stimulation causes vasoconstriction of pulmonary vasculature and vagal stimulation results in vasodilation. Dysfunction of autonomic nervous system plays an important role in the development of pulmonary hypertension (8, 9). Moreover, it has been demonstrated that pulmonary artery denervation could improve the hemodynamics and exercise capacity of patients with pulmonary hypertension (10–13).

Heart-rate recovery at 1 min (HRR1) after exercise is a widely recognized surrogate marker of cardiac autonomic function, which is defined as the change in heart rate (HR) from the maximum workload to 60 s after exercise cession (14). HRR1 is a non-invasive and less time-consuming measure, which can be quickly obtained from routine exercise testing [cardiopulmonary exercise test (CPET) or 6-min walk test], compared with other HR responses, such as HR variability measured with Holter electrocardiography. Previous studies have reported that HRR1 was associated with exercise tolerance, hemodynamics and prognosis in patients with pulmonary arterial hypertension (PAH) (group I pulmonary hypertension) (15–17). Additionally, Inagaki et al. also reported that HRR1 was correlated with hemodynamics in CTEPH (group IV pulmonary hypertension) (18). Recently, we reported that HRR1 could independently predict prognosis in patients with CTEPH (19). However, to the best of our knowledge, no one has systematically studied the interaction between BPA and cardiac autonomic function. The main objectives of the present study were to determine whether BPA could alleviate sympathovagal imbalance and whether baseline HRR1 could predict outcome of BPA.

MATERIALS AND METHODS

Study Design and Participants

This retrospective study was conducted in Fuwai Hospital, Chinese Academy of Medical Sciences (Beijing, China). The study protocol was approved by the Ethics Committee of Fuwai Hospital (Approval NO: 2020-1275). Written informed consent was obtained from each patient. We screened all patients with inoperable CTEPH who underwent BPA from May, 2018 to Jan, 2021. The establishment of CTEPH was based on the 2015 European Society of Cardiology/European Respiratory Society guidelines (4). The eligibility for BPA was assessed by a multidiscipline team, consisting of a surgeon specializing in pulmonary endarterectomy, an interventional cardiologist specializing in BPA and a physician specializing in pulmonary hypertension. By design, patients were excluded if they: (1) did not have baseline HRR1 data; (2) did not undergo right heart catheterization (RHC) at follow-up; (3) were using beta-blockers or other antiarrhythmic agents. The following clinical data were collected via an electronic medical record system by two independent reviewers: demographics, World Health Organization functional class (WHO-FC), N-terminal pro-brain natriuretic peptide (NT-proBNP), arterial oxygen saturation (S_aO_2), 6-min walk distance (6MWD), targeted therapy at baseline, anticoagulants, parameters derived from echocardiography, CPET and RHC, the number of BPA sessions, the number of dilated subsegmental vessels, and the time interval between baseline and reevaluation RHC. Any discordance was resolved by the supervisors (ZHZ and ZHL).

RHC and BPA Procedure

In our center, a single catheterization laboratory visit consists of one BPA session and two RHC measurement. The first RHC measurement was performed before the initiation of BPA procedure to acquire the baseline hemodynamics. The second RHC measurement was performed immediately after the BPA procedure to acquire immediate post-operative hemodynamics. The detailed protocols of RHC and BPA have been provided in our previous publications (20, 21). Briefly, RHC was performed to measure hemodynamics, including mixed venous oxygen saturation, right atrial pressure, right ventricular pressure, mPAP, pulmonary arterial wedge pressure (PAWP), cardiac output (calculated by Fick's method) and pulmonary vascular resistance (PVR). After RHC, we performed pulmonary angiography, in anterior-posterior and lateral (60 degree) projections, to acquire overall view of the filling defect. Subsequently, a 70 cm 6F-7F long sheath (Flexor® Check-Flo® Introducer; Cook Medical, Bloomington, IN, USA), via the right femoral vein, was inserted into the lobar pulmonary artery to introduce a 6F guiding catheter (Multi-purpose [Cordis Corporation, Bridgewater, New Jersey, USA] or Amplatz Left [Terumo® Heartrail™ II; Terumo Corporation, Tokyo, Japan] or Judkins Right Tokyo, Japan] or Judkins Right [Terumo® Heartrail™ II; Terumo Corporation, Tokyo, Japan]). Based on selective pulmonary angiography, a 0.014-inch guidewire (Hi-Torque Pilot 50; Abbot, Santa Clara, CA, USA) was passed across the target lesion. To reduce the risk of complications, a 2.0 × 20 mm balloon was used at initial dilation, while smaller balloons may also be used for subtotal or total occlusion lesions. The balloon size was gradually increased in the subsequent BPA sessions according to the reference vessel diameter. Inflation pressure was dynamically adjusted, and selective angiography was performed to confirm vascular filling. Assessment of WHO-FC, NT-proBNP, S_aO_2, 6MWD, echocardiography and CPET were performed within 7 days prior to and after each BPA procedure. Follow-up reevaluation,

including RHC and CPET, would be performed over 3 months after the last BPA session.

Cardiopulmonary Exercise Test

The detailed protocols of CPET have been provided in our previous publications (22, 23). Briefly, an incremental symptom-limited exercise test was performed by the same examiner on an upright cycle ergometer using the COSMED Quark CPET system (COSMED, Rome, Italy). Three minutes of rest were followed by 3 min of unloaded pedaling, and progressively increasing workload by 5–30 W/min in a ramp pattern to maximum tolerance. HRR1 was defined as the change in HR from the maximum workload to 60 s after the completion of CPET. Oxygen consumption at peak (VO_2@Peak) was defined as the highest 30-s average of oxygen consumption in the last minute of exercise. HR at peak represented the highest HR observed during the exercise protocol. HR at recovery was defined as the value of HR at the moment when exercise stopped. ΔHR was defined as (HR at peak—HR at rest). HR acceleration time was defined as the time taken to increase to 75% of ΔHR (3 min of rest was not included). Slope of increased HR was defined as 75% of ΔHR/HR acceleration time (18).

Definition of BPA Responders and Non-responders

According to the results of reevaluation RHC at follow-up (over 3 months after the last BPA session), patients were categorized as BPA responders or BPA non-responders. In line with previous publications (24), the BPA responders were defined as patients who met one or both of the following criteria: (1) mPAP $\leq$ 30 mmHg and (2) a reduction of PVR $\geq$ 30%. Correspondingly, patients with a mPAP $>$ 30 mmHg and a reduction of PVR $<$ 30% at follow-up were categorized as BPA non-responders.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range). Categorical variables are given as counts or percentages. Comparison between BPA responders and non-responders were made using an independent-sample *t*-test, the Mann–Whitney *U*-test or the Chi-square test, as appropriate. Two-way analyses of variance were used to compare HRR1 at baseline, after the first BPA session and at follow-up with Tukey's test for multiple comparisons. Correlations between HRR1 and other variables were examined by using Spearman correlation coefficient. The association between baseline HRR1 and BPA outcome was evaluated by using logistic regression model. Univariate logistic regression was firstly performed to identify potential predictors of BPA success. Subsequently, variables with clinical significance or $P < 0.100$ in univariate analysis were selected for multivariable logistic regression (enter method). Receiver operator characteristic (ROC) curve analysis was performed to determine the optimal cutoff of HRR1 in predicting BPA outcome. A two-sided $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 25.0 (IBM SPSS Corp.; Armonk, NY, USA) and Prism GraphPad 8 (GraphPad Software, LaJolla, CA, USA).

RESULTS

Patient Enrollment

One hundred and twenty six patients underwent BPA from May, 2018 to Jan, 2021. Of these patients, 37 were excluded for missing baseline HRR1 data ($n = 12$), no reevaluation RHC at follow-up ($n = 22$) and using beta-blockers or other antiarrhythmic agents ($n = 3$). Among the remaining 89 patients, a total of 206 BPA sessions were performed [2.0 (interquartile range, 1.0-3.0)/per patient], with 1343 subsegmental vessels dilated [14.0 (interquartile range, 7.5–19.0) /per patient]. According to hemodynamics at follow-up, 53 were categorized as BPA responders and 36 patients as BPA non-responders.

Baseline Characteristics

Baseline characteristics of BPA responders and non-responders are summarized in **Table 1**. Compared with BPA non-responders, responders had lower levels of NT-proBNP, higher HRR1 and underwent more BPA sessions. Of note, mPAP (50.8 ± 11.9 mmHg vs. 51.5 ± 10.9 mmHg, $P = 0.861$) and PVR (10.2 ± 4.4 wood units vs. 10.0 ± 3.6 wood units, $P = 0.855$) at baseline were comparable between BPA responders and non-responders.

We also compared baseline characteristics of the included and excluded patients. Both groups were comparable in terms of demographics, exercise capacity, cardiac function and morphology, hemodynamics, and targeted therapy at baseline except higher proportion of WHO FC I/II in the included patients (**Supplementary Table 1**).

Clinical Assessments at Follow-Up

The clinical status of BPA responders and non-responders at follow-up is presented in **Table 2**. Like baseline, BPA responders still had higher proportion of WHO FC I/II, lower levels of NT-proBNP, and higher HRR1 at follow-up. More importantly, BPA responders had higher S_aO_2, more favorable echocardiographic parameters [reflected by smaller right ventricular end-diastolic diameter/left ventricular end-diastolic diameter (RVED/LVED), greater left ventricular ejection fraction and lower tricuspid regurgitation velocity], lower VE/VCO_2 slope and better hemodynamics (reflected by higher mixed venous oxygen saturation, lower mPAP, higher cardiac index and lower PVR) than BPA non-responders, even though these variables were comparable at baseline between the two groups. Among BPA responders, 21 achieved a mPAP $\leq$ 30 mmHg and 4 achieved a mPAP $<$ 25 mmHg.

The Effect of BPA on HRR1

Figure 1 shows that, compared with baseline, HRR1 tended to increase within 7 days after the first BPA session in both BPA responders and non-responders, and this improvement persisted at follow-up.

Correlation Between HRR1 and Well-Validated Markers of CTEPH Severity

As shown in **Table 3**, HRR1 at baseline and follow-up were associated with NT-proBNP, VO_2@Peak, VE/VCO_2 slope, RVED/LVED, mPAP, and PVR. Furthermore, the absolute change

TABLE 1 | Baseline characteristics of BPA responders and non-responders.

Variables	All patients ($n = 89$)	Responders ($n = 53$)	Non-responders ($n = 36$)	*P*-value*
Age, years	58.4 ± 11.6	58.0 ± 11.9	58.9 ± 11.3	0.607
Female, n (%)	47.0 (52.8)	28 (52.8)	19 (52.8)	0.996
Body mass index, kg/m^2	24.0 ± 3.3	24.1 ± 3.4	23.7 ± 3.3	0.552
WHO FC				0.082
I or II, n (%)	37.0 (41.6)	26 (49.1)	11 (30.6)	
III or IV, n (%)	52.0 (58.4)	27 (50.9)	25 (69.4)	
NT-proBNP, ng/L	814.0 (195.7, 1780.5)	497.0 (107.2, 1450.0)	1052.0 (460.2, 2460)	**0.019**
S_aO_2, %	91.6 ± 3.1	91.9 ± 2.7	91.2 ± 3.7	0.380
6MWD, m	366.5 ± 110.5	381.6 ± 96.0	343.9 ± 127.4	0.124
Targeted therapy				0.399
None, n (%)	36 (40.4)	25 (47.2)	11 (30.6)	
Monotherapy				
ERAs, n (%)	6 (6.7)	3 (5.7)	3 (8.3)	
PDE-5is, n (%)	21 (23.6)	9 (17.0)	12 (33.3)	
sGCs, n (%)	16 (18.0)	8 (15.1)	8 (22.2)	
Combination				
ERAs+PDE-5is, n (%)	9 (10.1)	7 (13.2)	2 (5.6)	
ERAs+ sGCs, n (%)	1 (1.1)	1 (1.9)	0	
Anticoagulants				0.414
Warfarin, n (%)	57 (64.0)	31 (58.5)	26 (72.2)	
Rivaroxaban, n (%)	29 (32.6)	20 (37.7)	9 (25.0)	
Dabigatran, n (%)	3 (3.4)	2 (3.8)	1 (2.8)	
Echocardiography				
LVED, mm	41.0 ± 5.4	40.9 ± 5.8	41.0 ± 4.9	0.621
RVED, mm	32.3 ± 6.2	31.7 ± 5.9	33.1 ± 6.7	0.451
RVED/LVED	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.841
LVEF, %	65.0 ± 5.5	65.1 ± 5.8	64.9 ± 5.0	0.923
TRV, m/s	4.3 ± 0.6	4.3 ± 0.7	4.4 ± 0.6	0.543
Cardiopulmonary exercise test				
HR at rest, beats/min	77.7 ± 13.1	77.2 ± 12.2	78.5 ± 14.4	0.645
HR at peak, beats/min	124.3 ± 20.7	127.0 ± 19.1	120.4 ± 22.5	0.140
HR at recovery[a], beats/min	122.8 ± 24.4	126.6 ± 19.6	117.2 ± 29.5	0.076
HRR1, beats	16.0 (10.0, 22.5)	17.0 (11.0, 26.5)	13.0 (8.0, 17.0)	**0.018**
ΔHR[b], beats	46.1 ± 20.1	49.8 ± 20.5	41.9 ± 18.7	0.067
HR acceleration time[c], s	407.9 ± 135.6	429.6 ± 111.5	376.1 ± 161.1	0.068
Slope of increased HR[d]	0.09 (0.07, 0.11)	0.09 (0.07, 0.11)	0.08 (0.07, 0.11)	0.701
VO_2@Peak, mL/min/kg	12.5 ± 3.5	12.8 ± 3.9	12.0 ± 2.6	0.278
VE/VCO_2 slope	49.2 ± 9.5	49.1 ± 9.7	49.3 ± 9.2	0.969
Hemodynamics				
S_vO_2, %	69.2 ± 5.2	69.9 ± 5.1	68.2 ± 5.3	0.137
mRAP, mmHg	8.0 (6.0, 9.0)	7.8 ± 3.1	8.2 ± 3.8	0.708
mPAP, mmHg	51.1 ± 11.4	50.8 ± 11.9	51.5 ± 10.9	0.861
PAWP, mmHg	10.0 ± 3.2	9.3 ± 3.1	10.8 ± 3.3	0.050
Cardiac index, L/min/m^2	3.0 ± 0.7	3.0 ± 0.7	3.0 ± 0.7	0.635
PVR, wood units	10.1 ± 4.1	10.2 ± 4.4	10.0 ± 3.6	0.855
BPA procedure				
Number of BPA sessions	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	2.0 (1.0, 3.0)	**0.039**
Number of dilated subsegmental vessels	14.0 (7.5, 19.0)	14.0 (9.0, 22.5)	12.0 (7.0, 17.0)	0.115
Time interval[$], days	227.0 (117.0, 422.0)	287.0 (161.0, 486.5)	203.5 (100.8, 372.0)	0.122

*Data are presented as mean ± standard deviation, median (interquartile range) or number (percentage). BPA, balloon pulmonary angioplasty; ERAs, Endothelin receptor antagonists; HR, heart rate; HRR1, heart-rate recovery at 1 min; LVED, Left ventricular end-diastolic diameter; LVEF, Left ventricular ejection fraction; mPAP, Mean pulmonary arterial pressure; mRAP, Mean right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAWP, Pulmonary arterial wedge pressure; PDE-5is, Phosphodiesterase-5 inhibitors; PVR, Pulmonary vascular resistance; RVED, Right ventricular end-diastolic diameter; 6MWD, 6-min walk distance; sGCs, Soluble guanylate cyclase stimulators; S_aO_2, arterial oxygen saturation; S_VO_2, Mixed venous oxygen saturation; TRV, tricuspid regurgitation velocity; VE/VCO_2 slope, Minute ventilation/carbon dioxide output slope; VO_2@Peak, Peak oxygen consumption; WHO FC, World Health Organization functional class. [$] Time interval between baseline and follow-up. *Responders vs. non-responders. [a] The value of HR at the moment when exercise stopped. [b] HR at peak minus HR at rest. [c] The time taken to increase to 75% of ΔHR (3 min of rest was not included). [d] 75% of ΔHR/HR acceleration time. Bold values mean $P < 0.05$.*

TABLE 2 | Re-assessment of BPA responders and non-responders at follow-up*.

Variables	Responders ($n = 53$)	Non-responders ($n = 36$)	*P*-value
WHO FC			**0.023**
I or II, n (%)	48.0 (90.6)	26.0 (72.2)	
III or IV, n (%)	5.0 (9.4)	10.0 (27.8)	
NT-proBNP, ng/L	103.0 (56.9, 237.7)	356.0 (158.2, 800.9)	**0.001**
S_aO_2, %	93.7 ± 2.6	91.3 ± 6.2	**0.007**
6MWD, m	436.8 ± 86.2	417.3 ± 91.3	0.396
Echocardiography			
LVED, mm	44.4 ± 4.6	42.6 ± 4.0	0.097
RVED, mm	28.1 ± 4.9	30.2 ± 6.4	0.089
RVED/LVED	0.6 ± 0.1	0.7 ± 0.2	**0.027**
LVEF, %	66.4 ± 4.8	62.9 ± 5.7	**0.008**
TRV, m/s	3.6 ± 0.7	4.2 ± 0.6	**<0.001**
Cardiopulmonary exercise test			
HR at rest, beats/min	74.7 ± 13.3	75.9 ± 11.5	0.561
HR at peak, beats/min	126.9 ± 18.5	121.2 ± 21.0	0.155
HR at recovery[a], beats/min	123.6 ± 25.3	120.3 ± 20.8	0.247
HRR1, beats	24 (17.5, 32)	20 (13.0, 28.0)	**0.048**
ΔHR[b], beats	52.2 ± 17.1	45.3 ± 18.3	0.075
HR acceleration time[c], s	451.3 ± 53.8	419.3 ± 115.0	0.446
Slope of increased HR[d]	0.09 (0.07, 0.10)	0.08 (0.06, 0.10)	0.444
VO_2@Peak, mL/min/kg	15.0 ± 3.8	13.5 ± 3.6	0.081
VE/VCO_2 slope	41.3 ± 7.9	45.3 ± 7.6	**0.025**
Hemodynamics			
S_vO_2, %	72.6 ± 4.7	69.6 ± 5.7	**0.007**
mRAP, mmHg	6.5 ± 3.0	7.0 ± 3.3	0.536
mPAP, mmHg	34.9 ± 9.2	46.2 ± 10.5	**<0.001**
PAWP, mmHg	10.3 ± 3.4	9.9 ± 3.6	0.567
Cardiac index, L/min/m^2	3.5 ± 0.9	3.1 ± 1.0	**0.020**
PVR, wood units	5.1 ± 2.3	9.1 ± 3.4	**<0.001**
Decrease of mPAP, %	−31.8 (−39.4, −19.3)	−10.8 (−18.8, −16.3)	**<0.001**
Decrease of PVR, %	−45.3 (−62.0, −35.5)	−12.2 (−25.1, 0.1)	**<0.001**

*Data are presented as mean ± standard deviation, median (interquartile range) or number (percentage). BPA, balloon pulmonary angioplasty; HR, heart rate; HRR1, heart-rate recovery at 1 min; LVED, Left ventricular end-diastolic diameter; LVEF, Left ventricular ejection fraction; mPAP, Mean pulmonary arterial pressure; mRAP, Mean right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAWP, Pulmonary arterial wedge pressure; PVR, Pulmonary vascular resistance; RVED, Right ventricular end-diastolic diameter; 6MWD, 6-min walk distance; S_aO_2, arterial oxygen saturation; S_VO_2, Mixed venous oxygen saturation; TRV, tricuspid regurgitation velocity; VE/VCO_2 slope, Minute ventilation/carbon dioxide output slope; VO_2@Peak, Peak oxygen consumption; WHO FC, World Health Organization functional class. *Over 3 months after the last BPA session. [a]The value of HR at the moment when exercise stopped. [b]HR at peak minus HR at rest. [c]The time taken to increase to 75% of ΔHR (3 min of rest was not included). [d]75% of ΔHR/HR acceleration time. Bold values mean $P < 0.05$.*

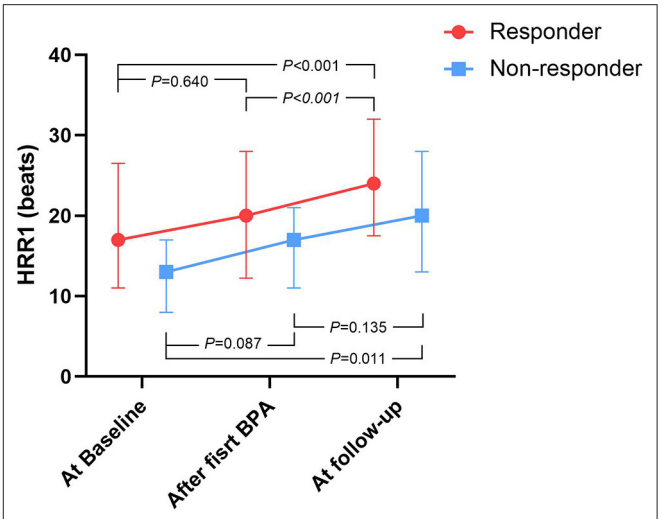

FIGURE 1 | The time course of HRR1 values during BPA procedure, stratified by BPA responders and non-responders. BPA, Balloon pulmonary angioplasty; HRR1, heart-rate recovery at 1 min. At baseline: within 7 days prior to the first BPA session, after first BPA: within 7 days after the first BPA session, At follow-up: over 3 months after the last BPA session.

of HRR1 from baseline to follow-up was associated with the absolute change of NT-proBNP, VO_2@Peak, VE/VCO_2 slope and RVED/LVED from baseline to follow-up. Additionally, the absolute change of HRR1 from baseline to follow-up was also associated with the number of BPA sessions, the number of BPA dilated subsegmental vessels, and the time interval between baseline and follow-up.

Predictors of BPA Responders

In univariate logistic regression, WHO FC, NT-proBNP, HR at recovery, HRR1, ΔHR, HR acceleration time, PAWP, the number of BPA sessions, the number of dilated subsegmental vessels, and the time interval from baseline to follow-up had a $P < 0.100$ (**Table 4**). Age, mPAP and PVR were also included in multivariable logistic regression (enter method) for their clinical significance. The number of dilated subsegmental vessels ($r = 0.861$, $P < 0.001$) and the time interval from baseline to follow-up ($r = 0.745$, $P < 0.001$) were excluded from multivariable logistic regression for their collinearity with the number of BPA sessions. Variables reflecting HR response to exercise (i.e., HR at recovery, HRR1, ΔHR and HR acceleration time) did not enter multivariable logistic regression simultaneously after consideration of sample size and collinearity. Finally, three to seven independent variables were included in multivariable logistic regression based on the number of events observed (25, 26). In model 1, HRR1 was adjusted for the number of dilated subsegmental vessels and PAWP. In subsequent models, HRR1 was further adjusted for the variables in model 1 plus age, NT-proBNP, WHO FC, mPAP, and PVR. In all these 6 multivariable logistic models, HRR1 remained as an independent predictor of BPA responder (**Table 5**). Similar analysis procedure was also performed for HR at recovery, ΔHR and HR acceleration time. However, no significant association was observed between these variables and BPA outcome (**Supplementary Table 2**).

Using ROC curve analysis, with the largest sum of sensitivity and specificity chosen, the cutoff value for HRR1 in predicting BPA responders was 19 beats, with an area under the curve of

TABLE 3 | Correlation between HRR1 and well-validated markers of CTEPH severity.

Variable	HRR1 at baseline	HRR1 at follow-up	ΔHRR1
NT-proBNP at baseline	$r = -0.422$, $\mathbf{P < 0.001}$		
NT-proBNP at follow-up		$r = -0.302$, $\mathbf{P = 0.006}$	
ΔNT-proBNP			$r = -0.225$, $\mathbf{P = 0.049}$
S_aO_2 at baseline	$r = 0.248$, $\mathbf{P = 0.019}$		
S_aO_2 at follow-up		$r = 0.172$, $P = 0.126$	
ΔS_aO_2			$r = 0.037$, $P = 0.750$
VO_2@Peak at baseline	$r = 0.540$, $\mathbf{P < 0.001}$		
VO_2@Peak at follow-up		$r = 0.410$, $\mathbf{P < 0.001}$	
ΔVO_2@Peak			$r = 0.293$, $\mathbf{P = 0.013}$
VE/VCO_2 slope at baseline	$r = -0.548$, $\mathbf{P < 0.001}$		
VE/VCO_2 slope at follow-up		$r = -0.481$, $\mathbf{P < 0.001}$	
ΔVE/VCO_2 slope			$r = -0.333$, $\mathbf{P = 0.004}$
RVED/LVED at baseline	$r = -0.250$, $\mathbf{P = 0.018}$		
RVED/LVED at follow-up		$r = -0.261$, $\mathbf{P = 0.018}$	
ΔRVED/LVED			$r = -0.089$, $P = 0.439$
mPAP at baseline	$r = -0.332$, $\mathbf{P = 0.001}$		
mPAP at follow-up		$r = -0.297$, $\mathbf{P = 0.007}$	
ΔmPAP			$r = -0.134$, $P = 0.245$
PVR at baseline	$r = -0.412$, $\mathbf{P < 0.001}$		
PVR at follow-up		$r = -0.311$, $\mathbf{P = 0.005}$	
ΔPVR			$r = -0.069$, $P = 0.557$
Number of BPA sessions			$r = 0.411$, $\mathbf{P < 0.001}$
Number of dilated subsegmental vessels			$r = 0.445$, $\mathbf{P < 0.001}$
Time interval*			$r = 0.298$, $\mathbf{P = 0.008}$

*BPA, balloon pulmonary angioplasty; CTEPH, Chronic thromboembolic pulmonary hypertension; HRR1, heart-rate recovery at 1 min; LVED, Left ventricular end-diastolic diameter; LVEF, Left ventricular ejection fraction; mPAP, Mean pulmonary arterial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PVR, Pulmonary vascular resistance; RVED, Right ventricular end-diastolic diameter; S_aO_2, arterial oxygen saturation; VE/VCO_2 slope, Minute ventilation/ carbon dioxide output slope; VO_2@Peak, Peak oxygen consumption. *Time interval between baseline and follow-up. Δ means the absolute change of these variables from baseline to follow-up. Bold values mean $P < 0.05$.*

0.643 (95% CI: 0.528–0.758). According to this cutoff value, 30 patients were classified into HRR1 $\geq$ 19 beats and 59 patients into HRR1 $<$ 19 beats. The number of BPA sessions [median (interquartile range): 2.0 (1.0, 3.0) vs. 2.0 (1.0, 3.0), $P = 0.561$], the number of dilated subsegmental vessels [median (interquartile range): 10.5 (7.0, 17.0) vs. 14.0 (9.0, 19.0), $P = 0.101$], and the time interval between baseline and reevaluation RHC [median (interquartile range): 267.5 (115.3, 425.3) days vs. 210.0 (115.0, 427.0) days, $P = 0.801$] were comparable between patients with HRR1 $\geq$ 19 beats and $<$ 19 beats. Compared with baseline, mPAP and PVR improved at follow-up in both HRR1 $\geq$ 19 beats and $<$ 19 beats groups (**Figure 2**). However, the proportion of BPA responders was significantly higher in patients with HRR1 $\geq$ 19 beats (80.0% vs. 49.1%, $P = 0.005$).

DISCUSSION

The most important finding of our study is that easily available and non-invasive HRR1 is a strong predictor of BPA outcome. Second, we found that HRR1 tended to increase within 7 days after the first BPA session, and this improvement persisted at follow-up, suggesting that BPA could alleviate sympathovagal imbalance. Last, our results showed that improvement in HRR1 was associated with improvement in the well-validated markers of CTEPH severity, indicating that HRR1 might serve as a biomarker which could monitor the efficacy of BPA sessions.

BPA Alleviated Sympathovagal Imbalance

As shown in **Figure 1**, HRR1 tended to increase within 7 days after the first BPA session and this improvement persisted at follow-up in both BPA responders and non-responders. Similarly, Huo et al. found that the administration of Ambrisentan continuously improved HRR1 in patients with PAH (27). We also found that HRR1 was associated with well-validated markers of CTEPH severity both at baseline and follow-up. More importantly, the change of HRR1 from baseline to follow-up was also correlated with the change of those markers. Therefore, easily available and non-invasive HRR1 might serve as a biomarker which could dynamically monitor the efficacy of BPA sessions.

In healthy subjects, HR is regulated by a sympathovagal balance (28, 29). During exercise, sympathetic activation and parasympathetic withdrawal both contribute to the increase of HR; after peak exercise, sympathetic withdrawal and parasympathetic reactivation both contribute to the recovery of HR (14, 30). Pulmonary hypertension results in right-sided

TABLE 4 | Univariate logistic regression analysis for BPA responders.

Variable	OR	95% CI	*P*-value
Age	0.993	0.957–1.031	0.712
Female	1.002	0.429–2.340	0.996
Body mass index	1.040	0.914–1.184	0.547
WHO FC	0.457	0.188–1.113	0.085
NT-proBNP	1.000	0.999–1.000	0.074
S_aO_2	1.074	0.936–1.232	0.308
6MWD	1.003	0.999–1.007	0.128
RVED/LVED	0.476	0.066–3.450	0.463
LVEF	1.005	0.929–1.086	0.905
TRV	0.810	0.413–1.587	0.539
HR at rest	0.992	0.960–1.025	0.641
HR at peak	1.016	0.995–1.038	0.142
HR at recovery[a]	1.017	0.997–1.037	0.088
HRR1	1.041	0.997–1.088	0.071
ΔHR[b]	1.021	0.998–1.044	0.071
HR acceleration time[c]	1.003	1.000–1.006	0.081
Slope of increased HR[d]	0.035	0.000–15.984	0.284
VO_2@Peak	1.075	0.943–1.226	0.277
VE/VCO_2 slope	0.998	0.953–1.045	0.931
S_vO_2	1.065	0.980–1.158	0.138
mRAP	0.964	0.851–1.092	0.566
mPAP	0.994	0.958–1.032	0.762
PAWP	0.857	0.741–0.990	**0.037**
Cardiac Index	1.106	0.600–2.038	0.747
PVR	1.014	0.911–1.127	0.802
Number of BPA sessions	1.545	1.067–2.236	**0.021**
Number of dilated subsegmental vessels	1.062	1.004–1.123	**0.037**
Time interval*	1.002	1.000–1.004	0.080

*BPA, balloon pulmonary angioplasty; HRR1, heart-rate recovery at 1 min; LVED, Left ventricular end-diastolic diameter; LVEF, Left ventricular ejection fraction; mPAP, Mean pulmonary arterial pressure; mRAP, Mean right atrial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAWP, Pulmonary arterial wedge pressure; PVR, Pulmonary vascular resistance; RVED, Right ventricular end-diastolic diameter; 6MWD, 6-min walk distance; S_VO_2, Mixed venous oxygen saturation; TRV, tricuspid regurgitation velocity; VE/VCO_2 slope, Minute ventilation/ carbon dioxide output slope; VO_2@Peak, Peak oxygen consumption; WHO FC, World Health Organization functional class. *Time interval between baseline and follow-up. [a]The value of HR at the moment when exercise stopped. [b]HR at peak minus HR at rest. [c]The time taken to increase to 75% of ΔHR (3 min of rest was not included). [d]75% of ΔHR/HR acceleration time. Bold values mean $P < 0.05$.*

TABLE 5 | Multivariable logistic regression analysis for BPA responders.

Model	Variable	OR	95% CI	*P*-value
1	HRR1	1.059	1.010–1.110	**0.017**
	Number of BPA sessions	1.802	1.173–2.771	**0.007**
	PAWP	0.824	0.700–0.971	**0.021**
2	HRR1	1.066	1.013–1.121	**0.013**
	Number of BPA sessions	1.881	1.199–2.950	**0.006**
	PAWP	0.826	0.700–0.975	**0.024**
	Age	1.018	0.972–1.065	0.453
3	HRR1	1.056	1.002–1.113	**0.041**
	Number of BPA sessions	1.896	1.198–3.002	**0.006**
	PAWP	0.816	0.689–0.968	**0.019**
	Age	1.017	0.971–1.066	0.463
	NT-proBNP	1.000	0.999–1.000	0.239
4	HRR1	1.057	1.001–1.116	**0.048**
	Number of BPA sessions	1.898	1.197–3.010	**0.006**
	PAWP	0.816	0.689–0.968	**0.019**
	Age	1.017	0.971–1.066	0.464
	NT-proBNP	1.000	0.999–1.000	0.270
	WHO FC	1.042	0.309–2.505	0.948
5	HRR1	1.061	1.004–1.122	**0.037**
	Number of BPA sessions	1.860	1.167–2.966	**0.009**
	PAWP	0.805	0.676–0.959	**0.015**
	Age	1.021	0.973–1.071	0.394
	NT-proBNP	1.000	0.999–1.000	0.216
	WHO FC	0.943	0.273–3.258	0.927
	mPAP	1.021	0.972–1.071	0.410
6	HRR1	1.062	1.004–1.123	**0.034**
	Number of BPA sessions	1.894	1.177–3.050	**0.009**
	PAWP	0.826	0.695–0.982	**0.031**
	Age	1.018	0.971–1.068	0.458
	NT-proBNP	1.000	0.999–1.000	0.126
	WHO FC	0.803	0.223–2.887	0.737
	PVR	1.121	0.952–1.319	0.169

BPA, balloon pulmonary angioplasty; HRR1, heart-rate recovery at 1 min; mPAP, Mean pulmonary arterial pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAWP, Pulmonary arterial wedge pressure; PVR, Pulmonary vascular resistance; WHO FC, World Health Organization functional class. Bold values mean $P < 0.05$.

heart failure, which is a syndrome affecting many organs rather than a condition of pure hemodynamic failure. Previous studies have reported sympathetic hyperactivity and parasympathetic hypoactivity in PAH (9, 31), which is considered as an adaptive mechanism for reduced cardiac output (8). The aforementioned mechanism could also be operational in CTEPH. Thus, it is possible that the impaired HRR1 observed in our study reflected potentially continued sympathetic activation and a lack of normal parasympathetic reactivation after peak exercise. We hypothesized that BPA ameliorated hemodynamics, improved right and left ventricular function, increased cardiac output and then alleviated sympathovagal imbalance (reflected by increased HRR1).

Baseline HRR1 Predicts the Outcome of BPA

To date, there is no widely recognized tools for predicting the efficacy of BPA prior to intervention. In the present study, we found that baseline HRR1 was a strong predictor of BPA outcome. Previous studies have demonstrated that both sympathetic hyperactivity and parasympathetic hypoactivity are associated with pulmonary vascular remodeling and right ventricular dysfunction (8, 9, 32). At baseline, BPA non-responders had lower HRR1 than responders, even though hemodynamics were comparable between both groups. At follow-up, BPA non-responders had reasonably worse hemodynamics and still had lower HRR1 than responders. This indicated that, both at baseline and follow-up, BPA non-responders had more severe sympathovagal imbalance than

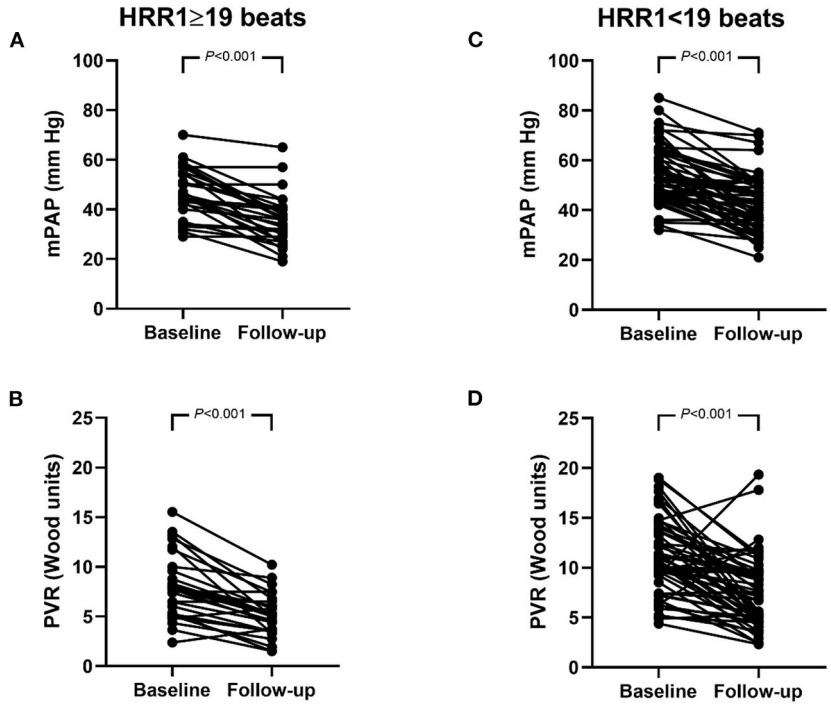

FIGURE 2 | (A–D) Hemodynamics at baseline and follow-up in patients with HRR1 ≥ 19 beats and < 19 beats. mPAP **(A)** and PVR **(B)** in patients with HRR1 ≥ 19 beats. mPAP **(C)** and PVR **(D)** in patients with HRR1 < 19 beats. At baseline, HRR1 ≥ 19 beats vs. HRR1 < 19 beats (mPAP: 46.2 ± 10.2 mm Hg vs. 53.5 ± 11.3 mmHg, $P = 0.004$; PVR: 8.0 ± 3.2 wood units vs. 11.3 ± 4.0 wood units, $P < 0.001$). At follow-up, HRR1 ≥ 19 beats vs. HRR1 < 19 beats (mPAP: 35.5 ± 10.0 mm Hg vs. 41.4 ± 11.3 mmHg, $P = 0.012$; PVR: 5.1 ± 2.1 wood units vs. 7.4 ± 3.6 wood units, $P = 0.003$). HRR1, heart-rate recovery at 1 min; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance.

responders. We speculated that severe sympathovagal imbalance at baseline (reflected by HRR1 < 19 beats) may increase the tone of pulmonary vasculature to a higher level, which weakens the efficacy of the future BPA sessions. Thus, for BPA non-responders with HRR1 < 19 beats at baseline, transcatheter pulmonary artery denervation might serve as a complementary therapy. Romanov et al. reported that, in patients with residual CTEPH after pulmonary endarterectomy, those underwent transcatheter pulmonary artery denervation had greater improvement in hemodynamics and exercise capacity than those treated with riociguat (13).

It should be stressed that we do not mean to imply that HRR1 < 19 beats is a contraindication for BPA. As shown in **Figure 2**, mPAP and PVR were also decreased significantly after BPA in patients with HRR1 < 19 beats. Our results should be interpreted as: when undergoing similar amount of BPA sessions and dilating similar amount of subsegmental vessels, the percentage of BPA responders were higher in patients with HRR1 ≥ 19 beats at baseline than that in those with HRR1 < 19 beats at baseline (80% vs. 49.1%, $P = 0.005$). Another issue is that the number of BPA sessions is relatively small for both BPA-responders and non-responders in the present study. Non-responders may turn into responders in the future BPA sessions. However, this does not undermine the clinical importance of our results. Because clinicians could anticipate that patients with HRR1 ≥ 19 beats at baseline may achieve a more favorable hemodynamic amelioration with less BPA session and lower medical costs than those with HRR1 < 19 beats.

LIMITATIONS

The main limitation of the study is the inherent biases of a retrospective study. Thirty-seven patients were excluded from the study. Nevertheless, we found that the baseline characteristics were comparable between included and excluded patients. Another limitation is that the autonomic function was indirectly assessed by using HRR1 as a surrogate marker in the present study. The interaction between BPA and the autonomic function needs to be further investigated by using gold standard methods such as the measurement of muscle sympathetic nerve activity.

CONCLUSION

BPA could significantly improve HRR1, which indicates the alleviation of sympathovagal imbalance. The change in HRR1 after BPA is associated with the change of well-validated markers of CTEPH severity, which suggests that HRR1 might serve as a biomarker for dynamically monitoring the efficacy of BPA sessions. Baseline HRR1 is a strong independent predictor of BPA outcome.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Fuwai Hospital (Approval No. 2020-1275). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZL and ZZ contributed to the conception of the study and are guarantors of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article. YZ and XL wrote the manuscript. QZh, QZe, TY, QJ, LY, AD, XM, and CA contributed to data collection. ZL, CX, and QL contributed to the acquisition of funding. All authors critically reviewed the manuscript for intellectual content and had final responsibility for the decision to submit for publication, contributed to data analysis, and interpretation.

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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.2022.795420/full#supplementary-material

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