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Women in heart failure and transplantation

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Women in heart failure and transplantation

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ORIGINAL RESEARCH
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Disproportionate Mitral Regurgitation Determines Survival in Acute Heart Failure

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Objectives: To assess the prevalence and impact of mitral regurgitation (MR) on survival in patients presenting to hospital in acute heart failure (AHF) using traditional echocardiographic assessment alongside more novel indices of proportionality.

Background: It remains unclear if the severity of MR plays a significant role in determining outcomes in AHF. There is also uncertainty as to the clinical relevance of indexing MR to left ventricular volumes. This concept of disproportionality has not been assessed in AHF.

Methods: A total of 418 consecutive patients presenting in AHF over 12 months were recruited and followed up for 2 years. MR was quantitatively assessed within 24 h of recruitment. Standard proximal isovelocity surface area (PISA) and a novel proportionality index of effective regurgitant orifice/left ventricular end-diastolic volume (ERO/LVEDV) >0.14 mm^2/ml were used to identify severe and disproportionate MR.

Results: Every patient had MR. About 331/418 (78.9%) patients were quantifiable by PISA. About 165/418 (39.5%) patients displayed significant MR. A larger cohort displayed disproportionate MR defined by either a proportionality index using ERO/LVEDV >0.14 mm^2/ml or regurgitant volumes/LVEDV >0.2 [217/331 (65.6%) and 222/345 (64.3%), respectively]. The LVEDV was enlarged in significant MR−129.5 ± 58.95 vs. 100.0 ± 49.91 ml in mild, [$p < 0.0001$], but remained within the normal range. Significant MR was associated with a greater mortality at 2 years {44.2 vs. 34.8% in mild MR [hazard ratio (HR) 1.39; 95% CI: 1.01–1.92, $p = 0.04$]}, which persisted with adjustment for comorbid conditions (HR; 1.43; 95% CI: 1.04–1.97, $p = 0.03$). Disproportionate MR defined by ERO/LVEDV >0.14 mm^2/ml was also associated with worse outcome [42.4 vs. 28.3% (HR 1.62; 95% CI 1.12–2.34, $p = 0.01$)].

Conclusions: MR was a universal feature in AHF and determines outcome in significant cases. Furthermore, disproportionate MR, defined either by effective regurgitant orifice (ERO) or volumetrically, is associated with a worse prognosis despite the absence of adverse left ventricular (LV) remodeling. These findings outline the importance of adjusting acute volume overload to LV volumes and call for a review of the current standards of MR assessment.

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INTRODUCTION

Acute heart failure (AHF) is associated with high mortality (1) and remains a substantial financial and healthcare burden (2). The recognition and prevention of precipitating factors, therefore, remain of the utmost importance (3). Acute and worsening of chronic degenerative mitral regurgitation (MR) (4, 5) is a recognized cause of AHF-related hospitalization (6) whereas the role of functional mitral regurgitation (FMR), secondary to cardiac remodeling and left ventricular (LV) dysfunction (7), is less established.

Functional mitral regurgitation (FMR) has a significant impact on morbidity and mortality (8). However, the complexity and heterogeneity of myocardial disease in heart failure (HF) and the subsequent alterations to the mitral valve apparatus have made the quantitative analysis of MR difficult. This has created disagreements between guidelines that suggest differing cut-offs for severe FMR (9, 10). Despite good prognostic value to these assessments (11), there has been no significant benefit from surgery and/or interventions based on these quantitative thresholds (12, 13).

It has become clear that the current standard of echocardiographic assessment, developed for primary MR, where the left heart has the advantage of intrinsic compliance (14) and time to compensate for volume overload (15), cannot be applied automatically to FMR without adjustments. There is emerging evidence that in this group of patients the volume loading from MR should be adjusted to the LV volume. A novel conceptual approach of using the ratio of MR effective regurgitant orifice (ERO) to left ventricular end-diastolic volume (LVEDV) has been suggested to explain differing outcomes in two recent, large, randomized, controlled trials of percutaneous mitral valve repair (16, 17) for patients with HF with significant FMR (18). There are ongoing calls for this approach to be validated in prospective studies (19). We have termed this value the proportionality index (PI).

The analysis of the implications of disproportionate MR has been investigated in individual retrospective assessments of both the MITRA-FR (20) and the COAPT (21) randomized-controlled trials alongside a combined appraisal (22). These assessments have provided conflicting results, with vigorous debate (23–25) and investigations as to the implications of disproportionality assessments based on either EROA/LVEDV $>$ 0.14–0.15 or regurgitant volume (RV)/LVEDV $>$ 0.2 (i.e., 20%) (26). This clearly calls for the assessment of this concept in a "real-world" clinical scenario faced by cardiologists and acute physicians.

Most previous studies have enrolled patients with *chronic* HF and optimized pharmacotherapy (17). Very little is known on the prevalence and significance of MR in patients presenting in *acute* HF. The handful of prospective studies investigating its role have not included either early or volumetric assessments and have mainly focused on stable patients (27, 28). Preliminary data from the European Heart Failure survey and US cohort studies suggest MR in hospitalized patients with HF is common (29, 30) but prognostic implications remain unclear. It is possible that MR is missed altogether in patients with AHF due to the dynamic nature of MR (31, 32), particularly if LV volumes remain within an accepted normal range. We, therefore, conducted a study to examine the prevalence and significance of MR in AHF and to determine whether proportionality indices would be effective at identifying patients who face adverse consequences of regurgitant mitral valves.

METHODS

Patients and Trial Design

This was a prospective observational study to assess the prevalence of significant MR in consecutive patients admitted with an acute or exacerbation of chronic heart failure (A/ECHF) over 12 months following a 1 month rolling-in period in a single center [St Peter's Hospital (SPH), Chertsey, UK]. Enrolment, data collection, storage, and analysis occurred at this site. Hospital coding data from 2013 to 2016 was used to estimate a recruitment target of 500 patients.

Patients who displayed signs or symptoms of AHF were screened according to the pre-specified study protocol (**Appendix 1** in Supplementary Material). Locations of assessment included the accident and emergency department, intensive care unit, high-dependency unit, acute medical unit, coronary care unit, respiratory ward, and care for the elderly ward. If A/ECHF was considered as the primary cause of admission following physician-led clinical examination, patients were consented and recruited into the study if bedside point-of-care brain natriuretic peptide (BNP) level was raised. They underwent transthoracic echocardiography (TTE) within 24 h of recruitment to assess cardiac and valvular function (**Appendix 2** in Supplementary Material).

Patients with sepsis, respiratory failure secondary to pulmonary causes, stable chronic HF with an alternative diagnosis, and existing in-patients at the start of recruitment were not included. Patients in whom echocardiography was not possible (deceased, did not consent or discharged) were excluded from further analysis. All recruited patients were followed up for 2 years.

Trial Oversight

The trial was designed by the physician-led executive committee in conjunction with Ashford & St Peter's Hospital Trust Research and Development team. The research protocol was approved by relevant institutional review boards and ethics committees and all

participants gave written informed consent. The study complied with the Declaration of Helsinki.

Data were stored electronically and were available for review by all authors. The first and last authors developed the manuscript for submission. The design and implementation of this project and the decision to submit for publication were by the last author. Statistical analysis was carried out by an independent organization with established expertise in the statistical analysis including government policy projects.

Study Data Collection

Diagnosis of AHF on admission was made by a dedicated study physician according to European Society of Cardiology (ESC) guidelines (10). BNP and TTE results were not disclosed to the emergency/acute clinical team. Demographic and past medical history data were identified from hospital records, while sex and ethnicity were self-reported by patients. Mortality data were recorded from the summary care record system used nationally by general practices in the United Kingdom and via the Evolve^{TM} (Kainos, United Kingdom) online software for in-patient deaths recorded by SPH. If unavailable, general practices and family members were directly contacted.

Point-of-Care BNP

Point-of-care BNP measurement was performed using i-STAT Point of Care (POC) Serum BNP analyzer (Abbott, Illinois, USA) with cut-off value >100 pg/ml. This POC system has displayed good clinical agreement at lower BNP values (33). BNP cartridges were acquired and stored according to manufactures guidelines.

Echocardiography

Echocardiography was performed using a dedicated G.E. Vivid S70 (GE Healthcare, Illinois, USA) machine. Images were stored and analyzed offline using EchoPac software version 201 (GE Healthcare, USA). Most of the TTE studies were performed by a single accredited operator according to study protocol (**Appendix 3** in Supplementary Material). Every study was analyzed by the primary operator and cross-checked by an expert in echocardiography. Standard echo parameters of left heart geometry: (LVEDV and left ventricular end-systolic volumes (LVESV), LA area (LAA) were measured. MR quantitative analysis was performed using the PISA method to derive MR ERO area and regurgitant volume (RV) (34). Significant MR was defined as MR greater than mild severity, with grading categorized according to ESC guidelines (34). Systolic pulmonary artery pressure (sPAP) was estimated from tricuspid regurgitant jet and jugular vein respiratory fluctuations.

Statistical Analysis

Data analysis was primarily carried out JB. Receiver Operator Curve analyses were carried out for the ERO and the PI (ERO/LVEDV). The optimum cut-off for the prediction of 24-month mortality was estimated by identifying the sensitivity and specificity associated with the maximum Youden Index. These cut-offs were then used as a binary determinant of proportionate vs. disproportionate MR. To evaluate volumetric assessments of proportionality in MR we also included the regurgitant volume/LVEDV and defined proportionate vs. disproportionate MR as $<$ or $>$ 0.2, as outlined in Namazi et al. (26).

Sociodemographic and baseline characteristics were summarized by severity group and overall for the complete analysis set. Categorical variables were reported as numbers and percentages and between-groups comparisons were compared using the chi-squared test or Fisher's exact test, as appropriate. Continuous variables were reported as means and standard deviations or as medians and interquartile ranges and compared using Student's *t*-test or the Mann–Whitney *U*-test.

For the primary analysis of 24-month mortality, unstratified Kaplan–Meier curves were constructed. Hazard ratios were estimated using an unadjusted Cox-regression model, with statistical significance being assessed using the log rank test. Secondary analyses were carried out using Cox-regression analyses adjusted for significant covariates. The selection of covariates to be included was based on initial multiple univariate regression analyses, modified according to clinical opinion from the research team. These were gender, age, body-mass index, and pre-existing diagnoses of chronic obstructive pulmonary disorder, hypertension, chronic kidney disease, ischemic heart disease, diabetes mellitus, and cerebrovascular disease. For all comparisons, the threshold of statistical significance was set at a two-sided α value of 0.05.

Data Storage

Enrolled patients had an objective, echocardiographic and clinical characteristics collected *via* a standardized collection form which was stored online in a password-protected database specifically devised for study by Metanoic Health Ltd., United Kingdom.

Data were entered by primary operators and double-checked by independent specialists. Histograms were performed on all continuous data to screen for statistical outliers using Statistical Package for the Social Sciences (SPSS) version 24 (IBM, New York, USA). Any outlying data points were then rechecked to screen for input errors or errors of measurement. The echocardiography data was retained on two separate hard drives to allow for off-site analysis and to reduce the risk of data loss in accordance with Good Clinical Practice research protocols.

RESULTS

With a 1-month run-in period, 616 consecutive patients presenting with symptoms of A/ECHF were assessed for eligibility for the MRAHF study from July 2016 to August 2017. About 447 (72.6%) participants were recruited. About 418 individuals were included in the final analysis after excluding the data from rehospitalization and three individuals lost to follow-up.

All patients were found to have MR (100%) and 434/447 (97.1%) patients had functional MR as their underlying etiology. Based on clinical interpretation of MR on echocardiography patients were divided into two groups: all patients with moderate and above severity of MR were included in group 1 (significant MR) whereas all other patients in group 2 had mild MR. There was a high prevalence of ESC

TABLE 1 | Baseline characteristics.

	All patients ($n = 418$)	Significant MR ($n = 165$)	Mild MR ($n = 253$)	p-value*
Demographics				
Age, mean (SD), y	78.7 (11.7)	79.3 (12.0)	78.3 (11.5)	0.395
Gender (male), n (%)	222 (53.1)	84 (50.9)	138 (54.6)	0.459
Race, *n* (%)				
White	390 (93.3)	150 (90.9)	240 (94.9)	0.110
BAME	28 (6.7)	15 (9·1)	13 (5.1)	0.110
BMI, mean: kg/m^2 (sd)	28.6 (8.06)	29.5 (8.82)	27.2 (6.52)	0.004
Comorbidities *n* (%)				
Coronary artery disease	152 (36.4)	65 (39.4)	87 (34.4)	0.300
Hypertension	232 (55.5)	89 (53.9)	143 (56.5)	0.602
Diabetes	130 (31.1)	41 (24.9)	89 (35.2)	0.026
Chronic kidney disease	189 (45.2)	73 (44.2)	116 (45.9)	0.733
COPD	61 (14.6)	18 (10.9)	43 (17.0)	0.085
Cerebrovascular disease	64 (15.3)	30 (18.2)	34 (13.4)	0.183
Presentation				
NYHA class, *n* (%)				
II	37 (8.9)	12 (7.3)	25 (9.9)	0.361
III	161 (38.5)	61 (37.0)	100 (39.5)	0.608
IV	220 (52.6)	92 (55.8)	128 (50.6)	0.299
ECG findings				
Sinus rhythm, *n* (%)	163 (39.0)	56 (33.9)	107 (42.3)	0.086
AF, *n* (%)	192 (45.9)	85 (51.5)	107 (42.3)	0.065
Paced, *n* (%)	39 (9.3)	15 (9.1)	24 (9.5)	0.891
Other rhythm, *n* (%)	18 (4.3)	5 (3.0)	13 (5.1)	0.300
Observations				
BPs, mmHg mean (sd)	136 (26.4)	133 (25.4)	138 (27.0)	0.040
BPd, mmHg mean (sd)	76 (16.9)	75 (17.7)	76 (16.9)	0.539
HR, bpm mean (sd)	89 (27.2)	89 (27.7)	90 (26.9)	0.663
SpO_2, % mean (sd)	95.0 (3.78)	95.2 (3.82)	94.8 (3.75)	0.209
Biochemistry				
Hemoglobin, g/l mean (sd)	122.5 (21.76)	121.6 (22.39)	123.1 (21.36)	0.486
Creatinine, μmol/l mean (sd)	120.0 (73.44)	126.9 (85.27)	115.6 (64.36)	0.148
eGFR, ml/min/1.73 m^2 mean (sd)	48.3 (14.56)	47.1 (15.74)	49.1 (13.72)	0.181
CRP, mg/dl mean (sd)	29.5 (42.74)	31.9 (44.09)	28.0 (41.88)	0.385
BNP, ng/l mean (sd)	1,363 (1254.2)	1,729 (1315.7)	1,124 (1153.9)	<0.0001

*BAME, Black, Asian, and minority ethnic; COPD, chronic obstructive pulmonary disorder; NYHA, New York Heart Association; AF, Atrial fibrillation; BPs, blood pressure systolic; BPd, blood pressure diastolic; HR, heart rate; SpO_2, peripheral capillary oxygen saturation; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; BNP, brain natriuretic peptide. *p-values are estimated using Mann–Whitney U-test for medians, N−1 χ^2 for proportions and independent samples t-test for continuous variables.*

guideline-defined significant MR in our cohort. 165 (39.5%) of enrolled patients had significant MR, 253 (60.5%) had mild MR.

There were broad similarities in demographics, comorbidities, and presenting features between patients with significant and mild MR (**Table 1**). The mean age across both groups was 78.7; 53.1% were males and 93.3% self-identified as "white" ethnicity. Patients were highly symptomatic −361 (91.1%) with NYHA class III/IV presentation but not in cardiogenic shock [mean blood pressure (BP) 136/76 mmHg]. Patients did not have features of severe anemia or infection. The overall BNP averaged 1,363 ng/l and this was higher in patients with severe MR (1,729 ng/l) compared to mild MR (1,124 ng/l) ($p < 0.0001$).

The medical therapy at index admission was similar between both groups (**Table 2**) except for angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARBs) which were less common in the group with significant MR [34.5 vs. 46.6% ($p = 0.032$)]. Both groups had an increase in the intensity of HF therapy at discharge. There was a higher rate of prescription of mineralocorticoid receptor antagonists in the significant MR group [30.3 vs. 20.9% ($p = 0.023$)]. Based on this, the clinical team, blind to study findings, provided better optimization of medications for patients with significant MR.

Quantitative assessment of MR on echocardiography indicated significantly higher EROA and regurgitant volume (RV) in significant MR (**Table 3**). LV volumes remained within

TABLE 2 | Medical therapy on admission and discharge.

	Medications on admission, *n* (%)			Medications on discharge, *n* (%)			Difference—discharge vs. admission*			
	Significant MR (*n* = 165)	Mild MR (*n* = 253)	*p* value** (significant MR vs. mild MR)	Significant MR (*n* = 165)	Mild MR (*n* = 253)	*p* value** (significant MR vs. mild MR)	Significant MR		Mild MR	
							n (% change)	*p*-value***	*n* (% change)	*p*-value***
ACEi/ARB	57 (34.5)	118 (46.6)	0.032	73 (44.2)	123 (48.6)	0.457	16 (10.1)	0.029	8 (3.7)	0.328
BB	91 (55.2)	140 (55.3)	0.865	126 (76.4)	184 (72.7)	0.252	41 (26.0)	<0.0001	47 (19.3)	<0.0001
MRA	26 (15.8)	25 (9.9)	0.081	50 (30.3)	53 (20.9)	0.023	26 (16.5)	<0.0001	28 (11.5)	<0.0001
Diuretic	89 (53.9)	118 (46.6)	0.180	135 (81.8)	191 (75.5)	0.053	51 (32.3)	<0.0001	75 (30.9)	<0.0001
CCB	27 (16.4)	50 (19.8)	0.352	17 (10.3)	44 (17.4)	0.051	−8 (−5.1)	0.134	−4 (−1.7)	0.652
Digoxin	21 (12.7)	28 (11.1)	0.638	28 (17.0)	54 (21.3)	0.304	9 (5.7)	0.108	26 (10.7)	<0.0001

MR, mitral regurgitation; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta-blocker; MRA, mineralocorticoid receptor blocker; CCB, calcium channel blocker.

**Results relate to 401 patients with paired admission/discharge data, so figures may vary from the difference of the values in the previous columns.*

***Within-group p-values are estimated using N−1 χ^2.*

****Between-group p-values are estimated using McNemar's test for paired proportions.*

TABLE 3 | Hemodynamic assessment by echocardiography.

	All patients (*n* = 418)	Significant MR (*n* = 165)	Mild MR (*n* = 253)	*p*-value*
MR assessment				
Qualitative assessment, *n* (%)				
Mild	253 (60.5)	0 (0.0)	253 (100.0)	–
Moderate	87 (20.8)	87 (52.7)	0 (0.0)	–
Severe	78 (18.7)	78 (47.3)	0 (0.0)	–
Quantitative assessment (QA)				
ERO, cm^2; mean (SD)	0.23 (0.150)	0.33 (0.153)	0.14 (0.063)	<0.0001
QA not performed, *n* (%)	87 (20.8)	4 (2.4)	83 (32.8)	
RV, ml; mean (SD)	32.5 (20.14)	47.8 (17/79)	17.8 (7.10)	<0.0001
QA not performed, *n* (%)	88 (21.1)	4 (0.6)	84 (33.2)	
Vena contracta, mm; mean (SD)	0.38 (0.127)	0.47 (0.108)	0.30 (0.085)	<0.0001
QA not performed	60 (14.4)	1 (0.6)	59 (23.3)	
Left-heart volumes and estimated systolic PA pressure				
LVEDV, ml; mean (sd)	111.7 (55.5)	129.5 (58.95)	100.0 (49.91)	<0.0001
LVESV, ml; mean (sd)	68.0 (46.83)	82.4 (50.25)	58.5 (41.90)	<0.0001
EF, %; mean (sd)	42.9 (14.96)	38.9 (14.30)	45.5 (14.84)	<0.0001
LAA, cm^2; mean (sd)	28.7 (8.21)	31.4 (8.47)	27.0 (7.56)	<0.0001
SPAP, mmHg; mean (sd)	52.7 (16.67)	57.2 (17.89)	49.7 (18.62)	<0.0001

QA, quantitative assessment; QA not performed due to either insufficiency or complexity of MR jets. MR, mitral regurgitation; ERO, effective regurgitant orifice; RV, regurgitant volume; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; LAS, left atrial size; SPAP, systolic pulmonary artery pressure; sd, standard deviation.

**p-values (significant MR vs. mild MR) are estimated using independent samples t-test.*

the normal range in both groups, however LVEDV [129.5 vs. 100.0 ml ($p < 0.0001$)] and LVESV [82.4 vs. 58.5 ml ($p < 0.0001$)] were greater in significant MR. The left atrium was also significantly larger [LAA 31.4 vs. 27.0 cm^2 ($p < 0.0001$)]. LV ejection fraction (LVEF) differed between the groups [38.9 vs. 45.5% ($p < 0.0001$)] but remained above cut-off level for HF with reduced EF. The estimated sPAP was 57.2 mmHg in significant MR vs. 49.7 mmHg in mild MR [$p < 0.0001$].

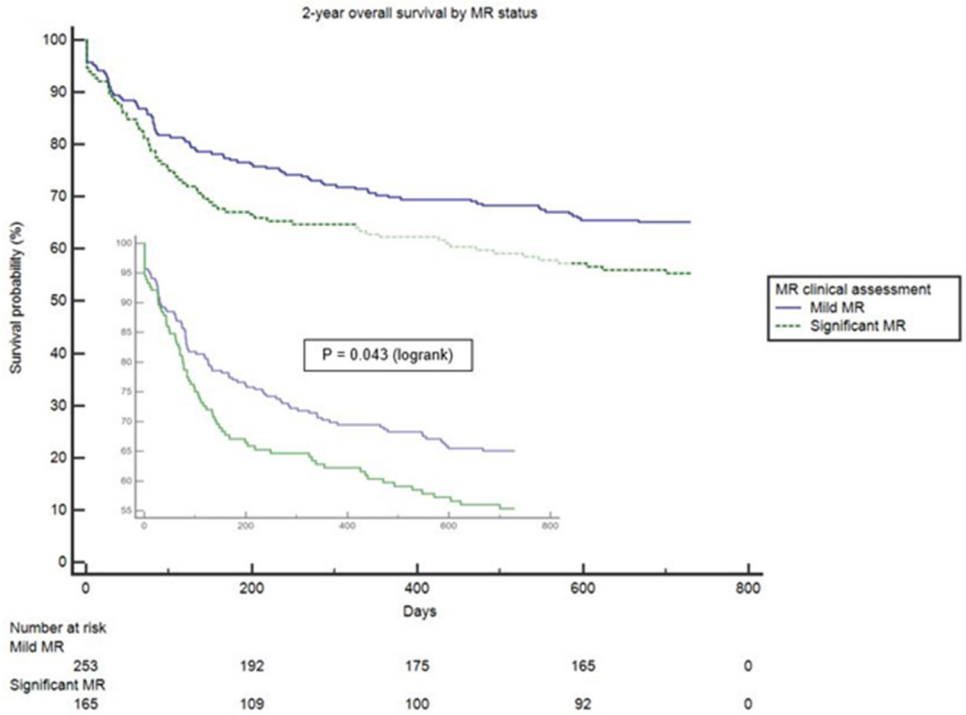

FIGURE 1 | Unadjusted survival curve of 2-year all-cause mortality comparing mild and significant MR.

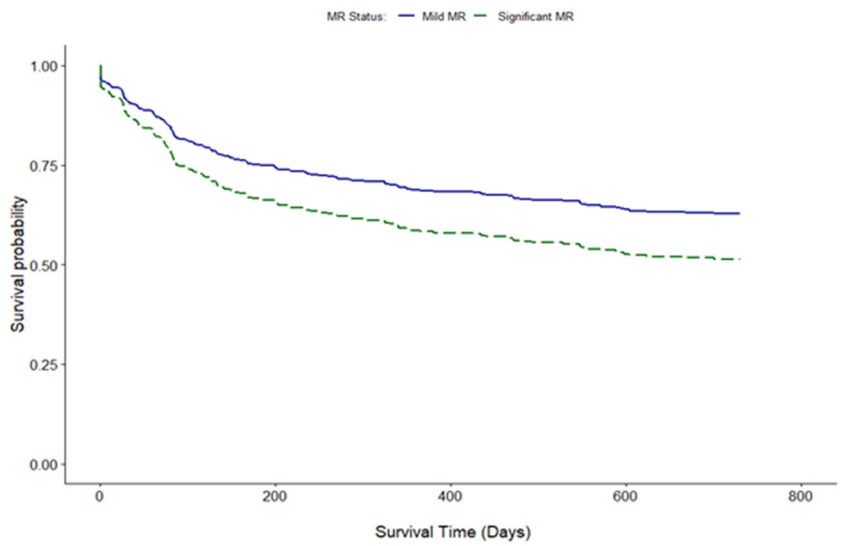

FIGURE 2 | Adjusted survival curve of 2-year all-cause mortality comparing mild and significant MR.

Quantitative assessment was not performed in a minority of mild MR individuals due to insufficiency of jets. Trivial (<4%) numbers of LV/sPAP measurements were not obtained.

Clinical interpretation of significant MR was an important differentiator in the long-term outcome. At 2 years, those with significant MR had 73 (44.2%) deaths compared with 88 (34.8%) in the mild MR group (hazard ratio 1.39 [CI 1.01–1.92], $p = 0.043$) (**Figure 1**). Cox-regression analyses adjusted for multiple covariates confirmed that significant MR is associated with a greater risk of mortality at 2-years [hazard ratio 1.43 (1.04–1.97), $p = 0.029$] (**Figure 2** and **Table 4**). Traditional echocardiographic grading of the severity of MR displayed a clear trend in survival

but was not able to predict significant differences between the three severity grades ($p = 0.081$) (**Figure 3** and **Table 5**).

Proportionality index (PI) cut-off was defined at 0.14 mm^2/ml by ROC analysis. Disproportionate MR was discovered in 217/331 individuals (65.6%). Regardless of the magnitude of volume overload, the presence of disproportionate MR was an important predictor of outcome from index event; there were 92 (42.4%) deaths compared with 32 (28.3%) in patients with and without proportionate MR [hazard ratio (HR) 1.62 (CI 1.12–2.34), $p = 0.010$] (**Figure 4** and **Table 5**). Cox-regression analyses adjusted for multiple covariates also confirmed that disproportionate MR is associated with a greater risk of mortality at 2 years [HR 1.54 (1.02–2.34), $p = 0.042$] (**Figure 5** and **Table 6**). Volumetric disproportionate MR (defined by RV/LVEDV > 0.2) was discovered similarly in 222/345 (64.3%) patients. There were 95 (42.8%) deaths in patients with disproportionate MR defined by regurgitant volumes, significantly more than the 39 (31.7%) with proportionate MR ($p = 0.045$).

TABLE 4 | Multivariable Cox-regression analysis of MR defined by ERO/LVEDV > 0.14 mm^2/ml.

Predictive variables	HR for OS	95% CI	*p*-value
ERO to LVEDV ratio (Ratio > 0.14 cm^2/ml)	1.54	[1·02, 2·34]	0·042
Gender–Male	1.04	[0.96, 0.72]	0.824
Age–Continuous	1.06	[1.03, 1.09]	<0.001
BMI–Continuous	0.99	[0.96, 1.02]	0.62
Known COPD–Yes	1.72	[1.07, 2.75]	0.024
Known hypertension -Yes	1.21	[0.83, 1.75]	0.326
Known CKD–Yes	1.81	[1.24, 2.63]	0.002
Known IHD–Yes	1.20	[0.83, 1.74]	0.329
Known diabetes–Yes	1.08	[0.71, 1.63]	0.723
Known cerebrovascular disease–Yes	0.74	[0.43, 1.27]	0.275

MR, mitral regurgitation; ERO, effective regurgitant orifice; LVEDV, left ventricular end-diastolic volume; COPD, chronic obstructive pulmonary disorder; BMI, body-mass index; HTN, hypertension; CKD, chronic kidney disease; IHD, ischemic heart disease; DM, diabetes mellitus; CVD, cerebrovascular disease.

DISCUSSION

This is the first "real-world" prospective study to assess the prevalence of MR in patients presenting with *acute* HF to an emergency department before the effect of intensive diuresis. In contrast to previous studies (30), patients with HF presenting with sepsis and other medical emergencies were excluded. Our study revealed that all patients requiring admission had some degree of MR. There was a high prevalence of traditionally defined clinically significant MR of moderate to a severe degree (39.5%), and disproportionate, MR defined by an index of proportionality defined by the ERO/LVEDV > 0.14 (65.6%).

Demographic and other clinical characteristics remained broadly similar between those presenting with significant and

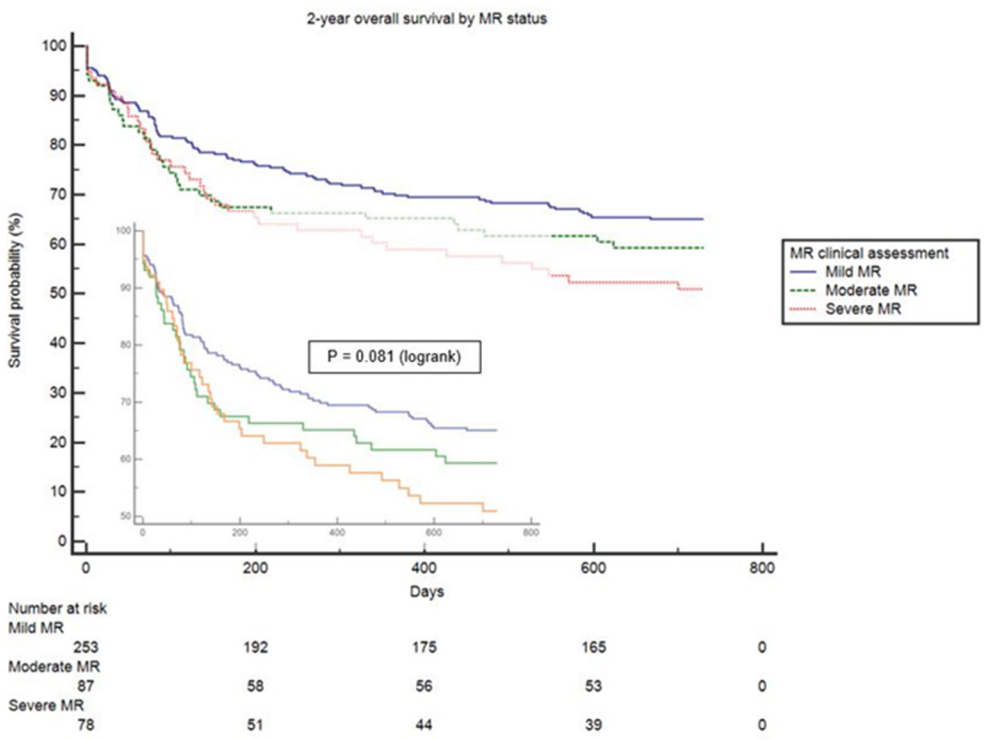

FIGURE 3 | Survival curves of 2-year all cause mortality comparing mild, moderate and severe MR.

TABLE 5 | Kaplan–Meier estimates for overall survival at 24 months.

	Clinical assessment of MR severity		
	Mild MR ($n = 253$)	**Significant MR ($n = 165$)**	
Deaths–*N* (%)	88 (34.8)	73 (44.2)	
Data censored–*N* (%)	165 (65.2)	92 (55.8)	
Kaplan–Meier estimated OS* mean months (95% CI)	17.6 (16.4–18.7)	15.6 (14.1–17.2)	
Hazard ratio			
Significant vs. mild (95% CI)		1.39 (1.01–1.92)	
p-value (Logrank)		0.043	
	Mild MR ($n = 253$)	**Moderate MR ($n = 87$)**	**Severe MR ($n = 78$)**
Deaths–*N* (%)	88 (34.8)	35 (40.2)	38 (48.7)
Data censored–*N* (%)	165 (65.2)	52 (59.8)	40 (51.3)
Kaplan–Meier estimated OS* mean months (95% CI)	17.6 (16.4–18.7)	16.1 (13.9–18.2)	15.1 (12.9–17.4)
Hazard ratio			
Moderate vs. mild (95% CI)		1.25 (0.83–1.85)	
Severe vs. mild (95% CI)		1.51 (1.00–2.33)	
Severe vs. moderate (95% CI)		1.22 (0.74–2.04)	
p-value (Logrank)		0.081	
	ERO/LVEDV assessment of MR severity		
	Mild (proportionate) MR ($n = 113$)	**Significant (disproportionate) MR ($n = 217$)**	
Deaths–*N* (%)	32 (28.3)	92 (42.4)	
Data censored–*N* (%)	81 (71.7)	125 (57.6)	
Kaplan–Meier estimated OS* mean months (95% CI)	18.9 (17.4–20.5)	16.0 (14.7–17.4)	
Hazard ratio significant vs. mild (95% CI)		1.62 (1.12–2.34)	
p-value (Logrank)		0.0097	

ERO, effective regurgitation orifice; LVEDV, left ventricular end-diastolic volume.

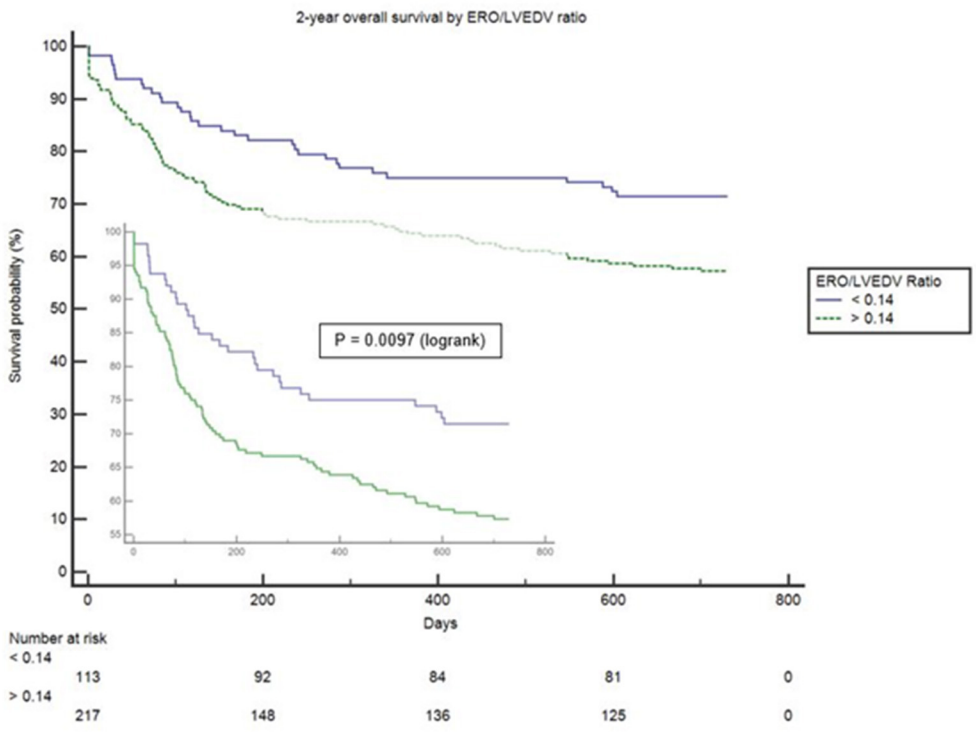

FIGURE 4 | Unadjusted survival curve of 2-year all-cause mortality comparing proportionate and disproportionate MR, defined by an ERO/LVEDV ratio.

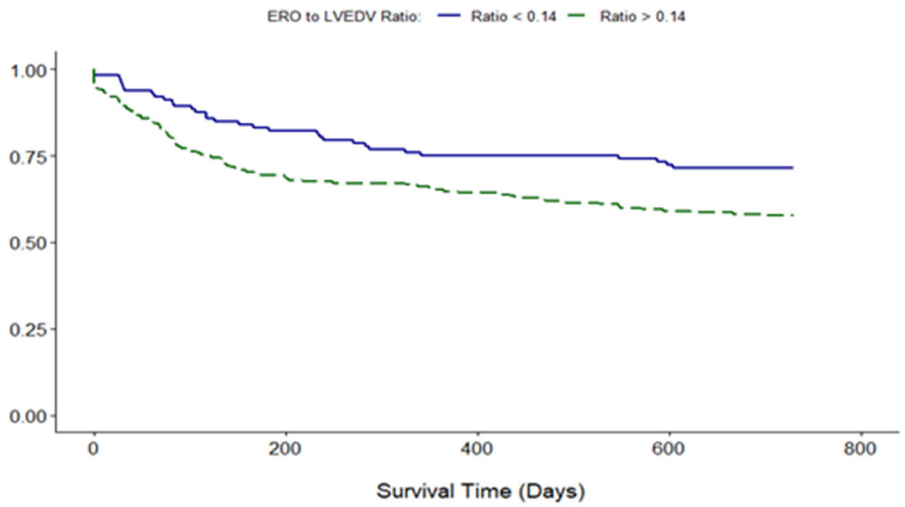

FIGURE 5 | Adjusted survival curve of 2-year all-cause mortality comparing proportionate and disproportionate MR, defined by an ERO/LVEDV ratio.

TABLE 6 | Multivariable Cox-regression analysis of significant MR for overall survival at 24 months.

Predictive variables	HR for OS	95% CI	*p*-value
Significant MR	1.43	[1.04, 1.97]	0.029
Gender	0.97	[0.70, 1.34]	0.9
Age	1.05	[1.03, 1.07]	<0.001
BMI	0.99	[0.96, 1.01]	0.2
Known COPD	2.08	[1.40, 3.08]	<0.001
Known HTN	1.20	[0.87, 1.67]	0.3
Known CKD	1.76	[1.26, 2.45]	<0.001
Known IHD	1.05	[0.76, 1.46]	0.8
Known DM	1.16	[0.81, 1.65]	0.4
Known CVD	0.84	[0.53, 1.32]	0.4

MR, mitral regurgitation; BMI, body mass index; COPD, chronic obstructive pulmonary disorder; HTN, hypertension; CKD, chronic kidney disease; IHD, ischemic heart disease; DM, diabetes mellitus; CVD, cerebrovascular disease.

mild MR. BNP, a well-established biomarker of ventricular disease severity in degenerative and functional MR (35), was the only distinguishing clinical parameter between patients with and without significant MR. However, there is no clear cut-off level for its use in AHF due to the heterogeneous nature of the myocardial injury. We, therefore, used portable, bedside echocardiography to identify and quantify MR. This was particularly important given that functional MR tends to be dynamic in nature and will likely settle with aggressive diuresis. Dynamic MR has been proven to have a prognostic impact in AHF (28) and we have expanded *early* hemodynamic assessment further by using volume-indexed parameters of MR.

Functional mitral regurgitation (FMR) is a distinct entity in terms of pathophysiology and prognostic implications (36) and to our knowledge, there is no consensus as to the timing of hemodynamic assessment of MR in AHF. Prior studies investigating MR in HF often require optimization of medical therapy before enrolment (17). Moreover, the severity and stage of underlying left heart geometric change due to ischemia, progressive myocardial disease, and/or LA enlargement makes it difficult to have a uniform approach for the assessment of left heart geometry (37, 38). MR is considered to be severe when the volume of chronic MR expands LV size beyond a given threshold—which has previously been determined to have a prognostic impact (10, 11). In our study, LV volumes were larger in patients with significant MR but remained within the normal range and significantly lower than in patients enrolled in percutaneous intervention trials for MR (16, 17).

The observed 2-year mortality in our cohort (38.5%) did not substantially differ from other AHF studies (1). The differences in short- and long-term mortality remained significant even after multivariate adjustments for comorbid conditions and demographics. Standard echo assessment (32) was useful but did not provide a clear separation point in survival between moderate and severe MR beyond clinical evaluation. Despite the absence of severe LV remodeling, patients with significant MR had higher mortality rates compared to the mild MR group despite similar (if not better) optimization of pharmacotherapy on index admission. At our center discharge medications suggested more intensive HF therapy in patients with significant MR.

When MR EROA was adjusted to LV volumes using a PI > 0.14 mm^2/ml we observed a rapid separation in survival from index admission for patients with disproportionate MR. We did not observe differences in either the prevalence or prognostic implications of using the EROA indexed to LVEDV as compared to the MR regurgitant volume.

Our study indicates that hearts that are disproportionately affected by MR carry a greater risk of mortality, suggesting MR is an active driver of poor outcomes. Our study suggests either

the EROA or RV is a clinically useful indexing parameter in the context of AHF. Subject to further confirmation by other outcome studies, our data asserts that functional MR should be assessed and managed completely differently to primary MR—using adjustments, namely, ratio/indexed parameters, rather than absolute volumetric analysis, to define thresholds for intervention in FMR patients.

The differences in the pathophysiology between primary and secondary MR (including the rate of change of atrial compliance) should therefore predicate adjustment of echocardiographic evaluation of regurgitant jets, transvalvular flow, the subvalvular apparatus, and the ventricle itself. We suggest that the current standards of cardiac assessment in HF should be updated to reflect the findings from this study and to lower the threshold of LV volumes for prognostically significant MR. This approach to the assessment of functional MR might become an important additional predictive tool to the current biomarkers such as BNP and cardiac troponin (39). This would be of particular benefit to individuals who could undergo surgical/catheter-based interventions to correct FMR.

The strengths of our study include the long-term follow-up, the consecutive enrolment of AHF presentations, and the small number of patients lost to follow-up. A limitation of our study is that it was undertaken at a single center where a majority of our patients self-identified as "White" ethnicity. However, interoperator variability in TTE is a well-characterized limitation of echocardiography and the single-center design of our study facilitated the use of a single operator in most echo assessments in our study, mitigating this limitation. We also did not adjust for differences between treatments in our groups because both admission pharmacotherapy and optimization at discharge occurred similarly between groups according to local and national guidelines. We assume that the difference in mortality would have been broader given more intensive HF therapy in patients with significant MR.

In conclusion, our prospective study demonstrated the high mortality of patients presenting in AHF, particularly those complicated by disproportionate MR. This approach of rapid MR evaluation might help identify those patients likely to benefit from interventions beyond pharmacological optimization. We consider these findings a significant "real-world" addition = to the ongoing debate on the management of disproportionate MR which has direct relevance to both acute physicians and cardiologists. Subject to further confirmatory studies, MR, particularly disproportionate, should not be ignored as a reflection of underlying poor LV performance but viewed as an active driver of poor outcome.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Anonymised/deidentified data will be made available for a period of 6 months from the publication of this article and made available at request with a signed data access agreement. Study protocol and statistical analysis plan will be made available from publication for a period of 6 months.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ashford and Saint Peter's NHS Foundation Trust. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB and AB developed the manuscript for publication. AB is a principal investigator and designed the concept. IB, DF, IJ, PS, and AB planned the study protocol. OL and EA were primarily responsible for echocardiographic data collection and analysis, supervised by AB. JS, JB, IJ, and MB were responsible for patient recruitment, database curation, and verifying the data along with the main statistical analysis which was implemented and designed by JB with clinical input from MB and AB. All authors had access to the database and all authors reviewed the manuscript before publication.

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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.2021.742224/full#supplementary-material

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

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ORIGINAL RESEARCH
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Driveline Features as Risk Factor for Infection in Left Ventricular Assist Devices: Meta-Analysis and Experimental Tests

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Background: Risk factors for driveline infection (DLI) in patients with left ventricular assist devices are multifactorial. The aim of this study was to analyze the correlation between mechanical driveline features and DLI occurrence.

Methods: A meta-analysis was conducted that included studies reporting DLI rates at 6 months after implantation of any of three contemporary devices (HVAD with Pellethane or Carbothane driveline, HeartMate II, and HeartMate 3). Further, outer driveline diameter measurements and *ex-vivo* experimental three-point bending and torsion tests were performed to compare the stiffness of the four different driveline types.

Results: 21 studies with 5,393 patients were included in the meta-analysis. The mean weighted DLI rates ranged from 7.2% (HeartMate II) to 11.9% (HeartMate 3). The HeartMate II driveline had a significantly lower maximal bending force ($Load_{max}$) (4.52 ± 0.19 N) compared to the Carbothane HVAD (8.50 ± 0.08 N), the HeartMate 3 (11.08 ± 0.3 N), and the Pellethane HVAD driveline (15.55 ± 0.14 N) ($p < 0.001$). The maximal torque ($Torque_{max}$) of the HeartMate II [41.44 (12.61) mNm] and the Carbothane HVAD driveline [46.06 (3.78) mNm] were significantly lower than $Torque_{max}$ of the Pellethane HVAD [46.06 (3.78) mNm] and the HeartMate 3 [95.63 (26.60) mNm] driveline ($p < 0.001$). The driveline of the HeartMate 3 had the largest outer diameter [6.60 (0.58) mm]. A relationship between the mean weighted DLI rate and mechanical driveline features ($Torque_{max}$) was found, as the the HeartMate II driveline had the lowest $Torque_{max}$ and lowest DLI rate, whereas the HeartMate 3 driveline had the highest $Torque_{max}$ and highest DLI rate.

Conclusions: Device-specific mechanical driveline features are an additional modifiable risk factor for DLI and may influence clinical outcomes of LVAD patients.

Keywords: left ventricular assist device (LVAD), mechanical features, risk factors, driveline infection, mechanical circulatory support (MCS)

INTRODUCTION

Heart failure remains among the main causes of morbidity and mortality worldwide with an increasing prevalence (1, 2). In recent years, left ventricular assist devices (LVADs) have become an established therapeutic option for end-stage heart failure (3) to support the circulation until myocardial recovery, as bridge to transplant, or as long-term destination therapy (DT) (4). Although LVAD recipients have excellent survival rates, postoperative adverse events can lead to impaired quality of life. The most common adverse events in the early and late periods after continuous flow LVAD implant are major infections (5). An infection rate of 9.1% in the first 3 months after LVAD implantation has been previously reported for pump-related percutaneous driveline infection (DLI) (6), that can lead to pain at the driveline exit site (DLES), an increase of medical costs, and even to stroke (7–9). Consequently, DLI is further the primary cause of readmission in LVAD patients (4). The development of DLI is multifactorial, with several reported risk factors such as increased body mass index (BMI) (10–13), history of diabetes mellitus (DM) (10), and an exposed velour (10, 14–16). The probability of developing a DLI seems to rise with the duration of LVAD support (17–19) and reaches a peak 6 months after implantation. This could be related to the increased activity of patients after hospital discharge (20) and the associated increase of trauma at the DLES, which was previously reported as one of the major initiators for DLI (21). Bending or torsion of the driveline is common during daily activity, e.g., changing of clothes, light exercises, or turning around while sleeping, which could lead to trauma at the DLES (4, 21) and rigid materials and large diameters of the driveline could exacerbate this problem. However, there is only limited knowledge about how driveline features such as diameter and stiffness of contemporary devices affect DLI occurrence. Therefore, this study aims to quantify and compare device-specific mechanical driveline properties of three LVADs with four different drivelines and to correlate them with DLI occurrence.

MATERIALS AND METHODS

Meta-Analysis

This meta-analysis is in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. (22).

Data Source and Search Strategy

Two independent reviewers used the databases PubMed and SCOPUS in October 2021 with the search terms "Driveline Infection AND Left Ventricular Assist Devices," "Driveline Infection AND LVAD," "Driveline infection AND HeartMate 3," "Driveline infection AND HVAD," "Driveline infection AND HeartWare," and "Driveline Infection AND HeartMate II" to identify studies assessing DLI data of LVAD patients (**Figure 1**). Since the HVAD Carbothane driveline did not receive FDA approval until 2019 (23), and no studies were found in the database, an additional manual research was performed. The literature search was not limited to the strict PICO format, as this would likely have excluded relevant articles, particularly retrospective cohort studies without a control group.

Study Selection and Data Extraction

The outcomes of interest were either a numeric DLI rate at 6 months or a freedom from DLI Kaplan-Meier curve of at least one of the three devices and the sample size. Exclusion criteria included case reports, review articles, non-English articles, records with wrong devices and records with unsuitable DLI rates (e.g., DLI rates stated as events per patient years). Extracted data included the study period, study design, the device, the DLI rate, the cohort's Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) classification, DM, age, BMI, DT indication, gender, and implant technique characteristics. The WebPlotDigitizer (Version 4.4, Ankit Rohatgi, 2020) was used to extract the 6 months DLI rate from the Kaplan-Meier curve. A random-effects model was used and for each device type, the extracted DLI rates were weighted with the Schmidt-Hunter method depending on their sample size and used to calculate a mean weighted DLI rate. The evaluation, organization, and analysis of suitable literature sources were done using the software Review Manager (RevMan) (Version 5.4.1, The Cochrane Collaboration, 2020).

Study Quality Assessment

Studies were assessed for methodologic quality using the risk of bias tool described in the Cochrane Handbook for Systematic Reviews (24). This tool enables subjective assessment of bias across six domains, including selection, performance, attrition, detection, and reporting.

Experimental Driveline Analysis

Sample Selection

An assortment of new and clinically used driveline samples without velour cover were analyzed. Eleven Pellethane HVAD (Medtronic Inc, Minneapolis, MN, USA), eleven Carbothane HVAD (Medtronic Inc), two HeartMate II (Abbott Inc, Chicago, IL, USA), and six HeartMate 3 (Abbott Inc) were used, and all measurements were repeated five times for each driveline specimen.

Three-Point Bending Test

An experimental three-point bending test (**Figure 2A**) was conducted, based on the standard EN ISO 178:2019 08 01 (25) using a BOSE® LM1 ElectroForce test bench system (Bose Corp. MN, USA) with an integrated displacement transducer. A 3D-printed design with a support span of 30 mm was used, with the radii of the supports and the loading nose being 2.5 mm. A 225 *n* load cell Type WMC-50-543 (Bose Corp. MN, USA) was mounted in line with the motor shaft. The measurement process was performed with the software WinTest® (Version: 7.1.2014- 04.04, Bose Corp. MN, USA) allowing movements of the linear motor and simultaneous recording of time, load, and displacement. The drivelines were bent to a total displacement

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; DLES, driveline exit site; DLI, driveline infection; IQR, interquartile range; LVAD, left ventricular assist device; DM, diabetes mellitus; DT, destination therapy.

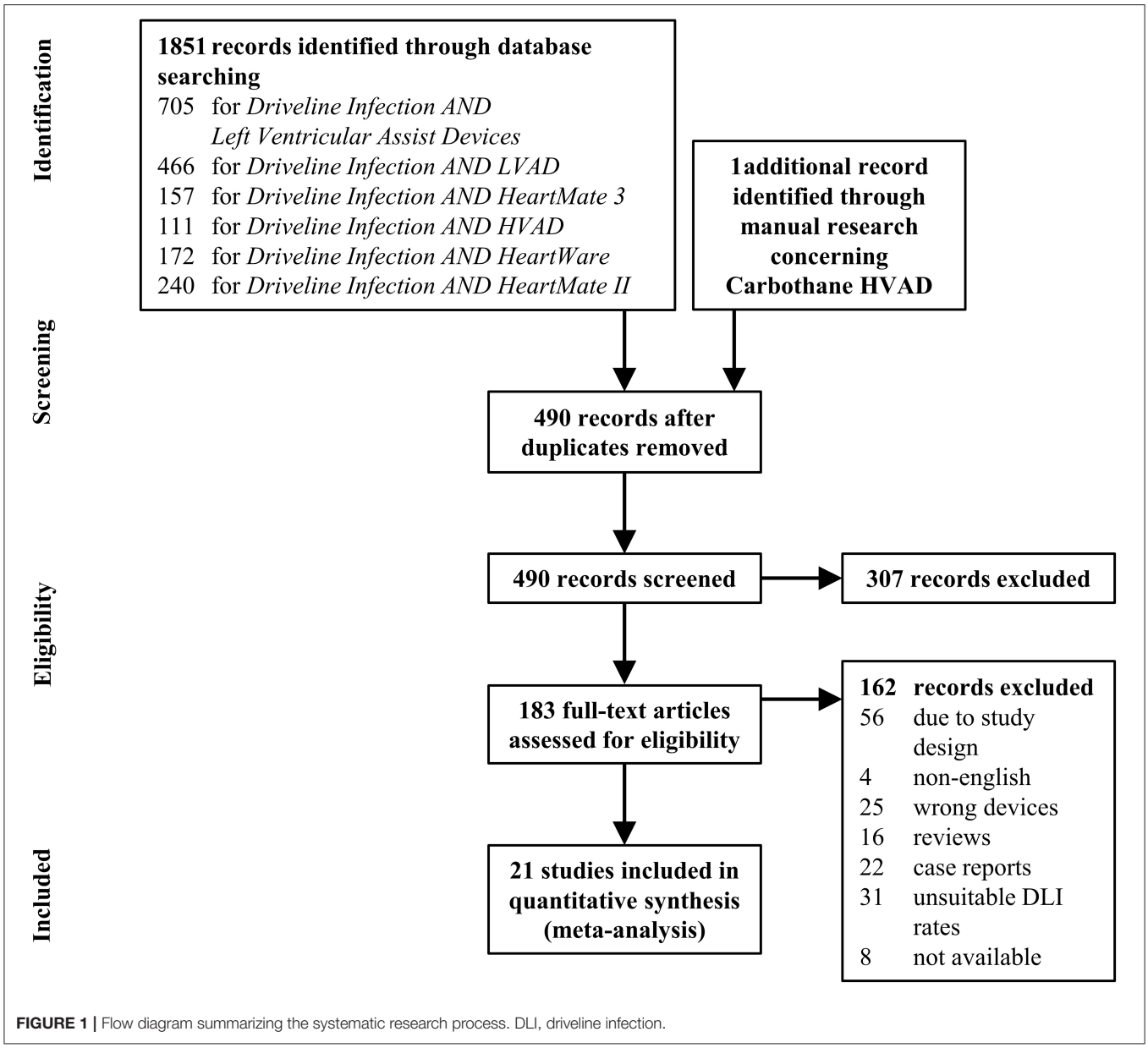

FIGURE 1 | Flow diagram summarizing the systematic research process. DLI, driveline infection.

of 12 mm with a bending velocity of 1.5 mm/s to measure the maximal bending force $Load_{max}$.

Torsion Test

The torsion test (**Figure 2B**) was modified from the standards EN 50289-3-10:2005 11 01 (26) and EN ISO 25539- 2:2019 06 01 (27). The drivelines were clamped vertically into a custom-made torsion testing apparatus with a free length of 12 cm. An Arduino Uno R3 (Adafruit Industries, New York, USA) was used to operate a 42SHDC3025-24B stepper motor (Anet Technology Co., Ltd., Shenzhen, CHN) to twist the driveline (720°) with an angular velocity of 100°/s. An iron bar was attached at the lower end of the driveline which was mounted in a tube as a duct and a lever arm with 10 cm was attached. When the stepper motor twisted the driveline, the lever arm pressed against a bar mounted on a RFS® 150 XY sensor (Honigmann Industrielle Elektronik GmbH, Gevelsberg, DEU) to measure the maximal torque ($Torque_{max}$). The DS1103 PPC Controller Board and the software ControlDesk (Version: 5.0, 2013, dSPACE GmbH, Paderborn, DEU) were used for the simultaneous recording of time and torque.

Statistical Analysis

Descriptive statistics are reported as mean ± standard deviation for normally distributed continuous variables and as median and interquartile range (IQR) for non-normally distributed values. Normal distribution was assessed by the Shapiro-Wilk test. One-way analysis of variance (ANOVA) or Kruskal-Wallis tests were

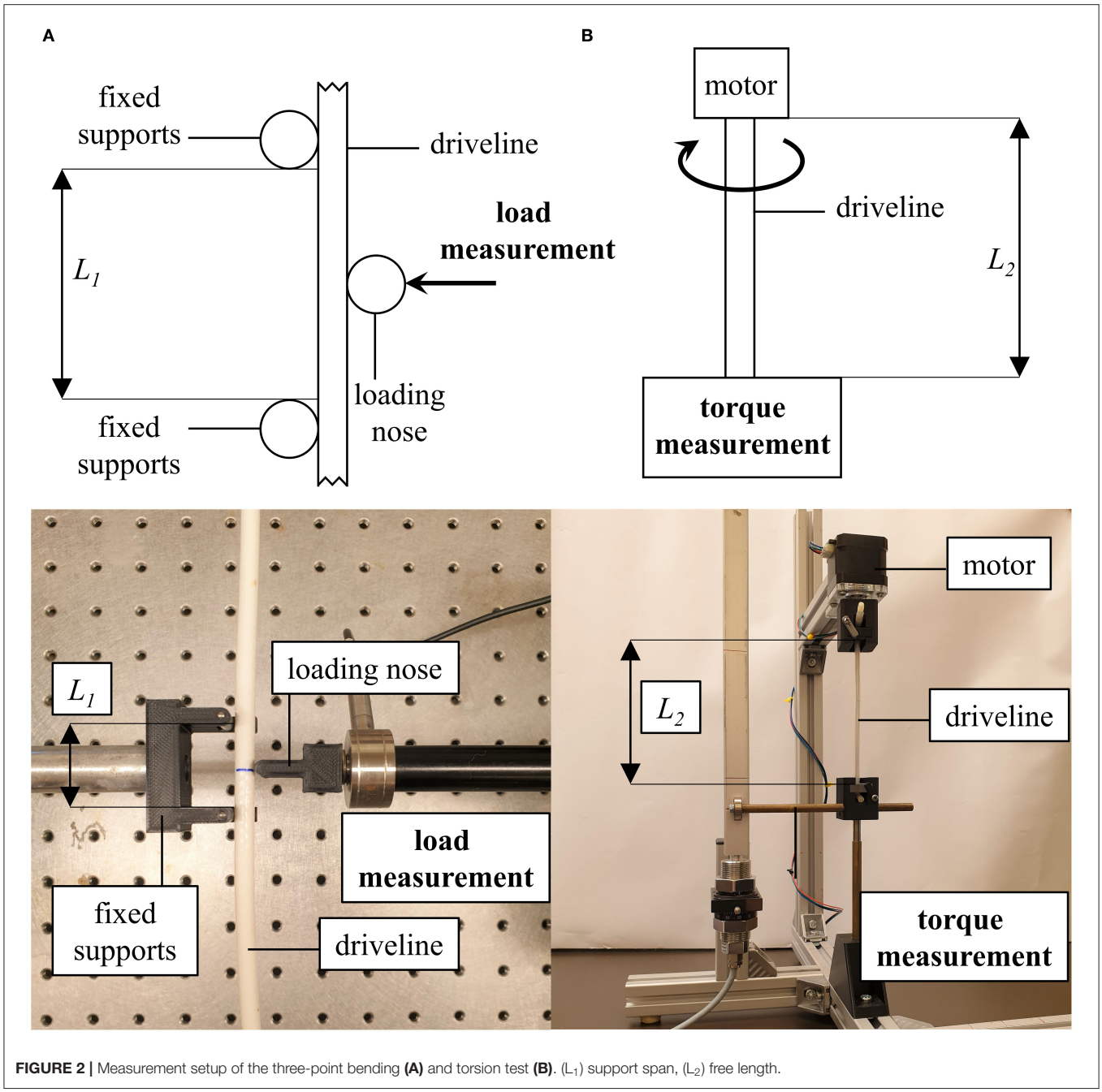

FIGURE 2 | Measurement setup of the three-point bending **(A)** and torsion test **(B)**. (L_1) support span, (L_2) free length.

used to test continuous variables ($Load_{max}$ and $Torque_{max}$) between the four driveline groups. When statistical significance was found ($p < 0.05$), *post-hoc* analyses were performed. Therefore, a Levene's test was used to check the homogeneity of variance with a significance level of $p < 0.05$. If homogeneity of variance was present, a Bonferroni-test was performed, otherwise a Games Howell test for normally distributed groups was used. In both cases, the significance level was set to $p < 0.05$. For non-normally distributed values, a pairwise comparison was performed with a Bonferroni correction, and the significance level was set at $p = 0.0125$. Statistical analysis was performed by SPSS for Windows Release 26.0.0 (SPSS Inc, Chicago, IL, USA) and MATLAB R2020a (The MathWorks Inc, Natick, MA, USA).

RESULTS

Meta-Analysis

Of the 490 full-text articles screened, $n = 20$ articles fulfilled the inclusion criteria and reported DLI rates at 6 months following LVAD implantation in one or more of the included device types (**Figure 1**). Manual search on DLI rates of patients supported with the Carbothane HVAD driveline revealed $n = 1$ abstract.

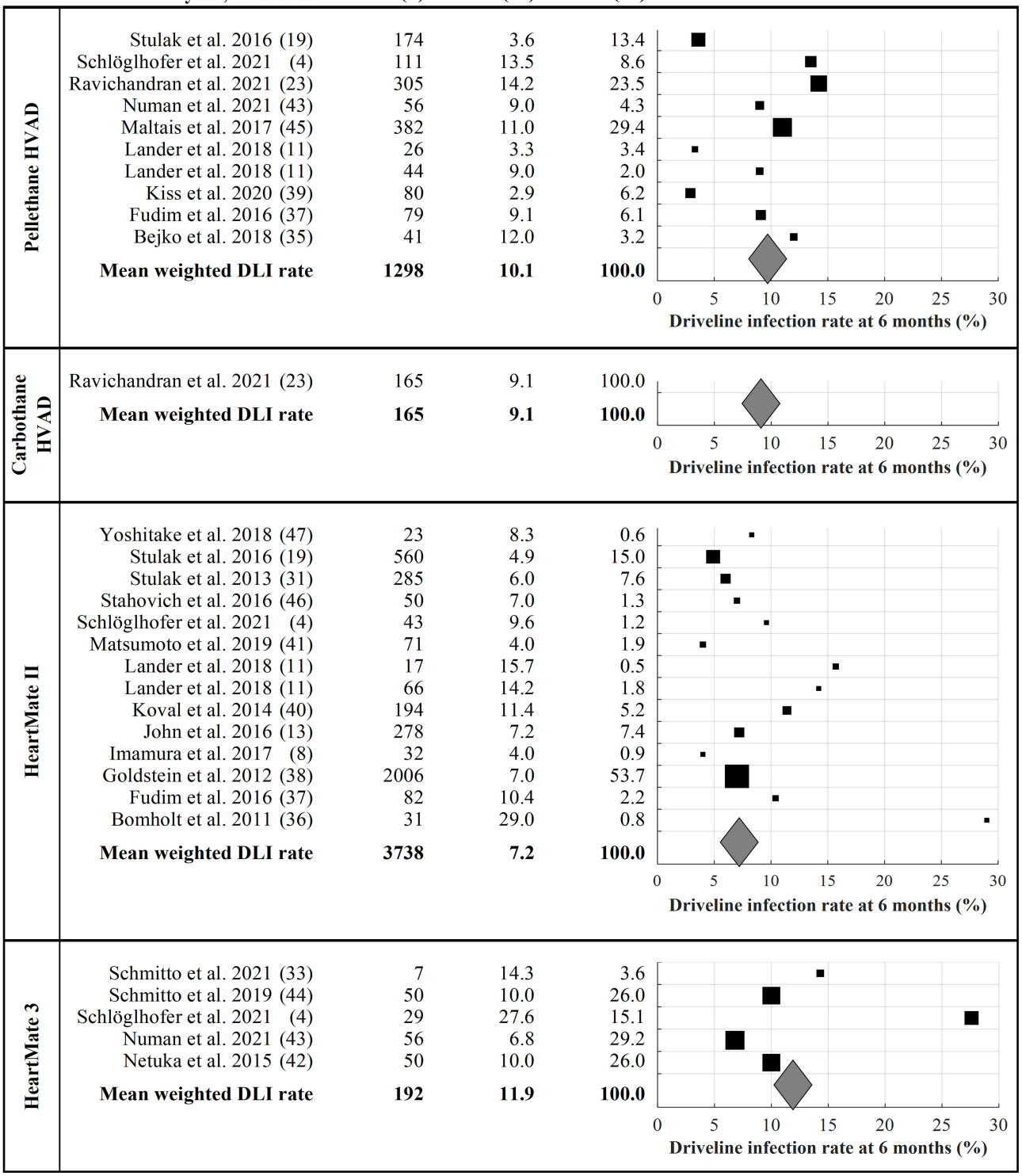

FIGURE 3 | Summary of all included and analyzed studies. The black marks represent the DLI of the study, the size of the black marks refers to the number of included patients. The mean weighted driveline infection rate for each device is represented by gray diamonds. DLI, driveline infection rate.

TABLE 1 | Summary of the mechanical features of the analyzed drivelines.

	$Load_{max}$ [N]	$Torque_{max}$ [mNm]	Diameter [mm]
Pellethane HVAD	15.55 ± 0.14	94.62 (3.89)	4.8 (0.0)
Carbothane HVAD	8.50 ± 0.08	46.06 (3.78)	4.8 (0.0)
HeartMate II	4.52 ± 0.19	41.44 (12.61)	6.0 (0.0)
HeartMate 3	11.08 ± 0.30	95.63 (26.6)	6.6 (0.58)

Values are either presented as mean ± standard deviation for normally distributed groups or as median (interquartile range) for non-normally distributed groups. $Load_{max}$, maximal bending force; $Torque_{max}$, maximal torque.

In total, 5,393 patients were included in the final meta-analysis. The most studies ($n = 14$) and included patients ($n = 3738$) were identified for the HeartMate II. The mean weighted DLI rates ranged from 7.2% (HeartMate II) to 11.9% (HeartMate 3). The final 21 articles, including the DLI rate after 6 months for each study and the mean weighted DLI rate for each driveline type are summarized in **Figure 3**. The overall mean weighted DLI rate including all studies was 8.1%. Of the included studies with reported patient characteristics, INTERMACS Class 1 ranged from 0 to 41.0%, age was between 38 ± 13 and 62.4 ± 8.3, BMI ranged from 20.4 ± 3.5 and 29.7 ± 6.23, 13.0% to 43.8% suffered from DM, 11% to 100% received their LVAD as DT, and 65.4% to 93% were male (see **Supplementary Table 1**).

Assessments of study quality and risk of bias are summarized in **Supplementary Table 2**. In the fast majority of studies, a low risk for performance (85.7%), detection (100%) and reporting (90.5%) bias was found. Moderate selection bias was more common (23.8%), whereas the attrition bias was rated as low (66.6%) or unclear (23.8%) in most studies.

Experimental Driveline Analysis

In total, 30 driveline samples were analyzed and **Table 1** summarizes their mechanical features. Among the four observed driveline types, Carbothane HVAD and Pellethane HVAD had the smallest diameter, with 4.8 (0.0) mm. The least rigid driveline in the three-point bending test was the HeartMate II ($Load_{max} = 4.27 \pm 0.07$ N), whereas the Pellethane HVAD driveline had a significantly higher $Load_{max} = 13.56 \pm 0.08$ n ($p < 0.001$). The stiffness of each driveline type is shown in **Figure 4A**. Significant differences ($p < 0.001$) were found between all groups. In the torsion tests (**Figure 4B**), the HeartMate II driveline had the lowest $Torque_{max}$ [41.44 (12.61) mNm] and the HeartMate 3 driveline had the highest $Torque_{max}$ [95.63 (26.60) mNm]. Further, the HeartMate 3 driveline $Torque_{max}$ was significantly higher ($p < 0.0125$) compared to the Carbothane HVAD and the HeartMate II drivelines. Comparable results were found between the Carbothane HVAD and the HeartMate II ($p = 0.95$) as well as between the Pellethane HVAD and the HeartMate 3 drivelines ($p = 0.69$).

Relationship of DLI Rates and Driveline Features

Figure 5 summarizes the relationships between the mechanical characteristics of the four different drivelines from the *ex-vivo* experimental study and the mean weighted DLI rates at 6 months. No relevant association between the mean weighted DLI rate and the driveline diameter (**Figure 5A**) or the $Load_{max}$ of the three-point bending test (**Figure 5B**) was found, respectively. There was an apparent relationship between $Torque_{max}$ of the torsion test and the mean weighted DLI rate (**Figure 5C**); The HeartMate II driveline had the lowest $Torque_{max}$ and lowest DLI rate, whereas the HeartMate 3 driveline had the highest $Torque_{max}$ and highest DLI rate.

DISCUSSION

DLI is one of the most common adverse events in the early and late phases after LVAD implantation (1). The development of DLI is multifactorial, with several reported non-modifiable risk factors like DM (10), age (12, 16, 28) or exposed velour (10, 14–16), and, on the other hand, modifiable risk factors (29), such as BMI (10–13), patient lifestyle and activity following hospital, discharge and the associated increase in trauma at the DLES (21). To the best of our knowledge, only one other study has reported the correlation between mechanical driveline features and DLI rates of LVAD-patients (8), but data for contemporary devices are missing. Therefore, the aim of this study was to quantify and compare device-specific driveline characteristics of the HVAD, HeartMate II, and HeartMate 3 as an additional modifiable risk factor associated with DLI, both *in-vivo* and *ex-vivo* (**Figure 6**).

As previously reported (20), DLI peaks 6 months after LVAD implantation. In this meta-analysis, we found a DLI rate of 8.1% at 6 months, making DLI one of the major adverse events after LVAD implantation. The mean weighted DLI rate was highest with the HeartMate 3 (11.9 %), compared with the Pellethane HVAD (10.1%), the Carbothane HVAD (9.1%), and the HeartMate II (7.2%). Therefore, regardless of patient demographics and center-specific DLES care protocols, the HeartMate II may have positive mechanical driveline features compared to other commercially available LVADs. The approaches for the development of the four contemporary LVAD drivelines investigated in this study are diverse, and different materials are used. Whereas the HeartMate II driveline consists of a soft silicone-based outer layer enveloping an inner jacket made of polyurethane wrapped around a fiber core made of polyethylene (30), the HeartMate 3 driveline has a silicone-based outer layer wrapped around a fiber layer of braided aramid enveloping a polytetrafluoroethylene layer (4). HVAD drivelines consist of an inner silicone lumen enveloped by either Pellethane® or with the new Carbothane® design (23).

Consequently, our *ex-vivo* experimental study showed significant differences in driveline stiffness between all devices ($p < 0.001$) as assessed by the $Load_{max}$ of the three-point bending test (**Figure 4A**). Even though there was no obvious relationship between $Load_{max}$ and the mean weighted DLI rate, the HeartMate 3 driveline with the largest diameter had the highest DLI rate (**Figure 5B**). This is in accordance with the findings of Imamura et al., who reported that the HeartMate II driveline had only 20–25% of stiffness and a smaller diameter compared to other devices (EVAHEART, and DuraHeart) and the

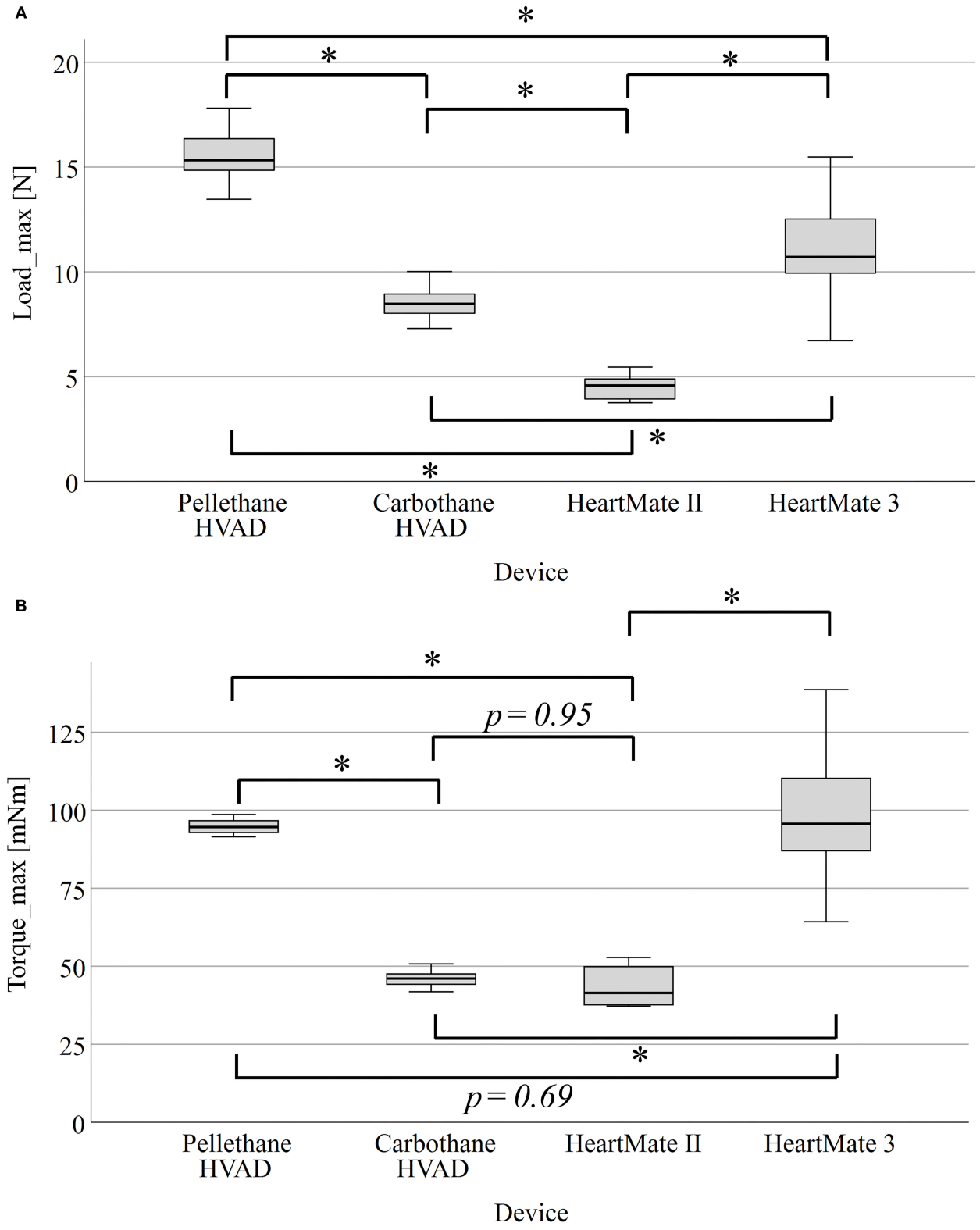

FIGURE 4 | Boxplots of $Load_{max}$ of the three-point bending test of four different driveline types **(A)** and $Torque_{max}$ of the torsion tests **(B)**. *$p < 0.001$. $Load_{max}$, maximal bending force; $Torque_{max}$, maximal torque.

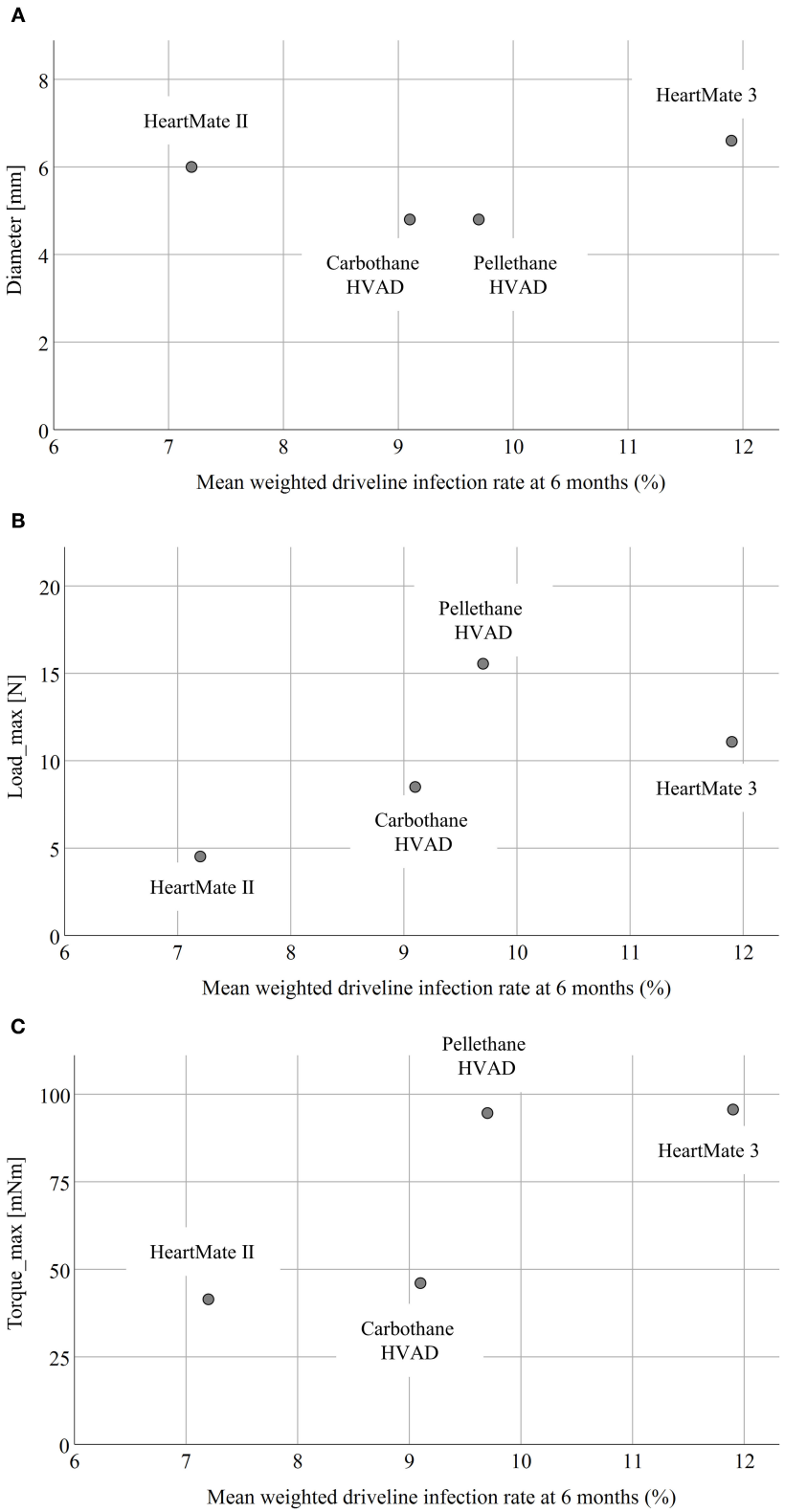

FIGURE 5 | Correlation between the diameter **(A)**, $Load_{max}$ **(B)**, and $Torque_{max}$ **(C)** and the mean weighted driveline infection rate at six months. $Load_{max}$, maximal bending force; $Torque_{max}$, maximal torque.

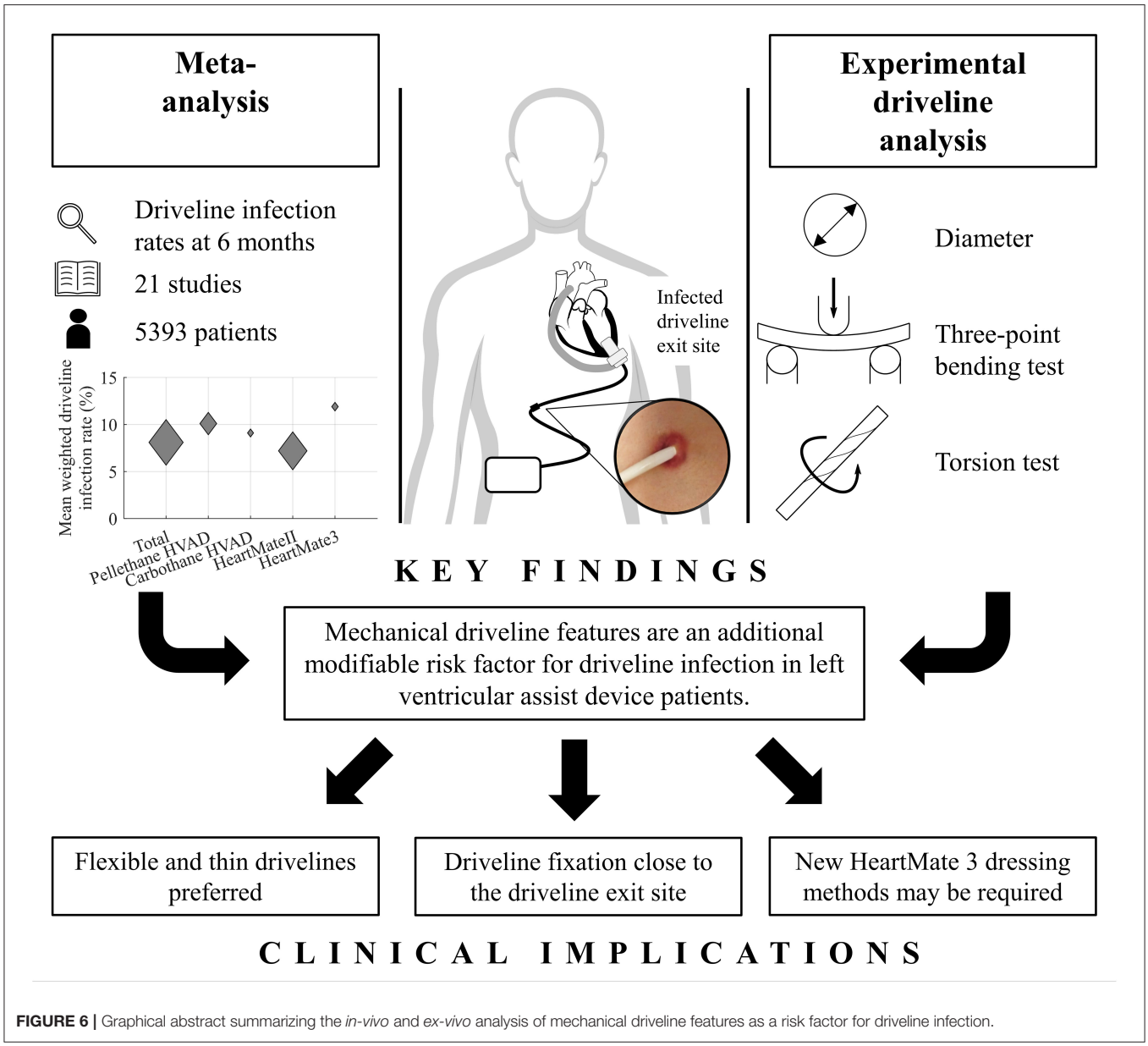

FIGURE 6 | Graphical abstract summarizing the *in-vivo* and *ex-vivo* analysis of mechanical driveline features as a risk factor for driveline infection.

highest DLI-free rate among those three devices (8). However, the key finding of this study was the hypothesis-generating apparent relationship between higher $Torque_{max}$ of the torsion test and the increased DLI rates (**Figure 5C**). Therefore, this parameter seems to be a crucial marker for further technical improvements, as driveline torsion is a frequent event in the daily life of LVAD patients, potentially exerting additional force on the DLES and thus leading to trauma-induced DLI as the adherent interface between the velour of the internal part of the driveline and the patient's tissues is critical for the protection against entry of microorganisms and subsequent infection (31). These findings could be relevant to clinical practice, as mechanical features are a modifiable risk factor and exploring more flexible and thinner drivelines would be a simple means to prevent DLI. Based on our *ex-vivo* results and in relation to the clinical DLI rates resulting from the meta-analysis, the most important feature of a LVAD driveline seems to be high flexibility (in terms of low $Torque_{max}$), followed by low stiffness ($Load_{max}$), and minimal thickness (diameter). Although the Medtronic HVAD was recently withdrawn from the market, the development of the new Carbothane driveline appears to be the first step in the multi-faceted strategies to reduce DLI and, by extension, a risk factor for one of the most feared and devastating complications during LVAD support—stroke (7–9). Since the relationship between driveline mechanical properties and DLI rates appears moderate, driveline features are certainly not the "only" risk factor, but are definitely a previously unknown additional factor in DLI development. Therefore, the technical improvement of

the mechanical properties of drivelines or even the elimination of them by transcutaneous energy transfer systems (32) should be a high priority in the future development of LVADs. In addition, the development of transcutaneous energy transfer systems will make disappear the need for periodic driveline repairs or for exchanging the HeartMate 3 modular cable—which was necessary in 50% of long-term patients as their active lifestyles caused the cable to deteriorate (33). Thus, the HeartMate 3 modular cable is both a curse and a blessing—the connector enables these necessary driveline exchanges, but this design feature may also be the reason for the higher DLI rates as the rigid modular connector might apply additional traction on the DLES compared to the other devices. Therefore, the results of our study lead to the hypothesis that the overall HeartMate 3 driveline design, including modular connector, is unfavorable, but the findings contrast with the MOMENTUM 3 final report (34), which found no significant but numerically higher 2-year DLI rates with HeartMate 3 (23.3%) vs. HeartMate II (19.4%).

Finally, it should be mentioned that with the HeartMate 3 as the only commercially available LVAD, new DLES dressing methods may be required, including additional binders or anchoring devices (4), e.g., to fix the driveline and the modular connector in a U-shape directly as close as possible at the DLES as well as the rigid connector on the skin to minimize driveline movement and trauma to prevent DLI. The design of next-generation LVAD peripherals should therefore possibly have a combination of external helix pump cable from the controller to the modular connector to absorb additional forces, followed by the most flexible and thin driveline possible to the DLES and implanted pump.

Limitations

This study has limitations that should be considered when interpreting the results. The meta-analysis was limited to 21 articles (35–47), including only one multicenter study that reported DLI rates of the Carbothane HVAD. Differences in study design, patient characteristics and selection, and center specific DLES care protocols might vary between centers, so we cannot exclude previously reported factors affecting the occurrence of DLI at all. In addition, experimental mechanical testing was limited to a rather modest number of drivelines ($n = 30$), including new and clinically used ones without velour cover or modular driveline connectors (HeartMate 3). The effects of chemical and physical aging of drivelines used *in-vivo* on stiffness were not investigated in this study.

CONCLUSION

Device-specific mechanical features of the driveline are an additional modifiable risk factor for the development of DLI and may influence clinical outcomes of LVAD patients.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MK, MS, and TS developed the concept and design, performed the statistical analysis, and funding was secured by DZ, GL, HS, and TS. MK and TS drafted the article. MK, A-KS, JR, DW, and CM collected the data. All authors performed critical revision of the article and approved the final 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.2021.784208/full#supplementary-material

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A Preclinical Rat Model of Heart Failure With Preserved Ejection Fraction With Multiple Comorbidities

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Heart failure with preserved ejection fraction (HFpEF) is a common complex clinical syndrome for which there are currently few evidence-based therapies. As patients with HFpEF very often present with comorbidities comprising the metabolic syndrome, we hypothesized, that metabolic syndrome could lead over time to the development of diastolic dysfunction and HFpEF. Obesity-prone rats were exposed to high-fat diet and compared to obesity-resistant rats fed with standard chow. Phenotyping of metabolic syndrome, associated with echocardiographic and cardiac hemodynamic measurements, was performed after 4 and 12 months. Blood and myocardial tissue sampling were performed for pathobiological evaluation. High-fat diet in obesity-prone rats elicited metabolic syndrome, characterized by increased body and abdominal fat weights, glucose intolerance and hyperlipidemia, as well as increased left ventricular (LV) systolic pressure (after 12 months). This was associated with LV diastolic dysfunction (assessed by increased LV end-diastolic pressure) and pulmonary hypertension (assessed by increased right ventricular systolic pressure). Echocardiography revealed significant concentric LV hypertrophy, while LV ejection fraction was preserved. LV remodeling was associated with cardiomyocyte hypertrophy, as well as myocardial and perivascular fibrosis. Circulating levels of soluble ST2 (the interleukin-1 receptor-like) markedly increased in rats with HFpEF, while plasma NT-proBNP levels decreased. RNA-sequencing analysis identified clusters of genes implicated in fatty acid metabolism and calcium-dependent contraction as upregulated pathways in the myocardium of rats with HFpEF. High-fat diet during 12 months in obesity-prone rats led to the development of a relevant preclinical model of HFpEF with multiple comorbidities, suitable for investigating novel therapeutic interventions.

Keywords: heart failure with preserved ejection fraction, diastolic dysfunction, metabolic syndrome, group 2 pulmonary hypertension, soluble ST2, RNA sequencing

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is a growing clinical and public health problem, accounting for approximately half of all cases of heart failure and associated hospital admissions (1). HFpEF is clinically defined as heart failure with normal ejection fraction and diastolic dysfunction, characterized by impaired relaxation and increased diastolic stiffness (2). All patients with HFpEF exhibit increased left ventricular (LV) filling pressure and reduced exercise tolerance (3, 4). However, pathophysiological mechanisms underlying disease progression of HFpEF are complex and remain poorly understood.

Although diastolic dysfunction is highly prevalent in HFpEF, the complex clinical phenotype that characterizes this syndrome stems from the presence of multiple cardiac and non-cardiac interrelated comorbidities, commonly seen in metabolic syndrome. These include obesity, systemic arterial hypertension, hyperlipidemia and *diabetes mellitus*, altogether contributing to the impairment of the cardiovascular reserve (5, 6). Furthermore, systemic inflammatory burden has been suggested to greatly contribute to HFpEF pathogenesis (7–9). HFpEF is more frequent in elderly patients (10, 11) and is closely associated with the presence of arterial hypertension as well as overweight or obesity (12). Moreover, up to 80% of patients with HFpEF develop pulmonary hypertension, which is associated with worse outcome and increased mortality (13).

HFpEF is a major unmet medical need and there is an urgent demand for new therapeutic approaches and strategies targeting mechanisms specific to HFpEF. However, definitive experimental evidence supporting these mechanisms has not emerged owing to limited success in developing a comprehensive preclinical model recapitulating the intricate pathophysiology of HFpEF and its global clinical picture. So far, evidence-based clinical therapies have failed to improve clinical symptoms, prognosis and mortality of HFpEF patients (8, 14, 15).

Because most HFpEF patients harbor multiple comorbidities, we propose a multiple-hit model created by inducing HFpEF in rats through metabolic stress (using a high-fat diet associated with a genetic predisposition) and prolonged follow-up timeline as a secondary stressor, to develop an animal model of HFpEF closest to human pathophysiology.

MATERIALS AND METHODS

Animals and Blood and Tissue Sampling

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine of the *Université Libre de Bruxelles* (Brussels, Belgium; protocol acceptation number: 656N). Applicable guidelines were followed in accordance with the "Guide for Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication eighth edition, update 2011).

Diet-induced obesity-prone (OP) and obesity-resistant (OR) Sprague-Dawley rats were raised using selected breeders obtained from Charles River Laboratories Inc. (Wilmington, MA, USA). These rats were used because their propensity to develop diet-induced obesity due to polygenic inheritance, thus closely mimicking the obesity in humans (16). Four-week-old OP ($n = 10$ and $n = 14$ in 4- and 12-month protocols respectively) and OR ($n = 9$ in both 4- and 12-month protocols) rats were enrolled in the study and kept under controlled environmental conditions (21°C, 60%-humidity atmosphere and 12-h light/dark cycles) along the protocol. OP rats were fed with a high-fat diet with 45% of energy from lipids and 17% from sucrose (473 kcal/100 g; D1245; Research Diets, New Brunswick, NJ, USA), whereas OR rats continued on standard rat chow (339 kcal/100 g with 5% of energy from lipids and 2% from sucrose; A03 Safe diets, Augy, France) for 4 and 12 months. Water and chow were given *ad libitum*. Body weight and food intake were monitored every week during the last 5 weeks of the protocols, to carefully characterize weight gain and energy ingestion. Food intake measured as food consumed per gram/day and calorimetry was evaluated.

As illustrated in **Figure 1A**, at the end of the protocols (at 4- and 12-month time points), OP and OR rats were evaluated by echocardiography and invasive hemodynamic measurements. Before sacrifice, blood samples were drawn from the femoral vein, placed in EDTA-coated tubes and centrifuged (for 15 min at 1,500 g) to collect plasma for biochemical analyses and evaluation of adipokine and cardiac biomarker levels. Under deep anesthesia (isoflurane 5%), rats were finally euthanized by exsanguination (via section of the abdominal aorta). Adipose deposits from visceral, retroperitoneal, epididymal and mesenteric sites, and the hearts were rapidly harvested, weighted and processed for analysis. Hearts were snap-frozen in liquid nitrogen and stored at −80°C for RNA-sequencing (RNA-seq) analysis or, after a 24-h fixation in 10%-neutral buffered formalin, were embedded in paraffin for histopathological evaluations. Heart weights were normalized to tibial length to assess cardiac hypertrophy independently of body weight.

Biochemical Analysis

To analyze potential alterations in glucose metabolism, a glucose-tolerance test was performed by intraperitoneal injection of glucose ($2\,g.kg^{-1}$ in saline) after overnight fasting. Glucose levels ($mg.dL^{-1}$) were measured, with a portable glucometer (Contour XT kit, Bayer HealthCare, Loos France), from a drop of blood taken at the tip of the tail before (0 min) and at 15, 30, 60, 90, 120 and 150 min after glucose administration. Glucose tolerance was obtained from the area under the curve (obtained from the serum glucose concentration *vs.* time points), using Graphpad Prism 9.0 (Graphpad software, San Diego, California, USA).

Concentrations of triglycerides, total cholesterol and HDL-cholesterol were determined using Cobas8000® in plasma samples, according to manufacturer's instructions. Plasma concentration of LDL-cholesterol was calculated.

Plasma Levels of Adipokines and Cardiac Biomarkers

Plasma concentrations of leptin and adiponectin were determined with rat leptin (KRC2281; Thermo Fisher

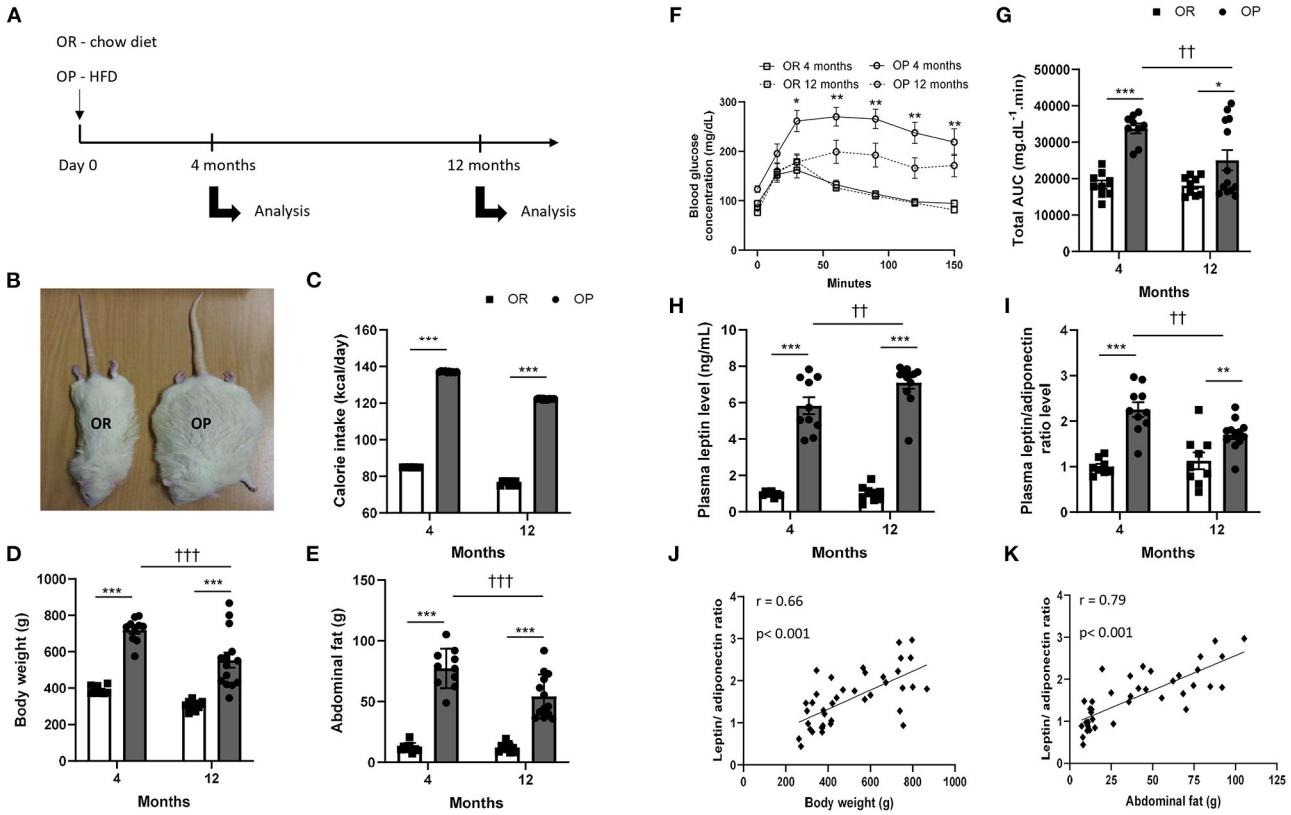

FIGURE 1 | High-fat diet fed obesity-prone (OP) rats present all characteristics of metabolic syndrome after 12 months. **(A)** Experimental design. Obesity-resistant (OR) and obesity-prone (OP) rats were respectively maintained on normal standard chow or high-fat diet for 4 and 12 months and then analyzed. **(B)** Representative illustrative picture of high-fat diet fed obesity-prone (OP; on the right) vs. normal chow-fed obesity-resistant (OR; on the left) rats at 12 months. **(C)** Averaged daily calorie intake evaluated along the 4 and 12 months of the study, in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant rats (OR; white bars). Body **(D)** and total abdominal fat weights **(E)** of high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant (OR; white bars) rats at 4 and 12 months. Glycemic response during fasting glucose tolerance test **(F)** and corresponding total area under the curve [AUC; (**G**)] in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant (OR; white bars) rats at 4 and 12 months. Data are presented as mean ± SEM (n = 8–14 rats per group). *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, ***$p < 0.001$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats; $^{\dagger\dagger}0.001 < p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, 4- vs. 12-month high-fat diet in obesity-prone (OP) vs. normal chow in obesity resistant (OR) rats. Plasma adipokine profile in high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant (OR) rats after 4 and 12 months. **(H)** Plasma leptin levels and **(I)** leptin-to-adiponectin level ratio in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant (OR; white bars) rats at 4 and 12 months. Data are presented as mean ± SEM (n = 8–14 rats per group). ***$p < 0.001$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats; $^{\dagger\dagger}0.001 < p < 0.01$, 4- vs. 12-month high-fat diet in obesity-prone (OP) rats. Correlations between plasma leptin-to-adiponectin ratio and different characteristics of metabolic syndrome, including body weight **(J)** and total abdominal fat weight **(K)**. Data of all experimental groups and both 4- and 12-month protocol duration were gathered and analyzed together using a parametric Pearson's correlation coefficient analysis.

Scientific, Waltham, MA, USA) and adiponectin (RRP300; R&D Systems, Minneapolis, USA) enzyme-linked immunosorbent assay (ELISA) kits respectively, according to manufacturers' instructions. Results were presented as the mean value of duplicated experiments.

Plasma levels of cardiac biomarkers, including N-terminal (NT) pro-B-type natriuretic peptide (BNP) and interleukin-1 receptor-like 1 (IL-1RL1, commonly called sST2), were evaluated using rat NT-proBNP (MBS012301; MyBioSource, San Diego, USA) and sST2 (MBS9348955; MyBioSource, San Diego, USA) ELISA kits respectively, according to manufacturers' instructions. Results were presented as the mean value of duplicated experiments.

Echocardiography

Transthoracic 2D, M-mode and Doppler echocardiography was performed using a digital ultrasound machine (Vivid-7, GE Healthcare, Etat, USA) equipped with a 12-MHz phased array transducer (Hewlett Packard, Palo Alto, CA) to assess cardiac structure and function. Animals were sedated with an intraperitoneal injection of ketamine ($24\,mg.kg^{-1}$) and medetomidine ($0.32\,mg.kg^{-1}$) and placed in right and left lateral recumbent positions on a heating pad to control body temperature. Sedation was confirmed by lack of response to firm pressure on one of the hind paws. Electrocardiogram was monitored via limb leads throughout the procedure. All measurements were obtained by the same observer

according to methods recommended by the American Society of Echocardiography currently applied to humans (17).

Two-dimensional M-mode and pulsed-wave Doppler echocardiography was performed in the right parasternal (long- and short- axis) and subcostal views. Diastolic (d) and systolic (s), septal (SWT) and posterior wall thickness (PWT) and LV diameters (LVID) were measured in M-mode from a LV short axis view at the level of *chordae tendinae* and fractional shortening (FS) was calculated. LV ejection fraction (LVEF) was derived using the Teichholz formula. To estimate LV hypertrophy, relative wall thickness ((SWTd + PWTd)/LVEDD), relative wall area (LV epicardial short-axis area—LV endocardial short-axis)/LV epicardial short-axis area) were calculated. LV mass was calculated using the American Society of Echocardiography recommended formula: LV Mass = 1.05 (5 /6 × A × L), where A (epicardial area—endocardial area) is planimetered short-axis area obtained at the papillary muscle level, and L is the LV length (apex to mid-mitral annulus plane) obtained from the parasternal long-axis view normalized for tibial length, to assess LV weight independently of body weight. Aortic flow was measured from the subcostal window to calculate forward stroke volume and cardiac output. All parameters were measured for at least three heart beats, at end-diastole and end-systole. An averaged value for each animal was used.

Hemodynamic Measurements

Invasive hemodynamic evaluation through close-chest LV and right ventricular catheterization was performed in spontaneously breathing rats under anesthesia using a rodent anesthesia system (Minerve, Esternay, France; isoflurane: 4% for induction, 2% during surgery and 1% while performing hemodynamic measurements). Left carotid artery was cannulated with a microtip pressure catheter (rodent catheter 1.6F, Transonic, The Netherlands), which was gently placed in the middle of the left ventricle. LV end-systolic and end-diastolic pressures were measured. LV end-systolic pressure was used for systemic vascular resistance calculation. Thereafter, the microtip pressure catheter was moved and placed in the middle of the right ventricle through the right external jugular vein cannulation. Correct anatomic placement was confirmed by respective pressure contours. After, right ventricular systolic pressure was measured. Hemodynamic data were recorded and analyzed with a ADV500 PV data acquisition system (Transonic, AD Instruments, The Netherlands) after stabilizing the pressure line for each animal.

Histological Analysis—Cardiac Morphometry

Three-micrometer myocardial sections were taken along the transversal axis of the heart and stained with hematoxylin-eosin for overall morphological analysis, as previously described (18). Briefly, mean cross-sectional areas of cardiomyocytes were calculated by measuring at least 50 cells for each myocardial sample (at a 400-fold magnification in light microscopy). From these hematoxylin-eosin-stained myocardial sections, structural vascular density (number of cardiac vessels/mm^2) was evaluated using at least 20 randomly selected microscopic fields (at a 200-fold magnification in light microscopy) of myocardial sections. All morphological analyses were performed using a LEICA DFC425C camera and LEICA DM2000 microscope (Leica Microsystems; Heerbrugg, Germany) and Image J analysis software (imagej.nih.gov/ij/) in a blind fashion by two independent investigators. Averaged values of cross-sectional areas of myocardial cells and of number of cardiac vessels were used as indicators of cardiomyocyte size and myocardial vascular density respectively.

Myocardial interstitial and perivascular fibrosis were evaluated by Masson's Trichrome and by Picrosirius red staining to assess the presence of collagen accumulation and fibrosis within myocardial sections. Picrosirius red staining was also observed under polarized light microscopy.

Quantification of Nitric Oxide (NO) Metabolite (Nitrite) Production

Four mm-length segments of freshly collected thoracic aorta were incubated during one hour with Krebs solution-Henseleit solution (118 $mmol.L^{-1}$ NaCl; 4.7 $mmol.L^{-1}$ KCl; 1.2 $mmol.L^{-1}$ $MgSO_4$; 1.2 $mmol.L^{-1}$ KH_2PO_4; 2.5 $mmol.L^{-1}$ $CaCl_2$; 25 $mmol.L^{-1}$ $NaHCO_3$; 5.1 $mmol.L^{-1}$ glucose; Merck, Darmstadt, Germany) in a 24-well cell culture plate. Supernatants were collected and the levels of NO were measured indirectly by the determination the nitrite levels, using the Measure-iT^{TM} High-Sensitivity Nitrite Assay kit (Molecular Probes, Eugene, USA) according to the manufacturer's instructions. Briefly, collected supernatants were ultra-filtered through a 10 000-molecular weight cut-off filter to eliminate proteins (VWR; Leuven, Belgium). Fluorescence was measured in triplicate with a microplate reader at 365/450 nm. Nitrite concentrations were obtained by referring to a standard curve realized in parallel and expressed in $mol.L^{-1}$.

RNA Extraction and RNA-Seq Data Acquisition

Total RNA was extracted from three randomly chosen snap-frozen myocardial tissue samples from 12-month OP and OR rats, using TRIzol reagent (Invitrogen, Merelbeke, Belgium), followed by a chloroform/ethanol extraction and a final purification using QIAGEN RNeasy® Mini kit (QIAGEN, Hilden, Germany), according to manufacturer's instructions. RNA sample concentration was determined with a standard spectrophotometer Nanodrop® (ND-1000; Isogen Life Sciences, De Meern, The Netherlands) and RNA integrity was assessed by visual inspection of GelRed (Biotium, Hayward, California)-stained agarose gels. RNA sample quality was finally checked using a Fragment Analyzer 5200 (Agilent, Santa Clara, CA, USA).

RNA-seq was performed at the Brussels Interuniversity Genomics High Throughput core (www.brightcore.be). Briefly, indexed cDNA libraries were obtained using the TruSeq RNA sample preparation kit (Illumina, San Diego, CA, USA) following the manufacturers' instructions. The multiplexed libraries were loaded on a Novaseq 6000 (Illumina) using a S2 flow cell and sequences were produced using a 200 Cycle Kit. Approximately 25-million paired-end reads per sample were mapped against the *rattus norvegicus* (Rnor 6.0) reference genome using STAR

software (version STAR_2.5.3a) to generate read alignments for each sample. Annotations Rnor 6.0.103.gtf were obtained from ftp.Ensembl.org. After transcripts assembling, gene level counts were obtained using HTSeq tool (version HTSeq_0.9.1).

Gene Ontology and iDEP Pathway Analysis of Differentially Expressed Genes

Hierarchical clustering and differentially expressed genes between high-fat diet fed OP rat and standard chow fed OR rat group were obtained using the integrated website application for analysis of RNA-seq data iDEP (http://bioinformatics.sdstate.edu/idep/). The genes with a p-value < 0.05 and an absolute log-fold change > 1 were selected for further evaluation. The volcano plot was constructed using VolcaNoseR (https://huygens.science.uva.nl/VolcaNoseR/). Gene ontology (GO) and pathways enrichment analysis was applied to these differentially expressed genes using Enrichr (Mouse Pathways database) (http://amp.pharm.mssm.edu/Enrichr). In parallel, manual literature analysis was realized for each differentially expressed gene regarding their molecular function in the rats and implication in HFpEF. Heatmap visualization was realized using R software v4.0.3 (https://www.r-project.org). The corresponding detailed results of RNA sequencing analysis are available at the GEO number: GSE189190.

Real-Time Quantitative Polymerase Chain Reaction (RTq-PCR)

Reverse transcription was performed using random hexamer primers and Superscript II Reverse Transcriptase (Invitrogen, Merelbeke, Belgium), according to the manufacturer's instructions.

For RTq-PCR, sense and antisense primers were designed using Primer3 program for *rattus norvegicus* superoxide dismutase (SOD) 1 and 2, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and hypoxanthine phosphoribosyl transferase (HPRT) 1 mRNA sequences. To avoid inappropriate amplification of residual genomic DNA, intron-spanning primers were selected when exon sequences were known. For each sample, amplification reaction was performed in triplicate using SYBR Green PCR Master Mix (Quanta Biosciences, Gaithersburg, MD, USA), specific primers and diluted template cDNA. Result analysis was performed using an iCycler System (BioRad Laboratories). Relative quantification was achieved by normalization with the housekeeping genes, GAPDH and HPRT1.

Statistical Analysis

All data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using Graphpad Prism 9.0 (Graphpad, San Diego, California, USA). Intergroup comparisons were tested by two-factor ANOVA for multiple measurements and LVd mass results were tested with one-way ANOVA. When the F ratio of these analyzes reached a critical p-value < 0.05, comparisons were made with a parametric Student t-test followed by Bonferroni correction as *post-hoc* test. A value of $p < 0.05$ was considered as statistically significant; n represents the number of individual data.

Correlations were analyzed parametrically by the determination of the Pearson correlation coefficient and non-parametrically by the Spearman's rank correlation coefficient.

RESULTS

High-Fat Diet in Obesity-Prone Rats Leads to Metabolic Syndrome

General and Abdominal Obesity

At baseline, body weights of 4-week-old OP and OR rats were similar (93 ± 2 *vs.* 93 ± 1 g in OP *vs.* OR rats). During the last 5 weeks of 4- and 12-month protocols, food intake was recorded and corresponding daily calorie intake was calculated and averaged. Despite their high-calorie diet (45% kilocalories from lipids and 17% from sugars), OP rats showed a higher daily food consumption (**Figure 1C**). Total daily calorie intake was greater by 35% in OP rats eating a high-fat diet compared to standard chow-fed OR rats (**Figure 1C**) independently of protocol length. Consumption of a high-fat diet for 4 and 12 months in OP rats promoted higher body (**Figures 1B,D**) and abdominal fat (**Figure 1E**) weight gains. These markers of general and intra-abdominal (visceral) obesity were already present and even higher after 4 compared to 12 months (85- *vs.* 82%-increases in body weight and 541- *vs.* 348%-increases in abdominal fat weight after 4 *vs.* 12 months respectively, in OP compared to OR rats; **Figures 1D,E**).

Metabolic Profile

Plasma levels of triglycerides, total cholesterol, and low- (LDL) and high-density (HDL)-cholesterol, were increased after 4 months, and even more so after 12 months of high-fat diet in OP rats (**Table 1**), suggesting aggravated dyslipidemia over time. The ratio of LDL-to-HDL-cholesterol was 3- and 4-fold increased after 4- and 12-month high-fat diet respectively in OP rats compared to their lean counterparts (**Table 1**).

As illustrated in **Figure 1F**, fasting blood glucose levels were similar in OP rats fed with a 4- and 12-month high-fat diet compared to OR rats. In order to test the effects of high-fat diet on metabolic parameters in OP rats, we used an intraperitoneal glucose tolerance test to assess glucose sensitivity. Serum glucose levels at 30, 60, 90, 120 and 150 min were significantly higher in OP rats fed with high-fat diet during 4 months than those observed in standard chow-fed OR rats with an apparent lower clearance (**Figure 1F**). Serum glucose curves did not return to their basal glucose levels 150 min after glucose administration in both 4- and 12-month high-fat diet-fed OP rat groups. Total AUC of serum glucose levels between 0 and 150 min were shown for the four groups of rats in **Figure 1G**. The AUC of 4- and 12-month high-fat diet-fed OP rats were higher than those observed in corresponding OR rats, with maximal AUC-value after 4 months of high-fat dieting. This suggested that high-fat diet in OP rats induced glucose intolerance at 4 and 12 months and that this was more pronounced at 4 months.

Because adipokines play important roles in the pathophysiological link between dysfunctional adipose tissue and cardiometabolic alterations, we evaluated the effects of high-fat

TABLE 1 | Evolution of plasma lipid profile in high-fat diet-fed obesity-prone and in normal chow-fed obesity-resistant rats during 4 and 12 months.

	4 months			12 months			*p* 4- vs. 12-month high fat diet in OP rats
	Normal chow in OR rats ($n = 8$)	High fat diet in OP rats ($n = 10$)	*p*	Normal chow in OR rats ($n = 8$)	High fat diet in OP rats ($n = 14$)	*p*	
Triglycerides (in mg.dL^{-1})	90 ± 9	218 ± 27	***	131 ± 16	910 ± 136	***	†††
Total cholesterol (in mg.dL^{-1})	55 ± 2	109 ± 9	*	73 ± 4	214 ± 22	***	†††
HDL-cholesterol (in mg.dL^{-1})	44 ± 1	66 ± 5	**	54 ± 2	86 ± 6	***	††
LDL-cholesterol (in mg.dL^{-1})	10 ± 1	43 ± 7	***	19 ± 2	128 ± 19	***	†††
LDL/HDL ratio	0.23 ± 0.02	0.69 ± 0.14	*	0.35 ± 0.03	1.55 ± 0.20	***	†††

*Values are expressed as means ± SEM. *0.01 < p < 0.05, **0.001 < p < 0.01, ***p < 0.001, high-fat diet-fed in obesity-prone (OP) vs. normal chow-fed in obesity-resistant (OR) rats. ††0.001 < p < 0.01, †††p < 0.001, 12- vs. 4-month high-fat diet-fed in obesity-prone (OP) rats. HDL means high-density lipoprotein; LDL, low-density lipoprotein; OR, obesity-resistant rats; OP, obesity-prone rats.*

diet on plasma adipokine levels. Plasma concentration of leptin was increased in OP rats after a 4-month and much more after a 12-month high-fat diet (**Figure 1H**). The concentration of adiponectin was also increased in these animals (4.2 ± 0.6 *vs.* 1.8 ± 0.4 μg.mL^{-1} and 7.5 ± 0.9 *vs.* 1.6 ± 0.4 μg.mL^{-1} in high-fat diet-fed OP compared to OR rats after 4 and 12 months respectively; $p < 0.001$). Leptin-to-adiponectin ratio, which has been proposed as a marker of adipose tissue dysfunction (19), was increased by 2-fold in high-fat diet-fed OP rats after 4 months (**Figure 1I**). After 12 months, this ratio was slightly lower compared to 4 months, but remained higher in obese rats compared to their lean counterparts (**Figure 1I**). This leptin-to-adiponectin ratio was correlated to body (**Figure 1J**) and abdominal fat (**Figure 1K**) weights.

Systemic Hypertension

While LV systolic pressure was similar in both groups of rats after 4 months, high-fat diet was associated with an increase in LV systolic pressure after 12 months in OP rats fed with high-fat diet (**Figure 2A**).

Characterization of Metabolic Syndrome

All characteristics of metabolic syndrome, except alterations in LV pressures, were already present after 4-month high-fat diet in OP rats. Even if the phenotype of metabolic syndrome and associated metabolic characteristics were less exacerbated (than after 4 months), LV systolic pressure was markedly increased after 12-month protocol (**Figure 2A**), showing LV pressure overload.

Metabolic Syndrome Evolves to HFpEF Overtime

Hemodynamic Evaluation

High-fat diet in OP rats was associated with increased LV end-diastolic pressure after 12 months, while no change was observed after 4 months (**Figure 2B**). As illustrated in **Figure 2C**, high-fat diet in OP rats increased systemic vascular resistance by 24% after 12 months. Right ventricular catheterization using a closed-chest approach was performed to evaluate pulmonary pressure in these rats. After 4 and 12 months, metabolic syndrome resulted in the development of mild pulmonary hypertension (assessed by increased right ventricular systolic pressure) in OP rats fed with high-fat diet (**Figure 2D**).

LV Structure

Echocardiography was performed in the two groups of rats after 4 and 12 months to assess cardiac structure and function. As illustrated in **Table 2**, there was no difference in heart rates between groups at both 4- and 12-month time points. All echocardiographic parameters measured after 4 months were similar between OP rats fed with high-fat diet and their lean counterparts (**Table 2**). After 12 months, metabolic syndrome resulted in significant LV wall hypertrophy (**Figure 3**), as evaluated by increased LV mass (standardized for tibial length; **Figure 3A**), relative wall thickness (RWT) and relative wall area (**Table 2**; **Figures 3C,D**). This concentric LV hypertrophy assessed by echocardiography was confirmed, at autopsy, by whole heart (**Figure 3B**) and cardiomyocyte (**Figure 4**) morphometric analysis. Total heart weight (normalized to tibial length) was similarly increased after 4 and 12 months in OP rats fed with high-fat diet (**Figure 3B**). The LV of the 12-month high-fat diet-fed OP rats presented obvious cardiomyocyte hypertrophy, characterized by an increase in cell surface area compared to obesity-resistant rats (**Figures 4A**a1,2,**B**). In contrast, after 4-month high-fat diet, cardiomyocyte surface area was reduced compared to OR rats (**Figure 4B**). Because myocardial interstitial fibrosis and collagen deposition influence the mechanical properties of the myocardium, we used Masson's Trichrome (**Figure 4A**a3,4) and Picrosirius red (**Figure 4A**a5–12) staining to detect fibrotic area. LV fibrosis, characterized by interstitial and perivascular fibrotic deposition, was exacerbated in high-fat diet-fed OP rats after 12 months (**Figure 4Aa**5–12). Capillary density, determined as the number of capillaries/μm^2 of myocardial tissue, was evaluated in LV cross sections. No differences were observed between the four groups of rats (19 ± 3 *vs.* 19 ± 2 and 16 ± 2 *vs.* 16 ± 1 capillaries/mm^2 myocardial tissue section in OP *vs.* OR rats after 4 and 12 months respectively, p>0.05 for all comparisons). Consistently, capillary-to-cardiomyocyte ratio remained unchanged between groups (data not shown). Myocardial infiltration with inflammatory cells

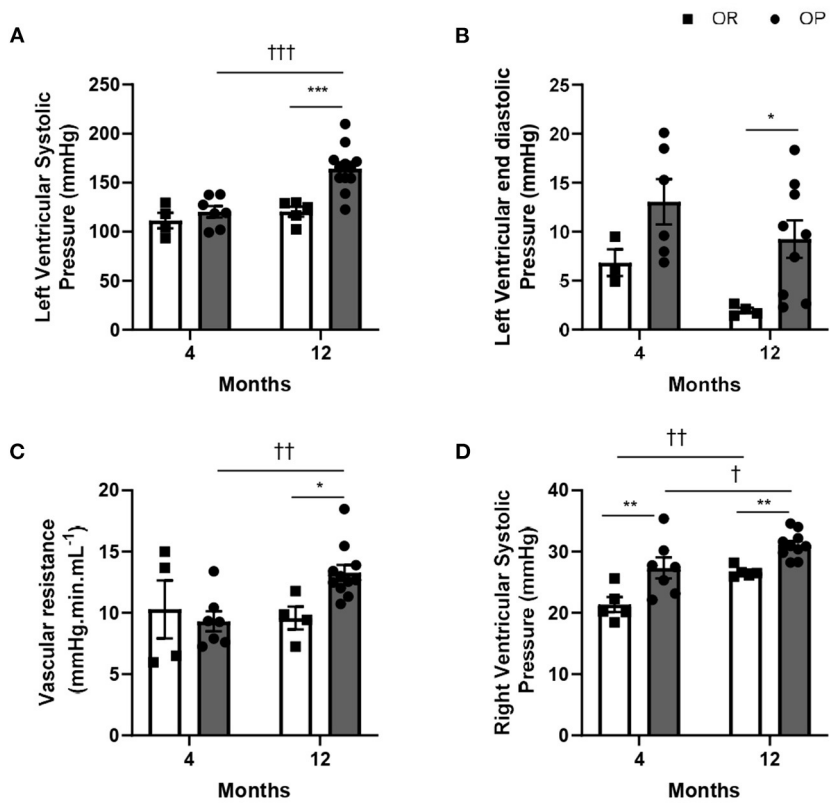

FIGURE 2 | Hemodynamic characterization of high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant (OR) rats after 4 and 12 months. **(A)** Left ventricular systolic (LVSP) and **(B)** end-diastolic pressures in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant (OR; white bars) rats at 4 and 12 months. **(C)** Total systemic vascular resistance was calculated as the ratio between the left ventricular systolic pressure (evaluated by closed-chest left ventricular catheterization) and the normalized cardiac output (evaluated by echocardiography) in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant (OR; white bars) rats at 4 and 12 months. **(D)** Right ventricular systolic pressure in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant rats (OR; white bars) at 4 and 12 months. Data are presented as mean ± SEM ($n = 8$–14 rats per group). *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, ***$0.001 < p$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats; $^{\dagger}0.01 < p < 0.05$, $^{\dagger\dagger}0.001 < p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, 4- vs. 12-month high-fat diet in obesity-prone (OP) or obesity-resistant (OR) rats.

(performed on hematoxylin-eosin-stained sections) was similar in all 4 groups of rats (data not shown).

LV Systolic Function

After 4 and 12 months, LV cavity sizes remained within the normal range and were similar in OP and OR rats, as assessed by unchanged LVIDd adjusted for tibial length. LVEF remained unaltered, with normal values above 60% (**Table 2**). No significant differences in FS (with normal values above 25%), stroke volume and cardiac output were observed (**Table 2**). Altogether, this strongly suggested preserved LV systolic function in OP rats with metabolic syndrome induced by a 12-month high-fat diet.

Cardiac and Endothelial Biomarkers

Circulating levels of cardiac diagnostic biomarkers for heart failure and cardiac dysfunction, including NT-proBNP and sST2, were evaluated in the two groups of rats at both time points. After 4 months, plasma levels of NT-proBNP were not significantly different between OP and OR rats, while NT-proBNP levels decreased after 12 months in rats with metabolic syndrome compared to their lean counterparts (**Figure 5A**). Plasma sST2 levels markedly increased in OP rats fed with high-fat diet during 12 months, while sST2 remained low after 4 months (**Figure 5B**). Plasma sST2 levels were correlated to LV relative wall area (**Figure 5C**) and LV systolic pressure (**Figure 5D**). As illustrated in **Figure 5E**, release of NO was decreased in thoracic aorta collected in 12-month high-fat diet fed OP rats compared to controls.

Pathobiological Myocardial Characterization

The RNA-seq analysis identified 165 differentially expressed genes between the 2 groups of rats after 12-month protocol (**Figure 6A**; **Supplementary Table 1** for detailed results). Analysis indicated that many of the genes that were upregulated in OP rats fed with high-fat diet during 12 months were implicated in energy substrate use (**Figures 6B,C**). Genes implicated in fatty acid synthesis, such as trimethyllysine hydroxylase-ε (TMLHE) and diacylglycerol kinase-γ (DGKG), and catabolism (3-hydroxybutyrate dehydrogenase-1; BDH1)

TABLE 2 | Two-dimensional and M-mode echocardiographic parameters of left ventricular structure and function in high-fat diet-fed obesity-prone and in normal chow-fed obesity-resistant rats during 4 and 12 months.

	4 months			12 months			*p* 4- vs. 12-month high fat diet in OP rats
	Normal chow in OR rats ($n = 8$)	High fat diet in OP rats ($n = 10$)	*p*	Normal chow in OR rats ($n = 8$)	High fat diet in OP rats ($n = 14$)	*p*	
Heart rate (in beats/min)	258 ± 13	257 ± 10	NS	227 ± 5	208 ± 8	NS	NS
Relative LV wall surface area	0.53 ± 0.01	0.52 ± 0.02	NS	0.54 ± 0.01	0.59 ± 0.02	*	††
LVEF (in %)	63 ± 2	68 ± 5	NS	63 ± 2	69 ± 3	NS	NS
FS (in %)	30 ± 1	34 ± 3	NS	30 ± 1	35 ± 3	NS	NS
LVIDd (in mm)	1.80 ± 0.04	1.77 ± 0.07	NS	1.78 ± 0.05	1.79 ± 0.08	NS	NS
LVRWT	0.42 ± 0.02	0.47 ± 0.03	NS	0.42 ± 0.02	0.51 ± 0.03	*	NS
Stroke volume (in mL)	0.26 ± 0.04	0.25 ± 0.02	NS	0.23 ± 0.01	0.28 ± 0.02	0.09	NS
Cardiac output (in mL/min)	15.3 ± 3.4	14.0 ± 1.3	NS	13.6 ± 0.4	13.2 ± 0.8	NS	NS

*Values are represented as means ± SEM. *0.01 < p < 0.05, high-fat diet fed in obesity-prone (OP) vs. normal chow fed in obesity-resistant (OR) rats. ††0.001 < p < 0.01 12- vs. 4-month high-fat diet fed in obesity-prone (OP) rats. Relative left ventricular wall surface area was calculated as follows: (LVAd sax EPI – LVAd sax PM)/(LVAd sax EPI), where LVAd sax EPI means left ventricular epicardial short-axis area at the papillary muscle tip level at end-diastole, and LVAd sax PM, left ventricular endocardial short-axis at the papillary muscle tip level at end diastole and was used to estimate left ventricular hypertrophy. Stroke volume was calculated as follows: (LVOT VTI x aortic cross-sectional area), where LVOT VTI means left ventricular outflow tract velocity time integral (used as a measure of cardiac systolic function and cardiac output) and the aortic cross-sectional area (in mm). Cardiac output was calculated with the formula (stroke volume x heart rate). LV means left ventricular; LVEF, left ventricular ejection fraction; FS, fractional shortening; LVIDd, left ventricular internal diastolic dimension (indexed for tibia length); LVRWT, relative wall thickness; OR, obesity-resistant rats; OP, obesity-prone rats; NS, not significant.*

were upregulated, as well as other molecules implicated in fatty acid transport, such as glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GPIHBP1) and lipocalin 2 (Lcn2) (**Figure 6B**). Creatine Kinase B (CKB), an enzyme implicated in energy homeostasis, was also upregulated (**Figures 6B,C**). As illustrated **Figure 6B**, the RNA-seq analysis also identified an upregulated cluster of genes implicated in calcium-dependent contraction, including glycoprotein (GPM6A), myosin light chain 1 (MYL1) and 9 (MYL9) and myomesin 3 (MYOM3), as well as a calcium voltage-gated channel subunit-α (CACNA1H) in 12-month high-fat diet fed OP rats. Arginine vasopressin receptor 1A (Avpr1a), a receptor implicated in both glycogenolysis and contraction was also upregulated in 12-month high-fat diet OP rats (**Figures 6B,C**). Myocardial mRNA expression of SOD1 and SOD2, two antioxidant enzymes were decreased in OP rats fed with high-fat diet during 12 months (**Figure 6D**).

Characterization of HFpEF

OP rats fed with high-fat diet during 12 months presented with elevated LV end-diastolic pressure (**Figure 2B**), global myocardial hypertrophy (**Figure 3**) and preserved LVEF (**Table 2**), with increased right ventricular systolic pressure (**Figure 2D**), which strongly suggested the development of HFpEF appearing secondarily to metabolic syndrome.

DISCUSSION

Given the limitations of current preclinical models, we developed a rat model of HFpEF that recapitulates the vast majority of the clinical features of the syndrome. Metabolic syndrome induced by prolonged high-fat feeding during 12 months in OP rats, leads to HFpEF characterized by increased LV filling pressures and diastolic dysfunction, adverse structural and functional LV remodeling (with cardiomyocyte hypertrophy and fibrosis), and preserved ejection fraction, together with elevated right ventricular systolic pressure. This was associated with increased circulating levels of sST2, while NT-proBNP levels remained low. At pathobiological level, myocardial RNA-sequencing analysis identified clusters of genes implicated in fatty acid metabolism and calcium-dependent contraction, as the most disrupted pathways in rats with HFpEF.

In patients, HFpEF is characterized by signs and symptoms of heart failure with evidence of cardiac structural and functional abnormalities consistent with the presence of LV diastolic dysfunction, in the presence of a normal LV ejection fraction (20). Although systolic function was preserved in the present experimental model, there was an increase in LV diastolic pressure. This is consistent with the pivotal criteria in establishing a diagnosis of HFpEF which remains in the evidence of LV filing pressure elevation, indicative of a diastolic dysfunction or impaired ability of the heart to fill during diastole, in presence of a non-dilated LV and a preserved LV ejection fraction (in the absence of significant mitral regurgitation) (21, 22). However, one limitation of the present study is that it was not possible to perform traditional measures of LV diastolic dysfunction in rats, including E/A, E/e' and Doppler flow velocities. Although the precise mechanism leading to myocardial stiffening remain undetermined, LV stiffness is thought to have active and passive components. Cardiac remodeling, including myocardial hypertrophy, inflammation and fibrosis, have been shown to play crucial roles in the pathophysiology of HFpEF, all leading to an impaired LV relaxation ability (23, 24). In the present study, LV remodeling, characterized by significant concentric LV hypertrophy and myocardial fibrosis (both interstitial and perivascular), was found in high-fat diet-fed OP rats, ultimately

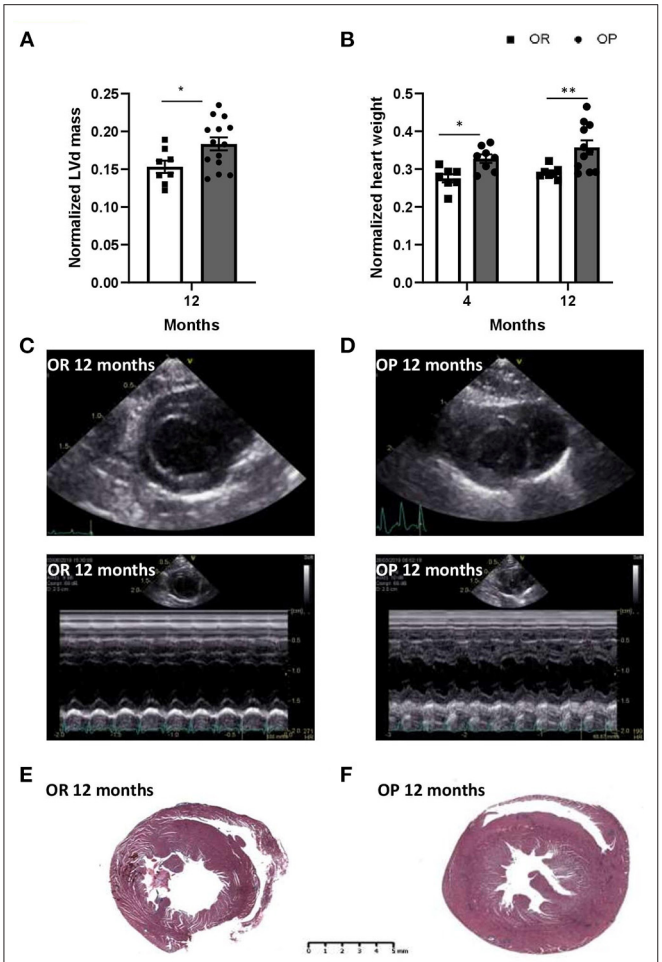

FIGURE 3 | High-fat diet-fed obesity-prone (OP) rats display, after 12 months, key alterations found in clinical HFpEF. **(A)** Echocardiographic evaluation of left ventricle mass normalized to tibial length (in mm) in high-fat diet fed obesity-prone (OP; black bars) vs. normal chow fed obesity-resistant (OR; white bars) rats at 12 months. **(B)** Ratio of heart weight (in g) to tibial length (TL; in cm) in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant rats (OR; white bars) at 4 and 12 months. Data are presented as mean ± SEM ($n = 8$–14 rats per group). *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats. Representative left ventricular bi-dimensional (top) and M-mode echocardiographic (bottom) tracings in normal chow fed obesity-resistant [OR; **(C)**] and high-fat diet-fed obesity-prone [OP; **(D)**] rats at 12 months. Images are representative of 10 independent rats. Representative Masson Trichrome-stained myocardial macro cross-sections at ventricle level from normal chow-fed obesity-resistant [OR; **(E)**] and high-fat diet-fed obesity-prone [OP; **(F)**] rats at 12 months. Scale bar: 5 mm.

leading to a loss of LV compliance and increased LV filling pressures (25).

Even if we found diastolic dysfunction (assessed by increased LV end-diastolic pressure) and significant alterations in LV structure, together with preserved LVEF, assessment of signs and symptoms of heart failure remain difficult (or even impossible) in rodents, as already discussed (26, 27). They are known to interfere with normal animal behavior and exercise capacity, although these may not be specific to HFpEF. In the present study, we did not evaluate exercise capacity *per se*, but we noticed that high-fat diet fed OP rats with HFpEF were more sedentary and moved less in their cages than their lean counterparts. Recently, two diagnostic algorithms, the HFA-PEFF (28)and the and H_2FPEF scores (11) used as novel clinical standards for defining the key clinical features of HFpEF, have been transposed to experimental models of HFpEF (26). Here, we found a HFA-PEFF score of 4 (corresponding to diastolic dysfunction and alteration in LV morphological aspects without increased natriuretic peptide levels) and a H_2FPEF score of 5 (related to the presence of obesity, hypertension, pulmonary hypertension and diastolic dysfunction) corresponding to an intermediate and a high score for HFpEF respectively. These 2 scores validated and confirmed the translational value of the present experimental model of HFpEF.

Obesity and associated metabolic risks have been proposed as a major driver of LV diastolic dysfunction and HFpEF, contributing to systemic inflammation and subsequent myocardial remodeling (7, 29, 30). Obesity has direct and indirect effects on the heart, including increased myocardial load associated with plasma volume expansion, worsening of systemic arterial hypertension, LV hypertrophy and increased aortic stiffness (31). In the present study, a worsening of the cardiovascular disease was observed with increasing age in high-fat diet-fed OP rats, even if their body weight was not increasing. HFpEF is also recognized to be an age-related disease (11). Heart failure is indeed disproportionately distributed among elderly individuals, as over half of all patients hospitalized with heart failure are older than 75 years, with 50% presenting with diastolic dysfunction (32). The present experimental model, obtained in genetically obesity-predisposed rats on a 12-month high-fat diet, recapitulates key comorbidities observed in patients with HFpEF, such as obesity, early metabolic derangements and pressure overload. After 12 months, OP rats on high-fat diet developed signs of HFpEF, characterized by elevated LV end-diastolic pressure and LV hypertrophy, while LV ejection fraction was preserved. LV systolic function remained normal, whereas diastolic function was impaired, which strongly suggests an early-stage of HFpEF after 12 months.

Systemic hypertension is one of the main underlying conditions, leading to HFpEF (33, 34). Hypertension, which causes broad changes in inflammation and metabolism, can cause myocardial stiffness and diastolic dysfunction (35). Insulin resistance may also be a key factor underlying the link between obesity and HFpEF (36), as the central driver of systemic microvascular inflammation and subsequent myocardial dysfunction (7). In the present study, LV pressure overload and glucose intolerance were present, when diastolic dysfunction and HFpEF were observed. Obesity is known to amplify comorbidities, such as insulin resistance and hypertension, partly through the adipose tissue which is capable of releasing regulatory factors, such as adipokines involved in promoting a global systemic pro-inflammatory state. Consistently, the risk of all-cause mortality was significantly higher in patients with HFpEF with abdominal obesity than in those without abdominal obesity (37). Here, we found

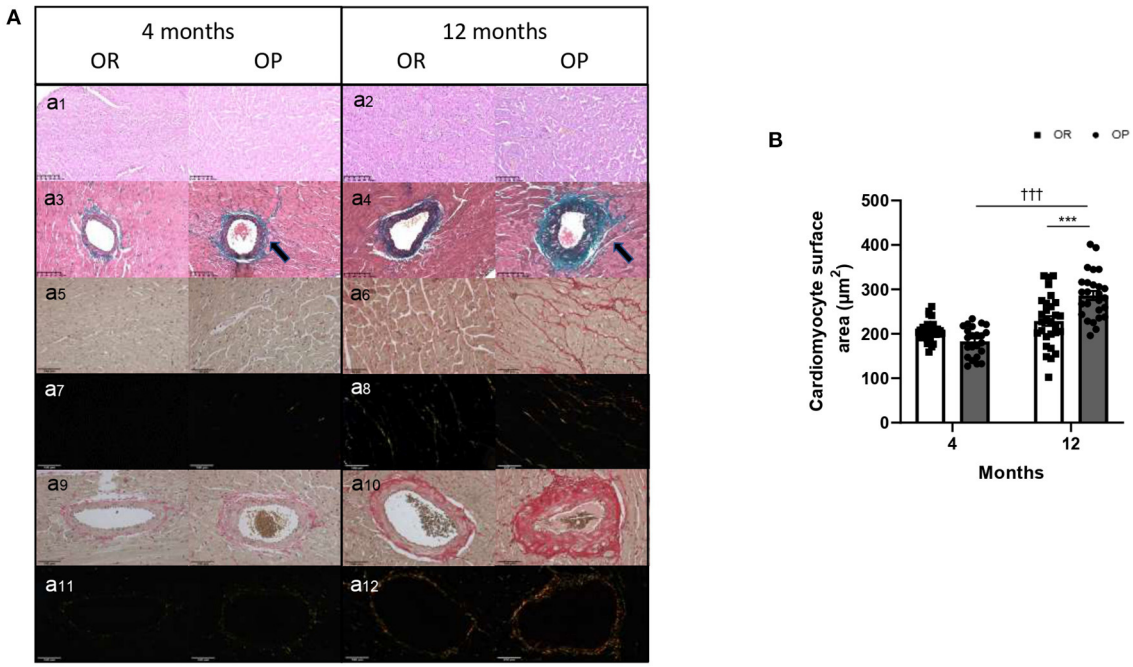

FIGURE 4 | Myocardial structure, including cardiomyocyte hypertrophy and fibrosis, in high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant (OR) rats after 4 and 12 months. **(A)** Representative images of hematoxylin and eosin-stained sections of left ventricle (a1,2) from high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant rats (OR) at 4 (a1) and 12 months (a2). Representative Masson Trichrome-stained sections of myocardial vessels (a3,4) from high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant rats (OR) at 4 (a3) and 12 months (a4). Trichrome Masson staining was performed to detect fibrotic areas [collagen fibers stained in green; indicated by arrows in a3,4]. Representative images of Picrosirius red-stained sections of left ventricle observed using classical and polarized light microscopy (a5–8 respectively) and of myocardial vessels (a9–12 respectively) from high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant rats (OR) at 4 and 12 months. Myocardial interstitial and vascular sections were obtained at 200-fold magnification. Scale bars: 100 µm. **(B)** Cardiomyocyte hypertrophy (assessed by cardiomyocyte area in square micrometer) in left ventricles from high-fat diet-fed obesity-prone (OP; black bars) and normal chow-fed obesity-resistant rats (OR; white bars) at 4 and 12 months. Data are presented as mean ± SEM (25 images for each animal; $n = 2$–3 rats per group). ***$0.001 < p$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats; $^{\dagger\dagger\dagger}p < 0.001$, 4- vs. 12-month high-fat diet in obesity-prone (OP) rats.

increased abdominal fat weight, together with increased levels of adiponectin and leptin, with high leptin-to-adiponectin ratio in OP rats on high-fat diet. This is consistent with previous data showing elevated levels of leptin and adiponectin in HFpEF (38). The impact of obesity on HFpEF pathophysiology encompasses hemodynamic, neurohumoral and inflammatory mechanisms, altogether contributing to the reduction of the normal relaxation ability of the LV as the ventricular wall becomes stiffer from increasing interstitial fibrosis.

Utilization of relevant biomarkers is of clinical interest, because HFpEF is often difficult to diagnose early. Natriuretic peptides, which are produced under cardiac pressure/volume overload and reflects the degree of myocardial stretching and dysfunction (39), represent the current gold standard of biomarkers for the diagnosis, management and prognosis of heart failure (40). In patients with HFpEF, natriuretic peptides are mainly elevated in patients with advanced diastolic dysfunction, but are frequently in the normal range in mild diastolic dysfunction (41). Extra-cardiac factors, such as age, obesity and renal function are known to influence its measurement. Moreover, natriuretic peptide testing has yielded false negative results in 20% of obese patients with heart failure (42), with a risk of a false-negative BNP value around 20%, and for NT-proBNP about 15% (43). In the present study, NT-proBNP levels remained low, probably due to the presence of obesity (also observed in humans), while circulating levels of sST2 were largely increased in animals with HFpEF. This is consistent with previous reports showing elevated circulating inflammatory and reduced cardiac stretch biomarkers in HFpEF (44–47). We did not evaluate the inflammatory biomarker level of C-reactive protein (CRP), but characterized level of sST2, a marker of inflammation and fibrosis, which has been suggested to play a crucial role in ventricular remodeling and fibrosis in the context of pressure overload (48). In patients with HFpEF, sST2 has been linked to LV functional impairment assessed by global longitudinal strain (49) and associated with patient outcome (50). sST2 may represent the recently proposed concept that a systemic pro-inflammatory state driven by coexisting conditions is the additional cause of myocardial remodeling and dysfunction leading to HFpEF (7, 8). Here, we found that serum sST2 levels were correlated with LV overload and hypertrophy, suggesting that elevated sST2 may reflect LV alterations in HFpEF. In this context, circulating sST2 levels could provide additional diagnostic value beyond NT-proBNP

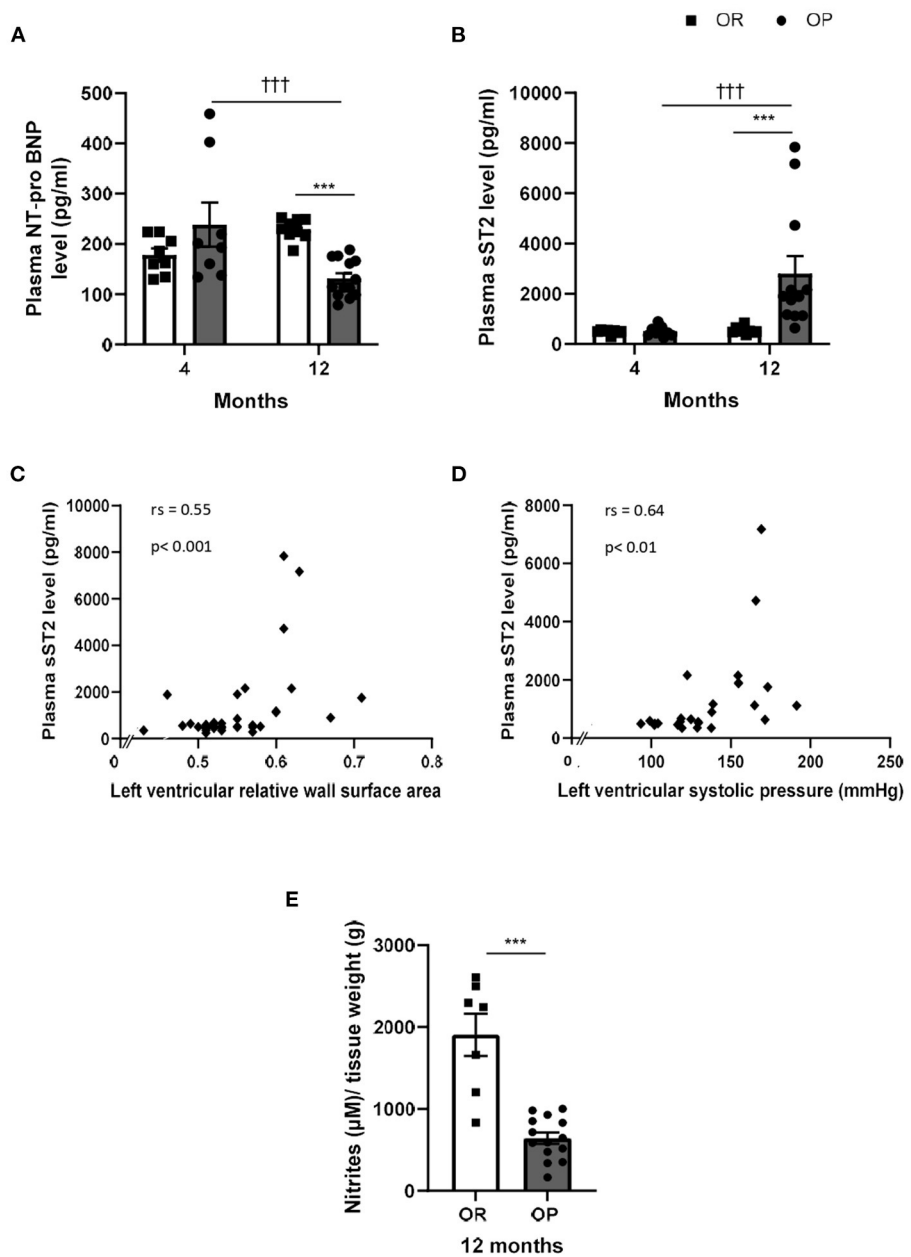

FIGURE 5 | Plasma cardiac biomarkers in high-fat diet-fed obesity-prone (OP) and normal chow-fed obesity-resistant (OR) rats after 4 and 12 months. Plasma levels of N-Terminal pro-B-type natriuretic peptide [NT-proBNP; **(A)**] and soluble suppression of tumorigenicity 2 [sST2; **(B)**] in high-fat diet-fed obesity-prone (OP; black bars) vs. normal chow-fed obesity-resistant rats (OR; white bars) at 4 and 12 months. Data are presented as mean ± SEM ($n = 8$–14 rats per group). $^{***}p < 0.001$, high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats; $^{†††}p < 0.001$ 4- vs. 12-month high-fat diet in obesity-prone (OP) rats. Correlations between plasma soluble ST2 levels and left ventricular structure and hemodynamic parameters, including left ventricular relative surface area **(C)** and left ventricular systolic pressure [LVSP; **(D)**] respectively. Data of all experimental groups and both 4- and 12-month protocol duration were gathered and analyzed together using a non-parametric Spearman's rank correlation coefficient analysis. Concentration of nitrites/NO **(E)** in supernatants of endothelium-intact thoracic aortic rings collected in 12-month high-fat diet-fed obesity-prone rats (OP; black bars) vs. normal chow-fed obesity resistant rats (OR; white bars) incubated during one hour with Krebs solution. Data are presented as mean ± SEM ($n = 9$–14 rats per group). $^{***}p < 0.001$ high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats.

levels for detecting LV diastolic dysfunction and early HFpEF, especially in obese patients.

Epidemiological studies have shown that pulmonary hypertension due to LV diastolic dysfunction, also referred to as pulmonary hypertension associated to HFpEF, is the most prevalent form of pulmonary hypertension associated to left heart disease (51, 52). Pulmonary hypertension is closely associated with worse outcome and mortality in

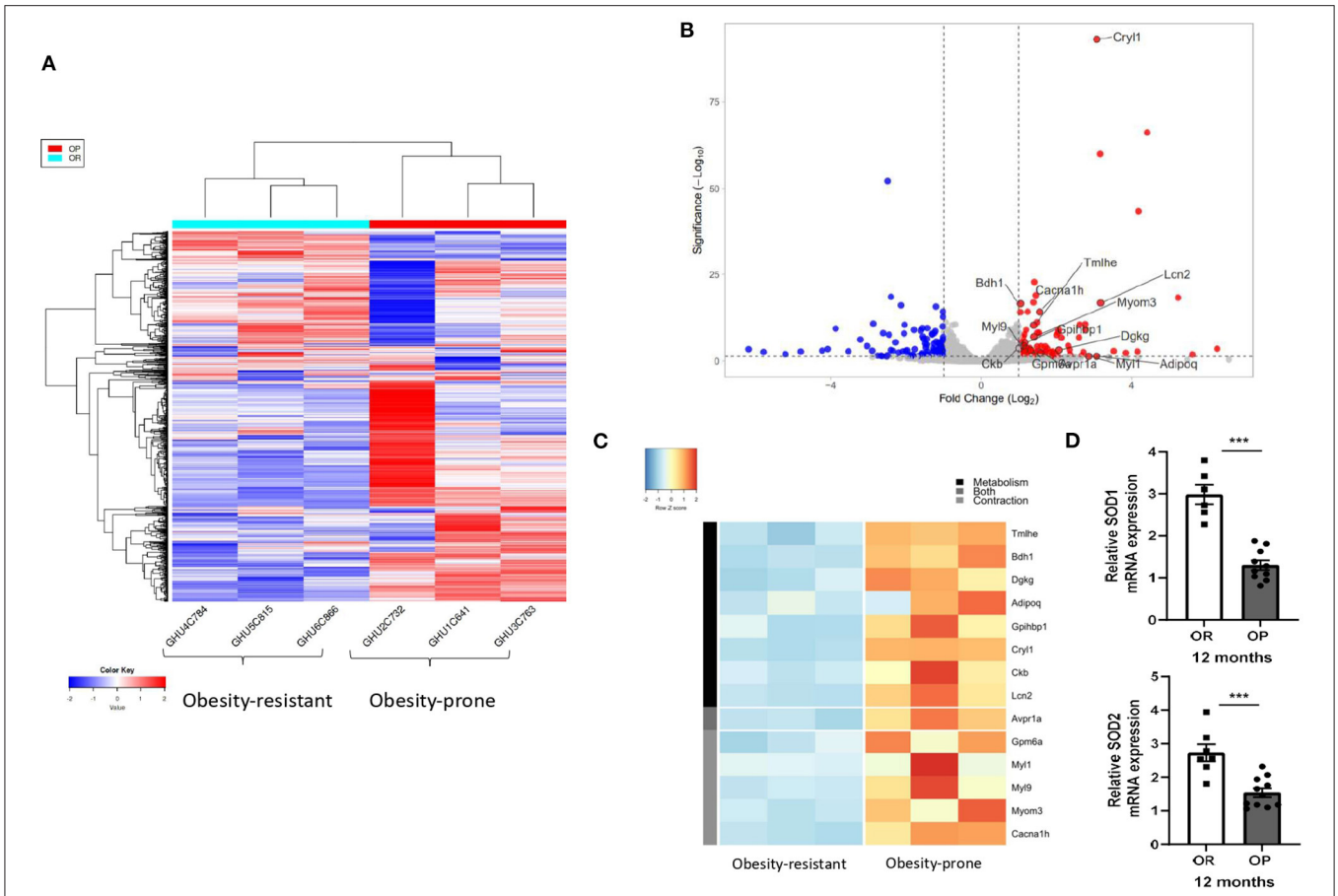

FIGURE 6 | Myocardial RNA sequencing (RNA-seq) data analysis identified clusters of genes implicated in fatty acid metabolism and calcium-dependent contraction in high-fat diet-fed obesity-prone (OP) after 12 months. Heat-map **(A)** showing hierarchical clustering of genes from RNA-seq in three randomly chosen normal chow-fed obesity-resistant rats (OR; on the left) and in three randomly chosen high-fat diet-fed obesity-prone rats (OP; on the right) at 12 months. Raw Z score are shown on the heatmap. Volcano plot **(B)** of RNA-seq transcriptome data displaying the pattern of gene expression values. Y-axis denotes – log10 *p*-values, while X-axis shows log2 fold change values. Volcano plot was generated using VolcaNoseR. Two clusters of differently expressed genes implicated in fatty acid metabolism and calcium-dependent contraction are represented **(C)** and full gene lists are available in **Supplementary Table 1**. **(D)** Relative mRNA expression of superoxide dismutase 1 (SOD1) and 2 (SOD2) in myocardial tissue samples from normal chow-fed obesity-resistant rats (OR; white bar) and from high-fat diet-fed obesity-prone rats (OP; black bar) at 12 months. Data are presented as mean ± SEM (n = 6–11 rats per group). ***$p < 0.001$ high-fat diet in obesity-prone (OP) vs. normal chow in obesity-resistant (OR) rats.

patients with HFpEF. In HFpEF, chronically elevated LV filling pressures cause a passive backward transmission of pressures in the pulmonary arteries, resulting in increased pulmonary arterial pressure and pulmonary vascular resistance (53). In addition, pulmonary hypertension exacerbates the LV diastolic dysfunction already occurring in the heart (54). Pulmonary artery pressure increased also with aging (55). The present experimental model recapitulates key clinical features known to be present in HFpEF patients who develop PH (26). The pulmonary artery pressure observed in 12-month high-fat diet fed OP rats was in the range of what is observed in HFpEF patients developing pulmonary hypertension (right ventricular systolic pressure around 46–51 mmHg) (52, 54).

In OP rats fed with high-fat diet during 12 months, exaggerated interstitial and perivascular fibrosis and cardiomyocyte hypertrophy were observed, while myocardial infiltration with inflammatory cells and capillary density remained unchanged. This cardiac hypertrophic phenotype was associated, at molecular level, with the upregulation of genes implicated in fatty acid use and in calcium-dependent contraction. These two gene clusters have both been implicated in mechanisms tempting cardiac adaptation to various stressors (24). Intriguingly, no inflammatory or cell survival markers were found. This strongly suggested that cardiac molecular signature in HFpEF reflects a myocardial response to extra-cardiac comorbidities, such as obesity, dyslipidemia, insulin resistance or hypertension, that may contribute to cardiomyocyte hypertrophy and altered contraction, which seem to ultimately concurring to the reduced compliance of the myocardium (56). A paradigm for HFpEF development was proposed, which identifies a systemic proinflammatory state induced

by comorbidities as the cause of myocardial structural and functional alterations. Indeed, this seems to induce myocardial microvascular endothelial inflammation and oxidative stress, which both contribute to reduced bioavailability of NO and decreased protein kinase G activity in cardiomyocytes. Resulting hypertrophy and stiffness in cardiomyocytes together with myocardial interstitial fibrosis played important roles in the development of diastolic dysfunction and HFpEF (7). Here, we found decreased expression of SOD1 and 2, which are first line antioxidant enzymes of defense against reactive oxygen species (ROS). Myocardial alteration in ROS elimination in HFpEF rats could probably contribute to myocardial oxidative stress. In HFpEF rats, we also showed decreased thoracic aorta release of NO, strongly suggesting endothelial dysfunction in these rats. However, the link between oxidative stress and endothelial dysfunction should be further investigated in next studies to understand better the pathogenesis of diastolic dysfunction in metabolic syndrome-associated HFpEF.

Given the heterogeneity in the HFpEF syndrome, any animal model of HFpEF only resembles a certain proportion of the patients. In the present study, we chose to develop an experimental model of HFpEF associated to obesity. Previous studies have already described obesity-based experimental models of HFpEF in rodents (26, 27, 57), but most of them presented discrepancies compared to the human phenotype. Preclinical obesity models usually present with rapid uncontrolled hyperglycemia and insulin resistance secondary to type 2 diabetes (58, 59), which does not mimic the clinical situation of HFpEF (60, 61). In our experimental model of HFpEF associated to obesity, we found glucose intolerance (but no type 2 diabetes) with low fasting glucose levels, which was close to the human condition. Additionally, obesity was mostly experimentally induced with specific genetic manipulations targeting the leptin signaling (58, 59, 62, 63). Because they do no exhibit similar abnormalities as do obese HFpEF patients, these experimental models are less pathophysiologically and clinically relevant. The originality of the present experimental model is that obesity naturally evolved with increasing age to HFpEF, mimicking the natural clinical overtime evolution of HFpEF in obese patients.

The present study validated a high-fat diet-induced rat model of early HFpEF developing pulmonary hypertension, which may offer a new avenue for testing potential mechanisms and therapeutic interventions, such treatment with the promising sodium-glucose co-transporter 2 (SGLT2) inhibitors that have shown recently beneficial effects in patients with HFpEF (64).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: www.ncbi.nlm.nih.gov/GSE189190.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the Faculty of Medicine of the Université Libre de Bruxelles (Brussels, Belgium; protocol acceptation number: 656N).

AUTHOR CONTRIBUTIONS

GH, LD, and KME conceived and designed study. GH, AH, AA, EH, PJ, GV, CW, and KME performed research. GH, LC, HL, PJ, CV, CD, and KME analyzed data. GH, LC, CD, J-LV, KME, and LD contributed new methods or models. GH and LD wrote the paper. All authors have given approval to 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.2021.809885/full#supplementary-material

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Lack of Usefulness of Donor-Derived Cell-Free DNA as a Biomarker for Cardiac Allograft Vasculopathy: A Prospective Study

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Background: Cardiac allograft vasculopathy (CAV) remains a major cause of morbidity and mortality among long-term heart transplant recipients. There is an unmet need for a non-invasive biomarker of CAV that could obviate the need to perform surveillance coronary angiograms in these patients. Our aim was to evaluate the performance of Donor-derived Cell Free DNA (dd-cfDNA) as a biomarker of CAV.

Methods: We prospectively measured dd-cfDNA levels in all patients undergoing routine coronary angiography >1 year after heart transplant at a single center. Endpoints included the association between dd-cfDNA levels and the presence CAV, according to several prespecified criteria.

Results: We included 94 heart transplant recipients, a median of 10.9 years after transplant. Coronary angiogram revealed CAV_0, CAV_1, CAV_2, and CAV_3 in 61, 19, 14, and 6% of patients, respectively. Comparison of dd-cfDNA levels in patients with CAV_0 and CAV_{1-2-3} (primary end-point) did not show significant differences (0.92%, IQR 0.46–2.0 vs. 0.46%, IQR 0.075–1.5, $p = 0.059$), nor did the comparison between patients with stable CAV (no new coronary lesions since previous angiogram, $n = 77$) and progressive CAV ($n = 17$); dd-cfDNA values 0.735% (IQR 0.195–2.0) vs. 0.9% (IQR 0.12–1.8), $p = 0.76$. However, we found an association between NTproBNP levels and CAV degree ($p = 0.017$). Dd-cfDNA levels did not correlate with NTproBNP ($\rho = -0.095$).

Conclusion: In this study, dd-cfDNA did not perform as a useful biomarker to avoid surveillance coronary angiograms for CAV diagnosis.

Clinical Trial Notation: Potential Role of Donor-derived Cell Free DNA as a Biomarker in Cardiac Allograft Vasculopathy, NCT 04791852.

Keywords: donor-derived cell free DNA, cardiac allograft vasculopathy, coronariography, biomarker, NTproBNP, cardiac troponin

INTRODUCTION

Cardiac allograft vasculopathy (CAV) remains the leading cause of long-term graft failure and a major cause of late death among heart transplant recipients (1). In spite of all the advances in the past years, its incidence has remained stable, affecting 25–30% of patients at 5 years and almost 50% after 10 years of transplant (2). In 2010, the International Society for Heart and Lung Transplantation (ISHLT) published a standardized consensus that classified it into four categories according to coronary angiography findings and cardiac allograft function (**Supplementary Table 1**) (3).

Owing to graft denervation, angina symptoms are very infrequent, and patients typically present with progressive heart failure or ventricular arrhythmias late in the course of the disease. Due to the poor prognosis it implies, it is of paramount importance to diagnose it at early stages, and clinical practice guidelines recommend an annual or biannual coronariography after heart transplant (4). However, coronary angiograms are an invasive technique with associated risks, and can cause significant patient discomfort. There is clearly a unmet need for a non-invasive biomarker of this entity that could obviate the need to perform surveillance coronary angiograms. Donor-derived Cell Free DNA (dd-cfDNA) has shown a good ability to rule out cellular rejection in heart transplant recipients (5–7), but its performance as a biomarker for CAV has not yet been tested.

The main objective of the FreeDNA-CAV study (Potential Role of Donor-derived Cell Free DNA as a Biomarker in Cardiac Allograft Vasculopathy, NCT 04791852) was to determine the ability of dd-cfDNA to detect asymptomatic CAV in a prospective cohort of heart transplant patients. Our main hypothesis was that allograft ischemia resulting from angiographic CAV would result in release of dd-cfDNA into the circulation.

MATERIALS AND METHODS

Design

FreeDNA-CAV was a single center, observational, prospective, cross-sectional, investigator-driven study. We prospectively obtained dd-cfDNA levels in all consecutive asymptomatic patients who underwent surveillance coronary angiogram more than 1 year after an orthotopic heart transplant in our center between January 2019 and January 2021.

Main exclusion criteria were: age under 18 or over 80 years old, multiorgan transplant, estimated glomerular filtration rate <30 ml/min/m^2, history of acute cellular rejection (ACR) $\geq$ 1R or antibody mediated rejection (AMR) in the previous 6 months, clinical suspicion of CAV (determined by the presence of heart failure, ventricular arrhythmias or ECG changes suggestive of myocardial ischemia), concomitant infection by Cytomegalovirus (CMV) or evidence of sepsis, inflammatory disease or neoplastic disease.

According to study protocol, all patients underwent on the same day: coronary angiogram, echocardiogram, electrocardiogram, blood sample extraction for dd-cfDNA quantification (%) and lab tests that included NT-proBNP, cardiac troponin, renal function, CMV PCR and anti-HLA antibodies, both donor-specific and non-donor specific (Luminex® assay).

Echocardiogram was performed at the Imaging Unit of our center. Restrictive cardiac allograft physiology was defined as symptomatic heart failure with echocardiographic or hemodynamic suggestive findings according to ISHLT guidelines (3).

Concomitant acute rejection was ruled out by endomyocardial biopsy (EMB) in those patients who underwent surveillance angiography on the 12th month after heart transplant or after reduction of baseline immunosuppression (according to local protocol). On the rest of the cohort, acute rejection was assumed absent based on the lack of symptoms, normal echocardiogram and negative anti-HLA antibodies (Luminex® assay). A cut-off value of median fluorescent intensity (MFI) $<$ 3,000 was considered negative for this purpose.

Baseline immunosuppressive therapy in our center typically consists of a triple drug regimen, including Tacrolimus, Mycophenolate Mofetil (MMF) and low-dose prednisone. Steroid withdrawal is only performed in case of adverse events (approximately in 30% of patients) and is always monitored with periodic EMB. Statins are routinely prescribed in all heart transplant recipients, and anti-platelet agents are added in the presence of any degree of coronary disease.

The investigation conforms with the principles outlined in the Declaration of Helsinki. The study was approved by the local Institutional Review Board and all patients signed informed consent.

End-Points

Our primary endpoint was the association between dd-cfDNA levels and the presence of any degree of CAV (CAV_0 vs. $CAV_{1,\ 2,or\ 3}$), and to determine the discrimination ability of this biomarker in this situation using receiver-operator characteristics analysis.

Secondary end-points included association of dd-cfDNA with the different degrees of CAV (0, 1, 2, and 3), correlation of dd-cfDNA with NTproBNP and troponin I, and association of NTproBNP and troponin I with CAV presence and degree.

Two subgroup analysis were prespecified. First, patients were stratified by time since transplant into three groups: less than 5 years, 5–10 years and more than 10 years after heart transplant. Second, due to the insidious nature of CAV, a subgroup analysis according to the level of progression since the previous angiogram was performed. Patients with CAV_0, and those with absence of new coronary lesions since previous angiogram were considered stable CAV, and patients with new coronary stenoses were considered progressive CAV for this purpose.

Sample Processing and Quantification of %Donor-Derived Cell Free DNA

Test tubes for dd-cfDNA quantification were sent to Eurofins Megalab central laboratory in Madrid (Spain) *via* delivery courier on the same day of extraction. Once in there, two geneticists were in charge of registering the sample and checking that it met the requirements to be analyzed. Valid test tubes were then

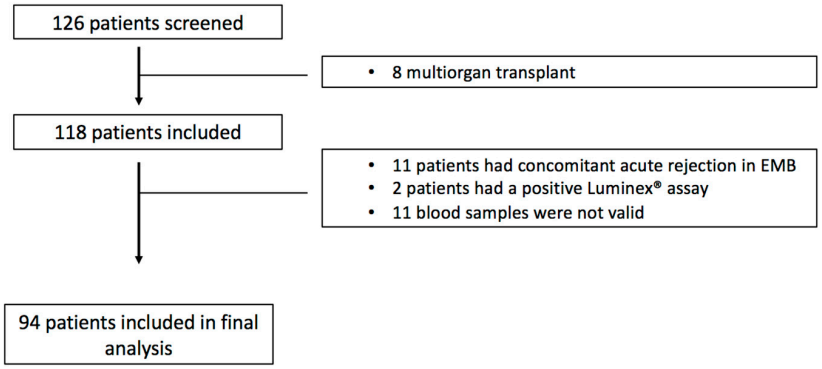

FIGURE 1 | Study flow-chart.

sent to Eurofins Genome laboratory in Rome (Italy), where the blood samples were processed and subsequently analyzed. All laboratory technicians and both genetists were blinded to the patient's identity and the angiogram results.

The percentage of dd-cfDNA was measured using Next Generation Sequencing (NGS) technology, which measures differential allele contributions in a panel of amplified single nucleotide polymorphisms (SNPs) to quantify dd-cfDNA in recipients, avoiding the need to genotype the donor (8).

A panel of more than 500 SNPs with high heterozygosity, low amplification error, low linkage, is selected for amplification and sequencing. Cell-free (cfDNA) is extracted from 1 ml plasma and is then amplified using Ampliseq protocol (Thermo-Fisher). Amplicons are sequenced using S5 NGS sequencer (Thermo-Fisher). An analysis pipeline incorporating a custom Next Generation Sequencing bioinformatics tools is used to align reads to the SNPs regions and determine the contribution of donor-derived sequences and calculate the percentage of dd-cfDNA. The sequencing depth is >1,000 unique reads per sample, with an average of 4,000 reads. Eurofins Megalab reports the fraction of donor-derived cfDNA as a percentage, with values over 0.7% being considered positive based on previous studies on acute rejection (9).

Coronary Angiograms

All coronary angiograms were performed at the cath lab in our institution by one experienced interventional cardiologist, who was blinded to the dd-cfDNA result. Coronary angiography results were classified according to ISHLT 2010 guidelines into four groups by the performing physician: CAV_0, CAV_1, CAV_2, and CAV_3 (3).

All studies were then reviewed by an independent interventional cardiologist, who acted as angiographic core lab and was blinded to the dd-cfDNA result and to the diagnosis made by the performing cardiologist. In case of disagreement with the original diagnosis, both interventional cardiologists reread the study, discussed the case and reached a consensus.

The angiographic core lab also reviewed previous angiograms and classified patients as stable CAV or progressive CAV, according to previously described criteria.

Statistical Analysis

Statistical analysis was performed using Stata/IC software v16.1. (StataCorp (10), *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC).

Categorical variables are presented as percentages and compared using the chi-square test or Fisher's exact test. Numerical variables are presented as median and IQR, and compared using Kruskal-Wallis and U Mann–Whitney tests.

To test the discrimination ability of dd-cfDNA in CAV, an area under the curve-receiving operating characteristic (AUC ROC) was estimated. We considered a good AUC ROC when it was above 0.7. The correlation between dd-cfDNA and other biomarkers (NTproBNP and Troponin I) was tested by means of the Pearson correlation coefficient.

RESULTS

From January 2019 to January 2021, a total of 126 heart transplant patients undergoing surveillance angiogram were screened for the study (**Figure 1**), and 94 patients were included in the final analysis.

Median age was 57 years (IQR 50–67), and 67% were men. Median time after heart transplant was 10.9 years (IQR 4.8–17.7). There were no statistically significant differences between patients with and without CAV regarding their baseline characteristics, except for NTproBNP levels and time after heart transplant, which were both significantly higher in patients with any degree of CAV. With respect to immunosuppressive treatment, patients with any degree of CAV were more likely to be on everolimus (14% vs. 40%, $p = 0.005$). **Table 1** summarizes baseline characteristics of the cohort.

Coronary angiogram revealed CAV_0 in 57 patients (61%), CAV_1 in 17 patients (19%), CAV_2 in 13 patients (14%) and CAV_3 in 7 patients (6%); thus, there was a total of 37 patients (39%) with any degree of CAV. Median dd-cfDNA values for each CAV group are shown in **Figure 2A**.

The dd-cfDNA levels did not differ significantly between patients with and without CAV, $p = 0.059$ (**Figure 2B**).

TABLE 1 | Baseline characteristics of the cohort.

	Total (*n* = 94)	CAV_0 (*n* = 57)	CAV_1, CAV_2 or CAV_3 (*n* = 37)	*P*-value
Male sex, n (%)	63(67%)	37(64.9%)	26(70.3%)	0.589
Median age (IQR)	57(50 – 67)	56(47 – 70)	57(50 – 65)	0.728
Hypertension, n (%)	69(73.4%)	42(73.7%)	27(73%)	0.939
Diabetes mellitus, n (%)	32(34.0%)	23(40.4%)	9(24.3%)	0.109
Dyslipidemia, n (%)	48(51.1%)	30(52.6%)	18(46.2%)	0.706
BMI ≥ 30, n (%)	18(19.6%)	11(19.3%)	7(20.0%)	0.934
Median Creatinine levels, mg/dl (IQR)	1.11(0.95 – 1.42)	1.11(0.98 – 1.37)	1.13(0.91 – 1.52)	0.999
Median Estimated GFR, ml/min/1.72 m2 (IQR)	67(47 – 82)	66.5(50 – 80)	67(46 – 82)	0.631
Median NTproBNP, pg/ml (IQR)	401(228 – 934)	354(184 – 567)	673(318.5 – 1, 616)	<0.01
Median Troponin I, μg/L (IQR)	0(0 – 0.02)	0(0 – 0.009)	0(0 – 0.02)	0.389
Echocardiogram				
Median LVEF,% (IQR)	60.5(56.8 – 65.2)	61(56.8 – 67.3)	60(56.3 – 62)	0.09
Median E/A (IQR)	1.85(1.6 – 2.25)	1.9(1.6 – 2.3)	1.8(1.5 – 2.1)	0.394
Median dd-cfDNA levels,% (IQR)	0.8(0.17 – 2)	0.92(0.46 – 2)	0.46(0.075 – 1.5)	0.059
Median time from heart transplant, years (IQR)	10.9(4.8 – 17.7)	9.8(4.1 – 13.6)	15.9(8.9 – 20.5)	0.004
Immunosuppressive treatment, n (%)				0.332
Calcineurin inhibitors	85(90.4)	54(94.8)	31(86.1)	0.148
MMF/Azatioprine	67(71.3)	44(77.2)	23(65.7)	0.230
Everolimus	22(23.4)	8(14.0)	14(40.0)	0.005
Prednisone	66(70.2)	42(77.8)	24(68.6)	0.005

IQR, interquartile range; BMI, body mass index; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; dd-cfDNA, donor-derived cell-free DNA; MMF, micophenolate mofetil.

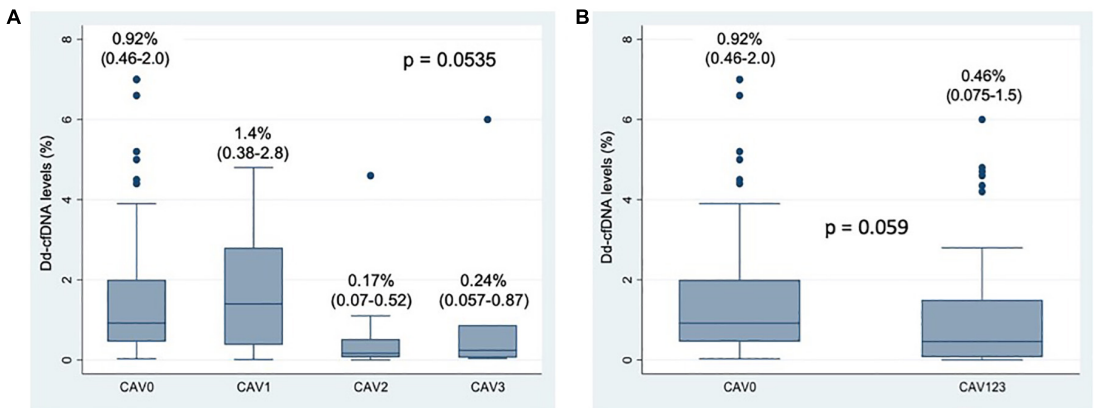

FIGURE 2 | (A) Box-plot of Donor-derived Cell Free DNA levels according to CAV degree. Numerical data represent median dd-cfDNA values and their interquartile range. **(B)** Box-plot of Donor-derived Cell Free DNA levels according to CAV presence (CAV0 vs. CAV 1, 2, or 3). Numerical data represent median dd-cfDNA values and their interquartile range.

The AUC ROC curve for the diagnosis of CAV confirmed once more the lack of ability to predict the presence of any degree of CAV (AUC ROC = 0.38) (**Figure 3**).

Subgroup Analysis

Time Since Transplant

There were no statistically significant differences between levels of dd-cfDNA in patients with or without CAV amongst the three prespecified subgroups: less than 5 years (p = 0.95), 5–10 years (p = 0.14) and more than 10 years after heart transplant (p = 0.16) (**Figure 4A**).

Cardiac Allograft Vasculopathy Progression

A total of 17 patients were classified as having progressive CAV (18%). No significant differences were found between patients with stable CAV (n = 77) and progressive CAV (n = 17), p = 0.76 (**Figure 4B**).

Performance of Other Biomarkers and Correlation With Donor-Derived Cell Free DNA

We found a significant association between NTproBNP levels and increasing degrees of CAV (p = 0.0169). Median levels for each

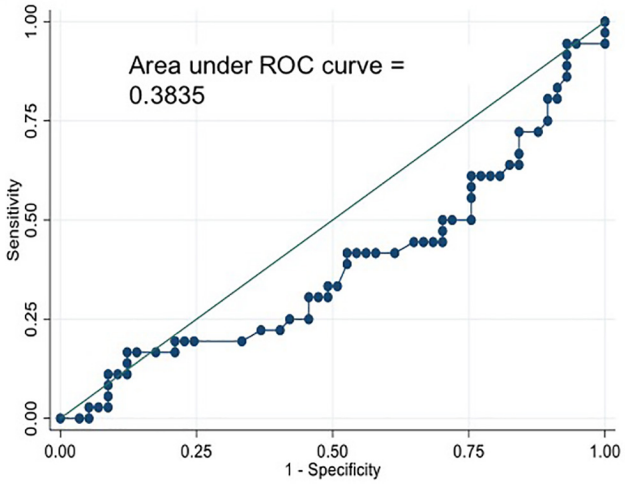

FIGURE 3 | Area Under the Curve Receiver Operating Characteristics (AUC ROC) curve for the diagnosis of CAV0 vs. CAV123.

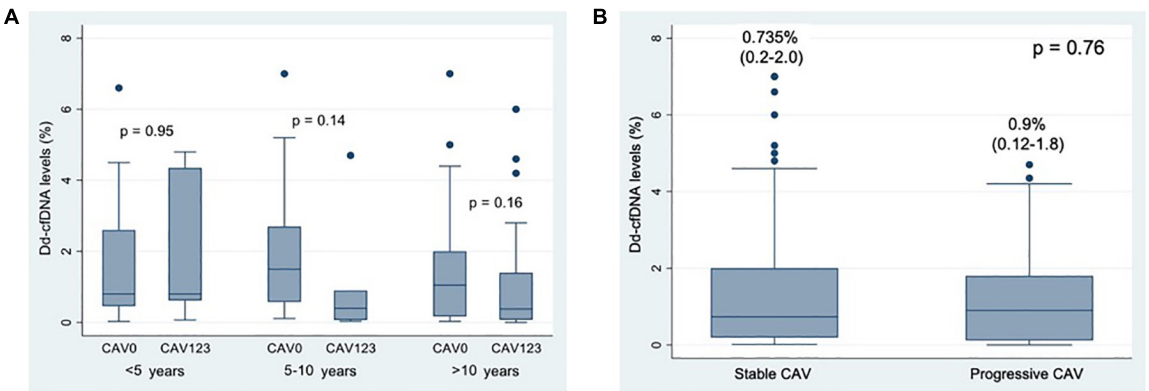

FIGURE 4 | (A) Box-plot of Donor-derived Cell Free DNA levels according to time after heart transplant. **(B)** Box-plot of Donor-derived Cell-Free DNA according to CAV progression. Numerical data represent median dd-cfDNA values and their interquartile range.

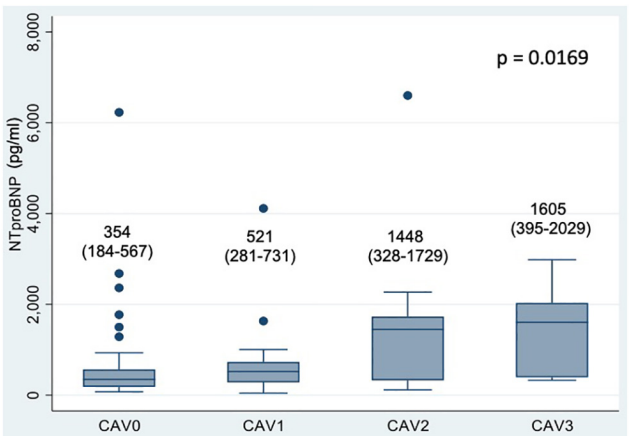

FIGURE 5 | Box-plot of NTproBNP levels according to CAV degree. Numerical data represent median NTproBNP values (pg/ml) and their interquartile range. An outlier value of NTproBNP 26 350 pg/ml in the CAV_0 group was excluded from the figure but included in the analysis.

CAV group are shown in **Figure 5**. There was also a significant difference between CAV_0 and CAV_{1-2-3} patients: 354 pg/ml (IQR 184–567) vs. 673 pg/ml (319–1,616), $p < 0.01$, with an AUC-ROC of 0.66 (**Supplementary Figure 1**). For an optimal cut-off point of 250 pg/ml, negative predictive value was 80%. There were no statistically significant differences between both groups in variables known to influence NT-proBNP levels, such as age, gender, obesity or renal insufficiency. No correlation was found between dd-cfDNA and NTproBNP levels ($\rho = -0.095$, $p = 0.38$).

A subset of 50 patients underwent Troponin I determination. We did not find an association between TnIc and the different degrees of CAV ($p = 0.86$), nor with dd-cfDNA levels ($\rho = -0.096$, $p = 0.51$).

DISCUSSION

To our knowledge, this is the first prospective study exploring the relation between dd-cfDNA and CAV. Even though we included a significant number of patients, and the incidence of CAV was similar to that described in previous registries (2), we could not find an association between the aforementioned biomarker and the presence or degree of CAV. Although, this could be interpreted as a "negative" study, we still think it is of great interest due to the unmet need of a non-invasive biomarker for this entity (11).

The presence of donor-specific DNA in plasma of solid organ transplant recipients was first described by Dennis Lo et al in 1998 (12). Since then, there has been an increasing interest in this technique, as the release of dd-cfDNA in the recipient's blood secondary to cell damage in the graft makes these molecules potential biomarkers of graft health (13). Most of the research so far has been focused on its ability to rule out acute cellular rejection. However, a recent review by K Kush points out the need to explore its potential use as a biomarker for CAV (14).

The data published to date in this field is rather scarce. Holzhauser et al found a borderline significantly higher proportion of patients with CAV (defined as Stanford III-IV or angiographic disease) in the subset of patients with dd-cfDNA above the median ($p = 0.047$) (15). Of note, DSA were present in 27% of the high dd-cfDNA group. This means that AMR, a condition known to increase dd-cfDNA levels, could not be ruled out, which could potentially explain the difference with our results. On the other hand, another study performed in 66 pediatric patients using a method that does not require genotyping the donor, found that ddcf-DNA levels were not significantly higher in samples associated with CAV (0.27% vs. 0.55%, $p = 0.057$) (16). These findings are in line with our results. The ongoing SHORE registry (NCT 03695601) will hopefully add more data to the question of a correlation between dd-cfDNA and CAV.

The evidence for dd-cfDNA as a biomarker of acute cellular rejection (ACR) in heart transplant recipients has already been established (5, 6).

Therefore, it seemed reasonable to speculate that allograft ischemia resulting from CAV would result in release of dd-cfDNA into the circulation. However, the pathophysiology of CAV differs from that of ACR. Some studies have implied immune-mediated pathways (chronic immune response, acute rejection) whereas others have involved non-inmunological factors, such as classical cardiovascular risk factors or CMV infection (17, 18).

This multifactorial etiology could potentially explain the results of our study. Even though CAV is associated with graft damage, this injury is more insidious and episodic than that of acute rejection. Moreover, CAV is clinically silent until late stages, averting the recognition of critical time-points in which necrosis occurs. Our hypothesis is that dd-cfDNA rises only during subclinical acute ischemic episodes, in the same way as cardiac troponin. Both are not usually elevated in patients with chronic ischemic cardiomyopathy. Unfortunately, the design of our study does not allow to draw inferences about the performance of the biomarker throughout the different stages of the disease.

Overall dd-cfDNA values in our study were higher than those reported in previous studies using slightly different techniques (5, 6). However, our median time after heart transplant was 10.9 years, and there is no evidence yet on the "normal" values of this biomarker at that stage post-transplant. In any case, due to the fact that all samples were tested in the same laboratory and with the same method, our conclusions should still be valid.

We chose to evaluate the performance of dd-cfDNA only in asymptomatic patients because it is in this subset of patients where the biomarker would be of most utility. We feel that, in symptomatic patients or with high suspicion of CAV, it would be advisable to perform an angiogram regardless of the levels of dd-cfDNA. However, this design choice (made on clinical grounds) might have reduced the effect size and, there, the power of our study to detect a significant association between dd-cfDNA and coronary allograft vasculopathy. Thus, this might partly explain the "negative" results of this study.

Neither of the subgroup analyses (by CAV severity and time since transplantation) revealed a meaningful relation with dd-cfDNA.

Correlation With Other Biomarkers

Our study showed a significant association between NTproBNP and the presence and severity of CAV. In spite of its wide availability, very few studies have focused on natriuretic peptides as biomarkers of CAV. In the study by Mehra et al., BNP was associated with the development of CAV (defined as coronary artery stenosis $\geq$ 40%), and cardiac deaths were significantly more prevalent in the subset of patients with BNP $\geq$ 250 pg/ml (19). The study by Arora et al confirmed the prognostic value of NTproBNP in heart transplant recipients, but only predicted CAV when combined with C-reactive protein (20).

Even though in our series we found a good correlation between NTproBNP levels and the degree of CAV, its ability to rule out the disease was suboptimal, with a AUC-ROC below 0.7. Sensitivity and specificity were only fair, and we could not find a negative predictive value cut-off of use in clinical practice.

On the other hand, we measured cardiac Troponin I in a subset of patients, but no relationship between this biomarker and CAV could be found. This supports our hypothesis that

patients with angiographic CAV do not seem to have active myocardial necrosis during long periods of time.

Evidence regarding cardiac Troponin I as CAV biomarker is rather scarce. Labarrere et al found that patients with persistently elevated levels of this biomarker during the 1st year post-transplant had a significant higher risk for subsequent development of CAV (OR 4.3, $p < 0.001$) (21). However, there is no solid evidence relating Troponin I and coronary angiogram findings during late follow-up.

Limitations

This study has several limitations that must be taken into account. First of all, it is a cross-sectional study, so the kinetics of the biomarker during the development of CAV could not be studied. However, our main goal was to test the ability of dd-cfDNA as a substitute for surveillance coronary angiograms in asymptomatic patients, and in this sense our study appropriately addresses this question.

Secondly, we did not perform routine intravascular ultrasound (IVUS) in our study, limiting our ability to relate the biomarker with earlier stages of the disease. Correlation with IVUS would have required studies during the first post-heart transplant year, in which intimal changes with prognostic significance occur. Nonetheless, coronary angiograms is the preferred method for CAV surveillance due to its wider availability and proven prognostic value.

Last, but not least, only a minority of patients underwent simultaneous endomyocardial biopsy. Nevertheless, the presence of acute cellular rejection in the absence of clinical suspicion in a cohort of asymptomatic patients a median of more than 10 years after heart transplant is exceptional, and the performance of routine endomyocardial biopsy in this setting is not usual in our clinical practice.

CONCLUSION

In this single center study, donor-derived cell-free DNA was not associated with the presence of CAV. The search for a biomarker with a high negative predictive value that could obviate the need to perform periodic surveillance angiograms is still open.

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 Comité Etico de Investigación con Medicamentos del Hospital Universitario Puerta de Hierro Majadahonda. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MJ-B and JS-C participated in the research design, the writing of the manuscript, and data analysis. LP-G participated in the performance of the research and data analysis. FH-P and MG-B participated in the research design and the performance of the research. CA-S, MT-S, JO-D, and SM-S participated in the performance of the research. 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.2022.856600/full#supplementary-material

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The "Right" Definition for Post–Left Ventricular Assist Device Right Heart Failure: The More We Learn, the Less We Know

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Right heart failure is a major cause of morbidity and mortality following left ventricular assist device implantation. Over the past few decades, the definition proposed by the Interagency Registry of Mechanical Circulatory Support and Society of Thoracic Surgeons has continually evolved to better identify this complex pathology. We propose that the latest definition proposed by the Mechanical Circulatory Support Academic Research Consortium in 2020 will increase our recognition and understanding of this complex disease phenomenon.

Keywords: right heart failure (RHF), left ventricular assist device (LVAD), Mechanical Circulatory Support, Academic Research Consortium, proper definition

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INTRODUCTION

Right heart failure (RHF) is a major cause of morbidity and mortality following left ventricular assist device (LVAD) implantation. It is estimated to occur between 9 and 42% of patients following LVAD implantation depending on the diagnostic criteria use (1). Additionally, prediction or prevention of RHF post-LVAD is challenging given the historical lack of an RHF universal definition, complicated by heterogenous derivation and validation methodologies predominantly driven by large single or multi-center studies (1).

After continuous-flow LVADs were first approved for destination and bridge to transplantation strategies, the fourth annual Interagency Registry of Mechanical Circulatory Support (INTERMACS) in 2012 concluded that RHF "represents a major challenge to the successful application of continuous-flow technology and constitutes a major thrust of future INTERMACS research" (2). This declaration continues to hold true in 2022 despite improved VAD technology with 2-year survival nearing that of heart transplantation (3, 4). In fact, the most recent INTERMACS annual reports omitted the RHF post-LVAD in their outcomes, noting varying definitions and lack of consistency making an analysis unreliable (4–7). Herein, we propose that the latest definition proposed by the Mechanical Circulatory Support Academic Research Consortium (MCS-ARC) to diagnose RHF-LVAD will help mitigate the ongoing challenges encountered by the heart failure community in this decade-long quest to find the right definition for RHF post-LVAD.

TABLE 1 | 2008 INTERMACS definition for right heart failure.

Symptoms and signs of persistent right ventricular dysfunction
Central venous pressure > 18 mmHg with a cardiac index < 2.0 L/min/m^2
Right Ventricular Assist Device Implantation OR use of inhaled nitric oxide or inotropic therapy for a duration of more than 1 week at any time after LVAD implantation
Absence of elevated left atrial or pulmonary capillary wedge pressure (>18 mmHg), tamponade, ventricular arrythmias or pneumothorax

TABLE 2 | 2014 INTERMACS definition for right heart failure.

Definition: Symptoms or findings of persistent right heart failure characterized by BOTH of the following:
1. Documentation of elevated central venous pressure by: - Direct measurement with right atrial pressure > 16 mmHg OR - Findings of significantly dilated inferior vena cava with absence of inspiratory variation by echocardiography OR - Clinical findings of elevated jugular distention at least halfway up the neck in an upright patient
2. Manifestations of elevated central venous pressure characterized by: - Clinical findings of peripheral edema (>2+ either new or unresolved) OR - Presence of ascites or palpable hepatomegaly on physical examination or diagnostic imaging OR - Laboratory evidence of worsening hepatic congestion (total bilirubin > 2.0 mg/dl) or renal dysfunction (creatinine > 2.0 mg/dl)
3. If the patient meets definition of both criteria above then a severity scale for right heart failure will be graded utilizing post implant inotropes, inhaled nitric oxide or intravenous vasodilators, need for right ventricular assist device and timing from surgery.

CLASSIFICATIONS AND EVOLUTION OF THE DEFINITION

The INTERMACS started as a partnership among the National Heart, Lung, and Blood Institute, hospitals, and industry in 2006 (8). The first adverse event definition for RHF by INTERMACS is shown (9) (**Table 1**). This definition did not accurately describe the specific objective criteria needed to identify the signs or symptoms suggestive of RHF nor incorporate the biomarker or laboratory assessment in their diagnosis criteria. In addition, a cutoff of central venous pressure (CVP) of 18 mmHg may be too non-specific and not capture the degrees of RHF post-LVAD. In fact, many studies published their outcomes of "RHF post-LVAD" not using this specific definition but rather a modified definition of the initial proposal by INTERMACS giving recognition to the varying degrees of disease presentations. Arigiriou et al. reported, using inotropes for more than 14 days or discharge from hospital to home, on inotropes with specific inotropic drug dose criteria to further define a more precise definition for RHF post-LVAD (9, 10). Kormos et al. also reported outcomes based on the timing of inotropic initiation and duration following LVAD implantation, acknowledging the role of varying mechanisms that may be causing early and late occurrences of RHF post-LVAD (11). Importantly, none of these studies utilized the sole definition proposed by INTERMACS. More precise and clinically applicable definitions were clearly needed.

In 2014, a refined definition to include the time frame from surgery and more specific diagnostic criteria were proposed by INTERMACS (**Table 2**). This definition incorporated documentation of a lower CVP of 16 mmHg by heart catheterization or elevated CVP by imaging or physical exam assessments. Furthermore, manifestations of elevated CVP were needed either through physical exam (e.g., edema), imaging (e.g., ascites), or through specific laboratory markers (e.g., elevated bilirubin or creatinine). This definition was more inclusive; however, the heart failure community continued to use varying definitions to define RVF post-LVAD. Parameters such as low mixed venous oxygen saturation levels, varying degrees of elevated CVP, CVP/wedge ratio, need for right-sided VAD, assessments of tricuspid regurgitation, tricuspid annular motion on echocardiography among other clinical variables were included to define RVF (1, 12). Despite an updated and more inclusive definition in 2014, the application for a sole definition of RVF remained heterogenous by the LVAD community—appropriately—given recognition of patients with clinical findings suggestive of RVF that may not be included in the 2014 definition.

In 2018, the INTERMACS was acquired by the Society of Thoracic Surgeons. In 2020, the MCS-ARC proposed a more expanded and inclusive definition of RHF post-LVAD (13), recognizing the varying degrees of phenotypical presentations. This definition is focused based on timing from LVAD implantation and acuity of up-escalation of mechanical or non-mechanical support (**Table 3**). An elevated CVP is not needed for the diagnosis, rather the pieces of evidence of RVF through a physical exam, imaging, broadened elevated biomarkers or hemodynamic parameters from right heart catheterization were now included. The significant limitations that existed in the prior definitions are now improved to become more sensitive for disease recognition.

DISCUSSION

Right heart failure (RHF) post–durable left ventricular assist device (LVAD) remains its Achilles heel, given its myriad of phenotypical presentations both in terms of timing post-implant and severity. The definition of RVF as an outcome has been traditionally heterogeneous in both time frames (acute, early, and late) and diagnostic criteria, with varied elements such as the INTERMACS definition, RVAD implantation, and prolonged inotrope/vasodilator dependence. Unfortunately, even contemporary landmark clinical trials (e.g., Momentum 3) that report RHF following LVAD have modified the INTERMACS 2014 definition to include RVAD implantation, need for inhaled nitric oxide, or inotropic therapy for >1 week to make it less subjective (3). More recently, Rame et al. used the 2014 INTERMACS definition to report an incidence of late RHF at 5% for mild and moderate RHFs and 0.2% for severe RHF in 2021 (14). The actual incidence of late RHF post-LVAD is likely much higher than that reported in this study given the more sensitive and inclusive definition of RVF reported by the MCS-ARC. To

TABLE 3 | 2020 Academic Research Consortium definition for right heart failure.

Early acute right heart failure	Early post-implant right heart failure	Late right heart failure
Need for implantation of right ventricular assist device at time of left ventricular assist device implantation	(A) Need for implantation of right ventricular assist device <30 days of left ventricular assist device implantation OR (*B) Failure to wean from inotropic support or inhaled nitric oxide within 14 days following LVAD implantation or having to initiate this support within 30 days of implant for a duration of at least 14 days OR (C) Death occurring in patients within 14 days of LVAD implant who have not received an RVAD but who remain on inotropes or vasopressors at the time of death and meet criteria for the diagnosis of Right Heart Failure on the basis of the above clinical findings * For Criteria B: At least two of the following must be present: - Ascites - Peripheral edema (>2+) - Elevated central venous pressure (>16 mmHg) - Elevated jugular venous pressure atleast half way up the neck in an upright patient OR At least one of the following must be present: - Renal failure with creatinine > 2 × baseline value - Liver injury with at least 2× upper limit normal in AST/ALT - Total bilirubin > 2.0 - SVO2 < 50% - Cardiac index < 2.2 liter/min/m^2 - Elevated lactate > 3.0 mmol/liter - Reduction in pump flow of >30% from previous baseline in absence of cardiac tamponade, tension pneumothorax or other mechanical causes.	(A) Need for implantation of right ventricular assist device >30 days of left ventricular assist device implantation OR (*B) Hospitalization that occurs >30 days post-implant and which requires intravenous diuretics or inotropic support for at least 72 h.

our knowledge, no study has reported outcomes of RVF post-LVAD utilizing the 2020 contemporary definition recommended by the MCS-ARC.

Albert Einstein famously said that *if he had 1 h to save the world, he would spend 55 min defining the problem and only 5 min finding the solution*. As an LVAD community, we need a contemporary, objective definition of RHF that is not dependent on documentation of subjective physical exam findings and limited laboratory results. The 2020 MCS-ARC definition of RHF, includes laboratory (lactate, SVO2, liver, and renal function), clinical (need for RVAD, inotropes or inhaled nitric oxide within 14 days, low pump flow), and hemodynamic parameters (low cardiac index) in addition to the physical exam to define the severity of RHF, is certainly a step in that direction. Or is it?

Is the 2020 MCS-ARC definition the final evolution or will this too become obsolete? More importantly, will it be utilized by the LVAD community or will we continue to modify definitions according to our traditional behaviors? Should we evolve the definition to further prognosticate the severity of RHF and include other hemodynamic variables such as pulmonary artery pulsatility index, impact of severe valvular heart disease, or response and/or resistance to diuretics? Only one thing is sure in our field: the more we learn, the less we know.

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.

AUTHOR CONTRIBUTIONS

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

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

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The Relationship Between the Utilization of Arterial Blood Gas Analysis and Rehospitalization in Heart Failure: A Retrospective Cohort Study

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Background: The most common presentation of decompensated HF is dyspnea, and arterial blood gas analysis is an excellent tool for the decision-making process for most dyspneic patients. However, data on the prognostic value of ABG in HF patients are limited. Herein, a retrospective cohort study was conducted to investigate whether the utilization of arterial blood gas analysis was independently associated with re-hospitalization in patients with heart failure.

Methods: As a retrospective cohort study, the relevant clinical data of hospitalized patients admitted to Zigong Fourth People's Hospital, Sichuan, China from December 2016 to June 2019 with a diagnosis of HF were analyzed. The re-hospitalization within 6 months and the use of intravenous diuretic, nitrates, inotropes, or vasopressors were compared between patients with and without arterial blood gas analysis. We used a multivariable logistic regression model, propensity score analysis, and an inverse probability-weighting model to ensure the robustness of our findings.

Results: We included 1,605 patients with heart failure. The overall re-hospitalization rate within 6 months was 38.2%; it was 34.8% and 41.8% for heart failure patients with or without arterial blood gas analysis, respectively. In the inverse probability-weighting model, the use of arterial blood gas analysis was associated with a 26% lower re-hospitalization rate within 6 months.

Conclusion: The performance of arterial blood gas analysis is associated with a 6-month rehospitalization rate benefit in a general population of heart failure patients. This association warrants further investigation.

Keywords: arterial blood gas analysis, heart failure, PSM, IPTW, rehospitalization

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INTRODUCTION

Many tests and procedures utilized in the treatment of heart failure patients are unverified clinically. When the results are extremely likely to affect patient therapy, an arterial blood gas (ABG) sample should ideally be collected. The need to evaluate the adequacy of patient ventilation, measure the response to therapeutic or diagnostic treatments, monitor the severity and course of a documented

disease process, and examine acid-base status are all common indications for an ABG analysis (1). Indications for ABG analysis should, according to the existing literature, be based on the patient's clinical assessment (2). Arterial puncture for ABG analysis, on the other hand, is an invasive operation that might result in artery occlusion, digital embolization, digital ischemia, sepsis, local infection, pseudoaneurysm, hematoma, hemorrhage, and skin necrosis (3).

Routine ABG analysis is not recommended in individuals with heart failure in clinical practice. When a precise measurement of O2 and CO2 partial pressure is required, an ABG analysis should be conducted (i.e., patients with respiratory distress). Patients with cardiogenic shock should have their lactate and pH levels checked. However, there is a lack of evidence on the prognostic significance of ABG in HF patients. The purpose of this study was to investigate if there was an association between ABG analysis and the rehospitalization rate of heart failure patients.

PARTICIPANTS AND METHODS

Study Cohort

The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement was followed in the reporting of this study (4). We retrospectively analyzed the relevant clinical data of hospitalized patients admitted to Zigong Fourth People's Hospital, Sichuan, China from December 2016 to June 2019 with a diagnosis of HF (4, 5). Heart failure was defined according to the European Society of Cardiology (ESC) criteria (2). The study aimed to investigate whether ABG analysis contributes to reductions in re-hospitalization rates and other clinically significant changes in the management of heart failure patients independently. The ethics committee of Zigong Fourth People's Hospital gave its approval to the study (Approval Number: 2020-010). Because of the study's retrospective nature, informed consent was not required. The study adheres to the Helsinki Declaration. The ABG group consisted of patients who had arterial blood gas taken at the first day of admission, whereas the non-ABG group consisted of the rest of the patients. The content of the analysis included pH, standard residual base, standard bicarbonate, partial pressure of carbon dioxide, total carbon dioxide, methemoglobin, haematocrit blood gas, reduced hemoglobin, potassium ion, chloride ion, sodium ion, glucose blood gas, lactate, measured residual base, measured bicarbonate, carboxyhemoglobin, body temperature blood gas, oxygen saturation, partial oxygen pressure, oxyhemoglobin, anion gap, free calcium, and total hemoglobin.

Primary Outcome and Secondary Outcomes

The primary outcome was rehospitalization within 6 months. Rehospitalization was defined as the first rehospitalization within 6-month time period after discharge from the index hospitalization. Unfortunately, the reason of rehospitalization was not clarifed in this public database. The secondary outcomes included offering intravenous diuretic, nitrates, inotropes, or vasopressors therapy.

Statistical Analysis

All of the participants were subjected to descriptive analysis. Numbers and percentages were used to represent categorical variables. For normal distributions, continuous variables were reported as mean and standard deviation (SD), and for skewed distributions, median and interquartile range. For categorical, regularly distributed, and non-normally distributed continuous variables, we employed the chi-square test, one-way ANOVA, and Kruskal-Wallis tests, respectively.

Propensity score matching (PSM) (6) was used to minimize the effect of confounding variables like disease severity, which may lead to outcome bias. The propensity score was calculated using a multivariate logistic regression model and was based on the likelihood of a patient receiving blood gas analysis. With a caliper width of 0.02 and a one-to-one closest neighbor matching algorithm, The following variables were used to generate the propensity score: demographic characteristics, body Mass Index (BMI), pulse, mean arterial pressure (MAP), respiratory rate, cardiac function classification, medical history (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic obstructive pulmonary disease, diabetes, chronic kidney disease, acute renal failure), Charlson comorbidity index (CCI) score, white blood cell count (WBC), red cell distribution width (RDW), platelet count, albumin, sodium, potassium, chloride, blood urea nitrogen (BUN, creatinine, estimated glomerular filtration rate (eGFR), brain natriuretic peptide (BNP), troponin and creatine kinase. The degree of PSM was measured using a standardized mean difference (SMD). A value of <0.1 was deemed acceptable. Finally, 556 matched pairs were generated and used in further studies. An inverse probability of treatment weighting (IPTW) model was utilized to produce a weighted cohort using the calculated propensity scores as weights. The propensity score was then adjusted using univariable logistic regression.

All the analyses were performed with the statistical software packages R (http://www.R-project.org, The R Foundation) and Free Statistics software versions 1.3. A threshold of $p < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Population and Baseline Characteristics

We included 1,605 patients in our study cohort (**Figure 1**). Blood gas was analyzed for 51.3% of patients during their admission. The characteristics of the original cohort are summarized in **Table 1**. In the original cohort, a larger percentage of the ABG patients were male (45.4%), at an advanced age (41.3%), in NYHA cardiac function classification IV (35.6%) or Killip grade $\geq$II(79.7%) and had higher Pulse (86.91 $\pm$ 21.49), Respiration rate (19.30 $\pm$ 1.70), White blood cell ($7.71 \pm 3.77 \times 10^9/L$), Troponin (0.43 $\pm$ 2.72 pg/ml), BNP (1,392.51 $\pm$ 1,462.23 pg/ml), CCI score, consisted of more Peripheral vascular disease (6.4%), Chronic obstructive pulmonary disease (COPD) (16.3%), Diabetes (26.5%) and Chronic kidney disease (25.3%) than no ABG group. The opposite patterns were observed in Albumin and Sodium.

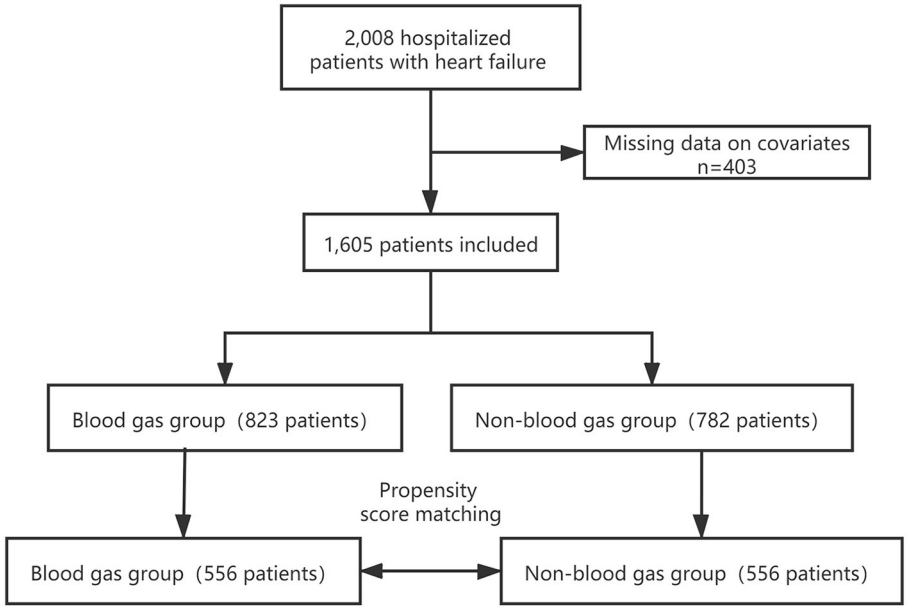

FIGURE 1 | Flow chart of the screening and enrollment of study participants.

Primary Outcome

The 30 variables were initially used to create a propensity score model. **Figure 2** illustrates the contributions of individual factors to the final propensity score. CCI score, Killip grade, history of COPD, respiration rate, and NYHA cardiac function classification are among the top variables: unsurprisingly, these covariates influence clinicians' judgments on whether or not to perform ABG analysis. IPW was used to normalize the differences between the ABG and no ABG cohorts based on the estimated propensity scores. Most of the weighted cohort variables were comparable or "balanced" across the groups with and without ABG analysis, as indicated in **Table 1**. The exceptions were respiration rate, history of COPD, and CCI score. A regression model was developed to adjust for these unbalanced covariates on the weighted cohort.

The rehospitalization rates were 41.8% (327/782) and 34.8% (286/ 823) for the no ABG group and ABG group in the original cohort, respectively. IPW demonstrated a significantly lower rehospitalization rate in the ABG group. The OR was 0.74 (95% CI, 0.61–0.91, $P = 0.004$). The propensity score-matched rehospitalization rates for the no ABG group and ABG group were 41.4 and 34.9%, respectively. In multivariable logistics regression, the OR was 0.76 (95% CI, 0.60–0.96, $P = 0.026$) (**Figure 3**).

Secondary Outcome Studies With Propensity Score Matching

We evaluated several secondary outcomes to investigate what factors could have contributed to the ABG analysis's advantages following PSM. There were several significant changes in secondary outcomes. In the beginning, ABG patients were considerably more likely to utilize intravenous diuretics (89 vs. 82.7%, $p = 0.003$). Second, ABG patients were significantly more likely to take intravenous nitrates (12.1 vs. 7%, $p = 0.006$). Finally, there was no significant difference in the use of intravenous inotropes or vasopressors in the two groups.

DISCUSSION

Patients with heart failure who are admitted to the hospital have a poor prognosis. Precise risk stratification might aid in the development of the best treatment options for these patients. Cardiogenic pulmonary edema causes pulmonary edema with impaired gas exchange (hypoxemia, hypercapnia), and insufficient cardiac output causes decreased tissue perfusion, leading to metabolic acidosis in HF patients with cardiogenic pulmonary edema. Thus, in high-risk HF patients, arterial blood gas analysis may be utilized as a comprehensive marker for backward and forward failure, and acid-base balance can be used to determine the general condition of heart function (7). However, there were conflicting opinions on the use of arterial blood gas analysis. According to Blum FE et al., a large reduction in the number of ABGs acquired in the intensive care unit has no detrimental influence on patient outcome or safety, and a reduction in the number of ABGs per patient allows for cost-effective patient care with a decreased risk of complications (1).

Dyspnea is the most prevalent symptom of decompensated HF, and ABG analysis is an excellent tool for most dyspneic patients' decision-making. Their risk classification might be based on the acid-base balance indicated by ABG. However, evidence is scarce on the prognostic significance of ABG in HF patients. In 588 AHF patients (prevalence of acidosis 8.5%), Minana et al. demonstrated that arterial PO_2, PCO_2, and pH upon admission were not associated with all-cause long-term

TABLE 1 | Baseline characteristics of participants.

Covariate	Original cohort			Matched cohort		
	No blood gas	Blood gas	SMD	No blood gas	Blood gas	SMD
n	782	823		556	556	
Age ≥ 80 years old (%)	264 (33.8)	340 (41.3)	0.156	205 (36.9)	204 (36.7)	0.004
Female (%)	479 (61.3)	449 (54.6)	0.136	326 (58.6)	321 (57.7)	0.018
BMI, kg/m^2 [mean (sd)]	22.04 (16.27)	21.42 (3.93)	0.053	21.38 (6.14)	21.49 (3.93)	0.021
MAP, mmHg [mean (sd)]	94.52 (15.58)	95.80 (16.00)	0.081	95.54 (15.82)	95.65 (16.10)	0.007
Pulse, /min [mean (sd)]	82.95 (20.74)	86.91 (21.49)	0.187	84.23 (20.65)	85.18 (21.14)	0.045
Respiration, /min [mean (sd)]	18.86 (1.63)	19.30 (1.70)	0.263	18.90 (1.43)	19.07 (1.36)	0.117
NYHA cardiac function classification (%)			0.252			0.093
II	148 (18.9)	128 (15.6)		91 (16.4)	102 (18.3)	
III	445 (56.9)	402 (48.8)		302 (54.3)	313 (56.3)	
IV	189 (24.2)	293 (35.6)		163 (29.3)	141 (25.4)	
Killip grade (%)			0.311			0.031
I	255 (32.6)	167 (20.3)		144 (25.9)	139 (25.0)	
II	386 (49.4)	435 (52.9)		295 (53.1)	294 (52.9)	
III	125 (16.0)	197 (23.9)		103 (18.5)	109 (19.6)	
IV	16 (2.0)	24 (2.9)		14 (2.5)	14 (2.5)	
Myocardial infarction (%)	46 (5.9)	68 (8.3)	0.093	37 (6.7)	40 (7.2)	0.021
Congestive heart failure (%)	720 (92.1)	764 (92.8)	0.029	516 (92.8)	501 (90.1)	0.097
Peripheral vascular disease (%)	30 (3.8)	53 (6.4)	0.118	28 (5.0)	28 (5.0)	<0.001
Cerebrovascular disease (%)	52 (6.6)	63 (7.7)	0.039	34 (6.1)	38 (6.8)	0.029
Chronic obstructive pulmonary disease (%)	57 (7.3)	134 (16.3)	0.282	52 (9.4)	35 (6.3)	0.114
Diabetes (%)	153 (19.6)	218 (26.5)	0.165	130 (23.4)	120 (21.6)	0.043
Chronic kidney disease (%)	163 (20.8)	208 (25.3)	0.105	125 (22.5)	123 (22.1)	0.009
Acute renal failure (%)	3 (0.4)	3 (0.4)	0.003	0 (0.0)	2 (0.4)	0.085
CCI score (%)			0.357			0.102
0	27 (3.5)	23 (2.8)		16 (2.9)	23 (4.1)	
1	347 (44.4)	270 (32.8)		218 (39.2)	231 (41.5)	
2	276 (35.3)	282 (34.3)		205 (36.9)	190 (34.2)	
3	111 (14.2)	185 (22.5)		97 (17.4)	90 (16.2)	
4	17 (2.2)	55 (6.7)		17 (3.1)	20 (3.6)	
5	4 (0.5)	7 (0.9)		3 (0.5)	2 (0.4)	
6	0 (0.0)	1 (0.1)		0 (0.0)	0 (0.0)	
White blood cell, $\times 10^9/L$ [mean (sd)]	7.04 (3.23)	7.71 (3.77)	0.189	7.23 (3.38)	7.26 (3.28)	0.011
RDW, % [mean (sd)]	14.91 (2.02)	14.82 (1.90)	0.047	14.85 (1.94)	14.85 (1.94)	<0.001
Platelet, $\times 10^9/L$ [mean (sd)]	142.61 (63.50)	146.44 (64.00)	0.06	143.42 (62.75)	146.12 (64.09)	0.042
Albumin, g/L [mean (sd)]	37.05 (4.95)	36.25 (4.86)	0.162	36.66 (4.83)	36.58 (4.91)	0.016
Sodium, mmol/L [mean (sd)]	138.60 (4.38)	138.07 (5.23)	0.109	138.58 (4.45)	138.42 (4.97)	0.034
Potassium, mmol/L [mean (sd)]	3.97 (0.65)	3.99 (0.74)	0.022	3.94 (0.62)	3.97 (0.74)	0.032
Chloride, mmol/L [mean (sd)]	102.17 (5.46)	101.76 (6.41)	0.07	102.34 (5.44)	102.10 (6.07)	0.042
BUN, mmol/L [mean (sd)]	9.54 (5.26)	9.67 (5.77)	0.024	9.41 (5.10)	9.38 (5.75)	0.004
Creatinine, μmol/L [mean (sd)]	110.50 (82.99)	108.80 (75.74)	0.021	105.70 (74.17)	108.15 (78.68)	0.032
eGFR, mL/min/1.73 m2 [mean (sd)]	67.28 (35.11)	69.53 (37.73)	0.061	69.67 (36.22)	69.83 (35.92)	0.004
BNP, pg/ml [mean (sd)]	1,189.64 (1,234.07)	1,392.51 (14,62.23)	0.15	1,259.75 (1,289.32)	1,318.20 (1,420.01)	0.043
Troponin, pg/ml [mean (sd)]	0.19 (1.12)	0.43 (2.72)	0.112	0.22 (1.31)	0.22 (1.50)	0.002
Creatine kinase, IU/L [mean (sd)]	122.23 (137.60)	148.11 (365.72)	0.094	105.70 (74.17)	108.15 (78.68)	0.032

BMI, body Mass Index; MAP, mean arterial pressure; NYHA, New York heart association; CCI, Charlson Comorbidity Index; BUN, blood urea nitrogen; Egfr, estimated glomerular filtration rate; BNP, brain natriuretic peptide.

mortality, discouraging the use of ABG for prognosis evaluation (8), whereas Alkalosis was associated with increased mortality in 621 AHF patients hospitalized to critical care units, according to Shirakabe et al. (9). Kato et al. demonstrated that the lower the PaCO2 level at admission, the greater the risk of long-term all-cause mortality in AHF patients (10). Meanwhile, according

to a study by Park et al. pH has additional predictive value and can be utilized to improve risk classification and management in high-risk AHF patients (7). These findings highlighted the clinical importance of ABG analysis at admission in AHF patients. The significant variances in the study populations may be the cause of the discrepancy. In this propensity score-matched cohort trial, ABG analysis recipients had a lower risk-adjusted rehospitalization rate within 6 months than patients who did not receive ABG analysis. This relationship was shown to be reliable in additional models.

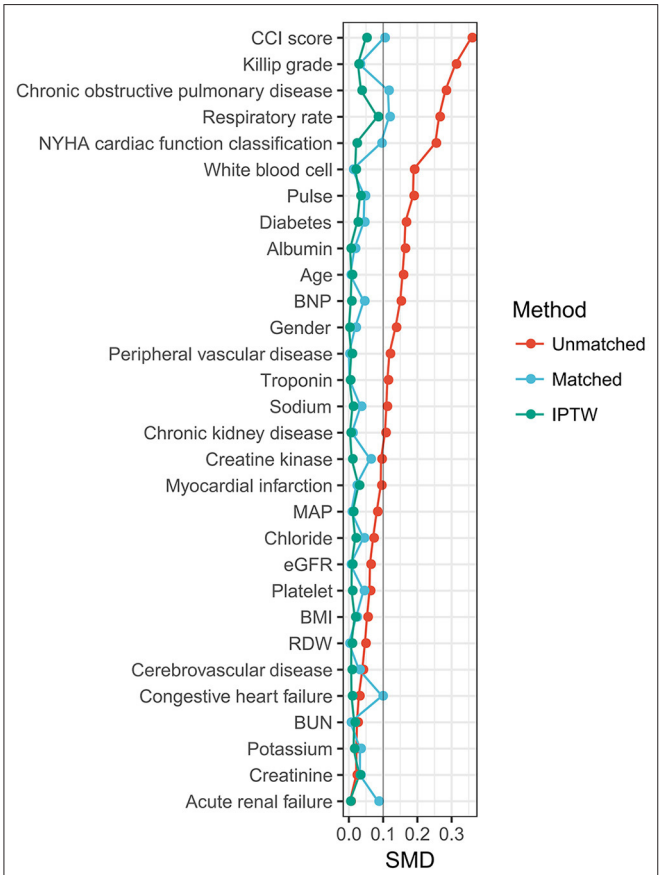

FIGURE 2 | Relative influence factor of covariates. The relative influence factor measures how discriminative the 30 covariates of the propensity score model are when predicting the likelihood of ABG performance.

Patients with ABG had a higher severity of illness score, more concomitant illnesses, and a higher BNP level in our study. Despite these indicators indicating a worse group of patients, we observed that after adjusting for confounding, patients who received ABG analysis had a substantially decreased rehospitalization rate after 6 months. There was an obvious selection bias in the study population since the criteria for ABG were not previously specified, and more "critical-looking" individuals were more likely to undergo ABG analysis.

Clinicians may choose to perform ABG tests early in the stay for patients with heart failure, considering the factors shown in **Figure 2**. We evaluated various factors between patients with and without ABG and tested several different hypotheses to explain the rehospitalization rate benefit. Intravenous diuretics and nitrates were used more often in the group who received ABG analysis. But it is not certain whether the ABG triggered the use of intravenous diuretics and nitrates or if it had already been in place. Whether the rehospitalization rate improvements are entirely due to the differences in intravenous diuretics and nitrates use is impossible to assess given the sample size. Our findings suggest that ABG may offer physicians information that might help them in the management of heart failure patients. The initial ABG may be used to identify individuals who are at the highest risk and require more invasive and intense therapy. We understand that observational database studies like this need meticulous, diverse, and rigorous statistical methodologies to deliver meaningful, trustworthy, and actionable findings. We believe that we have done so for the subject at hand, and plan to pursue further such analyses in the future to reduce the ambiguity of clinical decision-making in a complicated and complex context.

The findings should not be interpreted as the final and conclusive word on the value of ABG in the management of heart failure. The potential issues of residual confounding by variables not captured in the electronic health record, as well as the generalizability of the findings to other institutions,

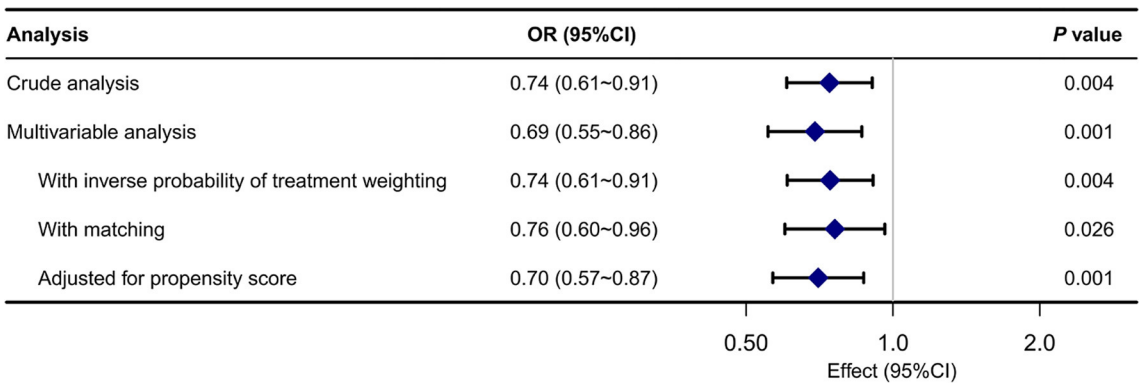

FIGURE 3 | Associations between ABG analysis and the outcome in the crude analysis, multivariable analysis, and propensity-score analyses.

require further investigation as an observational single-center study retrospectively performed on electronic health record data. Because practice patterns may have changed over the study period, the outcomes were not adjusted for the year of admission, which is a limitation of the analysis. For several analyses, prospective randomized trials will be required for confirmation.

CONCLUSION

In a broad population of heart failure patients, ABG analysis performance is associated with a 6-month rehospitalization rate benefit. The cause of this advantage is unknown; however, it might be related to the increasing use of intravenous diuretics and nitrates as suggested by ABG values. This relationship needs further investigation.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://doi.org/10.13026/8a9e-w734.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Zigong Fourth People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XZ: methodology and writing. YS and HZ: data curation and validation. HL and XJ: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Acute Cellular Rejection in Heart Transplant Patients: Insights of Global Longitudinal Strain, Myocardial Work, and an Exclusive Group of Chagas Disease

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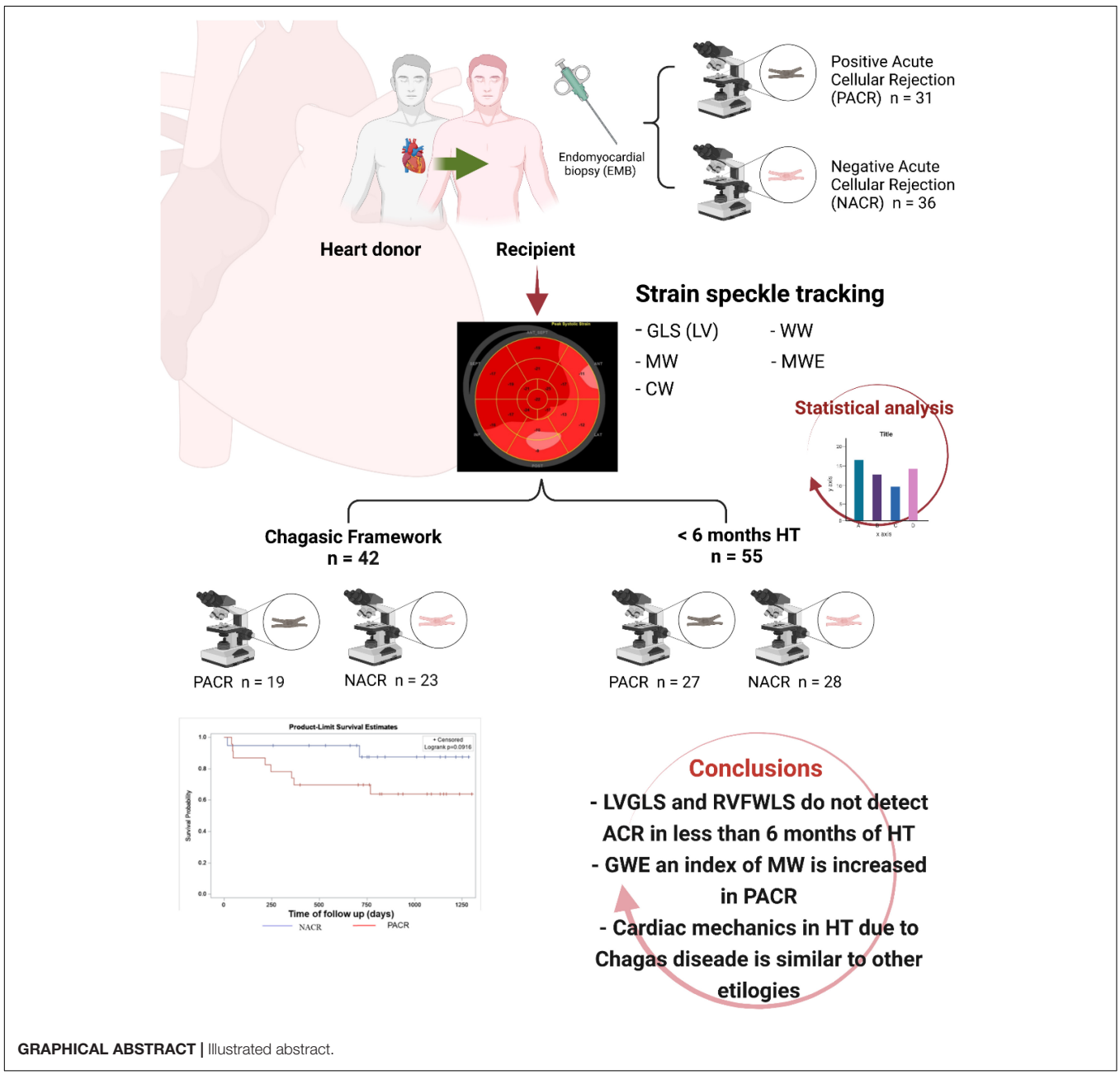
Background: Echocardiographic markers associated with asymptomatic acute cellular rejection (ACR) in patients with orthotopic heart transplant (HT) are still under investigation. The aim of our study was to determine clinical and myocardial strain imaging (MSI) variables evaluated by echocardiography associated with ACR in the first year of HT. A separate analysis was performed to compare variables during the first 6 months of HT, when ACR has a prevalence in 60% of patients. Another analysis evaluated an exclusive population with Chagas disease as the cause of HT.

Methods: We prospectively studied 67 patients with less than 1 year of HT, 36 patients without ACR (41% men, age 49 ± 12 years, 52% Chagas disease as the cause of heart failure), and 31 patients with ACR (59% men, age 55 ± 8 years, 74% Chagas disease as the cause of heart failure). Conventional echocardiographic measurements and MSI by global longitudinal strain (GLS) from the left ventricle (LV) and right ventricle free wall (RV-FWLS) and myocardial work (MW) from the left ventricle were obtained by experienced echocardiologists. Clinical variables, such as the presence of diabetes, hypertension, and immunosuppressant drugs, were compared between groups.

Results: HT patients with ACR were older and used more cyclosporine for immunosuppression. The positive ACR group had an increased relative wall thickness and LV mass index and similar LVGLS and RV-FWLS compared to the negative ACR group. Nevertheless, MW analysis observed increased global work efficiency (GWE) in positive ACR. Multivariate analysis identified older age, cyclosporine use, LV mass index, and GWE as independent predictors for detecting rejection. A separate analysis was performed for patients with less than 6 months of HT. Similar MSI was observed in both groups, with a trend for increased GWE in patients with ACR and significantly increased LV mass index in the ACR group. An exclusive group of Chagas patients as the primary cause of HT was analyzed, and similar MSI results for LVGLS, RV-FWLS, and MW were observed for both ACR and the no rejection groups. Additionally, the survival rates at 2 years were similar between the Chagas disease groups.

Conclusion: LVGLS and RV-FWLS were similar between patients with or without ACR in the first year after HT. Conversely, GWE, a derivative of LVGLS, and LV mass index were increased in positive ACR and could be markers for rejection. Increased LV mass index was also found in a subgroup analysis of patients less than 6 months after HT; however, MSI was similar regardless of ACR. For chagasic patients, rejection in the first year did not increase mortality at the 2-year follow-up, and MSI parameters were similar between patients with or without ACR. In a multivariate analysis to predict ACR, the independent parameters in this study were older age, cyclosporine use, LV mass index, and GWE.

Keywords: heart transplantation, echocardiography, rejection, strain imaging, endomyocardial biopsy



GRAPHICAL ABSTRACT | Illustrated abstract.

INTRODUCTION

Heart transplant (HT) is the gold standard treatment for end-stage heart failure. Important improvements in patient selection and perioperative management have mitigated frequent postoperative complications, and early survival has improved dramatically. Nevertheless, acute cellular rejection (ACR) is a major problem in the early period after HT, and approximately 25–32% of patients experience some graft rejection in the first year (60% within the first 6 months) (1, 2). This is a major cause of death among patients with HT, particularly chagasic recipients in developing countries, occurring in 10–14% of all patients with ACR, despite efforts to develop new immunosuppressive protocols (1).

Most patients with ACR are asymptomatic or have non-specific symptoms, some degree of neurohormonal activation occurs (3), and endomyocardial biopsy (EMB) continues to be the best method for diagnosis (4). However, EMB is an invasive method with significant complications, including perforation, pneumothorax, cardiac tamponade, arrhythmias, and damage to the tricuspid valve (1). The pursuit of non-invasive alternatives to ACR has been a goal in the first year of HT (1, 5).

Asymptomatic ACR is not usually related to a reduction in left ventricular ejection fraction (LVEF). Nevertheless, new technologies in echocardiography by tissue Doppler analysis and global longitudinal strain (GLS) by speckle tracking are important to detect early myocardial injury despite normal LVEF (5).

A recent study by Mingo-Santos et al. (6) suggested that global LV and RV free wall longitudinal strain (FWLS) could help rule out significant ACR during the first year after HT. Other studies found similar results for GLS (7–9). Combined with biomarkers, Cruz et al. (7) reported that patients with ACR had significantly lower values of LVGLS, RV-FWLS, and LV-Twist and higher levels of troponin I than patients without significant ACR.

In contrast, Ambardekar et al. (10) found no changes in myocardial strain and strain rate as assessed by 2D-STE on serial studies from patients with asymptomatic biopsy-proven rejection in the first year after HT, agreeing with the findings of Tseng et al. (9).

Therefore, as a consequence of these conflicting findings regarding GLS in HT, echocardiographic markers for ACR are still under investigation. Furthermore, myocardial work (MW), an index derived from strain/pressure curves, has never been reported in HT (11).

Interestingly, despite HT, patients with Chagas disease still have other aspects of the disease, such as impairment of the autonomic nervous system (12), which could alter the neurohormonal response generated by ACR and Chagas reactivation as a differential diagnosis for ACR (1).

The aim of our study was to determine clinical and myocardial strain imaging (MSI) as evaluated by echocardiography associated with ACR in the first year of HT. We show an important methodological framework for the analysis of echocardiography in the first 6 months within an exclusive Chagas disease population as the cause of HT.

PATIENTS AND METHODS

Patients

From January 2017 to December 2019, we prospectively included adult patients with less than 1 year of orthotopic HT at the Cardiology and Transplant Heart Institute, Brasília, Federal District, Brazil, in our study. Surveillance EMB was performed followed by a transthoracic echocardiogram on the same day, less than 4 h apart.

Patients were divided into two groups according to EMB results based on the 2004 International Society for Heart and Lung Transplantation (ISHLT) grading system (4): (1) without ACR (grades 0 and 1) and with ACR (grades 2 and 3). The HT group of rejection was compared to HT without rejection in terms of clinical and echocardiographic parameters.

Ethics Statement

This study was approved by the Ethical Committee of Cardiology and Transplant Heart Institute of Federal District, Brasília, Brazil, and the inscription number in Plataforma Brazil as a Certificate of Presentation of Ethical Appreciation is 65910517.0.0000.0026. All patients provided written informed consent to participate in this study.

Inclusion Criteria

We included patients with less than 1 year of orthotopic HT who came to our institution to undergo surveillance EMB and who were asymptomatic and hemodynamically stable. EMB and echocardiogram were performed on the same day. The protocol in our institution is to perform the echocardiogram less than 4 h after the EMB to diagnose any complications associated with the procedure (13) and before the discharge of the outpatients who only come for biopsy and follow-up.

Exclusion Criteria

Patients with an ejection fraction below 53%, Chagas disease reactivation, humoral rejection (4), irregular cardiac rhythm, inconclusive EMB (1), and a limited echocardiographic acoustic window were excluded.

Data Collection

Patients were enrolled at one routine follow-up EMB, only once within the first year of an orthotopic HT. In total, 71 patients were included, but 4 were excluded according to the exclusion criteria: 1 for poor acoustic window, 1 for inconclusive EMB (not enough material), 1 for Chagas reactivation, and 1 for ejection fraction <53%; 67 patients remained available for the study (**Figure 1**).

An endomyocardial biopsy was performed invasively through the femoral vein under fluoroscopy. Usually, a minimum of 3–5 segments were collected, aiming at the interventricular septal region, accessed by the right ventricle. The samples were analyzed by optical microscopy after hematoxylin and eosin (H&E) staining (1, 4). One pathologist blinded to the echocardiogram results analyzed all biopsies and classified cellular rejection according to the ISHLT grading system (4). Grades 0 and 1

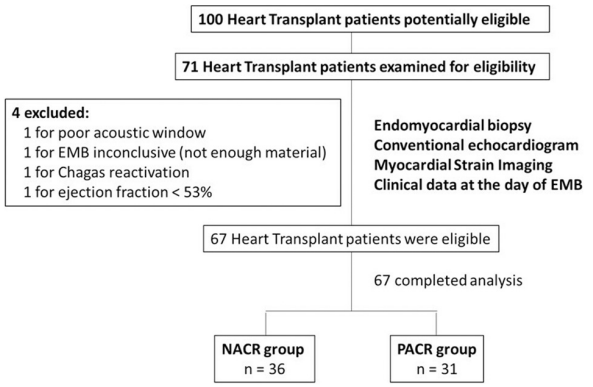


FIGURE 1 | Flowchart of HT patients with or without acute cellular rejection. This study included 67 patients who underwent HT. Patients with poor acoustic window, with EMB inconclusive (not enough material), with Chagas reactivation, and with ejection fraction <53% were excluded. In the end, 67 HT patients were included in this study. Among them, 36 patients were in the NACR group and 31 patients were in the PACR group. Statistical analysis for sample size was performed with power analysis based on mean and standard deviation from a similar study (7). HT, heart transplant; EMB, endomyocardial biopsy; NACR, negative acute cellular rejection; PACR, positive acute cellular rejection.

are considered representative of not having significant cellular rejection (1). Grades 2 and 3 represent significant cellular rejection, and changes in immunosuppressor medications are usually necessary (1). EMB was performed according to our institutional protocol once a week in the first month of HT, every 15 days in the second month, and monthly from 3 to 7 months, for a total of 9 biopsies at 6 months. After 7 months of HT, cardiac scintigraphy is recommended when possible, and EBM is performed only when scintigraphy is positive for inflammation (1).

Echocardiography was performed by three trained cardiologists on a commercially available ultrasound machine equipped with a 5 MHz probe (GE Vivid 9, GE Healthcare, Milwaukee, WI, United States). Cardiologists were blinded to the biopsy results until all data were analyzed.

Standard echocardiogram images were acquired according to the recommendations of the American Society of Echocardiography (14). LVEF was obtained by biplane Simpson's rule at apical 4- and 2-chamber views and LV mass was calculated using the equation proposed by Devereux et al. (14), indexed by body surface area. Relative wall thickness (RWT) was calculated as a ratio between 2 left ventricle posterior wall thickness and left ventricle end-diastolic diameter, with a value of >0.42 being considered abnormal (14). RV systolic function was assessed with conventional parameters recommended for routine clinical practice: tricuspid annular plane systolic excursion, systolic excursion velocity, and fractional area change obtained by M-mode, pulsed tissue Doppler, and two-dimensional echocardiography, respectively (14). Diastolic function was evaluated based on mitral inflow velocities (E and A), E/A ratio, annular mitral tissue Doppler velocities (e'/a'), and E/e' ratio (15). All conventional and MSI analyses were performed offline on a workstation by the software EchoPAC Version 2.02 (GE Vingmed Ultrasound, Norway).

Left ventricle MSI was analyzed by GLS obtained by 2D speckle tracking (16). We obtained three consecutive heart cycles from each of the apical views (apical 4-chamber, 3-chamber, and 2-chamber), with frame rates above 50 per second. Endocardial borders were manually traced in the end-systolic frame of the cardiac cycle, starting from the apical long-axis view, where it is simpler to identify the timing of aortic valve closure. The software generated a region of interest (ROI) of the entire myocardial thickness, which could be manually adjusted in width if necessary, and a moving image displaying the tracking. If the tracking was considered inaccurate, the operator could repeat the process, readjust the ROI, or select a new ROI. The software then divided the left ventricle myocardium into six segments, calculating segmental and GLS. The same process was repeated for the apical 4- and 2-chamber images and the GLS was determined by averaging local strains of all myocardial segments and displayed in the format of a polar map (**Figure 2**). Using the same images for GLS of the LV and obtaining the systolic and diastolic blood pressures automatically during the image acquisition, we obtained the MW (11). The software used for MW analysis incorporates the left ventricular pressure non-invasively estimated through a cuff into the left ventricle strain, giving the indices associated with pressure-strain curves. Along with segmental and global values for the MW index, additional indices are provided:

- Constructive work (CW): work performed by myocardial segments during systolic shortening, which contributes to left ventricle ejection.
- Wasted work (WW): systolic lengthening during contraction (opposite from what is expected at the time of cardiac cycle) and does not contribute to left ventricle ejection.
- Global work efficiency (GWE) is the ratio CW/WW reported as a percentage (0–100%) (**Figure 3**).

The rationale for analyzing MW is that GLS has some dependency on loading conditions, making it difficult to distinguish between abnormal GLS due to intrinsically reduced LV contractility and increased LV afterload. MW can reduce the afterload-dependent limitation of GLS by incorporating blood pressure into the analysis (17).

Right ventricle MSI was analyzed by longitudinal strain from the RV-free wall. Totally, three cardiac cycles were obtained from the apical 4-chamber view focused on the right ventricle (14), and temporal markers of opening and closure of the pulmonary valve were obtained from continuous wave Doppler from the pulmonary artery at the level of the pulmonary valve. The average of the three right ventricular free wall segments, basal, medial, and apical, was considered the RV-FWLS.

The electronic medical records of all subjects were reviewed on the day of the EMB and echocardiographic studies to compare clinical, demographic, and drug intake for immunosuppressor treatment. The clinical characteristics collected were age, primary heart disease that led to HT, weight, height, body mass

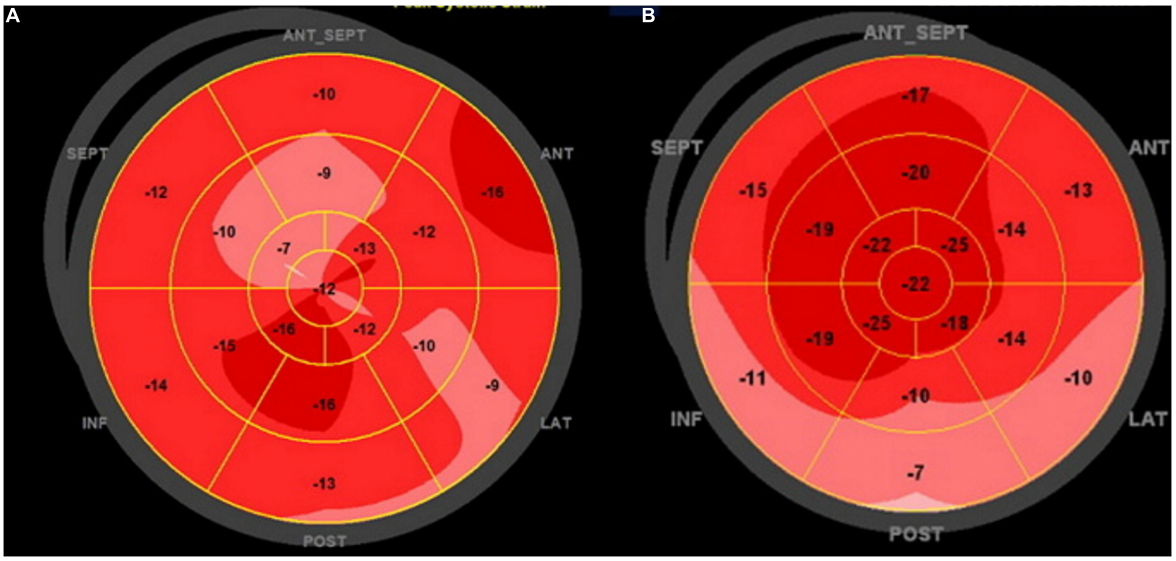


FIGURE 2 | Global longitudinal strain polar map from 17 segments: **(A)** patient with NACR and **(B)** patient with PACR. Numbers inside each segment represent segmental strains color-coded in shades of red, and lower values are lighter shades. The strain was reduced in PACR and NACR with an irregular patch pattern as observed in the **(A,B)**.

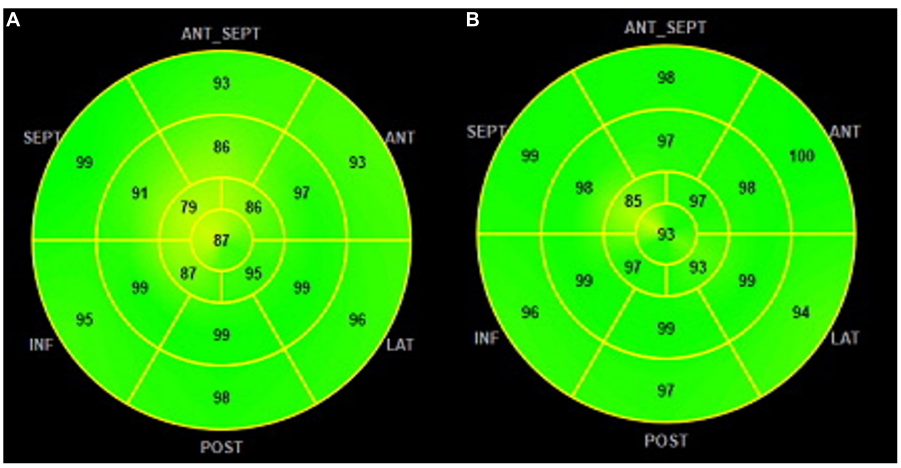


FIGURE 3 | GWE polar map from 17 segments: **(A)** patients with NACR having a mean GWE of 96% and **(B)** patients with PACR having a mean GWE of 92%. Numbers inside each segment represent segmental work efficiency color-coded in shades of green, and lower values are in yellow shades. GWE was slightly higher in PACR compared to NACR. GWE, global work efficiency.

index, blood pressure, heart rate, hypertension, diabetes, and date of transplant to EMB. The list of immunosuppressant drugs included mycophenolate, cyclosporine, tacrolimus, azathioprine, and prednisone.

Death Events Follow-Up for 2 Years

Patients with HT are routinely followed up at our hospital. Records of death events were followed prospectively for 2 years from the date of HT. The aim of this analysis was to check whether ACR could change mortality outcomes in the first year, mostly in chagasic patients, where autonomic dysfunction might be involved.

Data Analysis

The calculated sample size for each group before data collection was based on other studies with HT and GLS analysis (7). The number obtained for each group was 34 subjects. With 34 patients in each group, the study would have a power of 80% to detect a clinically important reduction in the GLS of 10%, considering the normal value of 21%, standard deviation of 4%, and 5% alpha.

Continuous variables are presented as the means (SD), and categorical variables are presented as the numbers (percentage of total). Continuous data were analyzed using the Student's *t*-test or the Mann–Whitney test when normality assumptions were not

met. Categorical variables were analyzed using either the Pearson χ^2 or Fisher's exact test.

The multivariate analysis consisted of the adjustment of Poisson regression models with robust variance for a positive biopsy result associated with clinical and echocardiographic variables, using the prevalence ratio and its respective confidence intervals as an effective measure. Poisson regression was used because it provides a better estimate of the prevalence ratios and consequently more accurately represents the effect measures for cross-sectional studies. The analysis was performed in two stages: univariate and multivariate. In both, prevalence ratios and their respective 95% confidence intervals were calculated. In the univariate analysis, the association between each independent variable and the occurrence of a positive biopsy result was verified, and those with $p < 0.25$ were selected for inclusion in the multivariate analysis. In the multivariate analysis, the models were built by consecutively excluding a variable from each complete model that had the highest p-value in the Wald test and readjusting and verifying the stability of the model after the removal of each variable to best fit the data. Once the final model was obtained, the variables that had been excluded after the univariate analysis were added one by one to the model, and the Poisson regression analysis was repeated to identify the variables that could contribute to the model in the presence of other variables. Multicollinearity between independent variables was assessed. The limit for the presence of multicollinearity was a tolerance indicator of less than 0.40.

A framework study of patients with Chagas heart disease as the primary cause of HT was performed (23 with rejection and 19 without ACR) due to the high prevalence of this etiology in this prospective cohort (63% of all 67 patients), and the evaluation of mortality by a Kaplan–Meier curve within 2 years of follow-up was constructed to compare survival with the log-rank test in patients with and without rejection at the first year. Another framework group analyzed was HT patients with less than 6 months of follow-up, where the frequency of EMB was higher according to the protocol used for ACR control in our institution.

RESULTS

Subsection A: Global Analysis

Patients were divided into two groups according to endomyocardial biopsy results: negative ACR (NACR) and positive ACR rejection (PACR). The clinical characteristics are described in **Table 1**. Patients with PACR were older (55 ± 8 vs. 49.3 ± 12 for NACR; $p = 0.03$) and were taking more cyclosporine (64 vs. 28% for NACR; $p = 0.0026$) and less tacrolimus (72 vs. 35% for NACR; $p = 0.0026$). A total of 80% ($n = 54$) of myocardial biopsies were performed in the first 6 months, and 20% ($n = 13$) were performed 6 months to 1 year from the date of transplant, probably related to the occurrence of a greater number of biopsies in the first 6 months, which was random. For primary heart disease, Chagas cardiomyopathy etiology was frequent but similar between groups (53% for NACR and 74% for PACR; $p = 0.57$).

Analyzing conventional echocardiographic parameters, we observed higher RWT and left ventricle mass index in patients with PACR (0.5 ± 0.1 vs. 0.44 for NACR; $p = 0.01$ for relative wall thickness; and 96 ± 27 g/m^2 vs. 89.4 ± 29 g/m^2 for NACR; $p = 0.045$). Although E/e' was lower in the PACR group (8.5 ± 4.6 vs. 11.9 ± 5.3 in NACR; $p = 0.01$), diastole could not be analyzed in more than 50% of patients due to technical problems, such as wave fusion (increased heart rate or abnormal atrial activation), making this result less reliable. All other echocardiographic conventional parameters were similar between groups and are displayed in **Table 2**.

The MSI parameters are described in **Table 3**. All parameters of MSI were similar, except for GWE, which was higher in patients with PACR (89.1 ± 5% vs. 85 ± 8.7% for NACR; $p = 0.03$).

A multivariate analysis was performed to assess variables that were independently associated with rejection, including clinical and echocardiographic characteristics. Based on the univariate analysis presented in **Table 4**, only the variables age, pretransplant diagnosis of myocardiopathy, BMI, systolic blood pressure, use of cyclosporine, end-diastolic posterior wall thickness, LV mass index, fraction area change of the right ventricle, and MWE

TABLE 1 | Clinical characteristics of heart transplant patients divided in NACR and PACR.

Characteristics	NACR ($n = 36$)	PACR ($n = 31$)	p-value
Age (years)	49.33 ± 12	55.19 ± 7.8	0.03
Gender female/male	25/11	15/16	0.08
SBP (mmHg)	129.17 ± 21	137.74 ± 26	0.14
DBP (mmHg)	83.67 ± 17	88.10 ± 16	0.29
HR (bpm)	84.78 ± 15	88.19 ± 13	0.32
Heigh (cm)	164.31 ± 7	163.90 ± 7	0.82
Weight (kg)	62.03 ± 10	65.51 ± 12	0.2
BSA (m^2)	1.67 ± 0.2	1.71 ± 0.2	0.35
BMI (cm^2/kg)	22.97 ± 3.6	24.40 ± 4	0.13
Time HT to EMB <6 m/>6 m	28/8	27/4	0.87
Hypertension			
Diabetes			
Primary heart disease			0.57
Chagas cardiomyopathy	19 (53%)	23 (74%)	
Idiopathic dilated cardiomyopathy	6 (18%)	4 (13%)	
Ischemic cardiomyopathy	3 (8%)	3 (10%)	
Valvular cardiomyopathy	2 (6%)	0	
Congenital cardiomyopathy	1 (3%)	0	
Postpartum cardiomyopathy	2 (6%)	0	
Non compaction cardiomyopathy	1 (3%)	11 (3%)	
CTRCD	1 (3%)	0	
Imunossupressor drugs			
Corticosteroids	34 (94%)	28 (90%)	0.52
Mycophenolate mofetil	25 (69%)	15 (48%)	0.08
Tacrolimus	26 (72%)	11 (35%)	0.003
Cyclosporine	10 (28%)	20 (64%)	0.003
Azathioprine	11 (30%)	16 (52%)	0.08
Time frame from HT to EMB (days)	89 ± 95	95 ± 98	0.52

NACR, negative acute cellular rejection; PACR, positive acute cellular rejection; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BSA, body surface area; BMI, body mass index; HT, heart transplant; EMB, endomyocardial biopsy; CTRCD, cancer-therapeutic-related cardiac dysfunction.

TABLE 2 | Conventional echocardiographic parameters.

Parameters	NACR (*n* = 36)	PACR (*n* = 31)	*p*-value
LVDD (mm)	10.03 ± 2.21	10.8 ± 2.09	0.16
PW (mm)	9.6 ± 1.84	10.6 ± 1.7	0.02
SW (mm)	43.7 ± 3.1	42.9 ± 4.9	0.48
Relative wall thickness	0.44 ± 0.1	0.50 ± 0.1	0.01
Ejection fraction (biplane Simpson) %	61.1 ± 8.8	64.5 ± 10	0.15
LV mass Devereux (g)	147.8 ± 43	162.0 ± 44	0.18
LV mass index (g/m^2)	89.4 ± 29	96 ± 27	0.045
RV diameter (mm)	33.8 ± 6	35.2 ± 4.5	0.28
TAPSE (mm)	12.5 ± 3.2	12.2 ± 2.4	0.67
Lateral annulus tricuspid SV (cm/s)	7.4 ± 2.0	7.2 ± 2.2	0.71
RV FAC %	42.3 ± 6.9	44.8 ± 9.5	0.22
LA volume index (g/m^2)	22.97 ± 3.6	24.40 ± 4	0.13
RA volume index (g/m^2)	22.7 ± 10.2	24.04 ± 7.9	0.55
E velocity (cm/s)*	76.8 ± 20.7	72.4 ± 24	0.49
e′ septal (cm/s)*	6.7 ± 2.1	7.2 ± 1.8	0.33
e′ lateral (cm/s)*	8.7 ± 3.0	10.04 ± 3.3	0.11
E/e′*	11.9 ± 5.3	8.5 ± 4.6	0.01

*NACR, negative acute cellular rejection; PACR, positive acute cellular rejection; LVDD, left ventricle diastolic diameter; PW, left ventricle posterior wall thickness; SW, septal wall thickness; LV, left ventricle; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change; LA, left atrial; RA, right atrial; E, mitral early systolic velocity; e′ septal, septal annulus early diastolic velocity; e′ lateral, lateral annulus early diastolic velocity. *Missing data due to technical limitations in 30% of NACR and 58% of PACR.*

TABLE 3 | Myocardial strain imaging parameters.

Parameters	NACR (*n* = 36)	PACR (*n* = 31)	*p*-value
LV GLS (absolute %)	12.1 ± 2.9	11.9 ± 2.7	0.83
RV FWLS (absolute %)	16.3 ± 5	16.5 ± 3.7	0.89
MWI (mmHg%)	1131.7 ± 469.4	1316.7 ± 508.2	0.12
GWE %	85 ± 8.7	89.13 ± 5	0.03
CW (mmHg%)	1395.9 ± 505	1541 ± 500	0.25
WW (mmHg%)	147.8 ± 43	162.0 ± 44	0.18

NACR, negative acute cellular rejection; PACR, positive acute cellular rejection; LVGLS, left ventricle global longitudinal strain; RV-FWLS, right ventricular free-wall longitudinal strain; WI, myocardial work index; GWE, global work efficiency; CW, constructive work; WW, wasted work.

were selected due to presenting $p < 0.25$ and were included in the complete model of the multivariate analysis. Applying this model, **Table 5** shows the results for significant parameters in the adjusted prevalence ratio. The summary of these findings is described as follows: for every 1-year increase in age (1.04 years with a CI of 1.02–1.07 for PACR; adjusted prevalence ratio; $p = 0.002$), the prevalence of a positive biopsy result increased by 4%. Patients who used cyclosporine had a prevalence of positive biopsy results 82% higher than those who did not use cyclosporine (yes for cyclosporine 1.82 with a CI of 1.06–3.1; adjusted prevalence ratio $p = 0.029$). For every 1 g/m^2 of indexed LV mass, the prevalence of a positive biopsy result increased by 1% (1 g/m^2 with a CI of 1.00–1.01; adjusted prevalence ratio $p = 0.04$). For each increase of 1 GWE unit, the prevalence of a positive biopsy result increased by 5% (1.05% with a CI of 1.00–1.11; adjusted prevalence ratio $p = 0.03$).

TABLE 4 | Univariate analysis for the presence of cellular rejection: tested parameters for gross prevalence ratios.

Parameters	GPR (CI)	*p*-value
Age (years)	1.03 (1.01–1.06)	0.0177
Cyclosporine use	–	0.029
No	1	–
Yes	2.18 (1.25–3.79)	0.0057
LV mass index g/m^2	1 (1.00–1.01)	0.10
MWE %	1.05 (1.00–1.11)	0.0298
BMI Kg/m^2	1.05 (0.99–1.02)	0.11
Hypertension	–	
No	1	
Yes	0.98 (0.51–1.88)	0.95
Diabetes		
No	1	
Yes	0.19 (0.03–1.22)	0.08
Lateral annulus tricuspid velocity (cm/s)	0.97 (0.85–1.10	0.66
TAPSE (mm)	0.98 (0.9–1.1)	0.71
RV FAC %	1.02 (0.99–1.05)	0.22
LV GLS% (absolute)	0.99 (0.9–1.1)	0.93
RV FWLS% (absolute)	0.99 (0.94–1.04)	0.67
RV FWLS% (absolute)	0.99 (0.94–1.04)	0.67

GRP, gross prevalence ratio; LV, left ventricle; WE, myocardial work efficiency; BMI, body mass index; TAPSE, tricuspid annular plane systolic excursion; RV, right ventricle; FAC, fractional area change; LVGLS, left ventricle global longitudinal strain; RV-FWLS, right ventricular free wall longitudinal strain.

TABLE 5 | Multivariate analysis for the presence of cellular rejection.

Parameters	APR (CI)	*p*-value
Age (years)	1.04 (1.02–1.07)	0.0002
Cyclosporine use	–	0.029
No	1	–
Yes	1.82 (1.06–3.1)	0.029
LV mass index g/m^2	1 (1.00; 1.01)	0.04
MWE %	1.05 (1.00; 1.11)	0.0298

APR, adjusted prevalence ratio; CI, confidence intervals; LV, left ventricle; MWE, myocardial work efficiency.

Interobserver variability was performed by comparing three different trained echocardiologist measures from 20 patients, and intraobserver variability was measured 1 month apart by one of the investigators. The intraclass correlation coefficient of LVGLS was 0.98 (95% CI = 0.94–0.99) for the interobserver variability coefficient and 0.88 (95% CI = 0.70–0.99) for intraobserver variability. The intraclass correlation coefficients of the RV-FWLS were 0.97 (95% CI = 0.94–0.95) for interobserver variability and 0.98 (95% CI = 0.95–0.99) for intraobserver variability. MW derived from GLS and the interobserver variability for each variable is described: GWI = 0.93 (95% CI = 0.84–0.97); GWE = 0.97 (95% CI = 0.93–0.99); GCW = 0.94 (95% CI = 0.85–0.97); and GWW = 0.92 (95% CI = 0.81–0.97).

Subsection B: Chagas' Study Framework

The most frequent cause of primary heart disease that led our patients to be subjected to HT was Chagas disease: 53% of NACR

and 74% of PACR. To investigate the behavior of this important group in many developing countries, where Chagas is a frequent cause of heart failure, a framework analysis was performed to verify whether ACR increases mortality in 2 years where we had 8 deaths in PACR and 2 in NACR. A Kaplan–Meier survival curve was built to verify whether patients with prior Chagas cardiomyopathy and rejection had an increased risk of death (**Figure 4**). Patients in this group had a similar age between PACR and NACR (56 ± 8 years; 54 ± 3 years; $p = 0.87$, respectively). Although there was an apparent trend for worse survival for PACR, in the follow-up of 2 years, survival was similar in both groups (log-rank $p = 0.09$).

Subsection C: Patients With Less Than 6 Months From HT to EMB Framework

A framework of patients with less than 6 months from HT to EMB was analyzed to verify whether echocardiographic parameters could discriminate NACR (28 patients) from PACR (27 patients) since a higher number of patients with less than 6 months was observed in this study (**Table 7**). Patients with PACR were older than those with NACR (49 ± 12 years and 55 ± 8 years, respectively; $p = 0.035$). Echocardiographic parameters were similar; only left ventricular mass index was higher in PACR, and GWE was borderline, with $p = 0.05$ for increased values in PACR (**Table 6**).

DISCUSSION

The main findings of this study are that LVGLS or RV-FWLS values were not different in patients with less than 1 year of HT regardless of positive ACR. Conversely, GWE, a derivative of LVGLS, and LV mass index are increased in patients with ACR. Additionally, MSI parameters in patients with less than 6 months of HT are similar between groups of PACR and NACR. Other important findings are that echocardiographic parameters of Chagas cardiomyopathy as the primary cause of HT were not different in ACR, and the presence of rejection did not impose a higher risk of death in a follow-up of 2 years. Equally important, a multivariate analysis of the observed variables was able to determine independent parameters associated with ACR, such as older age, GWE, use of cyclosporine, and increased left ventricular mass index.

Endomyocardial biopsy is the gold standard for ACR. However, some complications associated with the method are not uncommon, such as myocardial perforation, pericardial tamponade, and iatrogenic tricuspid valve injury (1, 13), and 20% of patients have inconclusive biopsies related to sampling errors and variability in results due to pathological findings interpretation (18). Considering the limitations of EMB and the patient's burden of undergoing nine biopsies in the first 6 months, non-invasive methods for the detection of ACR are of crucial interest.

Our study is the first to acknowledge MSI, conventional echocardiographic parameters, and clinical characteristics in the first year of HT. We highlight the analysis of specific groups, such as the first 6 months of HT, where ACR is reported in 60% of patients (2), and a population of primary Chagas disease as the cause of HT, where we observed that ACR at the first year in this group did not increase mortality in 2 years. In addition, for the first time, MW was described in HT, with some interesting results in GWE in patients with ACR.

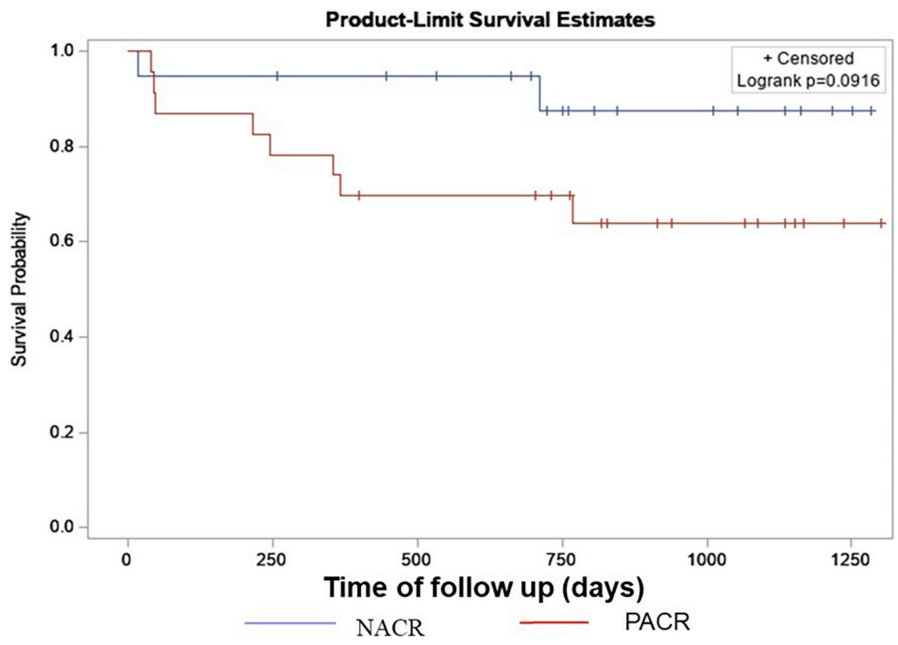


FIGURE 4 | Kaplan–Meyer survival curve for Chagas with NACR and PACR. Results from a log-rank test of censored survival. NACR, negative acute cellular rejection; PACR, positive acute cellular rejection.

TABLE 6 | Echocardiographic parameters for patients with Chagas heart disease as primary cardiomyopathy.

Parameters	NACR (*n* = 19)	PACR (*n* = 23)	*p*-value
LVDD (mm)	10.4 ± 2.1	10.5 ± 1.6	0.95
PW (mm)	9.79 ± 1.4	10.4 ± 1.6	0.20
SW (mm)	43.7 ± 3.1	43.7 ± 5.4	0.99
Relative wall thickness	0.45 ± 0.07	0.48 ± 0.08	0.19
Ejection fraction (biplane Simpson) %	59.8 ± 7	62.2 ± 9	0.54
LV mass index (g/m^2)	92.8 ± 28.5	97.04 ± 29.6	0.64
RV diameter (mm)	33.5 ± 6.4	35.6 ± 3.5	0.20
TAPSE (mm)	11.9 ± 3.2	12.1 ± 2.5	0.91
Lateral annulus tricuspid SV (cm/s)	7.4 ± 2.2	7.2 ± 2.4	0.74
RV FAC %	42.3 ± 6.9	44.8 ± 9.5	0.22
LA volume index (g/m^2)	37.8 ± 9	42.8 ± 10.5	0.12
RA volume index (g/m^2)	24.3 ± 10.2	26.5 ± 7.2	0.31
LV GLS (absolute %)	11.4 ± 2.4	11.9 ± 2.7	0.49
RV FWLS % (absolute %)	16.5 ± 3.8	16.9 ± 6.8	0.76
WI (mmHg%)	1053. ± 421	1307.6 ± 532	0.1
MWE %	83.8 ± 10	88.8 ± 5.5	0.05
CW (mmHg%)	1373.8 ± 517	1522.8 ± 520	0.35
WW (mmHg%)	221.5 ± 181	137.6 ± 69	0.34

NACR, negative acute cellular rejection; PACR, positive acute cellular rejection; LVDD, left ventricle diastolic diameter; PW, left ventricle posterior wall thickness; SW, septal wall thickness; LV, left ventricle; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change; LA, left atrial; RA, right atrial; E, mitral early systolic velocity; e' septal, septal annulus early diastolic velocity; e' lateral, lateral annulus early diastolic velocity; RV-FWLS, right ventricular free wall longitudinal strain; MWI, myocardial work index; MWE, myocardial work efficiency; CW, constructive work; WW, wasted work.

TABLE 7 | Echocardiographic parameters for patients with less than 6 months from heart transplant to EMB.

Parameters	NACR (*n* = 28)	PACR (*n* = 27)	*p*-value
LVDD (mm)	43.6 ± 23.4	42.1 ± 4.6	0.18
PW (mm)	9.9 ± 1.8	10.7 ± 1.7	0.09
SW (mm)	43.7 ± 3.1	43.7 ± 5.4	0.99
Relative wall thickness	0.46 ± 0.09	0.51 ± 0.09	0.03
Ejection fraction (biplane Simpson) %	62.1 ± 8.6	64.3 ± 10.9	0.44
LV mass index (g/m^2)	89.3 ± 30.7	95.8 ± 29.1	0.044
RV diameter (mm)	34.5 ± 5.9	35.1 ± 4.8	0.69
TAPSE (mm)	12.4 ± 3.1	12.3 ± 2.5	0.80
Lateral annulus tricuspid SV (cm/s)	7.4 ± 2.2	7.2 ± 2.4	0.74
RV FAC %	43.6 ± 7.1	44.9 ± 9.6	0.53
LA volume index (g/m^2)	43.4 ± 22.9	41.4 ± 10.7	0.68
RA volume index (g/m^2)	24.0 ± 11.1	23.5 ± 7.8	0.83
LV GLS (absolute %)	11.9 ± 2.3	11.7 ± 2.5	0.73
RV FWLS % (absolute %)	15.3 ± 6.8	16.2 ± 5.6	0.61
MWI (mmHg%)	1073.9 ± 441	1313 ± 542	0.08
MWE %	84.7 ± 9	88.6 ± 4.9	0.05
CW (mmHg%)	1298.2 ± 410	1537.5 ± 534	0.07
WW (mmHg%)	174 ± 125	151 ± 66	0.91

NACR, negative acute cellular rejection; PACR, positive acute cellular rejection; LVDD, left ventricle diastolic diameter; PW, left ventricle posterior wall thickness; SW, septal wall thickness; LV, left ventricle; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change; LA, left atrial; RA, right atrial; E, mitral early systolic velocity; e' septal, septal annulus early diastolic velocity; e' lateral, lateral annulus early diastolic velocity; RV-FWLS, right ventricular free wall longitudinal strain; WI, myocardial work index; MWE, myocardial work efficiency; CW, constructive work; WW, wasted work.

Badano et al. (5) recommended the analysis of baseline HT echocardiogram 6 months after the procedure due to ischemic injury and residual right ventricular dysfunction and performed comparisons in subsequent exams to better diagnose ACR. Conversely, the first 6 months are the most important for preventing cellular rejection, making the analysis of parameters in this time frame relevant.

Our data contrast with earlier work showing decreased LVGLS and RV-FWLS in a population with more than 6 months of HT (7) or a smaller population with a lower rejection frequency with less than 1 year of HT (6). However, Tseng et al. (9) observed similar results for LVGLS in a retrospective study that included patients with less than 1 year of HT without considering the period of time between transplant and EMB of less than or more than 6 months. Ambardekar et al. (10) also found no changes in myocardial strain or strain rate as assessed by 2D-STE on serial studies from patients with asymptomatic biopsy-proven rejection in the first year after HT, with no mention of differences in the time frame of 6 months. In both studies, the GLS values were similar to those in this study. These conflicting findings in GLS and ACR might be related to the time frame of transplant and EMB.

Despite the superiority of GLS over LVEF in the deeper evaluation of LV systolic performance, this technique is still limited by loading dependence (11, 16), affecting the correct measurement of myocardial contractile function in specific loading conditions, such as higher blood pressure (17) or increased neurohormonal activation associated with HT rejection (3). In this context, MW analysis, incorporating LV pressure, is less load-dependent than strain and therefore could provide incremental information in the setting of patients with HT. To the best of our knowledge, this is the first study to analyze MW in patients with HT and observe that, compared to normal individuals (19), the indices of MW are diminished in both PACR and NACR subjects (**Table 3**). Nevertheless, GWE is significantly higher in PACR subjects than in NACR subjects, including 1-year HT and borderline HT, if only less than 6 months of HT are included. A possible hypothesis for this finding is that patients with PACR have increased afterload as a consequence of neurohormonal activation (3) and slightly higher blood pressure; to maintain normal ejection fraction, the contractile efficiency must be compensated (20). This finding of higher GWE in PACR is more evident after 6 months of HT since before 6 months, some degree of ischemic injury might be present in both groups (5) as a confounding factor for rejection injury. Interestingly, in an elegant study by Tokodi et al. (20), GLS was impaired in elite swimmers, while indices of MW were completely normal, maintaining normal LV function, probably as a consequence of a better description of myocardial contractility. In summary, MW indices mighty precociously differentiate PACR compared to GLS in the first year of HT.

In accordance with previous studies, our data observed ventricular remodeling in HT with increased LV mass index and RWT (21), predominantly in PACR. This was likely related to

hypertrophy as well as edema caused by inflammation (5). RV parameters, such as FAC, tricuspid lateral annulus velocity, and TAPSE, were equally impaired in both groups, similar to the findings of Ingvarsson et al. (21).

In this study, the multivariable analysis identified independent predictors of ACR, such as age, predominant cyclosporine use as an immunosuppressor drug, LV mass index, and GWE. In contrast to previous studies, PACR occurred in older patients in this study. Normally, younger patients are more prone to ACR (22) as a consequence of a more active immune system (1). However, in our study, we observed an increased use of cyclosporine as a calcineurin inhibitor, which could be a confounding factor for the age variable, since older age could be associated with more cyclosporine utilization. Cyclosporine is known to be less effective than tacrolimus (23), making the presence of ACR more constant and possible statistical interaction of both variables, creating a bias in older subjects associated with increased ACR. In accordance with other studies, we confirmed an increased LV mass index and remodeling in PACR, likely associated with edema and hypertrophy in this group of patients (7, 21).

To the best of our knowledge, only one study has described MSI in HT with predominant chagasic cardiomyopathy as primary heart disease (7). Our study is the first to analyze a framework group with such characteristics and to observe similar conventional echocardiographic and MSI variables, both lower in PACR and NACR. In a Kaplan–Meier survival curve at the 2-year follow-up, the presence of ACR did not increase mortality in this group, despite possible impairment of the autonomic nervous system in these patients (12).

Strengths of the Study

The strength of this study was to scrutinize the time frame of EMB and describe variables for ACR with less than 6 months of HT when the majority of ACR occurs. We were able to demonstrate decreased and similar values of MSI in both the PACR and NACR groups. Additionally, for the first time, MW was described in HT as a possible early marker for PACR compared to GLS to detect rejection using GWE in a court with 80% of patients with less than 6 months of HT. Finally, a separate analysis of chagasic cardiomyopathy was performed to describe survival in 2 years and possible changes in MSI.

Limitations

The limitations of this study should be addressed. First, this was a single-center study with a small number of patients; nevertheless, a significant number of patients with ACR were enrolled compared to other studies. Another important comment is that GLS is an evolving method, and interobserver variability is high; thus, the values obtained in our study cannot be applied to other software for strain analysis. A multicenter study including other vendors and a higher number of patients must be performed. Our data could not determine the time frame where GLS is a marker for ACR; however, we were able to describe that MSI variables are not markers for ACR in a time frame of less than 6 months of HT.

CONCLUSION

In the first year of HT, LVGLS, and RV-FWLS by speckle tracking were not able to detect changes in patients with asymptomatic ACR. Conversely, GWE, a derivative of LVGLS, and LV mass index are increased in patients with ACR and could represent possible markers for ACR. Increased LV mass index was also found in a subgroup analysis of patients less than 6 months after HT; however, MSI was similar regardless of ACR. In a subgroup analysis of chagasic patients, MSI was similar between groups with or without rejection, and ACR at the first year of HT did not increase mortality at the 2-year follow-up. In a multivariable analysis, parameters such as older age, use of cyclosporine, increased LV mass index, and increased GWE were independent variables related to ACR in this study.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This research was approved by the Ethical Committee of Cardiology and Transplant Heart Institute, of Federal District, Brasília, Brazil and the inscription number in Plataforma Brazil as a Certificate of Presentation of Ethical Appreciation is 65910517.0.0000.0026. All patients signed an informed written consent to participate in this study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MO, FA, and AM were mainly responsible for the analysis of the data and manuscript writing. MO, AC, SL, and MQ were responsible for performing echocardiographic studies and MSI analysis. NM, SRA, SVA, and MP were responsible for data collection in medical records. MO and FA were responsible for the idealization of the study. All authors contributed to the article and approved the submitted version.

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The Relationship Between Body Mass Index and In-hospital Survival in Patients Admitted With Acute Heart Failure

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Background: The association between Body Mass Index (BMI) and clinical outcomes following acute heart failure (AHF) hospitalization is debated in the literature. Our objective was to study the real-world relationship between BMI and in-hospital mortality in patients who were admitted with AHF.

Methods: In this retrospective, multi-center study, we utilized the National Inpatient Sample (NIS) database to identify a sampled cohort of patients who were hospitalized with AHF between October 2015 and December 2016. Outcomes of interest included in-hospital mortality and length of stay (LOS). Patients were divided into 6 BMI (kg/m^2) subgroups according to the World Health Organization (WHO) classification: (1) underweight $\leq$19, (2) normal weight 20–25, (3) overweight 26–30, (4) obese I 31–35, (5) obese II 36–39, and (6) extremely obese $\geq$40. A multivariable logistic regression model was used to identify predictors of in-hospital mortality and to identify predictors of LOS.

Results: A weighted total of 219,950 hospitalizations for AHF across the US were analyzed. The mean age was 66.3 $\pm$ 31.5 years and most patients (51.8%) were male. The crude data showed a non-linear complex relationship between BMI and AHF population outcomes. Patients with elevated BMI exhibited significantly lower in-hospital mortality compared to the underweight and normal weight study participants (5.5, 5,5, 2,8, 1.6, 1.4, 1.6% in groups by BMI $\leq$19, 20–25, 26–30, 31–35, 36–39, and, $\geq$40 respectively, $p < 0.001$) and shorter LOS. In the multivariable regression model, BMI subgroups of $\leq 25 kg/m^2$ were found to be independent predictors of in-hospital mortality. Age and several comorbidities, and also the Deyo Comorbidity Index, were found to be independent predictors of increased mortality in the study population.

Conclusion: A reverse J-shaped relationship between BMI and mortality was documented in patients hospitalized for AHF in the recent years confirming the "obesity paradox" in the real-world setting.

Keywords: body mass index, BMI, acute heart failure, AHF, outcome

INTRODUCTION

Body mass index (BMI) is widely used for routine characterization of weight status in epidemiological and clinical research. However, it has limitations, for instance, not distinguishing fat from muscle mass (1–3).

According to the World Health Organization (WHO) classification, patients are considered (1) underweight when they have a BMI ≤18.5 kg/m^2, (2) normal weight with BMI levels 18.5–24.9 kg/m^2, (3) overweight BMI 25–29.9 kg/m^2, (4) class I obesity BMI 30–34.9 kg/m^2, (5) class II obesity BMI 35–39.9 kg/m^2, and (6) extremely obese BMI ≥40 kg/m (2, 4, 5).

Recent evidence suggests that obesity is associated with better outcomes in patients with heart failure (HF) (6). Multiple retrospective studies demonstrated conflict results. Some have shown a U-shape curve between BMI and long-term mortality in patients with acute HF (AHF), paradoxically protecting the obese population (7–17). In some analyses, the U-shape relationship would no longer be apparent after adjusting for confounding prognostic factors of mortality (7, 9). In other studies, the U-shaped BMI remained an independent predictor of mortality even after adjusting for other risk variables in the obese (13, 14) and underweight groups (15).

As the impact of obesity on AHF outcomes is still in debate. There is importance to understand the relationship between obesity and HF to find the optimal treatment goals for our patients. This is especially interesting in the light of the connection between obesity and HF with preserved ejection fraction, an important condition with many question marks. The current study aimed to describe the BMI distribution and its potential relation with in-hospital mortality outcome in six different BMI subgroups of patients with AHF in the large cohort of US database from the National Inpatient Sample (NIS) registry.

METHODS

Data Source

Pulled data from the NIS, the Healthcare Cost and Utilization Project (HCUP), and Agency for Healthcare Research and Quality (AHRQ) (4, 5) were used for the study. This study was exempt from institutional review by the Human Research Committee due to the NIS database, which only includes de-identified information.

The NIS is the largest collection of data on all-payer hospitalizations in the United States (US) and represents an approximately 20% stratified sample of all inpatient discharges from US hospitals (18). This includes records at the hospital level, such as hospital region, teaching status, bed size, and cost of hospitalization, and other data at the patient level, including demographic characteristics, primary and secondary diagnoses and procedures, comorbidities, and length of stay (LOS). LOS analysis included all cohort populations, i.e., discharged and those who died in the hospital. National estimates can be calculated using the patient-level and hospital-level sampling weights that are provided by the HCUP (4, 5).

For the purpose of this study, we acquired data for the years 2015 and 2016. During the study period, we used the International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) from the last quarter of 2015 and thereafter for reporting diagnoses and procedures in the NIS database. For each index hospitalization, the database presents a primary discharge diagnosis, a maximum of 14 or 24 additional diagnoses, and a maximum of 15 procedures. For our study, we limited our cohort to the time-period which used ICD-10 codes to convert the data because the ICD-10 coding system includes individual codes for all BMI values and ranges.

Study Population and Variables

We included patients 18 years or older with a principal diagnosis of AHF hospitalization based on ICD-10-CM codes starting with I50.21, I50.23, I50.31, I50.33, I50.41, I50.43, and I50.811 who have one of the Z68.x codes as I10-Dx1 to I10-Dx30. These codes represent the six subgroups in our study, namely, Z68, Z68.20–25, Z68.26–30, Z68.31–35, Z68.36–39, and Z68.4 (BMI ≤19 underweight group; BMI 20–25 normal weight group; BMI 26–30 overweight group; BMI 31–35 obese I group; BMI 36–39 obese II group; and BMI equal or ≥40 extremely obese group, respectively).

Patient demographics were collected from the database as follows: age, sex, and race. Prior comorbidities were identified by measures from the AHRQ. For the purpose of calculating the Deyo-Charlson Comorbidity Index (Deyo-CCI), additional comorbidities were identified from the database using ICD-10-CM codes. Deyo-CCI is a modification of the Charlson Comorbidity Index, containing 17 comorbid conditions of differential weights, with a total score ranging from 0 to 33 (detailed information on Deyo-CCI is provided in **Appendix Table 1**). Higher Deyo-CCI scores indicate a greater burden of comorbid diseases and are associated with mortality 1-year after admission (19). The index has been used extensively in studies from administrative databases, with proven validity in predicting short- and long-term outcomes (20, 21).

Our primary outcome in this study was in-hospital mortality. The secondary outcomes included length of stay in the hospital.

Statistical Analysis

The chi-square (χ^2) and Wilcoxon Rank Sum tests were used to compare categorical variables and continuous variables, respectively. The NIS provides discharge sample weights that are calculated within each sampling level as the ratio of discharges in the universe to discharges in the sample (22). We generated a weighted logistic regression model to identify independent predictors of in-hospital mortality. Our primary covariate of interest was BMI category, with normal weight, with the BMI 20–25 category used as the reference. Candidate variables included patient-level characteristics, Deyo-CCI, and hospital-level factors. We included all candidate variables that were associated with our primary outcome in our final multivariable regression model.

TABLE 1 | Frequency distribution of baseline characteristics by body mass index (BMI) group in patients with acute heart failure.

BMI groups Kg/m^2	≤19 Under-weight	20–25 Normal-weight	26–30 Over-weight	31–35 Obese I group	36–39 Obese II group	≥40 Extremely obese	Total	*P*-value
Unweighted[a]	2,784 (6.3)	2,529 (5.8)	3,545 (8.1)	7,159 (16.3)	6,248 (14.2)	21,725 (49.4)	43,990	
Weighted[b]	13,920	12,645	17,725	35,795	31,240	108,625	219,950	
Age group, %, years								<0.001
18–44	2.6	2.2	3.6	4.6	6.6	10.1	7.3	
45–59	7.5	8.5	14.0	18.8	23.2	29.9	23.2	
60–74	23.7	24.6	33.7	40.0	41.6	42.7	39.1	
>75	66.2	64.7	48.7	36.6	28.6	17.3	30.4	
Gender, %								<0.001
Male	61.8	48.7	44.3	44.3	46.8	56.1	51.8	
Female	38.2	51.2	55.6	55.7	53.2	43.9	48.1	
Race, %								<0.001
White	69.3	67.3	64.1	63.1	62.9	60.9	62.7	
Non-white	25.6	27.4	31.1	31.8	32.1	34.0	32.2	
Comorbidity, %								
Hypertension	38.3	36.4	38.5	41.6	43.9	43.5	42.1	<0.001
Diabetes mellitus	17.2	25.7	44.1	49.3	51.8	53.7	48.0	<0.001
Chronic renal disease	39.5	46.7	50.9	48.9	46.5	44.8	46.0	<0.001
Chronic obstructive pulmonary disease	46.6	37.3	41.3	45.0	47.6	52.7	48.6	<0.001
Peripheral vascular disease	23.5	25.4	25.8	24.1	21.3	15.8	19.8	<0.001
Atrial Fibrillation/Flutter	47.4	49.0	45.6	44.6	43.1	40.7	43.0	<0.001
Prior MI	14.0	16.3	18.4	17.8	14.5	11.3	13.8	<0.001
Deyo-CCI, %								<0.001
1	6.6	6.7	4.3	4.3	4.6	5.1	5.0	
2 or higher	93.4	93.3	95.7	95.7	95.4	94.9	95.0	
Outcome								
Mortality	5.5	5.5	2.8	1.6	1.4	1.6	2.2	<0.001
Length of Stay	6.46 ± 0.15	6.7 ± 0.15	5.9 ± 0.08	5.4 ± 0.08	5.4 ± 0.07	6.0 ± 0.05	5.9 ± 0.04	<0.001

BMI, Body Mass Index (Kg/m^2); Deyo-CCI, Deyo-Charlson Comorbidity Index; MI, Myocardial Infraction.
[a]*Represents the number of observations in the NIS database.*
[b]*Represents total national estimates after applying sampling weights.*

For all analyses, we used SAS® software version 9.4 (SAS Institute Inc., Cary, NC.), and a p-value < 0.05 was considered statistically significant.

RESULTS

Study Cohort

A total of 43,990 AHF hospitalizations across the US during 2015–2016 were included in the analysis. After applying the weighting method, these represented an estimated total of 219,950 AHF hospitalizations in patients with complete BMI data. Most patients (51.8%) were male, and the cohort's mean age was 66.3 ± 31.5 years.

AHF Patient Characteristics and Comorbid Presentation by BMI Group

Frequency distribution of baseline and clinical characteristics are presented in **Table 1** according to the six BMI-divided subgroups. The median BMI in the study was 39 (IQR: 32–43), with 79.9% of the patients with BMI above the normal (>25kg/m^2).

Of the AHF hospitalization, the majority of the patients were obese type I (16.3%), type II (14.2), and with morbid obesity (49.4%) (**Table 1**).

Female predominance, higher rate of patients with Deyo-CCI score above 2, diabetes mellitus 2, hypertension, and hyperlipidemia were found in patients with AHF with BMI higher than 25 kg/m^2. The lean patients with BMI ≤19 kg/m^2 were predominantly male and were the least diabetic.

The overall in-hospital mortality rate during the study period was documented at 2.2%.

The highest crude mortality rate was observed in the underweight and normal weight subgroup 5.5% in each group, with decreased mortality rate in patients with BMI 26–39 kg/m^2 (2.8%, 1.6%, 1.4%) in the obese groups, and then increase again in morbid obese patients, 1.6% (**Figure 1**).

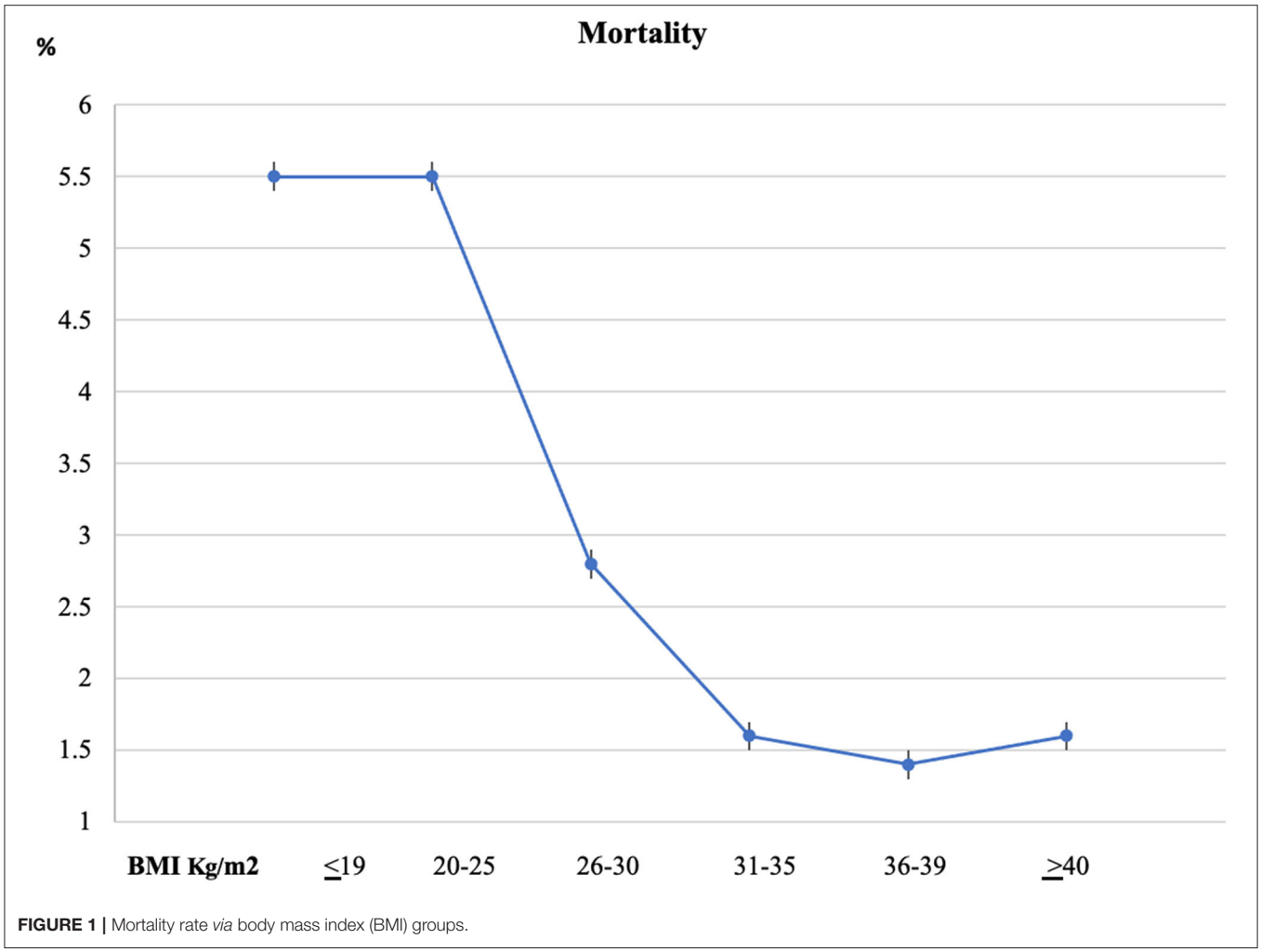

FIGURE 1 | Mortality rate *via* body mass index (BMI) groups.

Length of stay, like the mortality, was found to be greater in BMI<19 (6.46 + 0.15), BMI 20–25 (6.7 + 0.15), and BMI > 40 (6.0 + 0.05) (**Figure 2**).

Predictors of In-hospital Mortality

In the univariate analysis, we found a reverse J-shape association between survival with increasing BMI with lower mortality rate in obese patients with BMI > 25 kg/m^2.

Other baseline characteristics, such as older age, increasing Deyo-CCI score, white race, atrial fibrillation/flutter, peripheral vascular disease, and chronic renal failure, all increased the probability of in-hospital mortality ($p < 0.001$) (**Table 2**). In contrast, diabetes mellitus and hypertension were two protective comorbidities against mortality in patients with AHF ($p < 0.001$).

The reverse J-shaped correlation between BMI and mortality outcomes was again identified in the multivariable analyses (**Table 3**). A similar trend to the univariate model was also identified in the multivariate model of baseline characteristics. Older age >75 years, Deyo-CCI >2, white race, atrial fibrillation/flutter, peripheral vascular disease, and chronic renal failure all remained strong predictors of mortality after adjusting for potential confounders (**Table 3**). Furthermore, diabetes mellitus and hypertension remained protective against mortality ($p < 0.001$).

Predictors of Length of Stay (LOS)

The multivariable regression model for LOS is represented in **Table 4**. A similar reverse J-shape slope to that of mortality was observed for LOS, with the highest value for normal weight BMI 20–25 kg/m^2 [OR 5.51 (95% CI 5.24–5.78)] and lowest values for obese patients [OR 4.20 (95% CI 4.01–4.40), OR 4.24 (95% CI 4.04–4.44)] for BMI 31–35 kg/m^2 and 36–39 kg/m^2 obese groups, respectively, $p < 0.001$, (**Table 4**).

DISCUSSION

In this retrospective study benefiting from the NIS, the largest all-payer in-patient database in the US, we distinguished 219,950 patients to study the correlation between BMI and in-hospital mortality and LOS outcomes following AHF hospitalization. To our knowledge, this is a distinct main study examining the relationship between BMI and in-hospital mortality in patients with AHF.

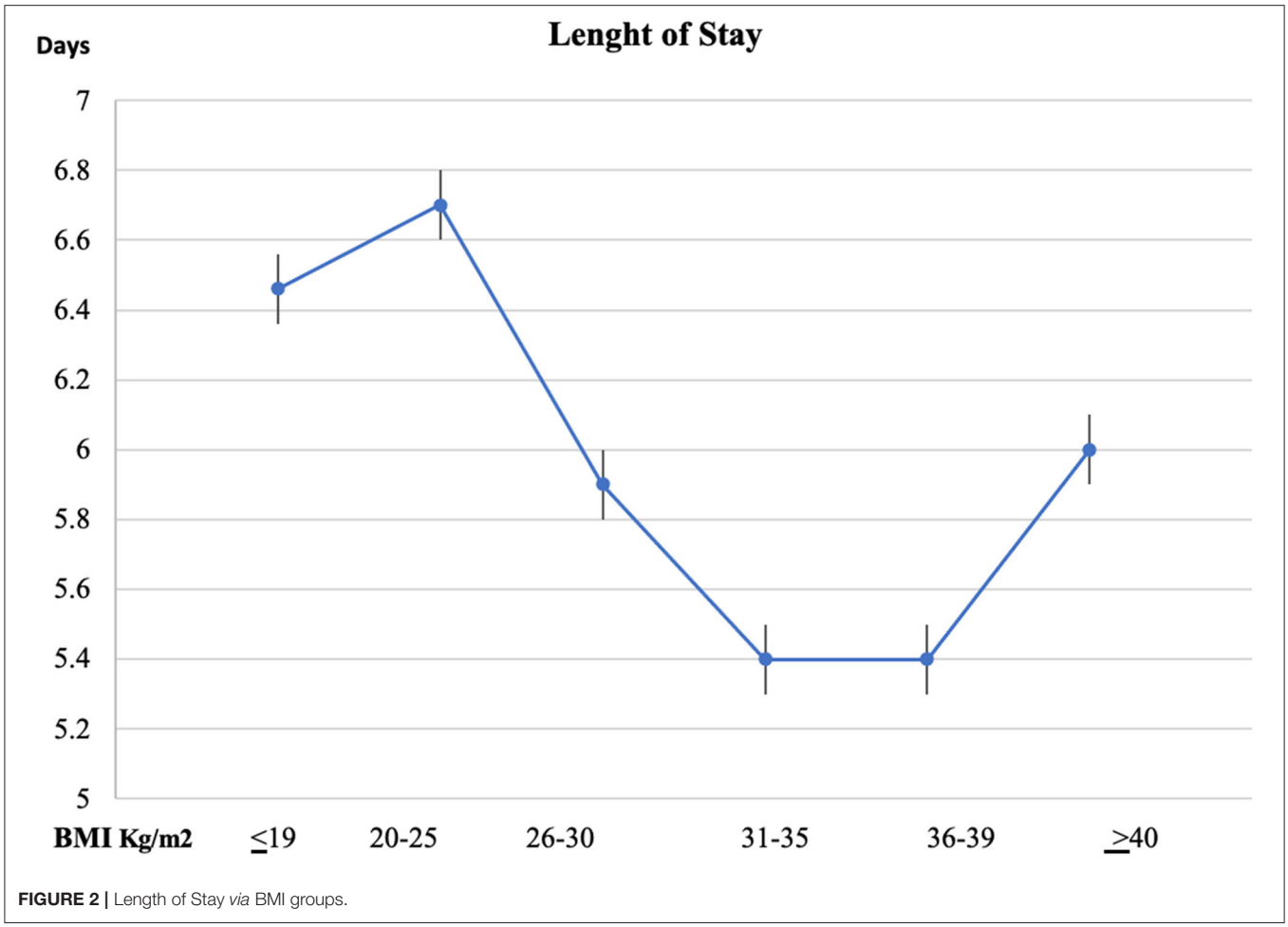

FIGURE 2 | Length of Stay *via* BMI groups.

This nationally analyzed dataset indicated a reverse J-shape correlation between BMI levels and in-hospital mortality during hospitalization of patients with AHF across the US during the years 2015–2016.

In patients that were hospitalized with AHF, elevated BMI was found to be an independent predictor of lower in-hospital mortality and shorter length of stay during the study period.

Lower mortality rates were detected in the BMI 31–35 kg/m^2 and BMI 36–39 kg/m^2 obese groups after correcting for confounding variables.

Body mass index is proven to be an independent risk factor for various cardiovascular conditions, such as atrial and ventricular arrhythmia, sudden cardiac death, stroke, acute coronary syndrome, and HF (23–25). Overweight and obesity were found to be risk factors for left ventricular remodeling and overt HF (23). Obesity has been consistently associated with left ventricular hypertrophy and dilatation, which are known precursors of HF (26–28).

Prior studies in patients with AHF examined the effect of different BMI subgroups on all-cause mortality and presented a U-shape prototype, with lowest mortality in the obese and the underweight patient populations (7–17). These studies suggested an obesity paradox in which BMI does not distinguish between metabolically healthy and metabolically unhealthy obesity (3). The obesity impact on morbidity and premature mortality can be underestimated and therefore may lead to incorrect clinical courses (3). Studies suggest that the obesity paradox is present in HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF) (29). A potential explanation is that BMI represents lean body mass rather than accurate body fat or fluid retention in patients with HF (2). In a recent study of HF, obesity was associated with a reduced risk of death, but this protective effect disappeared after adjusting for VO_2 max and b-type natriuretic peptide (BNP) levels (30).

In contrast with other studies that showed lower mortality rates in underweight patients (7–17), in line with our findings, Seko et al. also revealed that very low BMI was associated with a higher risk of mortality in patients with AHF (15). In their study, greater mortality risk was observed in underweight and severely underweight patients with AHF compared to the normal weight BMI, which remained significant even after adjusting confounders, while the lower mortality risk in the overweight and in the obese groups was no longer significant after these adjustment (15). The results of this study demonstrate the concept that the deleterious effect of cachexia, rather than the favorable influence of obesity [in the form of nutritional and

TABLE 2 | Univariate analysis for predictors of in-hospital mortality, 2015–2016.

Predictor	Odds ratio (95% CI)	*P*-value
BMI kg/m^2 group		<0.001
≤19	0.99 (0.89,1.10)	0.786
20–25	1.00 (reference)	N/A
26–30	0.49 (0.43,0.55)	<0.001
31–35	0.28 (0.25,0.31)	< 0.001
36–39	0.24 (0.21,0.27)	< 0.001
≥40	0.28 (0.26,0.31)	< 0.001
Age group, years		< 0.001
18–44	1.00 (reference)	N/A
45–59	1.54 (1.28,1.86)	<0.001
60–74	2.08 (1.74,2.48)	<0.001
≥75	4.43 (3.72,5.27)	<0.001
Deyo-CCI		<0.001
0	N/A	N/A
1	1.00 (reference)	N/A
2 or higher	1.39 (1.19,1.62)	<0.001
Gender		0.169
Male	1.00 (reference)	N/A
Female	1.04 (0.98,1.10)	0.169
Race		<0.001
Non-white	1.00 (reference)	N/A
White	1.74 (1.63,1.87)	<0.001
Comorbidities		
Atrial fibrillation/Flutter		<0.001
No	1.00 (reference)	N/A
Yes	1.90 (1.79,2.01)	<0.001
Chronic pulmonary disease		0.001
No	1.00 (reference)	N/A
Yes	0.91 (0.86,0.96)	0.001
Diabetes mellitus		<0.001
No	1.00 (reference)	N/A
Yes	0.73 (0.69,0.77)	<0.001
Hypertension		<0.001
No	1.00 (reference)	N/A
Yes	0.53 (0.50,0.57)	<0.001
Peripheral vascular disease		<0.001
No	1.00 (reference)	N/A
Yes	1.30 (1.22,1.39)	<0.001
Prior MI		0.670
No	1.00 (reference)	N/A
Yes	0.98 (0.90,1.07)	0.670
Chronic renal failure		<0.001
No	1.00 (reference)	N/A
Yes	1.53 (1.44,1.62)	<0.001

BMI, Body Mass Index; Deyo-CCI, Deyo-Charlson Comorbidity Index; MI, Myocardial Infraction.

TABLE 3 | Multivariable analysis for predictors of in-hospital mortality, 2015–2016.

Predictor	Odds ratio (95% CI)	*P*-value
BMI kg/m^2 group		<0.001
≤19	0.96 (0.86,1.07)	0.487
20–25	1.00 (reference)	N/A
26–30	0.55 (0.49,0.62)	<0.001
31–35	0.33 (0.29,0.37)	<0.001
36–39	0.29 (0.25,0.33)	<0.001
≥40	0.41 (0.37,0.45)	<0.001
Age group, years		<0.001
18–44	1.00 (reference)	N/A
45–59	1.52 (1.25,1.84)	<0.001
60–74	1.81 (1.50,2.18)	<0.001
>75	3.09 (2.56,3.73)	<0.001
Deyo-CCI		<0.001
1	1.00 (reference)	N/A
2 or higher	1.67 (1.41–1.97)	<0.001
Gender		0.004
Male	1.00 (reference)	N/A
Female	0.91 (0.86,0.97)	0.004
Race		<0.001
Non-white	1.00 (reference)	N/A
White	1.47 (1.36,1.59)	<0.001
Comorbidities		
Atrial fibrillation/Flutter		<0.001
No	1.00 (reference)	N/A
Yes	1.48 (1.39,1.58)	<0.001
Chronic pulmonary disease		0.046
No	1.00 (reference)	N/A
Yes	0.94 (0.88,1.00)	0.046
Diabetes mellitus		<0.001
No	1.00 (reference)	N/A
Yes	0.86 (0.81,0.92)	<0.001
Hypertension		<0.001
No	1.00 (reference)	N/A
Yes	0.59 (0.55,0.63)	<0.001
Peripheral vascular disease		<0.001
No	1.00 (reference)	N/A
Yes	1.16 (1.08,1.24)	<0.001
Prior MI		0.050
No	1.00 (reference)	N/A
Yes	0.92 (0.84,1.00)	0.050
Chronic renal failure		<0.001
No	1.00 (reference)	N/A
Yes	1.46 (1.37,1.55)	<0.001

BMI, Body Mass Index; Deyo-CCI, Deyo-Charlson Comorbidity Index; MI, Myocardial Infraction.

caloric reserve based on several studies (31–33)], are likely the main reason for the inverse correlation between BMI and HF outcome (4). In severe HF, tissue hypoperfusion and cardiac cachexia might contribute to the adverse outcomes (34). We cannot explain the increase in mortality and length of stay in the extremely obese, as they had similar comorbidities. A focus on this BMI sub-group should be the goal of future studies to better understand the mechanisms behind this phenomenon. Also, an additional investigation using body fat mass might add insight to these findings.

TABLE 4 | Multivariable analysis for predictors of length of stay (LOS), 2015–2016.

Predictor	Odds ratio (95% CI)	*P*-value
Age group, years		<0.001
18–44	4.47 (4.22,4.72)	N/A
45–59	4.81 (4.62,4.99)	0.005
60–74	5.03 (4.86,5.20)	<0.001
>75	4.84 (4.68,5.00)	0.003
BMI kg/m^2 group		<0.001
<19	5.09 (4.83,5.35)	0.012
20–25	5.51 (5.24,5.78)	N/A
26–30	4.78 (4.55,5.02)	<0.001
31–35	4.20 (4.01,4.40)	<0.001
36–39	4.24 (4.04,4.44)	<0.001
>40	4.89 (4.74,5.04)	<0.001
Gender		0.007
Female	4.86 (4.70,5.03)	0.007
Male	4.71 (4.55,4.87)	N/A
Race		0.872
Non-white	4.78 (4.61,4.95)	N/A
White	4.79 (4.64,4.95)	0.872
Deyo-CCI		<0.001
1	4.22 (3.96,4.48)	N/A
2 or higher	5.35 (5.24,5.46)	<0.001
Comorbidities		
Atrial fibrillation/Flutter		<0.001
No	4.50 (4.34,4.65)	N/A
Yes	5.35 (5.18,5.52)	<0.001
Chronic pulmonary disease		0.563
No	4.78 (4.63,4.93)	N/A
Yes	4.81 (4.64,4.99)	0.563
Diabetes mellitus		<0.001
No	4.84 (4.68,4.99)	N/A
Yes	4.51 (4.33,4.69)	<0.001
Hypertension		<0.001
No	5.41 (5.24,5.57)	N/A
Yes	4.36 (4.20,4.51)	<0.001
Prior MI		<0.001
No	4.83 (4.68,4.98)	N/A
Yes	4.25 (4.04,4.46)	<0.001
Peripheral vascular disorders		0.492
No	4.78 (4.63,4.93)	N/A
Yes	4.83 (4.63,5.02)	0.492
Renal failure		<0.001
No	4.61 (4.46,4.76)	N/A
Yes	5.51 (5.34,5.69)	<0.001

BMI, Body Mass Index; Deyo-CCI, Deyo-Charlson Comorbidity Index; MI, Myocardial Infraction.

A few mechanisms may explain the obesity paradox in acute HF. It appears that higher lean body mass may be protective by role of myocytes on vasculature and by favorable cytokines (2). Fat tissue has been shown to produce tumor necrosis factor-α receptors which may be protective (2). High circulating lipoprotein levels in obese subjects may bind and detoxify lipopolysaccharides that may play a role in the release of inflammatory cytokines (35, 36). Experimental studies suggested that leptin produced by fat tissue may have a protective effect in HF (37). In obesity, lower levels of adiponectin were detected, associating it with lower mortality in HF (38). Also, there might be a higher use of guidelines-guided therapy in patients with higher BMI, according to one study (9). Taken together, fat distribution, lean mass, and cardio fitness could play essential roles in determining the observed differences in the HF population (3).

This study has several limitations. The accessible NIS database is retrospective and, as such, contains discharge patient records which are susceptible to coding errors. The NIS dataset lacks comprehensive information regarding medication, blood testing, and other important markers, such as NT-proBNP, that are associated with adverse cardiovascular events which are markedly important confounding variables. We therefore cannot rule out residual confounding of the associations we observed. Furthermore, we could only capture events that occurred in the same index hospitalization. Our model did not check collinearity between the dependent variable. As such, we cannot exclude the multicollinearity of these variables. These limitations are compensated by the real world, nationwide disposition of the data, and modification of reporting bias introduced by selective publication of results from specialized centers.

In conclusion, our study results improve the available literature on the protective association of obese BMI subgroups with mortality following AHF hospitalization. We showed a reverse J-shaped relationship between BMI and mortality. Higher BMI was independently associated with protecting against mortality and decreasing LOS, while the mild and moderately obese patient subgroups (BMI 31–39) exhibited the lowest in-hospital mortality in this study. Accordingly, BMI should be addressed carefully and considered by differences in clinical profile and treatment and not solely by an effect of body composition as part of the risk assessment for in-hospital mortality in patients who are hospitalized for AHF.

DATA AVAILABILITY STATEMENT

The Publicly available datasets were analyzed in this study. This data can be found here: Agency for Healthcare research and quality, Nis database, link : hcup-us.ahrg.gov.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GE-G and GR: conceived the idea and design of the study and drafted the paper. MY: drafted the paper. SC: data analysis

and interpretation and provided revisions to the manuscript. HW: data interpretation and provided major revisions to the manuscript. DP, ER, IG, and RA: major provided revisions to the manuscript. OA: conceived the idea and design of the study, principal investigator, and provided revisions to the manuscript. All authors had major contribution.

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The corresponding author affirms that she has listed everyone who contributed significantly to the work. The corresponding author had access to all the study data, takes responsibility for the accuracy of the analysis, and had authority over the manuscript preparation and the decision to submit the manuscript for publication. The corresponding author confirms that all authors read and approved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.855525/full#supplementary-material

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Right Ventricular Dysfunction Predicts Outcome in Acute Heart Failure

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Aim: The severity of cardiac impairment in acute heart failure (AHF) predicts outcome, but challenges remain to identify prognostically important non-invasive parameters of cardiac function. Left ventricular ejection fraction (LVEF) is relevant, but only in those with reduced LV systolic function. We aimed to assess the standard and advanced parameters of left and right ventricular (RV) function from echocardiography in predicting long-term outcomes in AHF.

Methods: A total of 418 consecutive AHF patients presenting over 12 months were prospectively recruited and underwent bedside echocardiography within 24 h of recruitment. We retrospectively assessed 8 RV and 5 LV echo parameters of the cardiac systolic function to predict 2-year mortality, using both guideline-directed and study-specific cutoffs, based on the maximum Youden indices *via* ROC analysis. For the RV, these were the tricuspid annular plane systolic excursion, RV fractional area change, tissue Doppler imaging (TDI) peak tricuspid annular systolic wave velocity, both peak- and end-systolic RV free wall global longitudinal strain (RV GLS) and strain rate (mean RV GLSR), RV ejection fraction (RVEF) derived from a 2D ellipsoid model and the ratio of the TAPSE to systolic pulmonary artery pressure (SPAP). For the LV, these were the LVEF, mitral regurgitant $\Delta P/\Delta t$ (MR dP/dt), the lateral mitral annular TDI peak systolic wave velocity, LV GLS, and the LV GLSR.

Results: A total of 7/8 parameters of RV systolic function were predictive of 2-year outcome, with study cutoffs like international guidelines. A cutoff of $< -1.8\ s^{-1}$ mean RV GLSR was associated with worse outcome compared to $> -1.8\ s^{-1}$ [HR 2.13 95% CI 1.33–3.40 ($p = 0.002$)]. TAPSE:SPAP of > 0.027 cm/mmHg (vs. < 0.027 cm/mmHg) predicted worse outcome [HR 2.12 95% CI 1.53–2.92 ($p < 0.001$)]. A 3-way comparison of 2-year mortality by LVEF from the European Society of Cardiology (ESC) guideline criteria of LVEF > 50, 41–49, and $< 40\%$ was not prognostic [38.6% vs. 30.9 vs. 43.9% ($p = 0.10$)]. Of the 5 parameters of LV systolic function, only an MR dP/dt cutoff of < 570 mmHg was predictive of adverse outcome [HR 1.63 95% CI 1.01–2.62 ($p = 0.047$)].

Conclusion: With cutoffs broadly like the ESC guidelines, we identified RV dysfunction to be associated with adverse prognosis, whereas LVEF could not identify patients at risk.

Keywords: acute heart failure, RV failure, RV dysfunction, ejection fraction, strain, strain rate

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INTRODUCTION

Acute heart failure (AHF) is a leading cause of hospitalization (1, 2) and carries a substantial risk of short- (3) and long-term mortality (4, 5). Echocardiography remains an essential tool in the evaluation of systolic function in all hospitalized AHF patients (6) and can provide crucial management-changing information with swift bedside hemodynamic assessment.

Left ventricular ejection fraction (LVEF) is routinely used as a surrogate for global left ventricular (LV) performance, but the evaluation of LV function remains challenging. Strain imaging has confirmed that a preserved EF does not guarantee "normal" systolic function (7, 8) and there is a highly complex relationship between EF and mortality (9–11). Poor outcomes are observed in those with significantly impaired EF (i.e., < 35–40%) (10, 12) but are also seen in the older (13), more comorbid (14) population with preserved ejection fraction (i.e., > 50%).

This complexity is reflected in the changes within international guidelines which have aimed to identify prognostic LVEF cutoff values, including the recent incorporation of a "mildly reduced ejection fraction" within European guidelines (6, 15) and "borderline/improved" within American guidelines (16). However, the evidence for all the current cutoffs is derived from trials investigating neurohormonal downregulation (17–19) or cardiac resynchronization therapy in *chronic* HF (20). The role of LVEF in AHF remains poorly investigated.

Historically the importance of right ventricular (RV) dysfunction has trailed the LV (21, 22) despite growing evidence that RV systolic dysfunction is an independent predictor of outcome across a range of LVEF (23–27). In fact, in 2006, the underrepresentation of the RV prompted the convocation of a working group by the National Heart, Lung, and Blood Institute to highlight and promote the contemporary understanding of its importance in heart failure (HF) (21).

We hypothesized that RV, rather than LV, systolic dysfunction may be more closely associated with long-term outcomes in AHF. To investigate this, we retrospectively used the study cohort of a prospective, observational study—the Mitral Regurgitation in Acute Heart Failure (MRAHF) study—to evaluate a battery of guideline-suggested and innovative assessments of systolic function of both RV and the LV.

MATERIALS AND METHODS

Outline

The MRAHF study methods are previously published (28). In summary, this study was a prospective, observational cohort investigation over 12 months at a single center. A total of 418 consecutive individuals who presented with signs of heart failure and met objective criteria of AHF with raised point-of-care BNP level > 100 pg/ml were enrolled and all patients underwent comprehensive bedside echocardiography within 24 h of recruitment to confirm AHF. Alternative diagnoses were excluded with patients presenting with sepsis, pulmonary respiratory failure, and chronic heart failure excluded.

Participants were followed up for 24 months and assessed by all-cause mortality through the United Kingdom summary care record system and by the online software Evolve™ (Kainos, United Kingdom) for patient records, including death certificates, used at our hospital.

Trial oversight was by the Ashford and St Peter's NHS Trust Research and Development team and was approved by the institutional review board and ethics committee. All patients gave written informed consent before enrollment in the study. All authors had access to data and this manuscript for review. The experimental design and decision for publication were by Dr. A. Baltabaeva.

Echocardiography

Echocardiography was carried out with G.E. Vivid S70 (GE Healthcare, United States) and analyzed and stored using EchoPac v202.5 (GE Healthcare, United States). Exams were performed with a dedicated protocol (**Supplementary Appendix 1**). Off-line measurements were carried out by two echocardiographers with the British Society of Echocardiography (BSE) level II transthoracic echo (TTE) accreditation. Studies and measurements were cross-referenced by a consultant cardiologist with > 10 years of practice as an imaging expert, based at the Department of Echocardiography for a high-volume cardiothoracic surgical, transplant and tertiary referral center with the European Association of Cardiovascular Imaging (EACVI) accreditation.

The assessment of the left and right atrial and ventricular geometry, RV and LV systolic, and data on calculation of systolic pulmonary artery pressures were obtained using a standard TTE minimum dataset approach advocated by the BSE (29).

Right Ventricular Systolic Parameters

RV systolic function assessment parameters included were the tricuspid annular plane systolic excursion (TAPSE); RV Fractional Area Change (RV FAC); RV Tissue Doppler Imaging (TDI) peak systolic wave velocity (RV S'); two-dimensional RV ellipsoid ejection fraction (RVEF) (30); RV Global Longitudinal Strain (RV GLS), and mean strain rate (RV GLSR) as well as the TAPSE to Systolic Pulmonary Artery Pressure Ratio (TAPSE:SPAP).

The RV GLS was assessed by taking the mean of the peak systolic and end-systolic strain from the basal, mid, and apical RV free wall by off-line analysis on the EchoPac workstation (v202.5) using LV dedicated strain analysis. RV systole was defined as the period between pulmonary valve opening and closure. Deformation of the interventricular septum was not included. RV GLSR was obtained by averaging values from the peak strain rate before pulmonary valve closure and from the basal, mid, and apical free wall segments.

LV systolic function assessment parameters included the LVEF; LV mitral regurgitation $\Delta P/\Delta t$ (LV MR dP/dt) when available; LV TDI lateral mitral annular peak systolic wave velocity (LV S'); LV Global Longitudinal Strain (LV GLS), and strain rate (LV GLSR).

Statistical Analysis

Statistical analysis was carried out using MedCalc v.20.015 (MedCalc® Software Ltd., Belgium). Receiver Operator Curve

(ROC) analyses were carried out on the previously discussed RV and LV systolic function parameters. The optimum cutoff for the prediction of 24-month mortality was estimated by identifying the sensitivity and specificity associated with the maximum Youden Index.

A 24-month mortality analysis was carried out by constructing unstratified Kaplan–Meier survival curves using ROC and guideline cutoffs. Hazard ratios were estimated using an unadjusted Cox regression model, with statistical significance being assessed using the Log-rank test. For all statistical comparisons in this study, significance was defined as a 2-sided α-value of 0.05; 4 patients were lost to follow-up leaving 414/418 (99.0%) with available 2-year mortality data. Patients lost to follow-up were omitted from KM analysis.

Patients with poor acoustic windows and unmeasurable parameters were also omitted from the analysis. The numbers available for each RV parameter are as follows: TAPSE 411/414, RVFAC 409/414, SPAP 401/414, TAPSE/SPAP 398/414, 2D RVEF 412/414, RV end-systole GLS 346/414, RV peak GLS 346/414, RV average GLSR 346/414 and RV S' 398/414. For the LV: LVEF 411/414, LV S' 404/414, LV MR dP/dT 352/414, LV GLS 411/414 and LV GLSR 411/414.

If the unadjusted Cox regression model Log-rank tests from the ROC analysis failed to meet the 2-sided α-value of 0.05, further Cox regression models were constructed using ESC-guideline-specified thresholds, if not previously carried out (i.e., LVEF). Once again, statistical significance was assessed using the Log-rank test.

To determine whether the impact of LV and RV systolic assessment changes, and is independently associated with outcome, when analyzed as continuous variables instead of categorical variables with cutoffs, we constructed logistic regression models including 8 relevant cardiovascular demographic and clinical comorbidities previously included in the original results of the MRAHF study (28). These were age, gender, body mass index (BMI), previous history of cerebrovascular accident (CVA) or coronary artery disease, and a prior diagnosis of hypertension, diabetes mellitus, or chronic pulmonary obstructive disease (COPD).

These models included 2 continuous, global assessments of LV and RV systolic function, LVEF and RV FAC, or LVEF and RVEF. We included both a guideline-directed assessment (RVEF) and a more experimental assessment (2D RVEF) to cross-reference the importance of RV global systolic performance with any method of assessment. In these models, the dependent variable was the binary outcome of survival vs. mortality at 2 years. The Enter method was used where all variables were included in a single step. The significance level was defined at a threshold of 0.05 and the odds ratio was calculated with 95% confidence intervals.

RESULTS

Using the ESC guideline-suggested cutoffs of RV and LV systolic function, Kaplan–Meier estimates were constructed and are displayed in **Figure 1**. The cutoff of 1.7 cm for TAPSE was associated with a worse 2-year prognosis for impaired vs. preserved longitudinal RV systolic function [HR 1.57 95% CI 1.15–2.16 (p = 0.005)]. The cutoff of 35% for RV FAC was also associated with a worse 2-year prognosis for impaired vs. preserved FAC [HR 1.38 95% CI 1.00–1.89 (p = 0.049)]. Using an ESC-suggested 3-phenotype model of HF, LVEF was assessed in a 3-way comparison of > 50, 41–49, and < 50%, but these cutoffs were not significantly associated with outcome (**Figure 1** and **Supplementary Table 1**).

Assessments of RV and LV systolic dysfunction *via* ROC analyses (**Supplementary Table 2** and **Supplementary Figures 1, 2**) identified the maximum Youden index-associated criteria which were compared with guideline-suggested cutoffs where available (**Table 1**). There were broad similarities between the cutoffs derived from the MRAHF data ROC analyses and the guideline-recommended cutoffs, apart from the LV GLS.

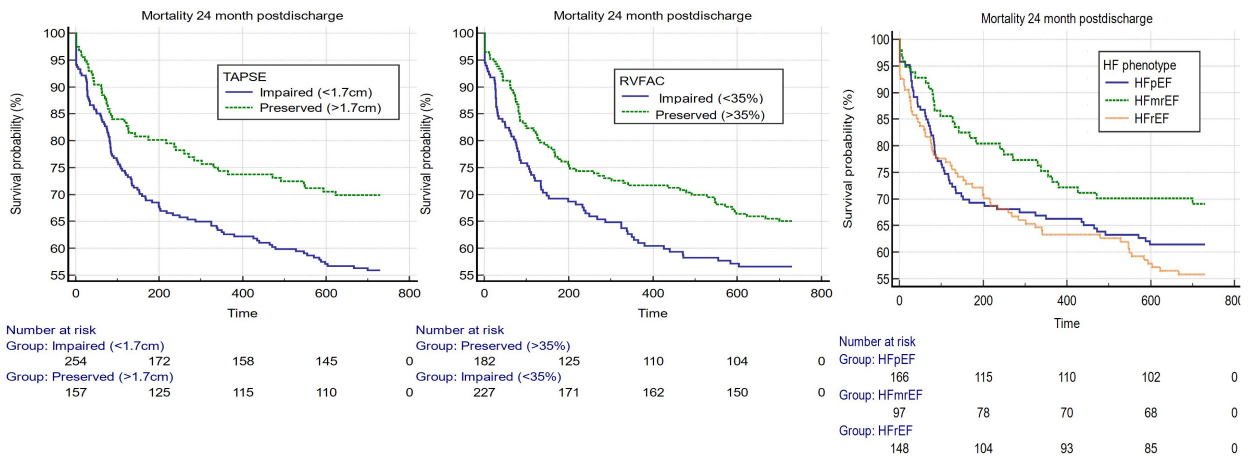

FIGURE 1 | Kaplan–Meier estimates 3 guideline-suggested cutoffs of the most used systolic assessments of both the right and left ventricles. From left to right, TAPSE (cutoff 1.7 cm) (p-value = 0.0051), RV FAC (cutoff 35%) (p-value = 0.049), and LVEF (cutoffs for heart failure with preserved ejection fraction (LVEF > 50%), heart failure with mildly reduced ejection fraction (LVEF 41–49%) and heart failure with reduced ejection fraction (LVEF < 40%) (p-value = 0.109).

TABLE 1 | Assessments of right ventricular (RV) and left ventricular (LV) systolic dysfunction.

Systolic assessment	Younden index cutoff	Guideline binary cutoff
Right ventricle		
TAPSE (cm) (n = 411)	1.6	1.7
RV FAC (%) (n = 409)	38.2	35
RV S' (m/s) (n = 398)	0.09	0.095
2D RVEF (%) (n = 412)	46.9	n/a
RV peak GLS (%) (n = 346)	−18.6	−20
RV end-systole GLS (%) (n = 346)	−18	n/a
Mean RV GLSR (s^{-1}) (n = 346)	−1.8	n/a
TAPSE:SPAP (cm/mmHg) [n = 398]	0.0268	n/a
Left Ventricle		
LVEF (%) (n = 411)	48	50*
LV S' (m/s) (n = 404)	0.06	n/a
LV GLS (%) (n = 411)	−6.32	n/a
LV GLS rate (s^{-1}) (n = 411)	−0.86	n/a
LV MR dp/dt (mmHg/s) (n = 352)	570	n/a

**ESC guidelines identify 50% as the cutoff for preserved EF but they do not suggest a binary cutoff for LVEF and instead delineate into 3 phenotypes - heart failure-preserved ejection fraction, heart failure with mildly reduced ejection fraction, heart failure with reduced ejection fraction. TAPSE, tricuspid annular plane systolic excursion; RV FAC, RV Fractional Area Change; RV S', RV Tissue Doppler Imaging (TDI) tricuspid annular peak systolic wave velocity; 2D RVEF, two-dimensional RV ellipsoid ejection fraction; RV inferior wall GLS, RV Inferior Wall Global Longitudinal Strain; TAPSE:SPAP, TAPSE to Systolic Pulmonary Artery Pressure Ratio; LVEF, left ventricular ejection fraction; LV S', LV TDI lateral mitral annular peak systolic wave velocity; LVGLS, LV global longitudinal strain; LV MR dp/dt, LV GLS rate (LV GLSR) and LV mitral regurgitation $\Delta p/\Delta t$.*

TABLE 2 | Assessments of right ventricular (RV) and left ventricular (LV) systolic dysfunction with binary cutoffs as determined by the criteria associated with the maximum Youden index.

Systolic assessment	Binary cutoff	Hazard ratio (95% CI)	*p*-value
Right ventricle			
TAPSE (cm)	≤1.6	1.50 (1.10–2.05)	**0.011**
RV FAC (%)	≤38.2	1.54 (1.13–2.11)	**0.007**
RV TDI S wave velocity (m/s)	≤0.09	1.26 (0.91–1.75)	0.170
RV peak inferior free wall GLS	>-18.6	1.67 (1.08–2.59)	**0.021**
RV end-systole inferior free wall GLS (%)	>-18	1.87 (1.18–2.95)	**0.008**
RV mean GLS rate (s^{-1})	>-1.8	2.13 (1.33–3.40)	**0.002**
2D RVEF (%)	≤46.9	1.50 (1.10–2.06)	**0.010**
TAPSE:SPAP	>0.0268	2.12 (1.53–2.92)	**<0.001**
Left ventricle			
LV ejection fraction (%)	>48	1.08 (0.78–1.50)	0.641
LV TDI S wave velocity (m/s)	≤0.06	1.22 (0.88–1.79)	0.231
LV GLS (%)	>-6.32	1.25 (0.88–1.79)	0.210
LV GLS rate (s^{-1})	≤-0.86	1.28 (0.89–1.83)	0.186
LV MR dp/dt (mmHg)	≤570	1.63 (1.01–2.62)	**0.047**

Hazard ratios indicate the hazard ratio associated with all-cause mortality at 2 years constructed from unadjusted Cox regression analysis, with p-values determined from the Log-rank test. Tricuspid Annular Plane Systolic Excursion (TAPSE); RV Fractional Area Change (RV FAC); RV Tissue Doppler Imaging systolic velocities (RV TDI S wave velocity), two-dimensional RV ellipsoid ejection fraction (2D RVEF); RV Inferior Wall Global Longitudinal Strain (RV inferior wall GLS); TAPSE to Systolic Pulmonary Artery Pressure Ratio (TAPSE:SPAP); LV mitral regurgitation $\Delta p/\Delta t$ (LV MR dp/dt). p-values in bold are statistically significant according to our threshold of 0.05.

The greatest differences between the MRAHF and guideline-discussed cutoffs were for LV GLS (−6.32 vs. c.−20%), which might be a reason the latter did not make into an official cutoff in chamber quantification release.

Of the 8 RV parameters assessed, 7 were significantly associated with worse outcomes (**Table 2**). The RV GLSR > -1.8 s^{-1} vs. < -1.8 s^{-1} exhibited the highest hazard ratio of 2.13 [95% CI 1.33–3.40 (p = 0.002)]. The only RV parameter which was not significantly associated with a worse outcome was the RV S' velocity $\leq$ 0.09 m/s vs. $>$ 0.09 m/s (p = 0.170). The unadjusted Kaplan–Meier curves of these cutoffs are displayed in **Figure 2**. An ESC guideline abnormality threshold of 0.095 m/s Lang et al. (31) was then assessed but was not significantly associated with the outcome (p = 0.169) (**Supplementary Table 3**).

Of the binary cutoffs of the 5 LV parameters only the MR dP/dt < 570 mmHg vs. > 570 mmHg was significantly associated with a worse outcome, hazard ratio 1.62 [95% CI 1.01–2.62 (p = 0.047)] (**Table 2**). The unadjusted Kaplan–Meier estimates of these cutoffs are displayed in **Figure 3**.

RV FAC and LVEF were included in a logistic regression model as continuous variables alongside 8 cardiovascular comorbidities previously included in the MRAHF 2-year outcome study (28) (**Supplementary Table 4**). RV FAC was an independent predictor of outcome (coefficient −0.02, odds ratio 0.98 [95% CI 0.96–1.00 (p = 0.028)] and LVEF was not (p = 0.794). Age, CKD, and COPD were also independent predictors of outcome. The model area under the ROC curve (AUC) was 0.744 (95% CI 0.699–0.786). To investigate if this effect was limited only to FAC, we also included another global assessment of RV systolic function with the ellipsoid assessment of RV ejection fraction, which was also an independent predictor of outcome OR 0.98 [95% CI 0.96–1.00 (p = 0.01)] (**Supplementary Table 5**).

DISCUSSION

This study characterized the prognostic impact of a battery of RV and LV systolic non-invasive assessments by echocardiography. The study-specific cutoffs for 13 parameters of systolic RV and LV function were broadly like the ESC guidelines, except for LV GLS. Overall, this study suggests that poor RV function determines outcome in AHF.

Most RV systolic assessments were associated with adverse outcome at a 2-year follow-up and this study confirmed the prognostic strength of standard parameters of RV function such as TAPSE and RVFAC which have well-established evidence in risk stratification for chronic heart failure patients (32–35). This study extends the more limited evidence for their role in prognosis in the setting of AHF (36) and adds weight to the application of current guideline cutoffs in this context.

RV longitudinal deformation obtained from the speckle-tracking technique has already been proven to be of prognostic importance despite load dependency (37) across a range of pathologies (38). RV strain proved to be a feasible

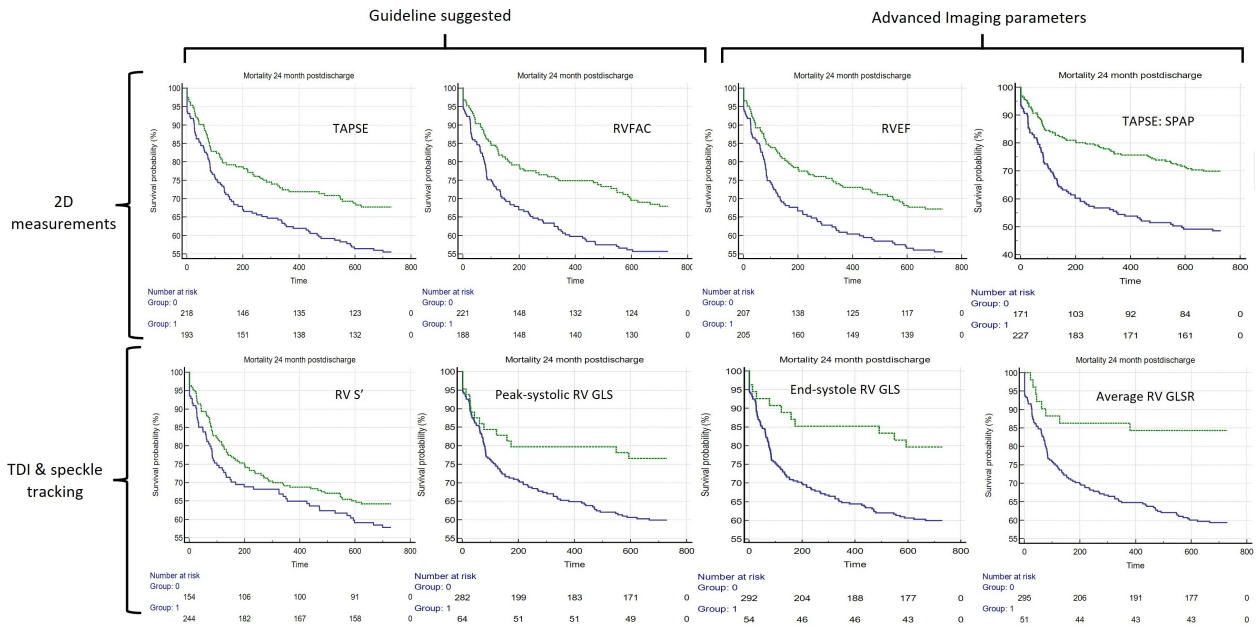

FIGURE 2 | Unadjusted Kaplan–Meier curves comparing 8 different assessments of RV systolic function, in all. The green lines represent the unimpaired systolic function and blue lines represent impaired systolic function. The echo parameter assessment of systolic function is displayed in the top-right corner of each (clockwise from top-left): TAPSE $\leq$ 1.6 cm, RV FAC $\geq$ 38.2%, 2D ellipsoid RVEF $<$ 46.9%, TAPSE:SPAP $<$ 0.0268 (cm/mmHg). RV S' $\leq$ 0.09 m/s, Peak systolic RV GLS $>$ −18.6%, End-systole RV GLS $>$ −18% and RV mean GLSR $>$ −1.8 s^{-} TAPSE, Tricuspid Annular Plane Systolic Excursion; RV FAC, RV Fractional Area Change; RV S' RV Tissue Doppler Imaging peak systolic velocity; RVEF, two-dimensional RV ellipsoid ejection fraction; RV GLS, RV Free Wall Global Longitudinal Strain; mean RV GLSR, mean RV free wall global longitudinal strain rate; TAPSE:SPAP, TAPSE to Systolic Pulmonary Artery Pressure Ratio.

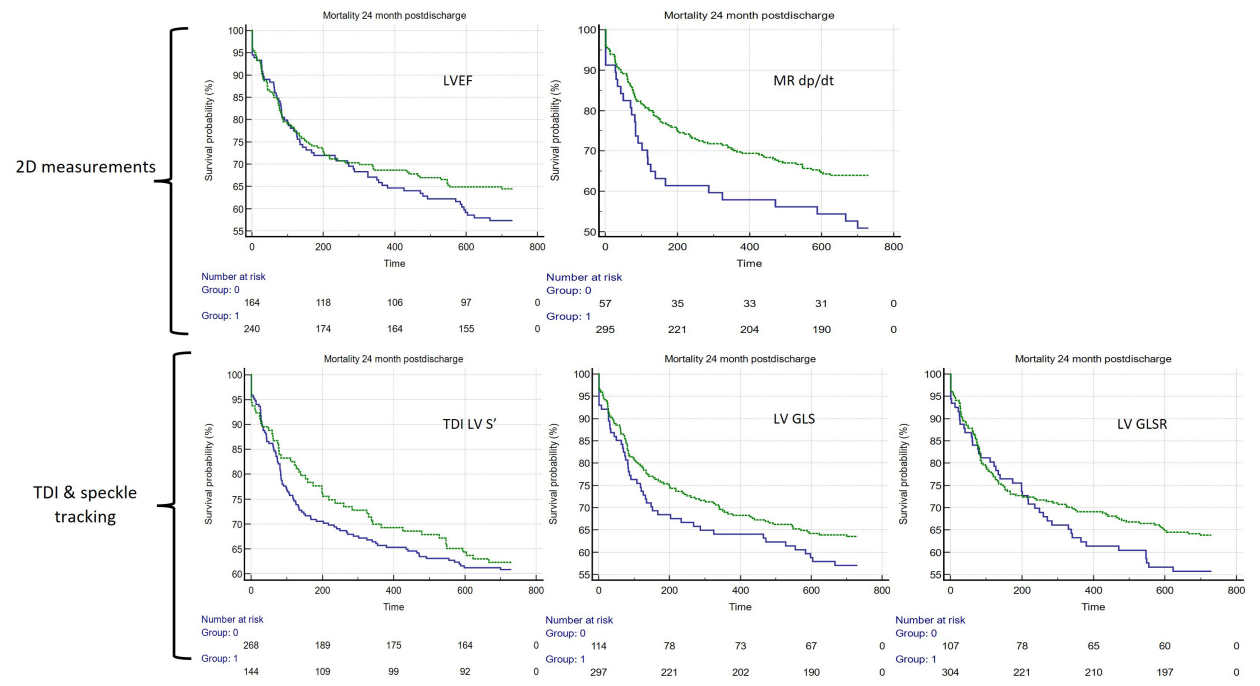

FIGURE 3 | Unadjusted Kaplan–Meier curves comparing 5 different assessments of LV systolic function. In all panels, green lines represent preserved systolic function and blue lines represent impaired function (clockwise from top-left): LVEF $>$ 48%, LV MR dp/dt $<$ 570 mmHg/s, LV TDI S' $\leq$ 0.06 m/s, LV GLS $>$ −6.32%, LV GLSR $>$ −0.86 s^{-1}. LVEF, LV ejection fraction; LV MR dp/dt, LV mitral regurgitation $\Delta p/\Delta t$; LV S', LV Tissue Doppler Imaging (TDI) lateral mitral annular peak systolic velocity; LV GLS, LV Global Longitudinal Strain; LV GLSR, LV global longitudinal strain rate.

and reproducible echocardiographic technique available in 83.5% of our cohort, all of whom were acutely unwell and breathless. It provided important additional information on RV mechanics which may be because RV longitudinal shortening is more important for systolic function than circumferential shortening (39).

In this analysis, we used RV free wall strain rather than RV global strain to exclude the septum which is largely influenced by LV myocardial function (31). Meanwhile, the strain rate, a non-invasive measure of myocardial contractility (40), was complimentary to deformation parameters and was able to discriminate those at risk of worse outcomes despite known problems with noise (41). To our knowledge, this is the first study to assess the role of RV GLSR in AHF. Given that our study data correspond well to international guidelines for RV systolic assessment, the study-specific cutoff of $-1.8\ s^{-1}$ may be of relevance to further research on the role of echo assessment of RV strain rate analysis.

To test the importance of RV function, we applied several other innovative assessments of RV systolic function which are not part of international guidelines. We applied a two-dimensional ellipsoid model to estimate RVEF which has previously been confirmed to be non-inferior to TAPSE and RV FAC in this cohort of patients (30). In our logistic regression model, it was independently associated with mortality.

We also included the TAPSE/SPAP ratio, a relatively novel estimate of the right ventricular-vascular coupling to adjust to afterload caused by the left heart disease. It has been suggested to predict the outcome in heart failure with preserved ejection fraction (HFpEF) (22) and pulmonary hypertension (42). In unadjusted analysis, our study data confirmed it as an additional value as a non-invasive prognostic parameter within the broad "all-comers" AHF setting of this study.

Given the good correlation of our study dataset to international guideline cutoffs, the cutoffs for more novel assessments such as 2D RVEF and TAPSE/SPAP ratios provided here may be of relevance for further research. Subject to confirmation by future studies in AHF, we think that 2D echo-derived RVEF and the SPAP/TAPSE ratio have the potential to play a significant role in clinical practice given both the ease of echo data acquisition and their potential prognostic significance.

RV S' was the only right-sided parameter that failed to reliably predict the outcome. This may be the result of angle-dependency which becomes an issue with RV remodeling and image acquisition in an acute setting where time limitations for scanning the unwell patient do not allow correct positioning of echo windows.

Unlike RV function parameters, LVEF and most of the LV systolic function parameters were not associated with the outcome when used either as a binary cutoff, or in a guideline-directed 3-way comparisons of HFpEF, heart failure with mildly reduced ejection fraction (HFmrEF), and heart failure with reduced ejection fraction (HFrEF).

The evaluation of ESC-suggested cutoffs for HFpEF, HFmrEF, and HFrEF echoed the results of retrospective analysis of the ASCEND-HF study (11) with a pattern of similarly poor, unadjusted, outcomes between HFrEF and HFpEF, with a slightly better prognosis of HFmrEF. Because of this complex relationship, LVEF remains a poor tool to predict outcomes in the setting of AHF, where there is a heterogeneous patient population who may present with advanced diastolic dysfunction, pulmonary hypertension, RV failure, or a combination of these, as examples. We feel the overly simplistic approach to relying on LVEF to assess global cardiac performance is outdated.

In the most recent guidelines of chamber quantification from EACVI/ESC, a value of −20% is identified to be suggestive of "healthy" myocardial strain (31) but no clear cutoff exists to identify poor LV longitudinal deformation, in part due to the heterogeneity of vendor and software measurements. In this study, a cutoff was substantially lower than what the guidelines suggest is "healthy" (−6%) and could not delineate those at risk of poor outcome. The role of GLS in AHF remains an important avenue for further investigation.

The only assessment of LV systolic function which displayed a discriminatory capacity for outcome was the MR dP/dt. This is a relatively load-independent reflection of global left ventricular contractility (43) which corresponds to the instantaneous pressure difference between the left atrium and LV (44). The cutoff suggested from the ROC analysis (570 mmHg) is broadly in keeping with the cutoff of 600 mmHg/s (45), indicating "severe LV dysfunction" because of advanced myocardial disease.

Limitations

There are several limitations of this study. It was conducted at a single center which limits the generalizability of its findings and further confirmatory studies are warranted. We have not further investigated LVEF as a continuous variable in the sub-set of individuals with significantly impaired EF (i.e., < 40%), where there is evidence for worsening outcome as LVEF deteriorates further (12). However, the worse outcome associated with severely low MR dP/dt (< 570 mmHg) is indicative of this.

Echo assessment of RV strain was adapted from the LV strain analysis software with timing used from pulmonary valve opening and closure, ideally, an LV-specific software should be used. We have also not carried out a multivariate analysis of every RV parameter which was shown to be predictive of the outcome of an unadjusted assessment. However, we selected two global assessments of RV systolic function (RVFAC and RVEF) and performed logistic regression analysis with cofounders selected from our previous MRAHF study results. We selected RVFAC as a guideline-directed and RVEF as a more experimental parameter.

Summary

In patients presenting with AHF, RV decompensation, confirmed by both load-dependent and independent parameters, is the main determinant of a long-term outcome. We have tested the novel parameters of RV function which proved to be feasible and significant as additional tools for standard RV assessment. This study suggests that a combination of left heart-induced afterload and depleted RV myocardial reserve plays an important role. This highlights the need to move past an overly simplistic reliance on the LV ejection fraction when assessing cardiac performance in

AHF. More attention should be paid to LV dP/dt when the MR jet is available for accurate assessment.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ashford and St. Peter's NHS Trust Institutional Review Board and Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AB developed the experimental design with inputs from IB, DF, IJ, and PS. MB and AB wrote the manuscript with editing available to all authors. MB carried out the statistical analysis. AB and MB made the final decision for submission. All authors had access to the manuscript and data before publication.

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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.2022.911053/full#supplementary-material

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Support Pressure Acting on the Epicardial Surface of a Rat Left Ventricle—A Computational Study

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The present computational study investigates the effects of an epicardial support pressure mimicking a heart support system without direct blood contact. We chose restrictive cardiomyopathy as a model for a diseased heart. By changing one parameter representing the amount of fibrosis, this model allows us to investigate the impairment in a diseased left ventricle, both during diastole and systole. The aim of the study is to determine the temporal course and value of the support pressure that leads to a normalization of the cardiac parameters in diseased hearts. These are quantified *via* the end-diastolic pressure, end-diastolic volume, end-systolic volume, and ejection fraction. First, the amount of fibrosis is increased to model diseased hearts at different stages. Second, we determine the difference in the left ventricular pressure between a healthy and diseased heart during a cardiac cycle and apply for the epicardial support as the respective pressure difference. Third, an epicardial support pressure is applied in form of a piecewise constant step function. The support is provided only during diastole, only during systole, or during both phases. Finally, the support pressure is adjusted to reach the corresponding parameters in a healthy rat. Parameter normalization is not possible to achieve with solely diastolic or solely systolic support; for the modeled case with 50% fibrosis, the ejection fraction can be increased by 5% with purely diastolic support and 14% with purely systolic support. However, the ejection fraction reaches the value of the modeled healthy left ventricle (65.6%) using a combination of diastolic and systolic support. The end-diastolic pressure of 13.5 mmHg cannot be decreased with purely systolic support. However, the end-diastolic pressure reaches the value of the modeled healthy left ventricle (7.5 mmHg) with diastolic support as well as with the combination of the diastolic and systolic support. The resulting negative diastolic support pressure is −4.5 mmHg, and the positive systolic support pressure is 90 mmHg. We, thereby, conclude that ventricular support during both diastole and systole is beneficial for normalizing the left ventricular ejection fraction and the end-diastolic pressure, and thus it is a potentially interesting therapy for cardiac insufficiency.

Keywords: cardiac assist device, cardiomyopathy, epicardial heart support, left ventricle, support pressure, fibrosis

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1. INTRODUCTION

Cardiovascular diseases remain the leading cause of death worldwide (1). If conservative therapy is inadequate due to the pathological condition of the heart, there are usually only two options remaining to treat this condition: cardiac assist devices or heart transplantation. However, there is a chronic shortage of donors. For example, in Germany, 320 patients received a donor heart in 2020, while the remaining 700 patients were still on the waiting list at the end of that year (2). While heart transplantation is a superior option in terms of survival and functional capacity, significant improvements in the field of cardiac assist devices have resulted in promising solutions that close the gap between availability and demand for donor's hearts. Over the last decades, various types of mechanical pumping systems, known as left ventricular (LV) assist devices, have been developed (3). They are used as an invasive form of therapy to directly support blood circulation. In principle, these systems mostly work to bypass the weakened heart. Such cardiac assist devices can relieve the load on the heart and become either a short- to medium-term bridge to treat cardiac insufficiency until possible transplantation (bridge-to-transplantation) or a permanent solution (destination therapy). Despite the rapid development of new devices and improvements in post-surgery survival and functional capacity nearing those of heart transplantation, there are still many problems to solve, including bleeding, thrombosis, strokes, and infections (3, 4).

For the most part, these problems are caused by the direct contact of blood with the cardiac assist device. Several approaches to avoiding direct blood contact, commonly referred to as direct cardiac compression devices, have been proposed to overcome such difficulties. The first bridge-to-transplantation based on a pneumatic compression cup (5) was followed by several other innovative approaches, e.g., (6–9). Review articles provide a more detailed overview of available direct cardiac compression devices (3, 10). For example, systems from AdjuCor GmbH (8) and CorInnova Inc. (9), currently in the preclinical testing phase, provide support during systole and are minimally invasive implants. The common principle of these devices is that they develop a pressure (force per area) that acts on the epicardial surface. Theoretically, a diseased heart can be thereby supported solely in the diastole, solely in the systole, or in both cardiac phases. However, most direct cardiac compression devices only work during systole. The objective of our computational study is to investigate (1) if an application of support pressure on the LV during both the diastolic and systolic phases is able to normalize the ejection fraction (EF) and left ventricular end-diastolic pressure (EDP) and (2) how much pressure is needed. The current study does not focus on the modeling of a particular cardiac assist device.

In general, the functionality of a direct cardiac compression device is mainly determined by the improvement in the pump function of a diseased heart. In the present study, it is quantified *via* the EF and EDP. However, the influence of a direct cardiac compression device on cardiac performance is not straightforward to compute, due to, e.g., the varying mechanical properties of active biological cardiac tissue that is undergoing complex deformations. The complex orthotropic tissue structure of the healthy myocardium, which can be modeled with different approaches (11–13), plays an important role. Furthermore, the amount of fibrosis in the ventricular wall strongly influences cardiac performance. Over the course of many heart diseases, there is a remodeling process that leads to an increase in fibrosis (14, 15). This is often independent of the triggering disease and can occur after cardiac volumetric pressure loading or ischemia (16). Various studies indicate a correlation between diastolic function and the amount of myocardial fibrosis (17, 18). Regardless of the functional impact of primary cardiomyopathy, the fibrosis progressively limits the diastolic function of the ventricle (19). As long as the condition can be systolically compensated, there is no reduction in the overall myocardial function (heart failure with preserved ejection fraction) (20). However, if the amount of fibrosis exceeds a certain level, the reduction in the diastolic function can no longer be systolically compensated, and the overall myocardial function is reduced (19). It has been shown that ventricular fibrosis inversely correlates with the ejection fraction, both in rats (21) and humans (17). Since the functional impairment resulting from the diastolic dysfunction due to myocardial fibrosis is the terminal stage of most cardiac diseases (17), a better understanding of the role of diastolic function and its relationship with the amount of fibrosis is important. We, therefore, calculated the effects of increasing fibrosis on cardiac function in a computational model of restrictive cardiomyopathy. Studies using postmortem mechanical testing in animal models after myocardial infarction have shown that the fibrosis leads to the stiffening of the cardiac tissue (22–25).

To optimally support a diseased heart *via* a direct cardiac compression device, two main factors play an important role: the time evolution of the force generated by the device and its maximum and minimum values. To date, how these factors influence cardiac function has not been investigated in detail. In this early research study, a computer simulation offers many advantages. First, it saves time compared to experimental testing. Second, there are beneficial synergies between computational modeling and simulation and experimental testing; for example, various parameters can be predicted that cannot be directly measured. Third, modeling and simulation can eventually be used to improve the adaptivity of such a system at a patient-specific level, making it a fundamental instrument in modern and future medicine. So far, a few finite element-based computational models that account for the coupling between a cardiac assist device and the heart have been developed. The existing work presents simulations for the ventricular pumps that are coupled with a univentricular (26) or biventricular heart models (27) *via* a cannula. A computational model for the innovative support system from AdjuCor GmbH is presented in Hirschvogel et al. (28). Recently, Chavanne (29) presented a simulation of a dielectric elastomer actuator-based aortic plaster interacting with a lumped parameter model for the heart.

The present study uses a finite element-based computer simulation. The study models an actively contracting rat LV with different amounts of fibrosis (30) and investigates the influence of direct cardiac compression devices supporting the LV on

cardiac performance during both diastolic and systolic phases. As the current study does not focus on modeling a particular cardiac assist device, for simplicity, the model represents a support pressure acting on the outer surface of the modeled LV, as depicted in **Figure 2A**. During diastole, a negative support pressure is modeled to facilitate the ventricular filling, whereas a positive support pressure in systole is applied to eventually support the blood outflow from the LV. In particular, we investigate whether a support pressure calculated as a difference between the modeled ventricular pressure in a fibrotic and healthy LV would lead to a normalization of cardiac function parameters. Subsequently, the maximum positive systolic and negative diastolic support pressures are optimized such that the EF, EDP, and left ventricular end-diastolic volume (EDV) of a control healthy rat LV are restored. In the present study, a simplified rat LV ventricle is computationally modeled.

2. METHODS

This section describes the computational model of the contraction of a rat LV and the associated numerical experiments.

2.1. Modeling

2.1.1. Balance Equations and Support Pressure Boundary Conditions

The field equation governing the state of the material point $\boldsymbol{X} \in \Omega_0$ at time t, $t \in [t_0, t_f]$ (t_0 and t_f are the initial and final times, respectively) can be formulated. The mechanical field equation is the balance of linear momentum together with the boundary conditions on the boundaries Γ_φ, Γ_1, and Γ_2:

$$0 = \mathrm{Div}[\mathbf{F} \cdot \mathbf{S}] + \boldsymbol{F}^{\varphi} \quad \text{in } \Omega_0, \tag{1}$$

$$\boldsymbol{\varphi}(\boldsymbol{X}, t) = \bar{\boldsymbol{\varphi}} \quad \text{in } \Gamma_\varphi, \quad \boldsymbol{T}(\boldsymbol{X}, t) = -p_i J \mathbf{F}^{-T} \boldsymbol{N} \quad \text{in } \Gamma_i,\ i \in \{1, 2\} \tag{2}$$

where $\mathbf{F}$ is the deformation gradient with its determinant $J = \det \mathbf{F}$, $\mathbf{S}$ is the second Piola Kirchhoff stress (PK2), $\boldsymbol{F}^{\varphi}$ is the external mechanical body force, $\boldsymbol{\varphi}$ is the displacement with prescribed value $\bar{\boldsymbol{\varphi}}$ on the boundary Γ_φ, $\boldsymbol{T}$ is the surface traction vector in the reference configuration, $\boldsymbol{N}$ is the outer unit normal in the reference configuration and p_i are the prescribed values of the pressures acting on the boundaries Γ_i, $i \in \{1, 2\}$. The pressure p_1 in the LV, obtained from the three-element Windkessel model (representing the interaction between the LV, aorta, and peripheral arteries), serves as a Neumann boundary condition on the endocardial surface Γ_1 whereas the support pressure p_2 serves as a Neumann boundary condition on the epicardial surface Γ_2. The basis of the LV (Γ_φ) is fixed in the longitudinal direction; additionally, the nodes on the outer basis are fixed in all directions (31).

2.1.2. Constitutive Equations

In the present study, the total PK2 is additively decomposed into the passive part $\mathbf{S}_{pas}$ and the active part $\mathbf{S}_{act}$ (31–35), namely

$$\mathbf{S} = \mathbf{S}_{pas} + \mathbf{S}_{act}. \tag{3}$$

Based on Martonová et al. (23), we model the LV as a mixture of the intact myocardium and fibrotic scar structure. The amount of fibrosis, *fib*, serves as a scaling factor. Furthermore, we assume that only the intact muscle tissue is able to contract (30, 36), and therefore the active part of the stress tensor is as well-scaled by the amount of fibrosis. The resulting PK2 reads as

$$\mathbf{S} = fib\, \mathbf{S}^s_{pas} + (1 - fib)(\, \mathbf{S}^m_{pas} + \mathbf{S}^m_{act}), \tag{4}$$

where the superscripts s and m correspond to the scar and intact myocardium and the subscripts *pas* and *act* to the passive and active parts of the PK2, respectively. In particular, by setting $fib = 0$, only the intact cardiac tissue is modeled. As proposed in Martonová et al. (23) for the passive part, the scar structure is modeled as a transversely isotropic material and the intact myocardium as an orthotropic material, according to Holzapfel and Ogden (37). The active contraction of the intact myocardium is modeled following the simple time-dependent approach from Pfaller et al. (38):

$$\mathbf{S}^{act}(t, \boldsymbol{f}_0, \boldsymbol{n}_0) = T(t)(\boldsymbol{f}_0 \otimes \boldsymbol{f}_0 + \nu \boldsymbol{n}_0 \otimes \boldsymbol{n}_0). \tag{5}$$

The temporal evolution of the active tension T depicted in **Figure 1** is obtained by using the parameters shown in **Table A1**. For the equations describing $T(t)$, we refer to the **Appendix** or the original study (38).

Based on the experimental evidence (39) and our previous study (30), in addition to the contraction in the fiber direction $\boldsymbol{f}_0$, reduced active stress along the cross-fiber direction $\boldsymbol{n}_0$ is added and scaled by ν in Equation (5). We note that the electromechanical coupling is omitted in this study as it would introduce further complexity and variability. However, the model can be coupled with a model for electrical excitation (38). The constitutive model is applied to the generic ellipsoidal rat LV based on the data from echocardiography. For more details regarding the geometry, fiber orientation, compressibility, and the Windkessel model serving as a boundary condition in Equation (1), we refer to previous study (35).

2.1.3. Diastolic Filling

The blood flow I between the left atrium and LV is given as

$$I = \frac{\Delta p}{R_{v1}}, \tag{6}$$

where Δp is the pressure difference between the left atrium and LV, and R_{v1} is the resistance of the atrioventricular valve. The pressure in the atrium p_a is modeled according to the following equation:

$$p_a(t) = \begin{cases} (1 + fib)p_{a1} & \text{if } t \le t_a \\ (1 + fib)(p_{a1} + p_{a2} \sin(t - t_a)) & \text{if } t_a < t \le t_{ed} \\ (1 + fib)p_{a1} & \text{if } t > t_{ed} \end{cases} \tag{7}$$

where p_{a1} and p_{a2} are the minimum and maximum atrial pressures, and t_a, t_{ed} model the onset of the atrial and ventricular

contraction, respectively. The resulting curve is shown in **Figure 1**. We note that the atrial pressure is as well-scaled by the amount of fibrosis, allowing us to model the higher EDP observed in rats with different amounts of fibrosis after myocardial infarction (40). To avoid an unlimited blood inflow from the atrium to the LV, the maximal EDV is restricted to that of the control rat.

2.2. Numerical Experiments

In the following, $p(t)$ defines the support pressure acting on the epicardial surface of the LV. Three numerical experiments are performed.

First, the amount of fibrosis is varied from 0% to 60% in order to compare the cardiac performance represented by the EF in the fibrotic rat LV at different fibrosis stages without a support pressure.

Second, a support pressure is applied in order to increase the diastolic and systolic performance of the diseased LV at different fibrosis stages. The support pressure is computed as a difference between the left ventricular pressure in the control and diseased LV at each time point during the cardiac cycle (**Figure 2B**), namely

$$p(t) = p^0_{LV}(t) - p^{fib}_{LV}(t), \tag{8}$$

where p^0_{LV} is the pressure in the control LV ($fib = 0$), and p^{fib}_{LV} is the left ventricular pressure in a fibrotic LV for different amounts of fibrosis. In these examples, we consider $fib \in \{0.1, 0.3, 0.5\}$.

Third, the support pressure displayed in **Figure 2C** and obeying the following equation is applied on exemplary fibrotic LVs, namely $fib \in \{0.3, 0.5\}$.

$$p(t) = \begin{cases} p_{min} & \text{if } t \le t_{ed} \\ p_{min} + \frac{p_{max}-p_{min}}{t_\Delta}(t - t_{ed}) & \text{if } t_{ed} < t \le t_{ed} + t_\Delta \\ p_{max} & \text{if } t_{ed} + t_\Delta \le t \le t_{es} \\ p_{max} - \frac{p_{max}-p_{min}}{t_\Delta}(t - t_{es}) & \text{if } t_{es} < t \le t_{es} + t_\Delta \\ p_{min} & \text{if } t > t_{es} + t_\Delta \end{cases} \tag{9}$$

As mentioned in the Introduction, three possibilities for supporting a weakened heart can be distinguished: pure diastolic, pure systolic, and combined diastolic and systolic support. For the first possibility, we aim to regain EDP^* and EDV^* of the healthy control LV (within given tolerances tol_1, tol_2). For the second and third possibilities, we additionally aim to nearly reach ESV^* of the healthy control LV. Note that EDP and EDV depend on the value of p_{min} only, i.e., $EDP(p_{min})$, $EDV(p_{min})$, while ESV depends on both values p_{min} and p_{max}, i.e., $ESV(p_{min}, p_{max})$. We started with the determination of the optimal negative support pressure during the diastole $p_{min} = p^*_{min}$ in Equation (9). In a second step, only systolic support is considered, i.e., p_{min} in Equation (9) is set to zero, and the maximal positive support pressure $p_{max} = p^*_{max-sys}$ is determined. In the last step, a combination of diastolic and systolic support is assumed. Therefore, p^*_{min} from the first step is fixed, and the optimal systolic support $p_{max} = p^*_{max}$ is to be found. We note that $p^*_{max-sys} \neq p^*_{max}$ hold in general, as the end-diastolic states are different in both cases. For these three steps, the following algorithm is performed:

1. Diastolic support: Find p^*_{min} so that $|EDV(p^*_{min}) - EDV^*| \le tol_1$ and $|EDP(p^*_{min}) - EDP^*| \le tol_2$ are fulfilled

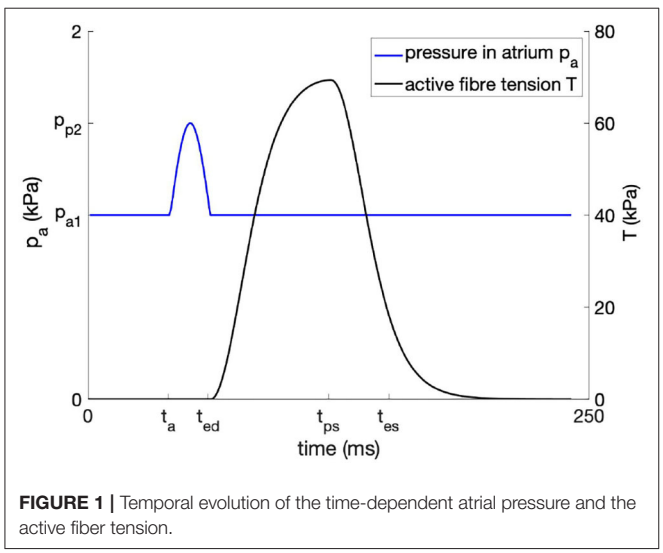

FIGURE 1 | Temporal evolution of the time-dependent atrial pressure and the active fiber tension.

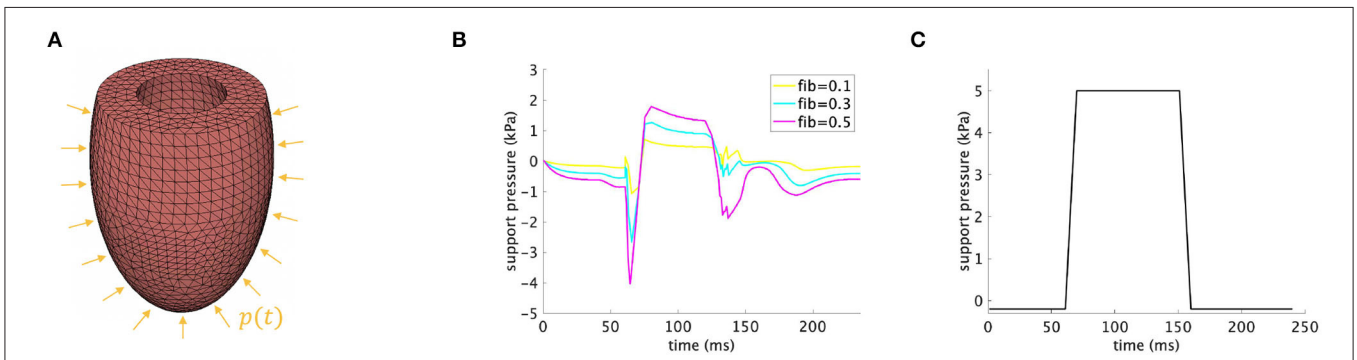

FIGURE 2 | (A) Discretized model of the rat LV with applied support pressure $p(t)$ on the epicardial surface. **(B)** Time-dependent support pressure according to Equation (8) for $fib \in \{0.1, 0.3, 0.5\}$. **(C)** Time-dependent support pressure according to Equation (9).

2. Systolic support: Set $p_{min} = 0$ kPa and find $p^*_{max-sys}$ so that $|ESV(0\text{ kPa}, p^*_{max-sys}) - ESV^*| \leq tol_1$ is fulfilled
3. Diastolic and systolic support: Set $p_{min} = p^*_{min}$ and find p^*_{max} so that $|ESV(p^*_{min}, p^*_{max}) - ESV^*| \leq tol_1$ is fulfilled

where the optimal support pressure $p^*(t)$ from Equation (9) is determined via the negative diastolic support pressure p^*_{min} and positive systolic support pressures $p^*_{max-sys}, p^*_{max}$, for the purely systolic support and systolic support combined with diastolic support, respectively.

3. RESULTS

Different quantities characterizing the cardiac performance are plotted for all simulations introduced in Section 2.2, including pressure volume loop, EF, EDV, ESV, EDP, and averaged end-diastolic hydrostatic stress over the domain, that is $\sigma_H = \frac{1}{n_{el}} \sum_{i=1}^{n_{el}} \sum_{j=1}^{3} \frac{\sigma^i_{jj}}{3}$, where σ^i_{jj} $(j = 1, 2, 3)$ are the diagonal components of the Cauchy stress in the i-th finite element and $n_{el} = 22846$ is the total number of the tetrahedral finite elements in the computational domain representing the LV; refer to **Figure 2A**. We note that EDP is the fluid pressure inside the cavity of the modeled LV, whereas the end-diastolic hydrostatic stress, computed according to the above formula, depends on the myocardial tissue structure and its volume change during the deformation (41). The latter can be interpreted as a measure of the force that drives fluid out of the myocardium and into the surrounding tissues. Positive hydrostatic stress means that the tissue is under extension and there is an increase in its volume (fluid flows into the myocardium), whereas negative hydrostatic stress implies that the myocardial tissue is compressed (fluid flows out of the myocardium).

3.1. Different Amounts of Fibrosis Without Support

Figure 3 shows the simulation results for scenarios with different amounts of fibrosis without any support pressure. Clearly, by increasing the amount of fibrosis, EF and EDV decrease, whereas ESV and EDP increase. For example, EF and EDP in the healthy model are 65.6% and 1 kPa (7.5 mmHg), respectively. These are close to the experimentally reported values in rats, which are slightly above the normal values in humans (35, 42, 43). By increasing the amount of fibrosis to 30% and 50%, EF reduces to 56.1% and 46.1%, whereas EDP increases to 1.6 kPa (12 mmHg) and 1.8 kPa (13.5 mmHg), respectively; refer to **Figures 3B,E**.

3.2. Support Pressure as the Difference With Respect to the Control Rat ($fib = 0$)

In **Figure 4**, changes in the cardiac function parameters are displayed for the rats with 10%, 30%, and 50% fibrosis in the LV. By applying a support pressure, computed according to Equation (8) and displayed in **Figure 2B**, EF, EDV, EDP, and end-diastolic hydrostatic pressure are at least partially improved, refer to **Figures 4B,C,E,F**, respectively. ESV remains nearly unchanged, refer to **Figure 4D**.

3.3. Constant Minimal and Maximal Support Pressure

Figures 5, **6** illustrate how the stepwise increase in the negative diastolic and positive systolic support pressure influences cardiac performance. The support pressure is increased until the EDP, EDV, and ESV of the control rat are reached up to tolerance. The resulting optimal values of the support pressure are $p^*_{min} = -0.5$ kPa (3.8 mmHg) and $p^*_{max} = 6$ kPa (45 mmHg) for $fib = 0.3$ and $p^*_{min} = -0.6$ kPa (4.5 mmHg), $p^*_{max} = 12$ kPa (90 mmHg) for $fib = 0.5$. For example, for the modeled case with 50% fibrosis in comparison with the control LV, the EF can be increased by 5% with only diastolic support, increased by 14% with only systolic support, and completely reach the value of the modeled control LV (65.6%) with the combination of the diastolic and systolic support. The end-diastolic pressure of 1.8 kPa (13.5 mmHg) cannot be decreased with only systolic support and can completely reach the value of the modeled control LV (7.5 mmHg) with only diastolic support as well as with the combination of the diastolic and systolic support. By increasing the value of the diastolic support, the end-diastolic hydrostatic stress becomes positive. This means that the myocardial tissue is extended and volumetric increase (possibly via a fluid inflow) is present.

4. DISCUSSION

The simulation results show that, with an increased amount of fibrosis, the cardiac performance is reduced; specifically, a reduction in EF is accompanied by increases in ESV and EDP, as shown in **Figures 3B,D,E**. These results mirror the experimental studies in rats after myocardial infarction, where the infarct size was determined as the percentage of the fibrotic scar in the LV (21, 40). As depicted in **Figure 3C**, for an amount of fibrosis of 20%, EDV decreases by 11%. With a further increase in the amount of fibrosis, EDV remains nearly constant due to the combination of the stiffer myocardium and a higher EDP, as shown in **Figure 3E**.

It is worth noting that the alterations in EDP and EDV are caused solely by changes in the passive material properties, whereas the value of ESV is influenced by both the change in the stiffness as well as the reduced maximum active tension, i.e., contractility, which is in Equation (5) scaled by the amount of fibrosis. The absolute value of the hydrostatic stress depicted in **Figure 3F** increases nearly linearly with the amount of fibrosis. Its negative value represents a mechanical compression in the cardiac tissue modeled as a continuum. Theoretically, a high negative hydrostatic stress together with a high EDP might lead to compression and the closure of arterioles supplying the heart and then eventually to an under-perfusion. A more elaborate computational model accounting for the heart perfusion is needed to interpret the results quantitatively.

Considering the support pressure resulting from the difference between the ventricular pressure in a healthy and diseased LV, **Figure 4C** shows that the support pressure is sufficient for reaching the control EDV. We note that the maximum possible EDV was limited to that of the healthy

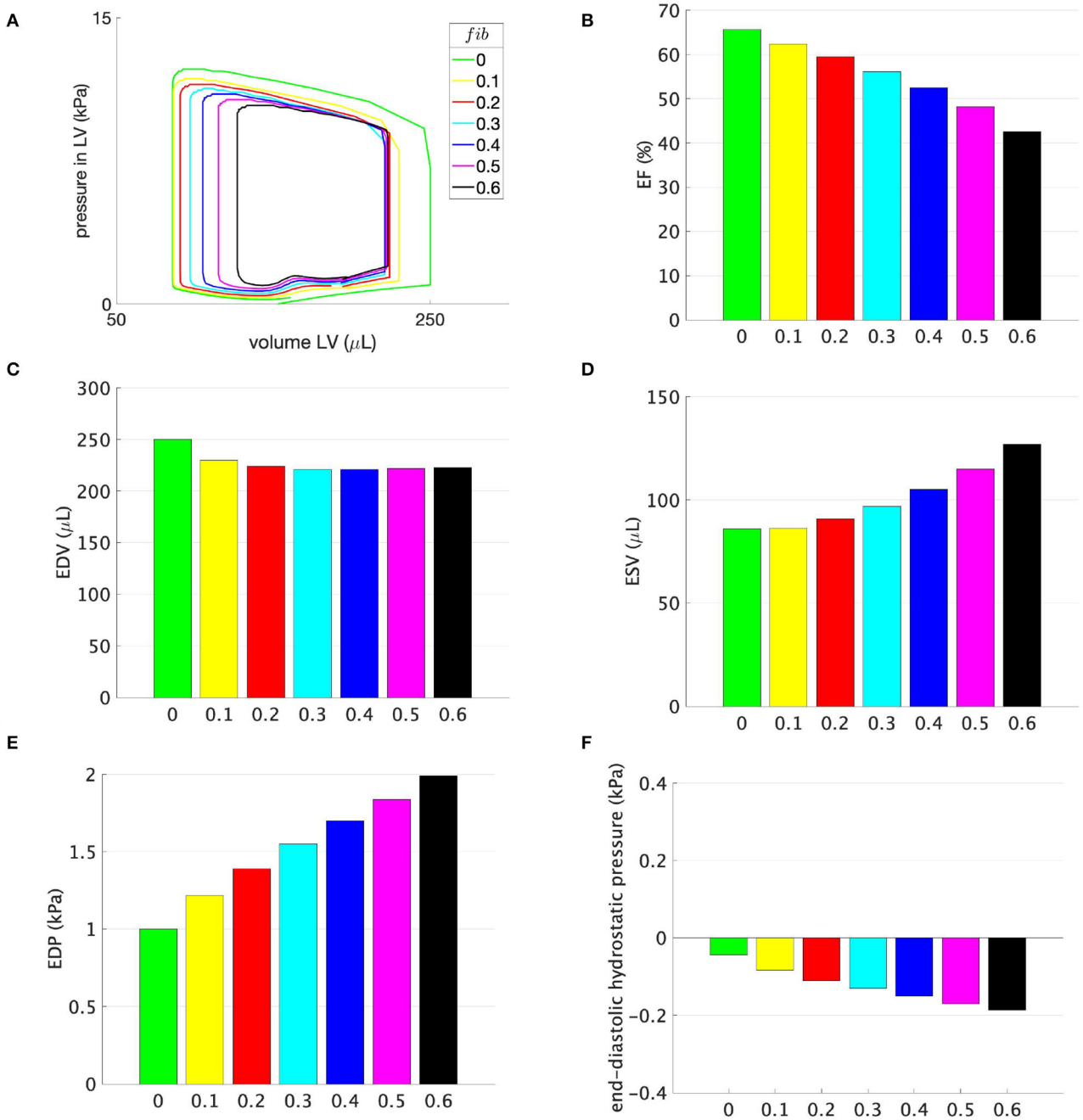

FIGURE 3 | (A) Pressure-volume loops, **(B)** ejection fraction (EF), **(C)** EDV, **(D)** ESV, **(E)** EDP, and **(F)** end-diastolic hydrostatic stress for models with different amounts of fibrosis *fib* displayed on the horizontal axis. No support pressure is applied.

rat. Therefore, even if the negative support pressure is higher than necessary, a normalization of the EDV is accompanied by a reduction in the EDP as depicted in **Figures 4C,E**. Due to the reduction in the EDP and enlargement of the LV, the hydrostatic stress becomes positive for all three fibrosis stages. This means that the tissue is under tension and better perfusion is expected. However, the observed decrease in ESV (**Figure 4D**) is marginal and only sufficient for the case $fib = 0.1$. Therefore, higher systolic support pressure is needed in order to reduce the ESV and eventually increase the EF, which is significantly below that of the control rat for higher amounts of fibrosis. For example, the resulting EFs are 61.1%, 55.2% for $fib = 0.3, 0.5$, respectively, compared to the control rat with EF= 65.6%.

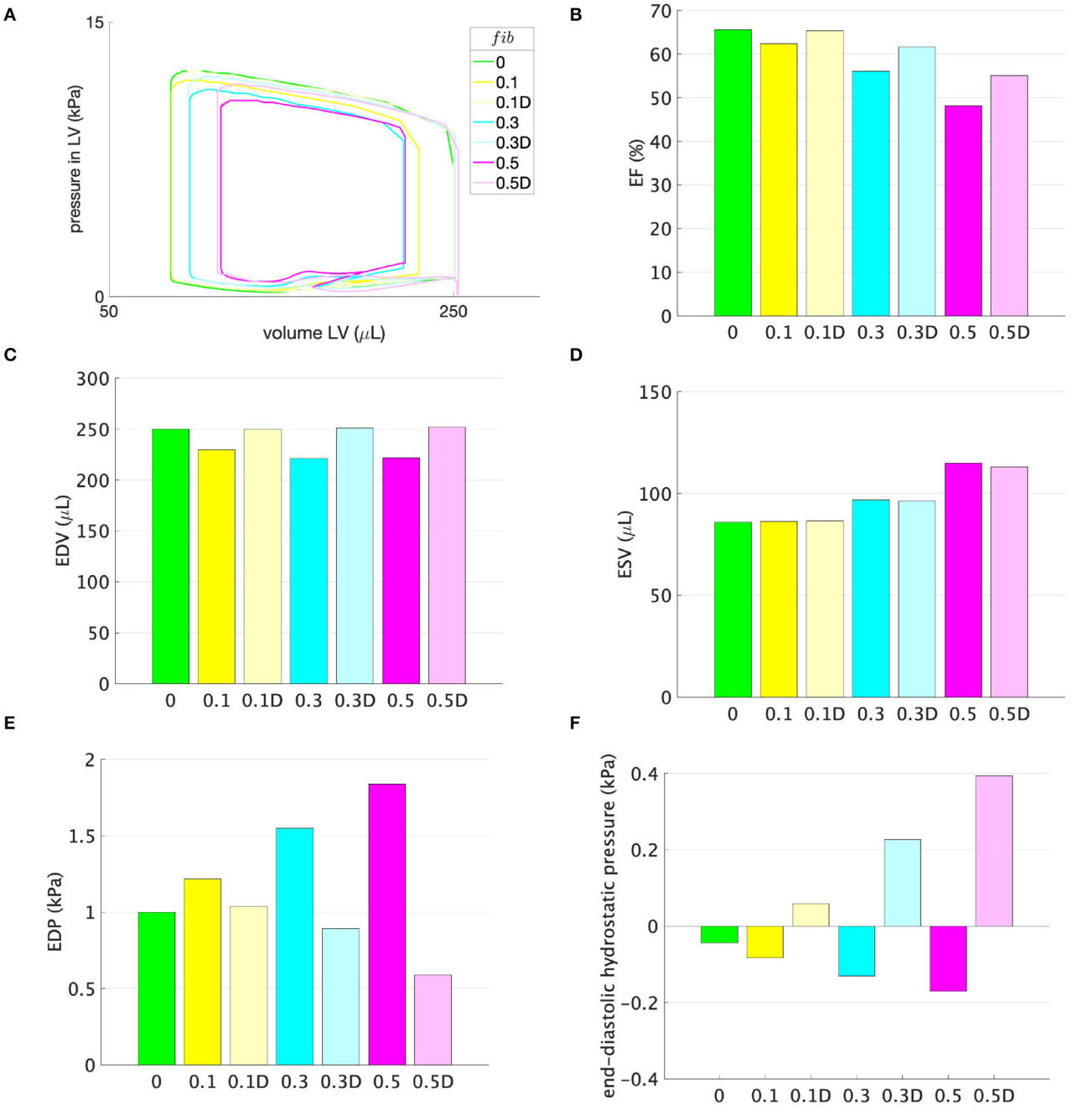

FIGURE 4 | (A) Pressure-volume loops, **(B)** EF, **(C)** EDV, **(D)** ESV, **(E)** EDP, and **(F)** end-diastolic hydrostatic stress for models with different amounts of fibrosis *fib* displayed on the horizontal axis. No support pressure is applied for models marked with the corresponding amount of fibrosis *fib* and displayed in opaque colors, whereas support pressure according to the function shown in **Figure 2B**, is applied for models with the suffix D displayed in transparent colors.

In the numerical test, that applied a constant minimum and maximum support pressure during the diastole and systole, respectively, the algorithm described in Section 2.2 is exemplarily performed for two fibrosis stages, namely $fib = 0.3, 0.5$. We started with supporting only the diastolic phase such that the negative value of the support pressure is increased until the desired EDV and EDP are reached up to a given tolerance. The EF can be increased by approximately 5% for both fibrosis stages, resulting in EF= 60.7%, 53.3% for $fib = 0.3, 0.5$, respectively. Even for the case with 50% fibrotic tissue, a relatively small support pressure of −0.6 kPa (−5.3 mmHg) is sufficient for regaining the desired EDV and EDP. Furthermore, as depicted in **Figure 7**, the compressive hydrostatic stress in the stiff fibrotic myocardium can be reduced and even changed into positive

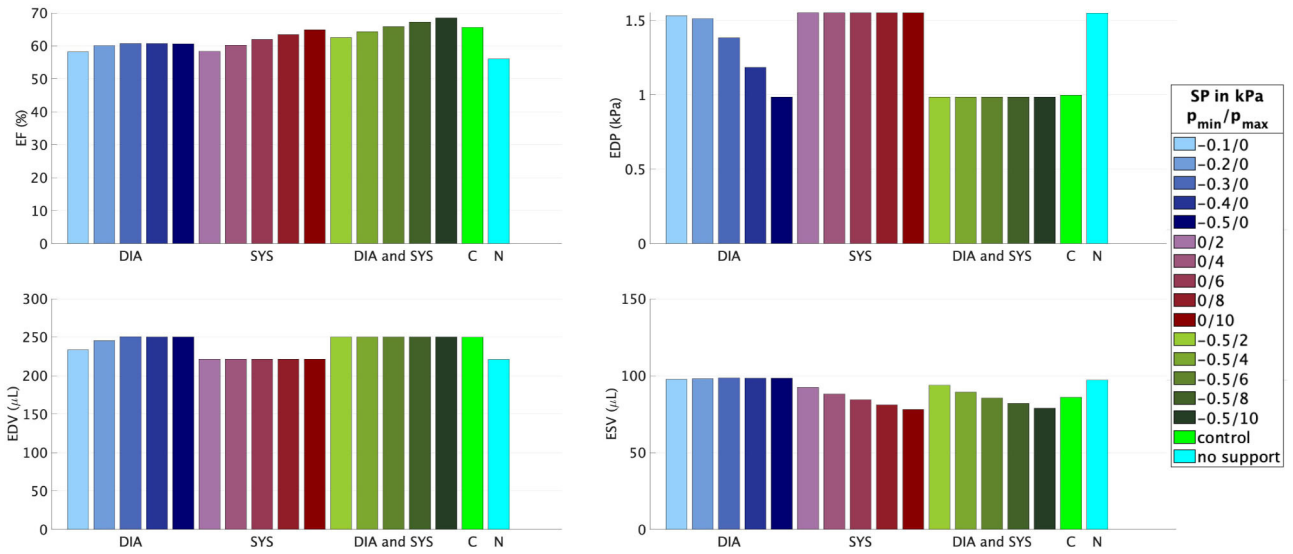

FIGURE 5 | Ejection fraction, EDP, EDV, and ESV for different combinations of the minimal p_{min} and maximal p_{max} values of the support pressure (SP) applied to the epicardial surface of the model with *fib* = 0.3. DIA, diastolic support (blue); SYS, systolic support (red); DIA and SYS, combined support (green); C, control rat LV (light green); N, rat LV with *fib* = 0.3 without any support (blue-green).

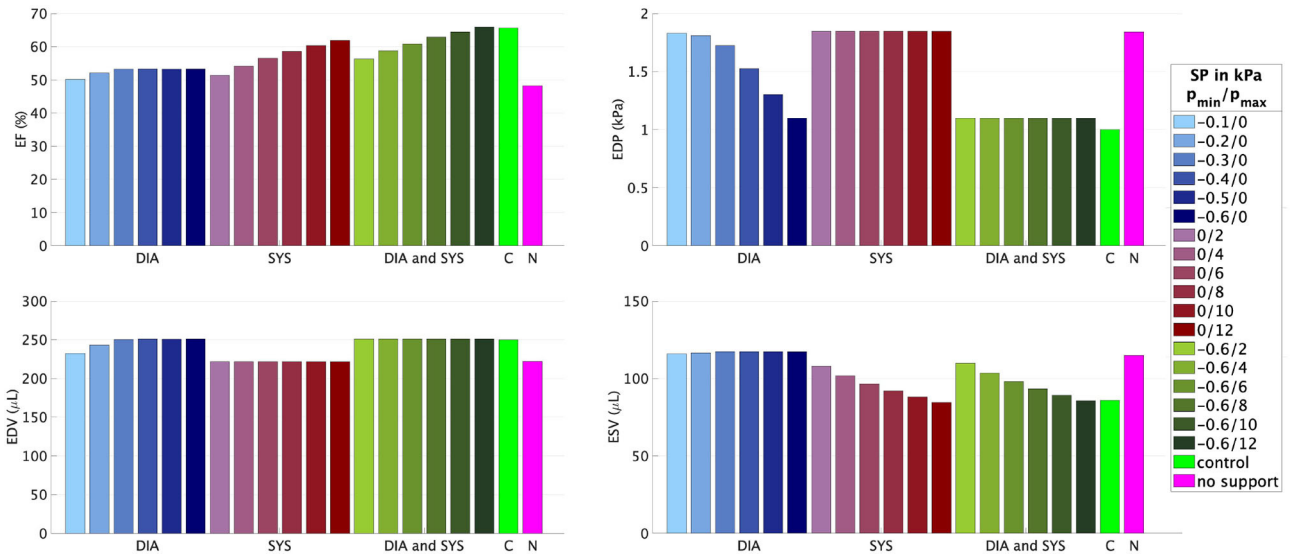

FIGURE 6 | Ejection fraction, EDP, EDV, and ESV for different combinations of the minimal p_{min} and maximal p_{max} values of the support pressure (SP) applied to the epicardial surface of the model with *fib* = 0.5. DIA, diastolic support (blue); SYS, systolic support (red); DIA and SYS, combined support (green); C, control rat LV (light green); N, rat LV with *fib* = 0.5 without any support (pink).

hydrostatic stress. We believe that this phenomenon could improve myocardial perfusion. However, as discussed above, this is currently only speculative and needs to be investigated. When only the systolic phase is supported, significantly higher positive support pressures are needed to normalize the systolic function, 6 kPa (45 mmHg) and 12 kPa (90 mmHg) for *fib* = 0.3, 0.5, respectively. Nevertheless, in this case, the desired EF of the control rat (65.6%) is not reached due to insufficient diastolic filling caused by the stiff fibrotic tissue. When using only the systolic support, a further increase in the support pressure would theoretically lead to the desired EF. However, the EDP would remain elevated, which has been identified as a potential predictor of heart failure (44–46). Whether solely the reduction in EDP would eventually lower

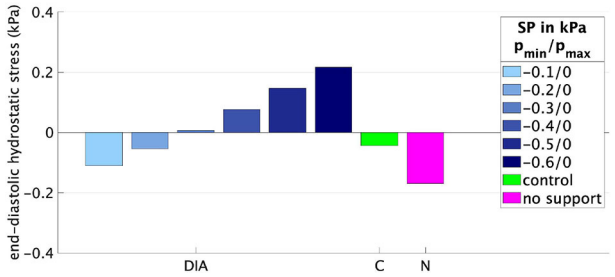

FIGURE 7 | End-diastolic hydrostatic stress for different support pressures (SPs) applied to the rat LV with *fib* = 0.5. DIA, diastolic support; C, control rat LV; N, rat LV with *fib* = 0.5 without any support.

the risk needs to be investigated further. The best option for normalizing EF and EDP turns out to be a combination of both, diastolic and systolic support. As demonstrated in **Figures 5**, **6**, complete normalization of the functional parameters can be reached.

The present study aimed to explore the potential usefulness of diastolic support during the cardiac cycle of a rat LV suffering from restrictive cardiac disease. A simple computational model based on an investigation of mechanical support acting on the outer surface of the LV was chosen. However, there are some limitations and possibilities for future model development. First, the present study investigated the rat heart model and, to date, is not clinically applicable. However, as pointed out in the Introduction, some innovative approaches have investigated possible direct compression assist devices for clinical use in humans. Second, since it is difficult to develop a model that accounts for all influencing factors causing heart insufficiency, we initially chose a model in which we can change both the diastolic and the systolic function of a ventricle by changing one parameter, namely the amount of fibrosis. Besides this, various other factors must be considered in the future to more realistically mimic the remodeling process. These include the effects of geometric changes (in particular those due to ventricular dilatation or hypertrophy), the fact that most failing hearts are rather dilated than restrictive (especially in Paediatrics), the fact that myocardial infarction and ischaemic heart disease are only one among multiple causes of heart failure, the influence of arrhythmias and synchronization, the ever-changing metabolic needs, the fluid status of the patient impacting the preload of the heart, and much more. Third, for a better understanding of the interaction between the heart and a direct cardiac compression device, instead of the prescribed support pressure, a complete direct cardiac compression device should be modeled. Here, one possibility would be to use mechano-active materials, such as biocompatible dielectric elastomer actuators (47) that compress and expand when a voltage is applied. Their relatively large (more than 40%) expandability would be beneficial for generating the support pressure needed during the diastole (48). Fourth, in future development, electromechanical coupling and in particular electromechanical feedback would possibly play a role with respect to the interaction of the heart and the direct cardiac compression device.

5. CONCLUSION

A computational model for different amounts of fibrosis in the rat LV is presented. Based on this model, we investigate how a support pressure acting on the outer surface of the diseased LV influences the cardiac performance quantified via the EF, EDP, and ESV, as well as the hydrostatic stress in the cardiac tissue. We conclude that a negative support pressure during diastole combined with a positive support pressure during systole can normalize the modeled diastolic and systolic function of the rat LV at different fibrosis stages. Although not investigated in this study, it is tempting to assume that the negative diastolic support pressure could potentially improve cardiac perfusion. Furthermore, we adjusted the value of the support pressure so that functional parameters of the healthy rat LV are restored. We conclude that cardiac assist devices without direct blood contact and with a simultaneous diastolic and systolic support functionality present a potentially interesting therapy for heart failure in restrictive LV physiology, as that is resulting from myocardial scars after ischaemic insults.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

DM mainly contributed to this study concerning the writing of the original manuscript, deriving the equations, modeling, simulation, and post-processing and visualization of the results. DH contributed to the fiber modeling and the revision of the manuscript. DB contributed to initial simulations and post-processing of simulation results. MW contributed to the acquisition of the publication fee support and advice for the medical part. SL and MA contributed to the conception and design of the study and the revision of the manuscript and provided the leadership responsibility for the technical and medical parts, respectively. MA contributed by writing and improving the medical parts of the manuscript. All authors contributed to the article and approved the submitted version.

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APPENDIX

Simulation Parameters

TABLE A1 | Material parameters used in the numerical examples.

Active stress	$t_{ed} = 60$ ms, $t_{ps} = 120$ ms, $\alpha_{min} = -0.1\text{ms}^{-1}$, $\alpha_{max} = 0.1\text{ms}^{-1}$, $\gamma = 25$ ms, T_{max}=70 kPa,
Passive stress	a = 1.665 kPa, b = 1.237 (-), a_f = 7.822 kPa, b_f = 0.008 (-), a_s = 0.0 kPa,
	b_s = 0.0 (-), a_{fs} = 1.342 kPa, b_{fs} = 9.178 (-)
Windkessel model	$R_p = 15$ kPa μL^{-1}ms, R_{v1} =0.1 kPa μL $^{-1}$ms, R_{v2} =0.067 kPa μL^{-1}ms,
	$C = 40$ μL kPa $^{-1}$ ms^{-1}, $t_a = 40$ ms
Support pressure	$t_{es} = 150$ ms, $t_\Delta = 10$ ms, $tol_1 = 1$ μL, $tol_2 = 0.1$ kPa

Active Tension

Based on the study by Pfaller et al. (38), the evolution of the active tension T reads as

$$\dot{T}(t) = -|a(t)|T(t) + T_{max}|a(t)|_+$$

with activation function a, the maximum value of the active stress T, and the function $|a(t)|_+ = \max(a(t); 0)$. The activation function $a(t)$ is modeled by

$$a(t) = \alpha_{max} \cdot f(t) + \alpha_{min} \cdot (1 - f(t))$$

with maximum and minimum activation rates α_{max} and α_{min}, respectively, and functions

$$f(t) = S^+(t - t_{ed})\Delta S^-(t - t_{ps}),$$
$$S^\pm(\Delta t) = \frac{1}{2}(1 \pm \tanh(\frac{\Delta t}{\gamma}))$$

with steepness γ and descending and ascending sigmoid functions S^+ and S^-, respectively. The indicator function $f \in (0, 1)$ indicates systole. The times t_{ed} and t_{ps} model the end-diastolic and the peak-systolic times, respectively.

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REVIEW
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Special Considerations in the Care of Women With Advanced Heart Failure

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Advanced heart failure (AHF) is associated with increased morbidity and mortality, and greater healthcare utilization. Recognition requires a thorough clinical assessment and appropriate risk stratification. There are persisting inequities in the allocation of AHF therapies. Women are less likely to be referred for evaluation of candidacy for heart transplantation or left ventricular assist device despite facing a higher risk of AHF-related mortality. Sex-specific risk factors influence progression to advanced disease and should be considered when evaluating women for advanced therapies. The purpose of this review is to discuss the role of sex hormones on the pathophysiology of AHF, describe the clinical presentation, diagnostic evaluation and definitive therapies of AHF in women with special attention to pregnancy, lactation, contraception and menopause. Future studies are needed to address areas of equipoise in the care of women with AHF.

Keywords: advanced heart failure, heart transplant, ventricular assist device, women, advanced therapies (ATs)

INTRODUCTION

Heart failure (HF) mortality is greater among women than men at all ages in US (1). In 2018, HF was the implicated cause of 83,616 deaths (38,487 males and 45,129 females) (1). Approximately 300,000 HF patients in the US currently have advanced HF (AHF), and an additional 5% will progress to advanced disease each year (2). Estimates of the prevalence of AHF varies from 5 to 25% between studies (2), and the exact proportion of women of reproductive age who have AHF is

unknown. AHF is associated with high morbidity and mortality, and huge healthcare-related costs, especially in the last year of life (3, 4). The 1-year mortality estimated by HF survival models is >20%−25% (4). In crude analyses, the heart transplantation (HT) rate for women and men were 0.789/100,000 and 2.33/100,000 respectively each year (5). Using data from the United Network for Organ Sharing and Centers for Disease Control and Prevention the HT to HF mortality ratio was 0.263 for women and 0.424 for men, supporting reports that irrespective of disease severity, less women than men receive a HT (5).

AHF is defined as the presence of progressive and/or persistent severe signs and symptoms of HF despite optimized medical, surgical, and device therapy (3, 6). While sex-related differences in epidemiology, risk factors, pathophysiology, response to therapies and outcomes in HF have been reported (7, 8), the unique characteristic of AHF in women and the influence of sex hormones has not been extensively described. Additionally, there is minimal data on female sex-specific cardiovascular risk factors in the evidence that directs current HF practice guidelines (9). When considering HF subtypes, women have a higher prevalence of HF with preserved ejection fraction (HFpEF) than men, which is rarely an indication for HT or left ventricular assist device implantation (LVAD) implantation (10). Among those with AHF and recurrent hospital admissions, women have a similar prevalence of HFpEF and HF with reduced ejection fraction (HFrEF), while most men with recurrent hospital admissions have HFrEF (5). Age-adjusted case fatality rates from the Atherosclerosis Risk in Communities study showed that HFrEF contributes to more mortality than HFpEF in women (11).

The management of AHF in women is complicated because detection by patients, their families and providers is often delayed (3). Recognizing AHF in women requires a thorough clinical assessment and risk stratification (4). Little is known about the impact of sex on HT allocation or the potential effect of gender bias on the decision-making process for other AHF therapies (12). Data from the organ procurement and transplantation network shows less women than men on the HT waiting list or receiving a HT, and this proportion further declines with age (**Table 1**). Younger women (<50 years) comprised only 9.6% of the total number of patients on the waitlist and 10.17% of total number who received a HT. The purpose of this review is to discuss the role of sex hormones on the pathophysiology of AHF, describe the clinical presentation, diagnostic evaluation and definitive therapies of AHF in women with specific attention to pregnancy, lactation, contraception and menopause, while considering barriers to treatment.

Abbreviations: AHF, advanced heart failure; HT, heart transplant; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; ISHLT, International Society of Heart and Lung Transplantation; LVAD, left ventricular assist device; MOMENTUM 3, Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3; PPCM, peripartum cardiomyopathy; REVIVAL, Registry Evaluation for Vital Information on Ventricular Assist Devices in Ambulatory Life; SHOCK, SHould we emergently revascularize Occluded Coronaries for cardiogenic shock; TMCS, temporary mechanical circulatory support.

PATHOPHYSIOLOGY OF ADVANCED HEART FAILURE IN WOMEN: THE ROLE OF SEX HORMONES

The pathophysiological mechanisms underlying HF involve the activation of structural, neurohumoral, cellular, and molecular pathways in response to myocardial injury in an attempt to maintain homeostasis (13). Sex hormones including estrogen, progesterone and testosterone modify some of the pathophysiological processes that promote HF progression in women (**Figure 1**). Estrogen modulates the expression of proteins that regulate vascular tone and response to myocardial injury, and influences ventricular contractile function, endothelial calcium metabolism, coronary calcification, coagulation and fibrinolysis, insulin resistance, inflammation and lipid oxidation (14). Through these actions, it limits cardiac remodeling and attenuates myocardial hypertrophy (15). Progesterone affects vascular tone by modulating calcium channel activity, inhibiting vascular smooth muscle proliferation and migration, and worsening the response to vascular injury (16, 17).

Androgen excess is associated with greater risk of heart disease in women, due to its adverse effects on the vasculature, lipoprotein levels and adiposity (16, 18, 19). Low androgen levels are also associated with atherosclerosis and coronary artery disease (19). Elevated testosterone promotes cardiac remodeling by causing myocardial hypertrophy, modulating the autonomic nervous system and regulating excitation contraction coupling through its effects on intracellular calcium levels (16). Physiological levels of testosterone improves endothelial function, peripheral vascular resistance and vasomotor tone, and its effects on the cardiovascular system depends on circulating estrogen levels and the peripheral conversion of testosterone to estradiol (19).

The effects of female sex hormones on HF pathophysiology is a continuum, that persists with progression to advanced disease, such that AHF is not an end-state but a dynamic condition where numerous mechanical, molecular, immunologic, ischemic, proarrhythmic, vascular, and musculoskeletal forces contribute to symptoms and continuing deterioration (20). There is increasing inability to meet the metabolic demands of end-organs and skeletal muscle, renal and hepatic dysfunction, and reduction in exercise capacity, cachexia, and fatigue (3). Estrogen, progesterone and testosterone receptors continue to activate cellular mechanistic cascades that modulate inflammation, apoptosis, vascular abnormalities and myocardial remodeling in response to worsening pathologic conditions in AHF (16).

CLINICAL PRESENTATION OF ADVANCED HEART FAILURE IN WOMEN

There is no specific event that marks the progression to AHF, instead a pattern of clinical findings may be the optimal indicator (3). AHF is characterized by recurrent hospitalizations, escalation of diuretics, intolerance or dose-reduction of guideline directed medical therapies, development of end-organ dysfunction,

TABLE 1 | Heart transplant waiting list and allocation according to sex and age-groups.

	Waiting list[a]		Number transplanted[b]	
Sex	**Male**	**Female**	**Male**	**Female**
Age-group				
18–34 years	239 (0.67)	120 (0.33)	4,790 (0.62)	2,964 (0.38)
35–49 years	489 (0.74)	168 (0.26)	12,297 (0.73)	4,499 (0.27)
50–64 years	1,126 (0.79)	293 (0.21)	30,321 (0.78)	8,480 (0.22)
>65 years	464 (0.83)	98 (0.17)	8,253 (0.82)	1,766 (0.18)
Total	2,318 (0.77)	679 (0.23)	55,661 (0.76)	17,709 (0.24)

Values are number (%) for the respective column.
[a]Based on organ procurement and transplantation network data as of June 1, 2022.
[b]US transplants performed: January 1, 1988–April 30, 2022.
Source: Organ procurement and transplantation network. Heart resources and services administration, US Department of health and human services. https://optn.transplant.hrsa.gov/data/.

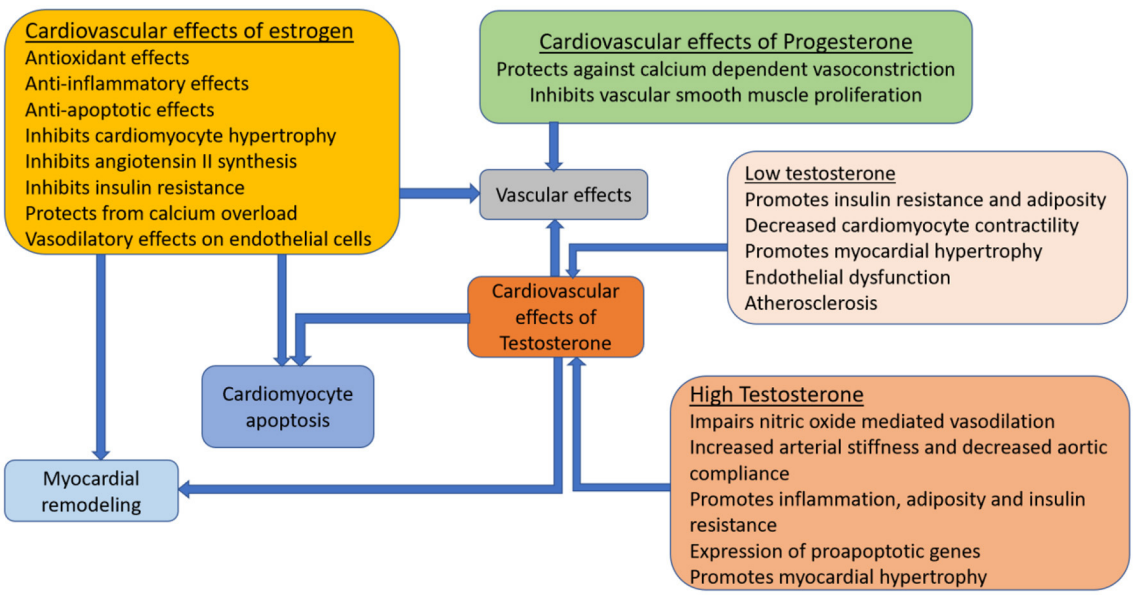

FIGURE 1 | Influence of sex-steroid hormones on the cardiovascular system. Cardiomyocytes and vasculature have receptors for major female sex-steroid hormones; estrogen, progesterone and testosterone. These sex-steroid hormones influence heart failure progression through cellular mechanistic processes that have direct and indirect effects on the myocardium and vascular tone.

cardiac cachexia, and refractory arrhythmias with or without device shocks (3). Recurrent hospitalization is a strong indicator of progressive decompensation as HF approaches its late stages (4). Failure to respond to conventional therapies, another manifestation of disease progression, presents with persistent functional impairment which can be nonspecific in elderly patients (3) and women. The absence of a sex-oriented assessment of disease severity could make the identification of AHF among women a persistent challenge for clinicians (21). However, the use of gender-specific risk prediction models did not improve the accuracy of predicting mortality risk in decompensated HF (22).

Women with AHF experience higher symptom burden, poor coping strategies, and greater prevalence of depression and social isolation than their male counterparts (23). As the disease progresses, its impact on functional status and quality of life is more debilitating among women, not just from HF alone, but the greater burden of comorbidities and older age of female HF patients (21). Frailty and cachexia, common features of AHF (4, 24), are more frequently seen in women (24). Physical frailty is characterized by worse symptom characteristics in women, and worse body composition characteristics in men (25). However, current frailty assessment tools are not sex-specific, and future research is needed to identify the ideal index for frailty assessment in women, and sex differences in reversibility of frailty with HF therapies (25). Women are also admitted less frequently than men for acute decompensated HF (1) which may lead to delayed recognition of advanced disease. Consequently, the

gender distribution of patients referred for AHF therapies likely does not represent the actual proportions of patients with AHF.

DIAGNOSTIC EVALUATION OF WOMEN WITH ADVANCED HEART FAILURE

The initial evaluation of AHF should be focused on excluding reversible causes and ensuring adequate treatment with maximally tolerated guideline directed medical therapies (26). For women with persisting features of hemodynamic instability or systemic hypoperfusion, with or without end-organ dysfunction, the evaluation process becomes more structured to establish candidacy for advanced therapies by identifying contraindications to heart transplantation (HT) or left ventricular assist device (LVAD) implantation (27). The evaluation process is both comprehensive and center-specific (26) and eligibility determined after review by a multidisciplinary selection committee.

Cardiopulmonary exercise testing provides objective information about cardiovascular reserve and prognosis (6). In women, a peak oxygen consumption $\leq$50% of expected is a recommended parameter for consideration for advanced therapies (28), since women exhibit better survival than men for any given peak oxygen consumption value (29). Invasive hemodynamics obtained from right heart catheterization provides information that guides specific pharmacotherapy and durable therapies by enabling precise assessment of filling pressures, pulmonary hypertension, cardiac output, and right ventricular performance (26). Sex differences in hemodynamics have not been systematically explored. However, in the SHOCK registry, cardiac power index, a strong predictor of mortality was significantly lower in women (30). Consequently, physiological differences between men and women must be acknowledged when interpreting functional testing. In the REVIVAL study, the 6-min walk test distance was significantly shorter in women than men by almost 40 m despite similar age and functional class (31). Cardiac biomarkers further improve risk stratification and selection for advanced therapies. Women with decompensated HF have higher natriuretic peptide levels than men for any given LV ejection fraction (32, 33), and natriuretic peptides are stronger predictors of HF-related mortality in women (34). Natriuretic peptides are influenced by adiposity, menopause and sex hormones, such that women have lower levels after the onset of menopause (35). It is unclear if sex-specific cut-offs in biomarker levels should be adopted for AHF prognostication (33).

LIFE SUSTAINING THERAPIES FOR WOMEN WITH ADVANCED HEART FAILURE

Gender disparities persist in the utilization of AHF therapies (36). In a multicenter retrospective analysis, 73.4% of referrals evaluating candidacy for advanced therapies were men (37). Women are allocated to less than a third of HT and LVAD in the US (38) and are under-represented in HF clinical trials (5, 7, 10).

Heart Transplantation

Despite having shorter waitlist times and greater HF-related mortality, women are less likely to receive a HT than men (39). Among patients transplanted yearly in US, women received only 26% while men received 74% of donor hearts (39). Gender disparities in HT are a consequence of fewer women being listed for transplant, greater waitlist mortality for women, less aggressive HF treatment in women, and organ allocation factors like allo-sensitization, which limits the availability of potential donors (39, 40).

Among patients who require hemodynamic stabilization prior to HT, women are more frequently bridged with inotropic support and less likely to receive mechanical circulatory support (MCS) as a bridge to transplantation (39). LVAD is an important bridging therapy that maintains cardiac function while awaiting HT, therefore, its underutilization could contribute to increased mortality during the pre-transplant period (40). However, it has also been reported that women who are supported with an LVAD as bridge to transplant have lower chances of HT than men, higher waitlist mortality, increased delisting for worsening clinical status and are less likely to be transplanted urgently (38, 41). Future studies are needed to explore the optimal waitlist strategy for women.

After HT, women tend to have better long-term survival than men, lower risk of coronary allograft vasculopathy and malignancy, but a higher risk of antibody-mediated rejection (39). Sex matching has less impact on early mortality among female transplant recipients, but, survival after 5 years is better among female recipients matched to female donors in comparison to women matched to male donors (8).

Left Ventricular Assist Devices

Regardless of the indication for implant, there are sex-related disparities in the utilization and outcomes of LVAD as bridge to transplant, bridge to recovery or destination therapy (8). Data from the INTERMACS registry involving 18,868 patients who received their first continuous flow-LVAD between June 2008 and December 2017, showed that women comprised only 21.1% of LVAD recipients mostly for a bridge to transplant indication (42). This disparity may be because women are referred later for advanced therapies (2), when they are no longer candidates for durable LVAD.

Despite mixed evidence, women appear to have similar complication rates as men with use of contemporary LVADs including in-hospital mortality, time to infection, post-operative bleeding, and device malfunction, however, stroke and early right ventricular failure are more common in women (38, 43). Female LVAD patients are at higher risk of both hemorrhagic and ischemic stroke, but the risk of hemorrhagic stroke is greater among women $<$65 years while ischemic stroke risk is greater among women $\geq$65 years (39). The factors that underlie gender differences in thromboembolic risk and responses to anticoagulation could similarly explain gender disparities in stroke risk after LVAD implantation and should be explored in future studies. Right ventricular failure is also more common in women than men after LVAD implantation, with some evidence

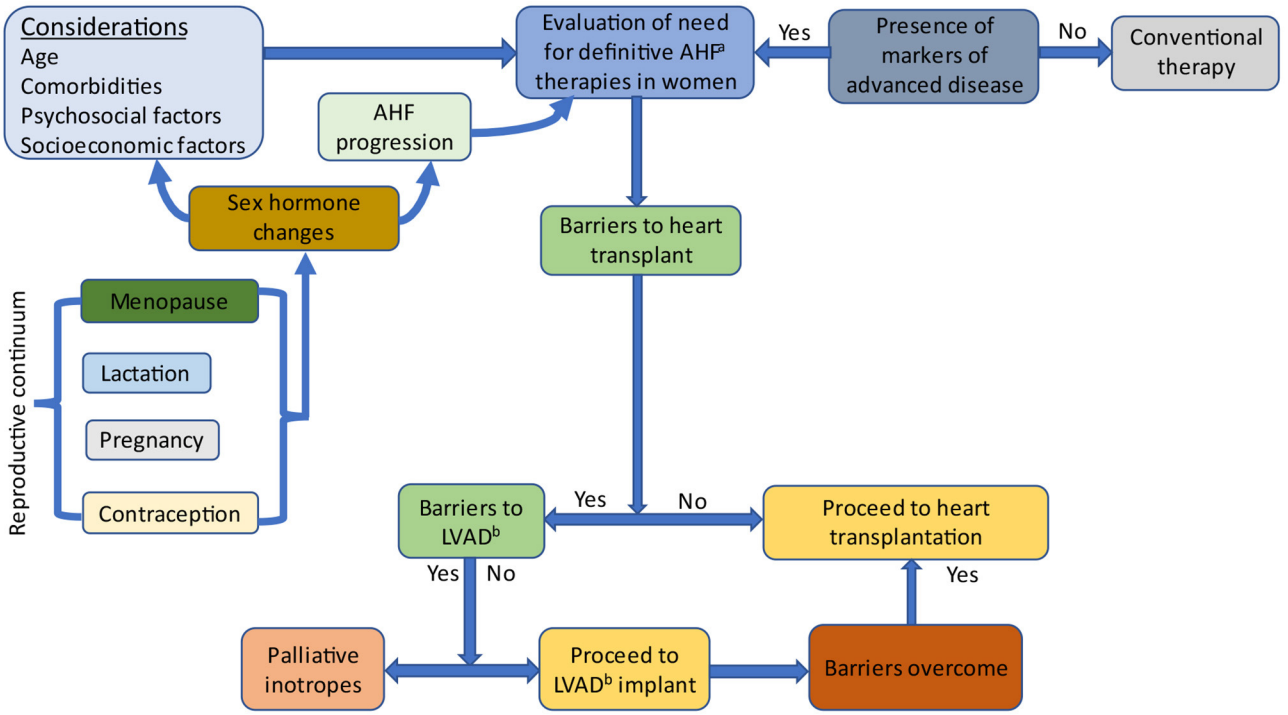

FIGURE 2 | Evaluation of women for advanced heart failure therapies. Sex-specific factors affect hormonal levels and include pregnancy, lactation, contraception and menopause. Sex hormones influence heart failure progression and factors which are considered when evaluating women for advanced heart failure therapies. AHF, Advanced heart failure; LVAD, Left ventricular assist device.

supporting later presentation and higher prevalence of non-ischemic cardiomyopathy as contributing factors (44).

Although many studies show few sex differences with LVAD usage, women with continuous flow-LVADs who were ≤49 years old were at increased risk of mortality in comparison to men of similar age in a study by Gruen et al. using the INTERMACS registry (42). In the same study, women had greater likelihood of adverse events including pump thrombosis, infection, bleeding and stroke (42). In another study, women with ischemic HF etiology had greater LVAD mortality risk than men (45). Other studies limited to continuous flow devices have shown comparable post-LVAD complication rates in both sexes (46). In an analysis of the National Inpatient Database by Ahmed et al. from January 2009 to December 2014 (mainly HeartMate II and HeartWare), there were no significant gender differences in in-hospital mortality or complications after LVAD implantation (47). It is unclear how gender biases in selection arising from differences in clinical severity or psychosocial issues could influence LVAD outcomes. To further address conflicting data, more sex-specific LVAD research is needed, especially limited to contemporary LVADSs like HeartMate 3 which has a lower rate of adverse events (44).

Temporary Mechanical Circulatory Support

Temporary MCS (TMCS) devices such as intra-aortic balloon pump, micro-axial LVADs, extracorporeal membrane oxygenation and TandemHeart can provide uni- or biventricular support to patients with AHF or cardiogenic shock. Current trends show a decrease in intra-aortic balloon pump use and increases in micro-axial LVADs and extracorporeal membrane oxygenation use in both sexes (48). Despite being sicker at presentation (44), TMCS is underutilized in women (49) and is associated with greater complication rates, including vascular complications that sometimes require surgical interventions (50). Women experience greater mortality from cardiogenic shock than men despite TMCS use (49). In acute myocardial infarction related-cardiogenic shock, women received TMCS support less frequently (48) even though Impella support prior to percutaneous coronary intervention is associated with greater survival benefits in women than men (44). Further research is required to explore sex-based differences, hormonal influences and potential anatomical considerations in TMCS utilization and outcomes.

Palliative Care and Inotrope Use

Palliative care is an interdisciplinary approach to patient management that focuses on reducing suffering and improving quality of life in serious illness such as AHF, for patients and their caregivers (51). Although current evidence shows an increasing trend in palliative care use in AHF, it remains underutilized in US with an estimated adoption rate of 6.2% in 2017 (52). There are sex disparities in response to palliative care interventions, with women experiencing less improvement in patient-reported outcomes than men (23). This may be because women with

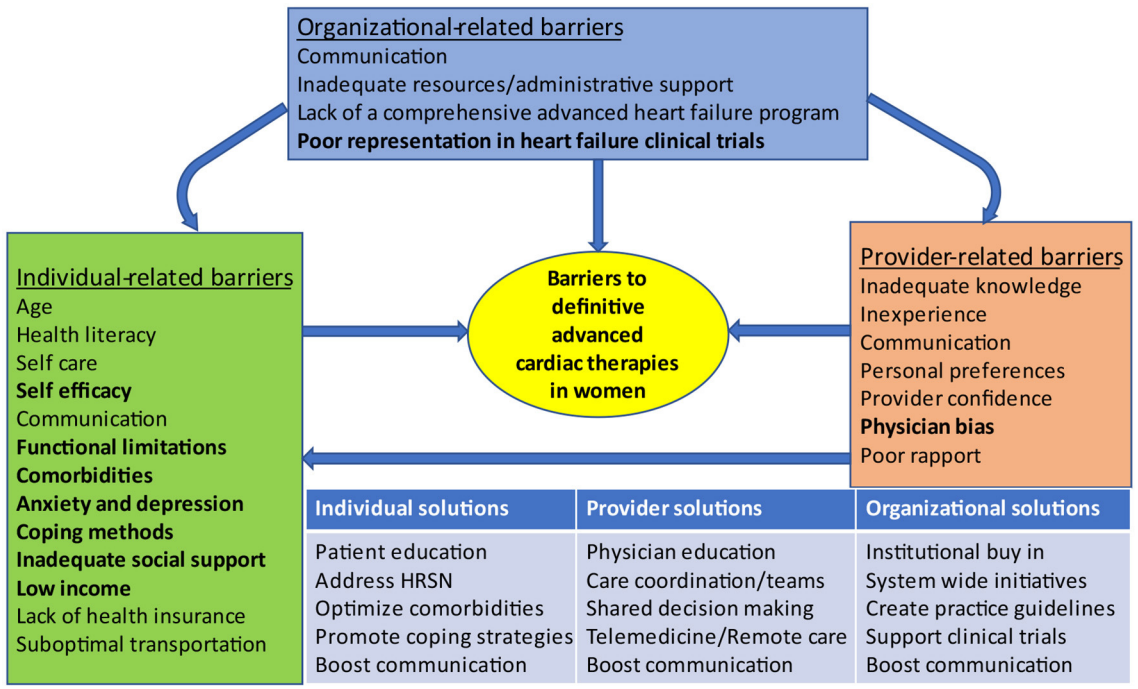

FIGURE 3 | Barriers to advanced heart failure therapies in women and proposed solutions. Organizational-related barriers can influence individual-related and provider-related barriers. Provider-related barriers can influence individual-related barriers. Factors that are more common in women appear in bold. HRSN, Health related social needs.

AHF have higher levels of distress before their symptoms are acknowledged and managed by their providers (23, 53). Despite excessive mortality associated with their use (2), palliative inotropes (milrinone or dobutamine) improve HF symptoms and decrease hospital admissions (54), making them an option for terminally ill patients who are not candidates for HT or LVAD. When used as a bridging strategy, men are over seven-times less likely to be successfully bridged to HT with long-term milrinone support than women (55). A sex-specific approach to the use of palliative care interventions is necessary to improve outcomes among women with AHF (23).

SPECIAL CONSIDERATIONS

The reproductive continuum spans contraception use, pregnancy, lactation and the menopausal transition, resulting in sex hormonal changes that affect HF development and progression (**Figure 2**). Irrespective of their life stage, similar considerations should be applied when evaluating women for AHF therapies.

Pregnancy

The progression of HF during pregnancy varies according to the underlying cardiomyopathy, and may be aggravated by physiological changes experienced during pregnancy (56). HF during pregnancy, although relatively rare (57, 58), is associated with increased risks of maternal and fetal complications (57) and is the most common cause of pregnancy-related death in developed countries (59, 60). Decompensation most commonly occurs during the second or third trimester, or shortly after delivery (56). HF in pregnancy may be due to pre-existing cardiac diseases such as congenital heart disease, non-ischemic cardiomyopathy, valvular disorders, hypertrophic cardiomyopathy or peripartum cardiomyopathy (PPCM) (56, 58). PPCM, an idiopathic cardiomyopathy with LVEF <45% that occurs toward the end of pregnancy or in early months after delivery, abortion or miscarriage, without other known causes of HF, is the most common cause of HF during pregnancy (56, 58, 61, 62). Irrespective of the underlying cardiomyopathy, women with severe functional impairment, moderate to severe LV dysfunction, hemodynamic load such as LV outflow tract obstruction and pulmonary hypertension should be counseled against pursuing pregnancy (59).

Women with AHF who become pregnant should be informed about the risk of deterioration, and therapeutic abortion offered to those with extreme risk in early pregnancy (60, 63). A multidisciplinary management strategy involving high risk obstetrics, neonatology, anesthesiology, HF cardiology, and cardiothoracic surgery should be pursued early in pregnancy (60, 64). The onset of hemodynamic instability and cardiogenic shock with need for inotropic or vasopressor use at any time during gestation should prompt referral to a tertiary center with capabilities for MCS and urgent delivery by cesarean section (63). Vaginal delivery should be considered if the woman is hemodynamically stable (60). However, in emergency situations, advanced therapies and drugs that are not recommended during

TABLE 2 | Future areas of study in the management of advanced heart failure in women.

Areas of equipoise in the management of advanced heart failure in women	Potential areas of future research
Hemodynamic instability	Identify sex-specific cutoffs that indicate hemodynamic compromise in men and women
Biomarker derangements	Explore the validity of sex-specific cutoffs for advanced heart failure prognostication
Exercise capacity	Investigate sex specific cut-offs in 6-min walk test, exercise duration and functional capacity
Frailty assessment	Explore the optimal strategy for frailty assessment among women with advanced heart failure
Waitlisting prior to heart transplant	Identify the optimal waitlist strategy for female patients in the pre-transplant period
Chronic inotrope use	Impact of chronic inotropes on sex-based clinical outcomes
Palliative care	Explore a sex-specific approach to the use of palliative care interventions
Temporary mechanical circulatory support	Evaluate sex-specific differences in the utilization and outcomes of temporary mechanical circulatory support
Anticoagulation strategy in LVAD	Explore optimal anticoagulation strategies in male and female LVAD patients
Referral for advanced therapies	Evaluate the role of a sex-specific risk stratification strategy in referrals for advanced cardiac therapies
Allocation of advanced therapies	Evaluate impact of interventions aimed at reducing inequities in allocation of advanced cardiac therapies

LVAD, left ventricular assist devices.

pregnancy should not be withheld. HT should be considered for patients who fail to recover after delivery despite maximal therapies (63). In PPCM, there is an increased potential for graft failure and death after HT in comparison to other HF etiologies (62), so HT should be reserved for women with refractory severe HF where LVAD is not possible or desirable, due to biventricular failure or severe initial right ventricular dysfunction (61). Women who desire pregnancy after HT should be counseled on the appropriate timing and management of pregnancy, and educated on the increased risk of cardiac allograft rejection and dysfunction, infection, and teratogenicity associated with use of immunosuppressive agents (63, 65). Pregnancy is not recommended in women supported with LVAD (66).

Lactation/Breastfeeding

Breastfeeding is associated with positive cardiometabolic changes including reduced insulin resistance, lower fasting glucose and blood pressure (67). Therefore, lactation may lower cardiovascular and HF risk. However, it is unclear if the reduction in cardiovascular risk factors could mitigate HF progression or ameliorate advanced disease. In PPCM specifically, prolactin suppression with bromocriptine (a dopamine agonist) was associated with greater LV functional recovery (68). The European Society of Cardiology recommends against breastfeeding when LV function is severely impaired but encourages breast feeding in women with mild systolic dysfunction (69). Guideline directed medical therapy can be used during lactation with careful attention to the safety profile of each medication and its possibility of being secreted in breastmilk (63, 66). The decision to pursue breastfeeding among mothers who are HT recipients should also be individualized and based on a risk-benefit analysis of the potential for immunosuppressive medications to be excreted in breast milk (65).

Contraception

Providers caring for women with AHF of reproductive age should inquire about contraceptive use, because pregnancy can lead to hemodynamic compromise (63). Contraceptive options include combined hormonal oral contraceptives, progestin-only formulations, intrauterine devices, barrier methods, hormonal implants and tubal ligation. Women with high-risk cardiac conditions should avoid combined hormonal contraception due to an increased risk of hypertension and stroke (70). Amongst women with AHF, intrauterine devices are the most appropriate contraceptive method (66). Following HT, there are additional concerns including drug-drug interactions with immunosuppressive medications (65). The ISHLT recommends against intrauterine devices due to an increased risk of expulsion in nulliparous women and concerns about increased risks of infection after HT (71). However, the Center for Disease Control and Prevention supports the use of intrauterine devices in women with complex medical conditions including solid organ transplant (72).

Menopause

There is accumulating evidence that the menopausal transition influences HF risk (73). Menopause is associated with metabolic derangements, inflammation and lipid abnormalities that promote an adverse cardiovascular risk profile and HF development (14). It is unclear if the increase in HF risk after menopause is predominantly due to hormonal changes that occur with the menopausal transition, or result from a higher prevalence of risk factors that occur with biologic aging (14). Even in the absence of biochemical markers of myocardial injury, early menopause is independently associated with HF development (74). The type of menopause also influences HF risk. When compared to those with natural menopause, women with surgical menopause have worse cardiovascular risk profiles prior to menopause, and the adverse changes in LV structure and function seen among them may be explained by their pre-surgical risk profile (75). Cardiovascular risk factors such as obesity and hypertension which affect HF progression influence both age at natural menopause and indications for surgical menopause (75). For instance, uncontrolled hypertension could trigger hospitalization, progression to advanced disease, and poor outcomes (76) in postmenopausal women with HF.

BARRIERS TO THERAPIES FOR WOMEN WITH ADVANCED HEART FAILURE

Barriers to AHF care prevail at the individual, provider and organizational levels (77) with well recognized gender differences (78) (**Figure 3**). Social determinants of health, including lack of health insurance, low income, and inadequate social support, are more prevalent among women, and contribute to physician bias in decision making, which promotes worse outcomes, delayed referrals and decreased access to advanced therapies for women (2). Self-care, an integral component of HF management is greatly affected by self-efficacy and functional status in women (79). Depression also negatively impacts self-care and is present in as many as 35% of HF patients (80). Depression, social isolation and poor support systems are recognized barriers to HF self-care in women (78). A woman's caregiving responsibilities may hinder her from seeking care due to conflicting priorities (38). Actual or perceived inadequacy in social support is an important barrier to equitable allocation of advanced therapies in women (10). African American women may be appraised more harshly (10), and are often perceived as having more financial and social challenges when compared to White patients and men (38). Strategies targeting barriers to advanced therapies in women must also be implemented at the individual, provider and organizational levels (**Figure 3**). Organizational policies especially those guiding the implementation of an integrated AHF program can influence individual and provider factors that affect candidacy for AHF therapies.

CONCLUSION AND FUTURE DIRECTIONS

HF progression is influenced by sex-specific risk factors which should be considered when evaluating women for advanced therapies. The management of AHF in women is highly complex and requires effective integration of conventional treatments, advanced therapies and palliative care to achieve optimal outcomes. There are persisting inequities in allocation of advanced therapies, and women are less likely to be referred for a HT or LVAD evaluation despite facing a higher risk of AHF-related mortality. Future studies should address areas of equipoise in the management of AHF among women (**Table 2**). Women should be given equal opportunities as men for inclusion in clinical trials on AHF.

AUTHOR CONTRIBUTIONS

IE proposed the study. IE, ED, EAH, VR, MK, AB, MB, MM, RR, AH, and CG contributed to design of the study and drafting the initial manuscript. IE, ED, EAH, VR, FB, TD, and SH contributed to editing and revising of the manuscript for intellectual content. EMH, FB, TD, and SH provided critical feedback. All authors approved the final version of the manuscript.

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Vascular rejection in cardiac allograft vasculopathy: Impact on graft survival

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Introduction

In the more recent years antibody mediated rejection (AMR) which is a major contributor to vascular rejection has evolved as the most important cause of morbidity and mortality in patients undergoing cardiac transplantation. Acute AMR in the first year would be concerning but a long term consequence of chronic AMR is cardiac allograft vasculopathy (CAV) which has devastating consequences accounting for ∼60% mortality (1–5).

AMR results largely from development of antibodies to the human leukocyte antigen (HLA classes I and II). Non-HLA antibodies have also been implicated in AMR in solid organ transplant recipients (6–8). Allosensitization can be due to a variety of causes including multiple blood transfusions, prior transplantation, human tissue allografts, and more recently the advent of durable mechanical circulatory devices. Prevalence of allosensitization has been noted in 30–50% women with >3 pregnancies due to pregnancy associated paternal antigens (9–13). This article addresses the impact of AMR, its consequences on survival of cardiac allograft recipients and the role of screening for donor specific antibodies (DSA), anti HLA antibodies as well as non-HLA antibodies in improving outcomes in this population.

Pathophysiological mechanisms underlying graft destruction during AMR

The pathophysiology appears to be largely driven by three major components- non-HLA antibodies, anti HLA antibodies as well as donor specific antibodies. The specific role of each of these antibodies in the pathophysiology is discussed below.

Non-HLA antibodies

The role of non-HLA antibodies in the pathogenesis of AMR remains less defined with limited data on their clinical significance, as well as pathophysiology of graft injury. A defined screening protocol for these antibodies should be executed post transplantation.

Non-HLA antibodies have been found to be directed against intracellular antigens. One of the mechanisms of graft destruction by non-HLA antibodies has been suggested *via* destruction of endothelial cells by DSA causing exposure of normally unseen autoantigens leading to production of non-HLA antibodies (14, 15).

Durable mechanical support could possibly trigger non-HLA antibodies. Patients bridged to transplant show higher serum reactivity. Such effects are also noted in patients who have undergone prior cardiac surgery and those with polyreactive antibodies (13, 16). It has been hypothesized that ventricular assist device (VAD) support causes polyreactive IgG antibodies directed to apoptotic cells and oxidized epitopes possibly due to non-specific B-cell activation and IgG sensitization and an inflammatory reaction concomitantly with PGD. Some of the antigens in particular that have been known to have antibodies in the AMR population are, vimentin, beta-tubulin, lamin A/C, and apolipoprotein L2. It is not clear how these mediate graft injury *via* their association with DSA during AMR (17).

It has also been noted that significant reactivity to non-HLA antigens was higher in patients who were positive for donor specific antibodies suggesting an association and synergy between the different types of antibodies. Elevated levels of polyreactive IgG antibodies have been associated with primary graft dysfunction (PGD) (16).

Patients diagnosed with AMR have recently been shown to have a reactive response to vimentin, beta-tubulin, lamin A/C, and apolipoprotein L2. Such increased reactive response to non-HLA antigens has been associated with AMR graft failure in solid organ transplants (18–22).

In the current literature a number of non-HLA antibodies have been shown to be involved in AMR (18–23). Non-HLA antibodies associate both with PRAs as well as DSA (23, 24). Patients with panel reactive antigen (PRAs) >10% during AMR showed increased reactivity to non-HLA antigens as compared to those who had no DSA or PRA. Positive PRA (>10%) without DSA suggests that circulating non-donor-specific anti-HLA antibodies (NDSA) or antibodies to non-HLA antigens could be the reason behind AMR and its impact on poor graft survival (24).

Antivimentin antibodies (AVA) have been implicated in AMR in animal models as well as human solid organ recipients. AVA is commonly seen in patients with autoimmune conditions like lupus/rheumatoid arthritis. Though vimentin is a cytoplasmic intermediary filament protein derived from the mesenchyme noted in leukocytes, fibroblasts, and endothelial cells it is often seen on the cell surface of cells serving as an autoantigen and eliciting an immune response in the autoimmune disease state especially on apoptotic neutrophils and T cells (25–31). AVA has been noted in both renal and cardiac transplant patients and notably in patients who develop cardiac allograft vasculopathy (CAV) (19, 32). Studies show an increased numbers of IL-17 secreting CD4+ T cells directed at vimentin and a reduction in IL-10 producing cells in patients with CAV, suggestive of vanishing tolerance to vimentin in patients with CAV (19, 33). Another important aspect of AVA in cardiac transplant patients is their differential response to immunosuppressive drugs. Mycophenolate mofetil (MMF) has been found to better in reducing AVA as well as HLA antibodies (34). In non-human primates AVA levels were not affected by cyclosporine treatment showing its ineffectiveness in this respect (27). The deleterious effects of AVA possibly occurs *via* complement fixation and a proinflammatory effect. Additionally, the interaction of AVA with neutrophils can lead to platelet activation causing a prothrombotic effect in the graft vasculature.

Other notable associations have been between anti cytoskeletal anti endothelial cell antibodies and cardiac allograft rejection and those against agrin, adipocyte plasma membrane-associated protein, Rho GDP-dissociation inhibitor 2 [ARHGDIB], Rho guanine nucleotide exchange factor 6, angiotensin-II type 1 receptor, endothelin type A receptor, lamin B1, BPI fold-containing family B member 1, peroxisomal trans-2-enoyl-coenzyme A reductase, phospholipase A2 receptor, protein kinase C zeta type, tubulin beta-4B class IVb in renal transplant patients suggestive of a significant role for non-HLA antibodies in AMR in solid organ recipients (35, 36).

Anti HLA antibodies

The activation of endothelial cells by anti HLA antibodies has been postulated to cause proliferation, cytokine production and leukocyte recruitment leading to graft injury and dysfunction. Checking pretransplant anti HLA antibodies should be done imperatively as part of the pre transplant work up algorithm because sensitization has been noted with increasing length of mechanical support. Interestingly, avoidance of perioperative leukocyte-filtered cellular blood product transfusions does not significantly decrease the incidence or degree of HLA sensitization. However, cellular blood product transfusions have been noted in some studies to reduce the possibility of alloimmunization which may reduce the problem in the patients bridged to transplantation (16, 17, 37).

The critical role of anti HLA antibodies in the pathogenesis of AMR and its ultimate impact on allograft survival leads credence for active surveillance of these antibodies pre and post transplantation. The anti HLA antibodies work *via* multifactorial mechanisms to destroy the graft. The most important of these is the complement activation cascade and formation of the membrane attack complex (MAC). Early proinflammatory proteins have recently been shown to drive the complement mediated injury. Other mechanisms include those mediated by the natural killer (NK) cells (38). Mechanisms independent of the complement system such as

antibody-dependent cell-mediated toxicity (ADCC) utilize NK cells, neutrophils and macrophages which bind antibody-coated target cells *via* DC16 resulting in activation of effector immune cells. In NK cells, perforin and granzyme B-mediated cytotoxicity are noted while macrophages use nitric oxide, TNF, and reactive oxygen species to elicit cellular damage (39–42).

Gender differences in alloreactivity is an important aspect that needs to be taken into account when screening for anti HLA antibodies pre transplantation. Female cardiac allograft recipients have a higher pretransplant diagnosis of idiopathic cardiomyopathy, increase levels of antinuclear antibodies, in addition to HLA-B8, DR3 haplotypes. Female heart transplant patients tend to have a shorter duration to the first episode of rejection, increased number, and frequency of rejection episodes, and tend to produce anti-HLA antibodies sooner than their male counterparts. Infection related mortality seem to be higher in female cardiac allograft recipients. Fatal infections have been in noted in female heart transplant patients due to increased cyclosporine levels. Another interesting observation has been that the incidence of CAV developing after the first year post-transplant was lower in females. Women tend to manifest an autoimmune state prior to transplant which may predispose them to a greater risk of allograft. An algorithm for early diagnosis and management including a more individualized approach will improve clinical outcomes in the female population (9).

Donor specific antibodies

DSA play a major role in development of AMR leading to loss of graft function and survival (3, 43–45). Presence of DSA are linked to increased graft loss and mortality. Interestingly preexisting DSA do not seem to affect survival significantly but *de-novo* DSAs (especially Class II) are associated with worse outcomes (46–50). Regardless of when DSA appear, early on or later, it confers a negative impact on graft outcomes. One of the important aspects of DSA is that they can escape detection from circulation because they can adhere to the graft and be just as detrimental. Presence of DSA can predict worse outcomes for the graft in the long term (51–54). AMR has been associated with CAV and presence of DSA has been associated with more severe CAV (55). DSA has been shown to cause endothelial activation leading to allograft dysfunction. Three to eleven percent of patients exhibit the presence of DSA at heart transplantation, interestingly *de novo* anti HLA II DSA seem to develop post-transplant in 10–30% of patients.

AMR and CAV

The role of AMR in pathogenesis of CAV is becoming more evident. AMR is frequently noted patients with CAV in grafts and asymptomatic AMR is associated with a higher risk for CAV and increased mortality. CAV is a progressive obliterative disease due to intimal proliferation and most often not amenable to percutaneous coronary interventions or cardiac surgical revascularization. It has been estimated that >10% of adult cardiac transplant patients carry a diagnosis of CAV at 1 year and >50% at 10 years post transplantation (55–58). The major cause of CAV is alloimmunity with multiple factors including non-immunological attributes of the donor and recipient such as age and medical comorbidities.

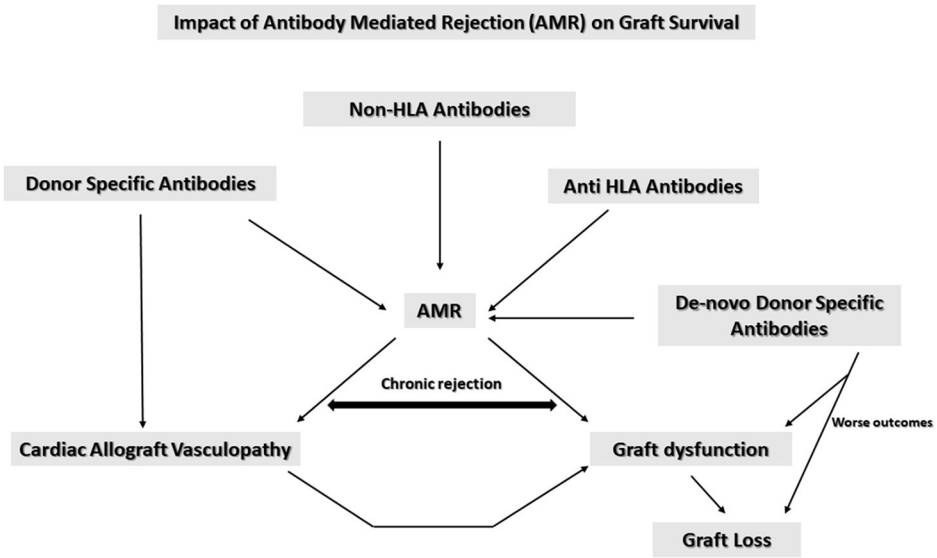

FIGURE 1
Impact of antibody mediated rejection on graft survival.

AMR and CAV have been increasingly associated, with AMR occurring more frequently in patients with CAV. Patients with asymptomatic AMR show higher risk for CAV. In addition to DSA involvement in AMR induced CAV direct role of complement activation also plays a significant role. Endothelial cell cells get damaged directly due to C4d complement activation and deposition. Targeting of NK cells and macrophages can lead to progression of plaque formation. DSA and complement activation in combination affect endothelial cells, platelets, and macrophages to accentuate the progression of CAV (59).

Discussion

In summary AMR is a primary cause for graft dysfunction and mortality. As summarized in Figure 1 anti HLA antibodies, non-HLA antibodies and DSA all contribute to AMR. AMR also is associated with CAV which in turn causes long term graft dysfunction and graft loss leading to significant morbidity and mortality. DSA are associated with CAV risk. Patients with anti HLA class II antibodies have a shorter time to CAV diagnosis (4 years approximately) vs. those with only anti HLA class 1 antibodies (7–8 years approximately). Patients with a mixture of antibodies to both classes I and II develop CAV within 2 years and are therefore at the highest risk (43).

Newer technologies using molecular biology techniques such as targeted amplification of donor-derived cell free DNA (dd-cfDNA) may be the beginning of a highly sensitive method of detecting AMR even before it can be detected by endomyocardial biopsy which is the current method of diagnosis. This technology is based on the premise that in the setting of acute rejection allograft cell death occurs resulting in elevated levels of dd-cfDNA in the recipient's circulation (60, 61). Such efforts to detect subclinical injury will help early diagnosis of AMR before possible graft injury. Application of molecular technologies for early clinical diagnosis may be the future for graft preservation.

Current literature attributes DSA a major role in determining the fate of the transplanted heart. In the past, DSA considered harmful were essentially those directed against ABO blood group antigens or donor HLA antigens. More recent literature shows antibodies directed against other molecules expressed by the donor organ such as anti-endothelial cell antibodies or antibodies against MHC Class I-related chain A (MICA), anti-endothelin-1 receptor type A (ETAR), Perlecan and anti-angiotensin II receptor type 1 (AT1R) are equally relevant. In addition to IgG, IgM is associated with worse graft outcomes in the long term. Additionally, DSA that are capable of activating the complement system produce worse outcomes than DSA which act *via* a complement-independent mechanism. Contrary to the earlier hypothesis *de-novo* are also associated with poorer outcomes. Currently patients with non-significantly low levels of DSA have been postulated to be safe to transplant with closer follow up of antibody levels which is becoming more feasible with new molecular technologies such as the use of dd-cfDNA (39, 62–67).

Monitoring for DSA and *de novo* DSA post-transplant could provide an early diagnostic strategy for early intervention and better outcomes for graft protection. However, due to lack of existing studies definitive algorithms for surveillance and treatment are non-existent. The surveillance strategies and treatment protocols vary from center to center. More studies are needed at a multicenter level to assess antibodies at pre and post-transplant at different time points to develop more definitive protocols for surveillance and treatment. Building registries would help with defining thresholds for intervention and modifying peri operative management. AMR still remains a challenge and largely responsible for graft loss in the present era. Definitive studies are required in this area to improve outcomes and graft dysfunction and loss.

Author contributions

NN conceived the idea and prepared the entire manuscript.

Conflict of interest

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

Publisher's note

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Women in mechanical circulatory support: She persisted!

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Many women physicians have blazed trails and played instrumental roles in advancing the field of Advanced Heart Failure (AHF), Mechanical Circulatory Support (MCS), and cardiac transplantation to its current recognition and glory. In contrast to other areas of cardiology, women have played an integral role in the evolution and emergence of this sub-specialty. Although the ceiling had been broken much later for women cardiothoracic (CT) surgeons in the field of AHF, the ingress of women into surgical fields particularly CT surgery was stonewalled due to pervasive stereotyping. The constancy, commitment, and contributions of women to the field of AHF and MCS cannot be minimized in bringing this field to the forefront of innovation both from technological aspect as well as in redesigning of healthcare delivery models. Integrated team-based approach is a necessity for the optimal care of MCS patients and forced institutions to develop this approach when patients with durable left ventricular assist devices (LVAD) began discharging from the hospitals to local communities. Women in various roles in this field played a pivotal role in developing and designing patient centered care and coordination of care in a multidisciplinary manner. While embracing the challenges and turning them to opportunities, establishing partnerships and finding solutions with expectations to egalitarianism, women in this field continue to push boundaries and subscribe to the continued evolution of the field of AHF and advanced cardiac therapies.

KEYWORDS

mechanical circulatory support (MCS), women in MCS, women in medicine, left ventricular assist device (LVAD), heart failure, CT surgeons, cardiologist

Introduction

In the traditionally male dominated profession of medicine, addressing gender disparities continues to spark passionate debate. Despite ongoing efforts and closing the gender gap among medical school matriculants, women have yet to achieve equality in multiple disciplines of medicine and surgery. In the United States,

approximately one in four trainees in cardiology and thoracic surgery is a woman, reflecting a significant gender gap in these specialties (1). The exception is seen in pediatric cardiology, where just over half of fellows have been women. The reasons for consistent underrepresentation of women in adult cardiology and cardiothoracic (CT) surgery fields are myriad. Women in these fields experience difficulties at multiple levels including (1) gaining entry (unlocking the door), (2) barriers to promotion, impediments to professional development, and delayed or limited career advancement (sticky floor), and (3) lack of leadership opportunities among institutions, professional societies, and editorial boards (glass ceiling) as represented in **Figure 1**. In spite of these challenges, women in these specialties have marched forward and redefined the fields with their professionalism, contributions and commitment, and constancy.

Evolution of AHF, MCS and transplant took a huge leap with the approval of LVAD implantation as a destination therapy (DT) and has transformed over the years from a primarily a surgically driven model to a multidisciplinary model due to the complexities involved in the care of these patients. As a lot of initial work in the field of MCS and transplantation was driven by CT surgeons, the role of women in the early stages of the field was paltry as the field of CT surgery was considered to be a man's field. Dr. Nina Starr Braunwald became the first woman to perform CT surgery in the United States breaking the sociocultural barriers and the pervasive stereotyping (2). It is the perseverance of pioneering women surgeons such as Drs. Nina Braunwald, Ann McKiel, and Nermin Tutunju that paved the path for women of future generations (3). These women blazed a trail and made CT surgery an achievable aspiration for other women who subsequently followed in their footsteps and in later years opened the window to surgical management of AHF including MCS and cardiac transplantation.

After the Randomized Evaluation of Mechanical Assistance for The Treatment of Congestive Heart Failure (REMATCH) clinical trial helped to establish LVAD implantation as a viable therapeutic option for patients with end-stage heart failure who are not candidates for cardiac transplantation, the role of heart failure cardiologists rose to prominence in the care of these patients and forced the evolution of this field as a distinct specialty (4). Conventional healthcare delivery models were challenged with LVAD patients living in the communities as the care of these patients required care coordination and multidisciplinary team (MDT) approach. The MDT care model of heart failure and MCS patients has been adapted by various medical and surgical specialties and has evolved into a standard of practice currently for patients with complex care needs.

While gender equity and parity have yet to be fully attained in this field, tremendous progress has nonetheless been made in this field as compared to other subspecialties of cardiology. Similar strides have been made in CT surgery over the past decade to reduce the gender disparity and recruit women through mentoring, advocacy, and sponsorship (5). In this review, we focus on the experiences and views of women cardiologists and CT surgeons in the field of MCS regarding the perceived barriers, challenges, growth opportunities and future directions for the women in this field.

Methods

Although this is a review, we followed an integrated approach and combined the information obtained through literature review and shared experiences from interviewees in presenting this article. For this review, we interviewed women in both current and past leadership positions in this industry. We sought to understand their motivations, challenges, and achievements to highlight the path of women in these fields over multiple generations. The interviewees included in this review were by no means intended to be an exhaustive list. We broadly categorized the interviewee's experiences into medical [advanced heart failure (AHF) cardiologists] and surgical (CT surgeons) perspectives based on the respective area of their professional practice. In addition, we reviewed the literature on women physicians in cardiology and, cardiothoracic surgery, medicine, and in academic medicine. We integrated the information derived from literature review with information shared by our interviewees due to the specific focus on the field of MCS. Although it is imperceptible to cleave AHF, MCS and transplantation, this article kept the primary focus on the role of women cardiologists and cardiothoracic surgeons in the field of MCS. We hope to illuminate the path and perseverance of these women to acknowledge their grit and dedication, as well as to provide a framework of success for future women of these fields. We have listed the Heart Failure Cardiologists and CT surgeons we spoke to with their current titles and leadership positions in alphabetical order as represented in **Table 1**.

Pioneers of advanced heart failure and mechanical circulatory support field

Over the past three decades, the emergence of the LVAD as a durable therapy for end stage heart failure revolutionized the field of AHF and MCS. This has transformed the operational framework of the field from a primarily surgeon driven model to a multidisciplinary team approach. This novel archetype brought the need for a new breed of cardiologists to facilitate the longitudinal care of patients with devices. Women cardiologists who found this specialty attractive due to complexity of the patients collaborated with surgeons, engineers, and industry,

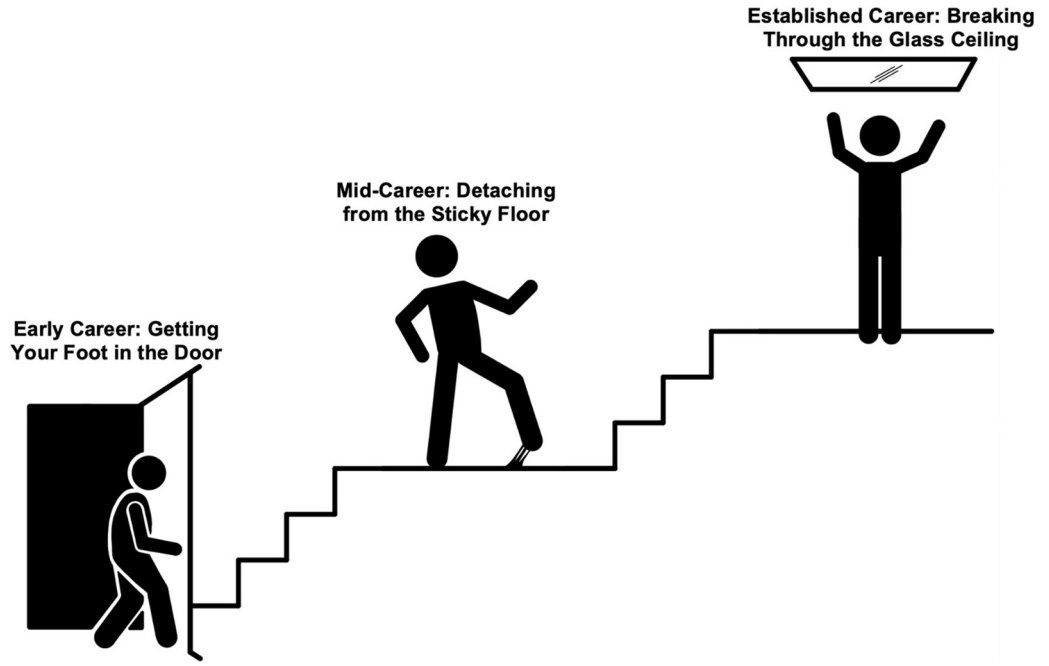

FIGURE 1
Stages of challenges facing women entering heart failure and CT surgery.

TABLE 1 List of interviewees for this review and their current affiliated institutions.

Name	Current institution	Area of expertise (practice)
Deborah Ascheim, MD	StrideBio, Inc.	AHF, MCS, and Transplant Cardiology
Geetha Bhat, MD	Penn State heart and Vascular Institute, Hershey, PA, United States	AHF, MCS, and Transplant Cardiology
Linda Bogar, MD	AdventHealth Transplant Institute, Orlando, FL, United States	CT surgery
Elizabeth Blume, MD	Boston Children's Hospital, Boston, MA, United States	Pediatric AHF, MCS, and Transplant Cardiology
Susan Brozena, MD	University of Pennsylvania Health System, Radnor, PA, United States(Retired)	AHF, MCS, and Transplant Cardiology
Margarita Camacho, MD	Newark Beth Israel Medical Center, Newark, NJ, United States	CT surgery
Jennifer Cook, MD	University of Cincinnati, Cincinnati, OH, United States	AHF, MCS, and Transplant Cardiology
Hannah Copeland, MD	Lutheran Hospital- Fort Wayne, IN, United States	CT surgery
Jennifer Cowger, MD, MS	Henry Ford Health, Detroit, MI, United States	AHF, MCS, and Transplant Cardiology
Amy Fiedler, MD	University of California San Francisco, San Francisco, CA, United States	CT surgery
Maya Guglin, MD, Ph.D.	University of Indiana Health, Indianapolis, IN, United States	AHF, MCS, and Transplant Cardiology
Amy Hackmann, MD	University of Texas Southwestern Medical Center, Dallas, TX, United States	CT surgery
Shelley Hall, MD	Baylor University Medical Center, Dallas, TX, United States	AHF, MCS, and Transplant Cardiology
Sharon Hunt, MD	Stanford University Medical Center, Stanford, CA, United States	AHF, MCS, and Transplant Cardiology
Mariell Jessup, MD	American Heart Association	AHF, MCS, and Transplant Cardiology
Manreet Kanwar, MD	Allegheny General Hospital, Pittsburgh, PA, United States	AHF, MCS, and Transplant Cardiology
Anuradha Lala-Trindade, MD	Icahn School of Medicine at Mount Sinai, New York, NY, United States	AHF, MCS, and Transplant Cardiology
Sharon Larson, DO, MS	University of Iowa, Iowa City, IA, United States	CT surgery
Seema Mital, MD	SickKids Research Institute, Toronto, Ontario, Canada	AHF, MCS, and Transplant Cardiology
Stephanie Moore, MD	University of Louisville, Louisville, KY, United States	AHF, MCS, and Transplant Cardiology
Salpy Pamboukian, MD	University of Alabama at Birmingham, Birmingham, AL, United States	AHF, MCS, and Transplant Cardiology
Brigitte Stiller, MD	University of Freiburg, Breisgau, Germany	AHF, MCS, and Transplant Cardiology
Nancy Sweitzer, MD, Ph.D.	Washington University School of Medicine, St. Louis, MO, United States	AHF, MCS, and Transplant Cardiology

which enabled them to develop partnerships, a fundamental key to building successful AHF and MCS programs and services. They established programs with their remarkable leadership, and team building skills and played instrumental roles in the evolution of this field. Noteworthy leadership in this field includes Dr. Sharon Ann Hunt, Dr. Hannah Valantine, Dr. Mariell Jessup, Dr. Lynne Warner Stevenson, Dr. Maryl Johnson, Dr. JoAnn Lindenfeld, Dr. Maria Generosa Crespo-Leiro, Dr. Donna M. Mancini, Dr. Maria Rosa Costanzo, Dr. Anne M Keogh, Dr. Heather Joan Ross, Dr. Geetha Bhat, Dr. Ileana Piña, Dr. Mary Norine Walsh, Dr. Roberta C. Bogaev, Dr. Nancy K Sweitzer, Dr. Emma Jane Birks, and Dr. Shelley Anne Hall. They have brought transformative changes that have shaped the field and defined the role of women in this field (6, 7).

Jean Rosensaft co-founded LVAD technology, Inc., in 1983 along with Dr. Adrian Kantrowitz and served as vice president of this bioelectronics research company that was focused on developing mechanical circulatory support (MCS) devices. A Japanese CT surgeon Dr. Chisato Nojiri joined the research team of Professor Willem Johan Kolff who was known as "father of artificial organs" at University of Utah after acquiring her Ph.D. in Japan. She focused on development of LVADs subsequently and developed a magnetically levitated implantable LVAD (8). It is the pathbreaking entry of Dr. Margarita Camacho in the clinical practice of AHF and MCS that has transformed the field for women CT surgeons. Brilliance, sedulity, and collective efforts of these women in various realms of the AHF and MCS propelled the field to its current distinguished status and pushed to create more space for women around them and for women of next generations.

Finding a path to the heart

Shaping the field

Dr. Sharon Ann Hunt is one of the originals in the field of AHF, MCS and Cardiac transplantation. She has witnessed the evolution of the field and informally addressed as "mother of transplant." Although she focused more on the care of heart transplant patients, she was involved in the care of Robert St. Laurent, a patient who underwent the first successful implantation of LVAD as a bridge to transplantation with a Novacor device. She wrote commentary for the REMATCH study, a landmark clinical trial demonstrating the survival benefit of LVAD as a destination therapy. Dr. Marriell Jessup is a thought leader and a pioneer in the field of AHF and has served in many roles that positively impacted the field. The efforts of pioneering physicians such as Drs. Sharon Hunt and Mariell Jessup culminated in recognition of AHF as a secondary specialty of cardiology. Dr. Jessup played a crucial role in developing the curriculum and establishing the standards of training and competency requirements for the heart failure subspecialty. Dr. Lynne Warner Stevenson, recognized as doyenne of MCS field played a crucial role in designing the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) for monitoring of outcomes and in developing INTERMACS risk profiles to better characterize the clinical risk of AHF patients (9). Dr. Birks's contributions to myocardial recovery of patients with LVAD are noteworthy (10). Dr. Hall organized the "Women in Transplantation and MCS" group and laid the foundation for collective action for advancement of women in this field. She played an instrumental role in bringing women in this field together and providing a platform for collaboration, support, and networking (11).

Dr. Chisato Nojiri, who dreamed of becoming a Nobel laureate in physics and later became a physician scientist, was deeply invested in the development of durable MCS devices. Later, she became the CEO of Terumo Heart Co., Ltd., and was successful in executing the development and commercialization of magnetically levitated implantable LVAD (DuraHeart) (12). The first woman cardiothoracic surgeon to implant a durable LVAD in clinical practice in the United States (presumably in the world) to our knowledge is Dr. Margarita Camacho. She opened the door for other women in the CT surgery field to join this very rewarding AHF and MCS realm of CT surgery. There are several female CT surgeons in the field currently and we compiled a list based on online search in alphabetical order, **Table 2**. The excitement, gratification, and cachet of offering a new life to someone with end stage heart failure and its impact on the patients and their families are the driving forces for women to enter the surgical specialty of AHF and MCS.

This section will delve into what inspired and persuaded the women we interviewed to join these teams and purse these fields. We interviewed physicians of different generations who are at different stages in their professional development with varied career trajectories including physicians in clinical practice, academic practice, and physician scientists. While the earlier generation physicians had latitude to design and

TABLE 2 List of female CT surgeons currently in the field of MCS (to our knowledge based on internet search).

CT surgeons in MCS field	Current affiliated institution
Linda Bogar, MD	AdventHealth Transplant Institute
Reshma Biniwale, MD	University of California Los Angeles
Margarita Camacho, MD	Newark Beth Israel Medical Center
Hannah Copeland, MD	Lutheran Hospital- Fort Wayne, IN
Amy Fiedler, MD	University of California San Francisco
Amy Hackmann, MD	University of Texas Southwestern
Sharon Larson, DO, MS	University of Iowa
Marzia Leacche, MD	Spectrum Health
Deyanira Prastein, MD	Rochester Regional Health
Alexandra Tuluca, MD	Einstein Healthcare Network
Katherine Wood, MD	University of Rochester

shape the field, later generations of interviewees touted their fortune to have many pioneering female physicians as their role models. Below we intend to highlight some of these trailblazing women whose work were inspiring to many of our interviewees. Comparable to this review, the list above by no means holds all female physicians to inspire and impact the field. These names represent some of the profound work that have progressed the practice of medicine for patients with AHF. Now having acknowledged some of these historical efforts, the remainder of this section will highlight themes that arose in our interviews with heart failure specialists and CT surgeons.

Perspectives of heart failure cardiologists

Few of the physicians we interviewed were successive generations of physicians or came from a medical background, but many had a passion to become a physician during their childhood. As heart failure was a relatively new branch of cardiology and a niche specialization, an interest in pursuing that fellowship came much later for most of the interviewees. Heart failure patients have chronic health needs facilitating longitudinal care allowing to build lifelong relationships with them. Dr. Mariell Jessup, Chief Science and Medical Officer of the American Heart Association and an Emeritus Professor of Medicine at the University of Pennsylvania School of Medicine, who is instrumental in carving out heart failure as a subspecialty stated that she derives motivation primarily from patients and patient stories (13). One physician informed us her drive to serve this population comes from the unique needs of care that this population has and shared her experiences of having to act as primary care physician for these complex patients. Where there are moments of immediate gratification when there is a noticeable clinical difference with institution of a temporary MCS device, there are other instances that demand patience and time to know the trajectory of clinical course and outcome. As the mortality in AHF is high, the field requires an ability to have end of life and advanced life care planning conversations with a patient's loved ones, a responsibility many of the women we interviewed held dearly.

Despite perceived limitations and challenges, heart failure as a specialty has been a rewarding experience to the physicians we spoke with. Given the high acuity in end stage heart failure, patient outcomes are not always favorable. Workdays are long and patient needs may arise at any hour of the day or night leading to grueling work hours and burnout. Though not all these challenges are unique to heart failure, there has been a decline in fellowship applicants to heart failure and transplant programs. In 2021, there were twice as many fellowship applications for interventional cardiology as for heart failure (2, 14). We inquired about this difference and our interviewees attributed this diminishing interest to multiple factors including the predominant nature of inpatient experience of heart failure during fellowship training, current model of pay structure based on relative value unit (RVU) billing system that is more lucrative for procedure-oriented cardiology secondary specialties in comparison to heart failure and perceptions of less autonomy in decision making due to MDT approach. Many women acknowledged the specialty had more delayed gratification compared to some other cardiology specialties and current models of value analysis and reimbursement do not effectively capture the efforts of heart failure cardiologists and the cognitive input required in taking care of these complex patients. In addition, heart failure cardiologists carry a significant emotional burden as the mortality of heart failure patients is higher than most cancers leading to channeling a portion of practice into end-of-life discussions.

Overall, there was an acknowledgment that no field is perfect. However, so many of our interviewees felt a strong calling to do such demanding work. They felt the reward of their career from the genuine passion they held to serve such unique heart failure patients.

Perspectives of cardiothoracic surgeons

Akin to the heart failure specialists we interviewed, many of the CT surgeons we spoke with did not come from generations of physicians but found a passion for surgery earlier in life. Most of the interviewees were motivated to become surgeons well before finding cardiac surgery, which then led them to MCS and transplant. Passion for this subspecialty partly came from a fascination with the heart itself. Some described the neatness of the heart being one of the few organs that we can physically feel functioning, while others were fascinated by the physiology of the heart. Comparable to other fields of surgery, there is an immediateness to the results of their work, but transplant and MCS are unique as these are some of few surgical subspecialties that involve putting something into the body rather than taking out. Many of the surgeons we spoke to enjoy the team-based approach used within MCS and transplant, and for the opportunity to collaborate with other specialties.

Great progress has been made in the technology and techniques of MCS and transplant and the surgeons we spoke with were excited about the accelerating pace of changes in the field and had varying opinions of the future path of MCS and transplant. Enthusiasm is on the upswing in the sphere of transplant as xenotransplantation has recently made headlines and donation after cardiac death (DCD) continues to expand into more institutions. The frontiers of MCS are being pushed to achieve better hemocompatibility, miniaturization, and thermal energy transfer systems with hope of developing a fully implantable LAVD free of external cables. There is boundless excitement regarding the potential for application

of artificial intelligence and machine learning in the clinical care of these complex patients and shift the paradigm towards precision medicine. Some felt that success of HeartMate 3 LVAD carved a future with greater use of MCS, while others felt the expansion of the donor pool and transplant techniques including xenotransplantation may open more avenues in the transplant arena. Regardless of the specialty's direction, some aspects are unlikely to change. Transplant cases can arise at any hour of the day and require significant coordination from procurement to transplantation leading to long and arduous days, presenting challenges in many surgeons' personal lives.

Though the work as a CT surgeon in MCS and transplant may be challenging, it was evident that the women we spoke with held genuine admiration for the field and enjoyment of the work they were performing. As one surgeon said, it is not for the faint of heart, but if you love it, go for it.

Sponsorship is the new mentorship

Perspectives of heart failure cardiologists

All women cardiologists we spoke to acknowledged the power and influence of mentorship in their professional development. While no one denied the significance of having a mentor that looks like you, the preference is unanimous among the interviewees for a quality mentorship over gender concordance. Many of the women we interviewed had predominantly male or only male mentors, often due to a lack of female leadership during the time of their training. They felt being seen for their work provided the greatest value as well as having mentors that brought them to the table. One example of this is seen with Dr. Elizabeth (Betsy) Blume current Director, Advanced Cardiac Therapies; Medical Director, Heart Failure Program at Boston Children's Hospital. Earlier in her career she was mentored by and collaborated with both cardiologist and surgeons who brought her into a multi-institutional study based on Pediatric Heart Transplant database that analyzed the outcomes of children bridged to heart transplantation with ventricular assist devices (15). Their collaboration continued and she played a major role in establishing Pediatric Interagency Registry for Mechanical Circulatory Support (PediMACS) (16). This inclusion and acknowledgment of her abilities allowed her a steppingstone for many future collaborations and leadership opportunities. Dr. Blume's story illuminates that quality mentorship does not only include setting an example but bringing mentees into the conversation and allowing them opportunities for authorship and leadership.

Expanding the role of mentorship into one of sponsorship brings inclusion and promotes their protégé. This idea was proffered by Dr. Salpy Pamboukian—Director, MCS Device Program; Co-section Head, Advanced Heart Failure, Cardiac Transplantation, MCS, and Pulmonary Vascular Disease at University of Alabama at Birmingham, while describing her own experiences as both a mentee and a mentor. The most transformative and powerful relationships were reaped when a mentor took an active interest in her career, "shepherding" her toward various opportunities. Many of the women spoke of the reciprocal relationship they cultivated as mentees and sought for as mentors.

Perspectives of cardiothoracic surgeons

The cardiothoracic surgeons we interviewed similarly felt that the quality of the mentorship was more impactful than the gender of the mentor. Two common themes we found that made mentorship impactful were belief and inclusion. Each interviewee described a unique experience of having to balance support and discouragement on their path from first deciding to pursue a career in medicine to training as a cardiothoracic surgeon. Regardless of any negativity they may have faced, their self-confidence, grit, and dedication helped in establishing quality mentor relationships. Though the poise previously described was not unique to any one surgeon we spoke to, Dr. Linda Bogar is one example of perseverance manifesting mentorship. She is a CT surgeon at AdventHealth Transplant Institute, Orlando, FL with a focus in heart and lung transplant and MCS, and previously served as the Surgical Director of Heart Transplantation and MCS at Thomas Jefferson University Hospital. Dr. Bogar found her calling to CT surgery late in the fourth year of her general surgery residency. Since the realization came after the deadline for the CT fellowship match, her chief at the time facilitated a 1-year "fellowship" as a substitute to prepare her for her actual CT fellowship the following year. This story highlights the reciprocal relationship seen in quality mentorship. Seeing and understanding Dr. Bogar's hard work and dedication, her mentor supported and believed in her to aid in her success. Many women that we interviewed felt that the investment in time and energy from their mentors came partly from a place of reciprocal respect. Belief in one's own capabilities and hard work created an environment for their mentor to both believe in them and put effort into their success. Every woman had at least one male mentor in their life invested in their growth and navigated supported opportunities for them. Most women of recent generations described being lucky enough to have at least one female mentor. Dr. Amy Fiedler, Assistant Professor of Cardiac Surgery at The University of California, San Francisco and member of the Presidential Leadership Scholars Program, discussed one such example of this female mentorship. Dr. Fiedler described the influence of cardiac surgeon, Dr. Jen

Walker, on her journey from being a general surgery resident to finding CT surgery. Dr. Walker not only encouraged Dr. Fiedler, but also made her realize that there was a space for someone like her as a woman in cardiac surgery. Though the sponsorship provided to Dr. Fiedler was impactful on her career, she posited that inclusivity and representation are crucial for success of the women entering such male dominated fields of medicine such as cardiology and CT surgery. Dr. Pamboukian summed up this concept in her statement "the ability to have a quality mentorship should not be dependent on race, ethnicity, age, or gender."

Wearing one hat at a time: Work-life balance

Perspectives of heart failure cardiologists

The most common response when asked about work-life balance is that no such thing exists. Many of the women we interviewed discussed the need to prioritize the different aspects of life at different times. This idea was well illustrated by Dr. Anuradha Lala-Trindade (Anu Lala), Director of Heart Failure Research, who serves in a leadership role at the Data Coordinating Center for the NHLBI CT Surgery Network, director of the fellowship program in AHF and Transplant at Mount Sinai, and a Deputy Editor at the *Journal of Cardiac Failure*. She described being moved by the notion that you can do it all, just not at the same time. Feeling one should be able to "wear all these different hats at all the same time" and expecting to excel at all one's different roles, is setting oneself up for frustration. Many of these women learned to focus on the different aspects of their life at different days and different times.

This prioritizing of responsibilities was echoed by Dr. Shelley Hall—current Chief of Transplant Cardiology, MCS and Heart Failure at Baylor University Medical center, President of Texas American College of Cardiology (ACC) and founded the Women in Transplant and MCS organization. She is the past Chair of UNOS cardiac committee and Thoracic and Critical Care Council of Practice, American Society of Transplantation (AST). She described how she often was unable to be as social at work as some of her male colleagues because each day she had to fulfill her duties as a physician before going home to her second job, motherhood. Dr. Hall and many other women we spoke to described the guilt that came with having a rigorous career as a heart failure cardiologist while being a mother. Some days they would be able to be a "great mom," being home for dinner, participating in school field trips, or baking cookies for class activities, while other days they would be a "great physician" by taking extra-call or completing research goals. Though they never failed to fulfill their duties as a care provider of AHF patients, many felt their gender posed unique challenges.

Seeking this work-life integration was also discussed by Dr. Deborah Ascheim, current Chief Medical Officer of StrideBio, Inc., and former Director of the International Center for Health Outcomes and Innovation Research's Clinical Trial Unit at the Icahn School of Medicine at Mount Sinai. Dr. Ascheim described her passion for working with critically ill heart failure patients in the ICU; however, the demands of the clinical practice combined with her clinical research activities were not always compatible with her own needs as a mother. Therefore, for a couple of years earlier in her career while serving as a heart failure cardiologist at Columbia University Medical Center, she carved her job in an albeit limited fashion to accommodate her personal needs even when such molding was not totally in line with her immediate professional goals. Dr. Ascheim felt this compromise may have sustained her longer in the field and opened future opportunities for her. She advised that women utilize negotiation skills to not only promote what they can offer their employer, but also to push and see what their employer can offer them. This synergy in the various hats these physicians wear, personally and professionally, are crucial for optimization of their abilities and overall wellbeing.

Perspectives of cardiothoracic surgeons

The cardiothoracic surgeons we interviewed preferred to rename work-life balance to work-life "integration." As one of the surgeons stated, "you have to take life one day at a time." Similar to the experiences of the heart failure cardiologists, the female surgeons we interviewed felt it is unrealistic and unhealthy to take on the many roles of professional and personal life simultaneously. One piece of advice that was offered to help with this integration was practicing and executing strong time management skills. One example of this was described by Dr. Hannah Copeland, current Surgical Director of Heart Transplant and MCS and Director of ECMO at Lutheran Hospital in Fort Wayne, Indiana. She would design her days to ensure a distinct presence in each of her roles as a mother, as a wife, and as a surgeon. Each morning she would wake-up at 3:00 AM to perform chart review, read literature, or complete other work tasks and workout, before her kids would wake up. She designated specific time for her professional and personal activities, and crafted her routine to allow her to be fully present with her children after work until their bedtime, while fulfilling her professional interests. Though many specialties of medicine, especially surgery, are not foreign to early mornings and long days, this example demonstrates the kind of time management and alterations many of these women needed to make for their personal lives and loved ones.

An unfortunate aspect of work-life integration in CT surgery is the need for sacrifice. All of the surgeons we spoke to recalled the challenge of turning away from aspects of their personal

life in order to adequately fulfill their duties and serve their patients. This can range from an inability to provide a reliable timeframe for family and friends to missing important family events. Though such sacrifice can at times be frustrating and difficult, in our interviews, we noticed an acceptance of what was required of them as surgeons without any regrets.

To be or not to be: One of the boys

The occupational minority status of women in cardiology and thoracic surgery is well known, and the effect of this is felt differently by each woman. Overall, many seemed to identify a progression over the years from needing to be "one of the boys" in order to become successful to nowadays where many trainees want to be acknowledged as a woman doing the same work as men. This evolution of the perspectives and experiences of female physicians in these sub-specialties may be attributable to various factors including societal changes toward career women, greater discussion of representation and salary equality, and generational changes in work-life priorities.

Perspectives of heart failure cardiologists

Earlier generations of physicians that we spoke to discussed how being one of the only women in their field was the *status quo*. One such woman is Dr. Sharon Hunt, one of the true pioneers of this subspecialty. As a fellow at Stanford University, Dr. Hunt and her three colleagues developed institutional guidelines for care of post cardiac-transplant patients (6). She served in various in leadership roles including Medical Director of the Heart Transplant Program at Stanford University and President of the International Society for Heart and Lung Transplantation (ISHLT) in 1996. She received several distinguished honors and awards including ISHLT Lifetime achievement award in 2012 and Hewlett award in 2013. She discussed the uniqueness of being one among the first to start treating post-cardiac transplant patients and the camaraderie she shared with her male colleagues. Being in an environment where energy was focused on the work being done and not the gender of the provider, created a space for her to be acknowledged as a capable, accomplished physician and not isolated by her gender.

Later generations of heart failure specialists encountered more gender-based stereotypes and shared experiences where their gender became more of a focus than their work. Overall, most physicians we interviewed were aware of underrepresentation of women leaders and role models in heart failure but did not use it as a deciding factor when choosing to enter the specialty. A few women we spoke to were motivated by the opportunity to close the gender gap; however, regardless of whether gender disparities were in the minds of these accomplished interviewees, everyone strongly felt that they should be recognized for their work independent of their gender. The diminished presence of women is not particularly limited to institutional and organizational leadership positions, but also evident at national conferences, industry sponsored events, editorial boards, and scientific societies. Dr. Nancy Sweitzer, Vice Chair of Clinical Research for the Department of Medicine at Washington University School of Medicine in St. Louis, and current editor-in-chief of *Circulation*: *Heart Failure* shared one such experience with us. She previously served as Chief of Cardiovascular Medicine and Director of the University of Arizona Sarver Heart Center. While attending a steering committee meeting for a heart failure device, she found herself to be the only woman on the committee and the only woman in the room that was not a member of the device company workforce. When she pointed this out to the members of the company, Dr. Sweitzer received a reply that they didn't know any women, to which she responded by writing them an almost 40-person list that later became known as "Nancy's List" within the company. In our interviews, we found a consensus that some of the modern issues with gender representation in heart failure came from a lack of trying to find women rather than overt exclusion. One's career goals can be accomplished, but today's culture may mean that those goals take longer to accomplish for women in this profession compared to their male colleagues. However, the goal for so many in the field can be best expressed by Dr. Sweitzer when she said she looks for the day where there are not men and women scientists, but just scientists.

Perspectives of cardiothoracic surgeons

Gender inequity is abound in CT surgery training programs, practice and organized CT surgery and there is a dearth of female role models. Dr. Margarita Camacho shared with us that she was the only female site principal investigator on several of the ventricular assist device (VAD) clinical trials in the United States for many years as AHF surgical field was dominated by men. She is the current Surgical Director of the Cardiac Transplant and Mechanical Assist Device Program at Newark Beth Israel Medical Center and previously served as Chair of the Society of Thoracic Surgeons Workforce on End-stage Cardiopulmonary Disease and President of the Society of Women in Thoracic Surgery. Dr. Camacho is just one example of only a handful of trendsetting women that forged a path in an era with no or limited female leaders. When asked about the gender-specific challenges she faced, she described an experience similar to Dr. Hunt. She discussed how her success came from focusing on her work and outcomes, which was also the focus of her male colleagues. She was not isolated because of her gender but acknowledged as a capable surgeon.

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Many physicians we spoke to from later generations discussed more gender-specific challenges in their career. Interestingly, almost all the women we spoke with described a mostly supportive environment during training but faced difficulties due to their gender when they entered into a faculty or staff position. These challenges ranged from micro-aggressions—such as being referred to by their first name at national conferences, while male colleagues in the same setting were referred to as "Dr. X"—to macro-aggressions like being told "why don't you just get married" when asking for more time in the operating room. These attitudes were never easy to handle, but each of these physicians found different motivations to push through, such as an overriding passion for their work or a desire to fill the gender gap. One powerful piece of inspiration came from Dr. Sharon Larson, current Surgical Director of the Extracorporeal Membrane Oxygenation (ECMO) Program at the University of Iowa and the first female cardiothoracic surgeon to practice in the state of Iowa. She spoke about patient outcomes being her greatest motivation for her work. During Dr. Larson's training she continuously strived to let her work speak for itself to focus the energy of her superiors on her role as a surgeon. If patients were being taken care of and skills were being developed, then "that is what [superiors] paid attention to and not necessarily the package from where the patient care and skill sets were coming from." Throughout her training, she would constantly seek feedback, but there were times where her gender, rather than her work, were the focus of the feedback. Though she never stopped striving to improve her skills, she started incorporating the feedback from her patients as her guiding force. "You can't be a physician without a patient." Becoming the first female CT surgeon in Iowa and later taking on her directorship role did create some gender-specific challenges; however, the motivation provided by gracious patients, some over many years, continues to ignite her passion for CT surgery.

Regardless of how their gender impacted their career or interactions with colleagues, every physician—heart failure specialists and CT surgeons—we interviewed agreed they wanted their work to speak for itself. For the physicians that do not want to be part of the "boys club," they do not desire special accommodations or privileges for their gender, but rather to be seen as equal for putting in the same work and having good patient outcomes. Though we saw a generational-based pattern in experiences, there surely exists differing intra-generational perspectives on gender. Some physicians we spoke to underscored the friendships and relationships they developed with their female colleagues during their careers within their institutions and across the organizations that fostered inclusion and branded themselves as "good girls clubs." Many of our interviewees agreed with the notion of women to women support, but also advised a need for genuine passion for the work, belief in oneself, and grit.

From being led to becoming a leader

Perspectives of heart failure cardiologists

Though women were incremental in the formation of the heart failure specialty, it has taken time to achieve greater representation of female leaders. Current trainees have many accomplished women to emulate; however, there is still more work to be done. The current and past leaders we interviewed were often goal-driven in their pursuit and execution of their leadership. Though these positions were earned through merit, gender-specific obstacles and barriers that these women encountered cannot be disregarded. We would like to highlight Dr. Brigitte Stiller for her groundbreaking contributions to the Berlin Heart in pediatric cardiology including development of institutional protocols and she became the first female president of the German Society for Pediatric Cardiology. Dr. Stiller reminisced her experience of this role as first female president was initially daunting as it was a position held by men for over 50 years. However, when the first meeting with her as president came, she remembered that when the men before her spoke, everyone was quiet and listened, and she knew to expect nothing less while she led. It was her male colleagues' responsibility to learn that there was a role change, and not for her to adapt to them. Dr. Stiller's described female leadership was powerful and poignant and never disrespected. She encouraged academic debate and differing opinions—understanding their benefit and importance—but made sure to keep conversations on topic. The confidence she learned came from a multitude of life experiences, but also her time in the ICU helped develop her skills in collaboration.

As the field of transplant and MCS transitioned from surgery-derived care to heart failure specialists, an MDT approach evolved. Although there may be some institutional variations, MDTs expanded with time and our interviewees played an integral role guiding their team members and developing common purpose. Dr. Stephanie Moore is the current Medical Director of the AHF Therapies Program at the University of Louisville and previously served as Program Director for Massachusetts General Hospital AHF Fellowship program. She described a strategically adaptive leadership depending on the hat she wears. She predominantly followed a coach-style approach while guiding MDTs and a mixed approach (coach and democratic styles) with her fellows in training (FIT). Dr. Moore believes that maintaining a positive spirit in these leadership roles is essential as her team is feeding off her energy and relying on her expertise and guidance. Most physicians we interviewed felt a need to lead by example, which some described as "being in the trenches" with your team. When specifically speaking about training fellows, Dr.

Moore and many other physicians equated the process to raising children. A gentle balance between supportive supervision and fostering autonomy is critical for the development of trainees to independently learn and grow from their mistakes in a safe environment. An essential element in leading a health care team or governing FIT is identifying a common goal by listening and understanding the perspectives of each team member and uniting the team around this common goal while aligning individual motivations to the common goal and nurturing individual needs and ambitions.

Perspectives of cardiothoracic surgeons

Many of the leaders in cardiac surgery we interviewed were recruited to their roles due to their niche skill set or a forte that may serve the institutional goal and/or serve their community needs. Building trust is a key element to succeed in a leadership position. One surgeon we interviewed was Dr. Amy Hackmann, previous Surgical Director of Lung Transplantation, ECMO, and MCS at Keck Hospital of University of Southern California and current Surgical Director of the ECMO program at UT Southwestern Medical Center. She emphasized the importance of gaining trust to provide strong patient outcomes. One way Dr. Hackmann builds such rapport is by creating a culture of strong communication. Patients with AHF are complex and all possible routes of treatment must be considered. A thoughtful approach to these options with careful assessment of the ratio of risk versus benefits and partnership with patients and their caregivers are required in decision making. It is important to listen, talk through problems and allow academic debate. In addition, Dr. Hackmann has always been motivated by patient outcomes and allows her work to speak for itself. Finding this self-confidence is a requisite when assuming leadership positions for effective team building and earning the trust of the team in one's competence and character is crucial for the success.

Complementary to trust building, these leaders of CT surgery also strive to create a culture of inclusivity. Bringing people to the table and increasing representation can strengthen the diversity of thinking and perspectives of an MDT. Representation is an important component of inclusivity, as well as attitudes. Many of these women highlighted this concept by discussing how they ensure to give credit when credit is due. Lastly, strong team culture comes from a leader understanding the individual attributes of each team player. Some of the surgeons discussed how they spent time understanding individuals' strengths and weaknesses. Some people possess skills of empathy but lack efficiency of work or have strong productivity but are prone to oversight. Acknowledging these strengths and placing these individuals in complementary roles builds a stronger, more cohesive team.

A path left behind

To leave behind a legacy is to make a lasting impact on the lives of those around you and generations to come. We interviewed women in a variety of stages in their career, who have either left a strong legacy behind or are continuing to build their legacy. Each interviewee was asked about the legacy they hope to leave behind, and we found the emergence of two themes: being remembered for their devotion to patients and educating their trainees.

Perspectives of heart failure cardiologists

As each of these physicians has established themselves as leaders in the field, they understand it is their responsibility to train the next generations of providers. For many of these women, having personally felt the impact of meaningful mentorship in their own careers, training goes beyond a responsibility but is also a passion. One such example of this passion was described by Dr. Maya Guglin, Medical Director of AHF, Heart Transplant and MCS Services at Indiana University and editor of The VAD Journal. The transition from practicing medicine in the Soviet Union to the United States had many challenges, but it was the belief and efforts of her mentors that helped her persevere and start on a path as a leader and an educator. Dr. Guglin experienced first-hand the difference a quality mentorship can make to a career and has made an effort to continue such support and encouragement. She hopes to leave a lasting legacy as an educator. "You leave part of your soul, part of your personality, part of your knowledge in the people you train." Many of the other physicians we spoke to aligned with Dr. Guglin's sentiments. They enjoy seeing the growth and ownership of those they have mentored.

These women gave a wide variety of advice to mentees, but the overarching theme was to follow their heart and pursue the passion. The work requires long hours which are physically and emotionally draining, but then love for your work will carry you through those hard days. One profound piece of advice was given by Dr. Jennifer Cowger, Medical Director of the MCS Program and Co-Director of the Cardiac Critical Care Unit at Henry Ford Health. She advised aspiring AHF and transplant specialists to define their careers and craft their path. In her words "your institution doesn't make your career; you make your career." This encompasses the grit and perseverance required of so many of these women throughout their careers. Many faced struggles—both gender and non-gender based—but in order to establish themselves as leaders of their field they leaned on their confidence in their abilities. The determination and grit put forth by these women, and so many more trailblazing women in the field, has left an inspiring path for future providers of AHF patients.

Perspectives of cardiothoracic surgeons

Continued education is a strong component of many CT surgeons' backgrounds due to the constantly evolving technologies of MCS and transplant. Some of the women we interviewed took this background a step farther and made it a priority in their mentorship. Understanding education can be more than just teaching new techniques and technologies but also inspiring youth to pursue such fields or establishing stronger patient-care cultures. One example of a surgeon who has worked to leave behind a legacy as a passionate educator is Dr. Margarita Camacho, who feels most proud of her work educating future generations. This passion ranges from training CT fellows to currently doing bi-monthly lectures for high school students at her local science center. Though education is a point of great pride for Dr. Camacho and so many other surgeons we spoke to, they derive most satisfaction from the positive patient experiences and outcomes and define their legacies. Dr. Camacho spoke about the joy she experiences seeing a transplant recipient doing well after surgery and it is this joy that can help get one through tough situations. She spoke of one of her mentors, thoracic surgeon Dr. Carolyn E. Reed, who kept thank you cards from patients in a shoe box. Dr. Reed would go to that shoe box to help her whenever she experienced cases with more emotionally taxing outcomes. Many of the surgeons we interviewed discussed how they wish to be known for their patient care.

The desire to leave a legacy of being a patient-centered physician was also an important component in their motivations to choose the field. One piece of advice was to ensure one truly has a passion for the work. As all the surgeons we interviewed discussed, CT surgery is incredibly demanding and can be very tough to balance with one's personal life. One needs grit, perseverance, and exceptional time management skills to succeed, but most importantly one needs passion for work to sustain in the field. As Dr. Camacho said, "passion will get you through the down days and make the good days exhilarating." Other surgeons agreed and felt it was important to think critically about how CT surgery fits into one's personal life in order to find balance and remain as mentally, emotionally, and physically healthy as possible. The surgeons we interviewed, and many more not in this piece, have prioritized others—their patients and mentees—to better clinical outcomes and further the abilities of future generations of surgeons.

FIGURE 2
Barriers to advancement for women in CT surgery and heart failure specialist.

Discussion

A medical school campus may not reflect gender imbalance in today's day and age; however, it is very apparent in clinical practice, societal conferences, and leadership summits (17). Despite unlocking the door and achieving tremendous progress over the past century, women continue to be underrepresented in traditionally male dominated fields such as CT surgery and cardiology at all levels with stark gaps in senior academic ranks, leadership, and executive positions. This issue is not isolated to the United States, as the literature reports similar gender-based discrepancies in Canada and across Europe (18, 19). While the magnitude of the gap may vary between the countries and continents, disparities based on gender and vertical segregation of women in cardiology and CT surgery are pervasive across the globe. Borelli et al. performed a gender-based analysis of the distribution of leadership positions within cardiology departments in a cohort of 23 European countries, finding significant disparities similar to the United States (20). Although different perspectives and hypotheses are postulated for these inequities, the underlying reasons are complex and multifold and differ a great deal depending on the domain of practice, type of practice and level of career. Cultural stereotypes and social expectations of women's role in family and society plays a role in these disparities. Some suppose that women tend to voluntarily drift away from the specialties that are more demanding due to their preference of wanting more flexibility to balance their domestic responsibilities, childcare needs, or caregiver responsibilities. Though this may be true in some women, it is unlikely to be the overarching reason. Heart failure field, despite being known for high intensity, acuity, and demanding work hours, attracts more women in cardiology than other procedure based subspecialties such as electrophysiology and interventional cardiology dispelling the myth of desire for flexibility as the reason for deterrence.

A systematic review of heart failure clinical trials by Whitelaw et al. revealed that women were underrepresented as authors without any significant change in the representation patterns in the past two decades. Based on randomized controlled trials studying heart failure published from 2000 to 2019 in high impact journals, women were represented in meager portions as lead (15.6%), senior (12.9%) and corresponding authorship (11.4%) roles (21). Homophily in the professional and societal circuits, lack of investment in diversity and inclusion, and lack of or limited institutional support and sponsorship from professional organizations and industry can undermine the professional development of women and perpetuates the entrapment of the sticky floor and glass ceiling. Nonetheless, losing a deserving, talented and high caliber physician for these reasons is a disservice to medicine, patients, and society. Investment in human capital to reduce the gender gap by addressing the barriers for entry and growth in these fields is essential to achieve gender equality and promote diversity, **Figure 2**. In contrast to male physicians and surgeons, the trajectories of women entering these rigorous fields differ greatly due to the meager presence of women mentors and role models as men have been at the center stages of these fields for decades. This leaves women in medicine less prepared to navigate the work environment in clinical practice and academic hierarchy as well as integrating into the fabric of professional societies. Several factors including implicit and explicit biases, attitudes and traditional norms pose challenges for women in traversing the crucial organizational structures to ascend in their careers, climb the leadership ladder, attain senior ranking positions, and grow in organized medicine. A complex interplay of several of these factors make women more susceptible to imposter syndrome. Acknowledging the existence of an imbalance and understanding its etiology is complex, we must then ask: how do we move forward? The responsibility of leveling the field of AHF not only falls on aspiring women physicians and surgeons but is also in the hands of members of all genders, generations, and geographies. It is essential for the institutions and organizations to define operating models for partnerships to advance principles of equality, diversity and inclusion and promote professional development of women in this field, **Table 3**. Although no strategy is exclusive to any career level, we broadly categorized strategies for women to advance their professional growth and presented a model by career stages as represented in **Figure 3**.

TABLE 3 Strategies to improve representation and professional advancement.

Individual	Institutional and organizational	Professional societies and editorial boards	Policy and oversight
Seeking mentors, sponsors, and allies	Incentivize and promote diversity in recruitment and foster inclusive culture	Promote and facilitate diversity and gender balance in societal activities	Policy and legal frameworks to support inclusive and equitable culture
Amplification through networking and social media	Programs to identify, acknowledge and address negative attitudes and stereotypes	Diverse and gender balanced panels and editorial boards	Incentivize diversity and recruitment and promotion
Utilizing digital and social media tools for peer promotion and self-promotion	Establish women leadership councils (WLC) and women resource groups (WRG)	Engage women and active sponsorship of women	Promote gender diversity in leadership ranks
Seeking education and training programs to navigate identity shifts with career advancement	Institutional training programs to increase the awareness and training on: - gender stereotyping - conscious and unconscious biases	Establish and sponsor women sections	Establish policies to promote gender neutral compensation structures and close gender-based wage gap
Seizing opportunities	Leadership development and career advancement programs geared toward women	Scholarships and coaching programs for women	Mandate salary equity reviews
Building peer support community and strategic partnerships	Structured programs and pathways for mentoring women		
Acknowledging and addressing feelings of "Impostorism"	Promote work culture to facilitate work-life balance and/or Breadwinner-caregiver responsibilities		
Resilience training	Active sponsorship of women and build diverse pipeline		
Engaging with women sections in professional societies and organizations			
Gaining agency			

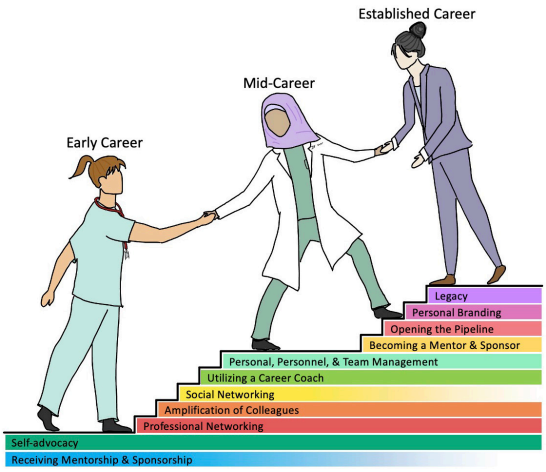

FIGURE 3
Strategies for aspiring and current women of heart failure and CT surgery can utilize to persevere through gender-based challenges.

For women early in their career, mentorship is critical to get their foot in the door. While gender concordance is not an essential element in mentorship, it is of value to have relatability when seeking advice on gender specific challenges. While the benefits of mentorship are vast and undeniable, it is not sufficient to achieve equality. One size does not fit all, and women need advocates, coaches, allies, and sponsors to aid in professional advancement. Amplification is one of the methods that can be utilized to promote the work of women in their early careers. The power of social media can be harnessed to promote crowdsourcing in a goal directed manner for this purpose as reflected in the social media campaigns #NYerORCover Challenge launched by Dr. Susan Pitt and #ILookLikeASurgeon set in motion by Dr. Heather Logghe (22). The connectivity supplied by social media can also promote opportunities for FIT and/or early career women to join peer support groups or engage in a wider range of professional networking (23, 24). Such methods of encouragement and inclusivity are also being practiced outside of the United States. Pompili et al. highlight the efforts made by the European thoracic surgery community to develop mentorship programs as well as female surgical associations and conferences (25). In addition, organization such as the Pink International Young Academy of Cardiology provides support and mentorship for aspiring female cardiologists internationally (26).

Mid-career women often face the sticky floor concept, where obstacles arise with ascending to higher levels of leadership. Though institutional and organizational cultures play a role in this, women tend to have a confidence gap and question their abilities and underestimate themselves. A change in the attitudes and actions of leaders are warranted within both specialties—of all genders—, our interviews elicited some strategies women may apply. Seeking the advice and guidance of a career coach can empower women to minimize salary gaps, negotiate better contracts for work-life integration, and offer strategies to build their leadership skills. In addition, professional development courses and workshops, leadership courses and certifications and creating networking opportunities will help acquire skill sets and sharpen their approach to professional growth. Creating a diverse and just culture that allows the physicians to reach their best potential and facilitating an environment that allows recruitment and retention of best physicians regardless of sex is critical for success of any healthcare organization.

Women with established careers are likely to have the confidence and fortitude to step outside of the box and exercise their leadership skills and influence the attitudes and practices of their institutions. Institutional and organizational legacy is infested with sexism, racism, and elitism, promoting inequality. This places women at a disadvantage as they are prone to second-generation gender bias and prevents them from securing higher ranks and C-suite positions, keeping the proverbial glass ceiling intact. Women in senior positions often face "double-bind" and often need to shift their choice of words, salary gap despite their accomplishments and struggle with imposter syndrome. Confidence, finding allies, building alliances with key personnel at institutions and organizations as well as creating a brand of their own can help steer their careers and help build a legacy they want to build. Most importantly, they can build pipelines and empower other women. Advocating for a culture of inclusivity that is based off potential and not old notions of gender or racial stereotypes can enhance diversity and a shift in culture of the organization.

Women in these fields continue to swim against the tide to reach their goals and continue to move the needle despite the constraining forces. However, the leaky pipe—a metaphor for the dwindling number of female HF cardiologists and CT surgeons as they progress through their field—must be flushed for a harmonious and healthy landscape. While it can be argued that it is a voluntary choice of women to join the branches of medicine that offer more flexibility and drift away from branches that hinder the work-life balance, it is important to critically analyze why women who are ambitious enough to pursue medical school and training are choosing not to do so in their careers.

Limitations

This article is generated based on review of information via web search and interviews. The accuracy of the information

depends on the veracity of representation of the information on web-based platforms. Unintentional lapses in conceptual understanding and interpretation may limit the representation of views expressed by interviewees.

Conclusion

The fields of AHF, MCS, and Transplant are truly unique callings. The long hours, difficult conversations, and high acuity of cases in both medicine and surgery demand a true passion for the field and the work. Though the heart failure specialty has a stronger history of including women relative to cardiothoracic surgery, both experience underrepresentation. Countless female heart failure specialists and CT surgeons have persevered for the betterment of younger generations of physicians and their patients. As described by Dr. Manreet Kanwar—current Medical Director of the Cardiac Transplant and MCS Program and co-director of the Division of Heart Failure and Pulmonary Hypertension at the Cardiovascular Institute at Allegheny Health Network—the equities some women are privileged to today comes from standing on the shoulders of Giants who changed the status quo and paved path for future generations. Women face a higher bar for acceptance due to stereotyping, implicit and explicit biases and may be subjected to extra scrutiny and judgment in establishing their validity in the workspace. Gender mainstreaming by taking gender perspectives into account with the goal to achieve gender equality in dialog, advocacy, resource allocation, planning and policy development is central to achieving gender equality. Leveling the field is a responsibility of all and purposeful and deliberate actions by individuals, institutions and organizations are required to reset these deeply ingrained norms and attitudes. Reengineering of organizational frameworks and policies are essential to build complementary support system to empower and promote women along with collective efforts to shift culture, practices and facilitate an equitable pipeline to achieve desired demographic shifts in these conventionally male dominated spheres of medicine and surgery at the highest ranks including leadership and C-suite positions.

Author contributions

MM involved in conceptualization, project administration, and supervision. KB and MM involved in the planning, execution, writing, and critical appraisal of this manuscript. FK involved in writing and critical appraisal of this manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Glossary

Imposter syndrome: an internal belief that one is not as competent as others how one is perceived externally.

Second generation gender bias: practices that are applied to all genders, therefore do not overtly appear sexist, but promote the values of the dominating gender.

Double bind: in gender bias, occurs when two opposing standards are placed on an individual, but both exist with negative connotations (e.g., a collaborative woman is seen as weak and non-assertive, but an assertive woman is seen as aggressive).

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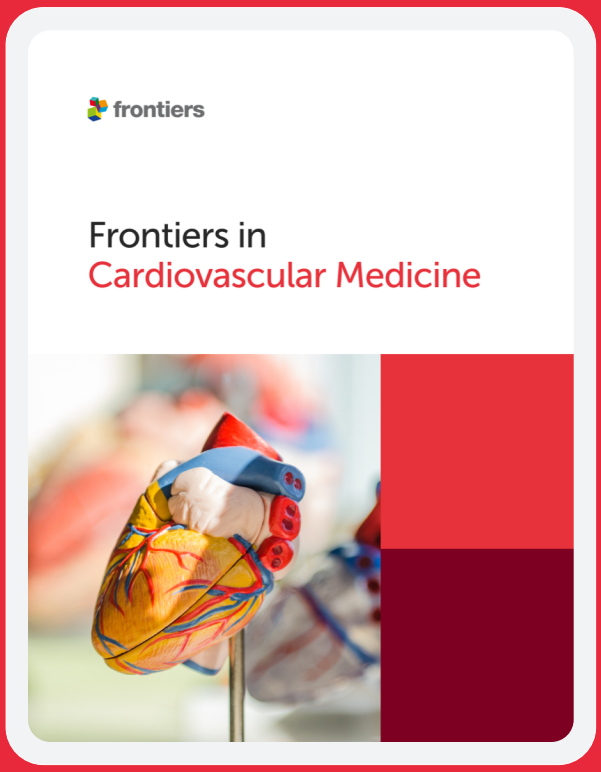

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