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RESEARCH ARTICLE | *Mechanisms of Respiratory Modulation of Cardiovascular Control*

Biomechanical and neuroautonomic adaptation to acute blood volume displacement in ischemic dilated cardiomyopathy: the predictive value of the CD25 test

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Acanfora D, Casucci G, Ciccone MM, Scicchitano P, Lonobile T, Chiariello L, Maestri R, Pedone C, Provitera V, Nolano M, Incalzi RA. Biomechanical and neuroautonomic adaptation to acute blood volume displacement in ischemic dilated cardiomyopathy: the predictive value of the CD25 test. *J Appl Physiol* 129: 1173–1182, 2020. First published September 17, 2020; doi:10.1152/japplphysiol.00514.2019.—We aimed to examine biomechanical and neuroautonomic adaptation to blood volume displacement induced by tilt test in patients with previous inferoapical/inferolateral (IA-IL) or basal/apical septal (BS-AS) myocardial infarction (MI). Twenty-four patients with heart failure (HF) and previous IA-IL MI and 30 patients with HF and previous BS-AS MI were enrolled. All patients underwent head-up tilt test, radionuclide ventricular function monitoring (VEST), sympathovagal balance evaluation, and chronotropic 25-dose isoproterenol infusion test (CD_{25}). Physiopathological reactions to stress-tests were assessed in both groups. Follow-up lasted 36 mo. IA-IL patients showed lower stroke volume (SV), cardiac output (CO), and left ventricle ejection fraction (LVEF) compared with BS-AS. End-diastolic volume decreased in IA-IL group ($F = 3.1$, $P = 0.043$) more than in BS-AS group during tilt test. The time trend of end-systolic volume, SV, CO, LVEF, and peak filling rate were similar in the two groups. Norepinephrine (IA-IL supine→tilting 499.5 (SD:28.8)→719.3 (SD:41.5) pg/mL vs. BS-AS supine→tilting 533.9 (SD:33.3)→768.8 (SD:47.9) pg/mL; $P < 0.001$) and epinephrine plasma concentrations (IA-IL supine→ tilting 125.7 (SD:9.8)→193.7 (SD:9.6) pg/mL vs. BS-AS supine→ tilting 118.8 (SD:8.9)→191.7 (SD:10.2) pg/mL; $P < 0.001$) increased in both groups. Low-to-high frequencies ratio significantly increased in IA-IL and decreased in BS-AS patients. CD25 was similar in IA-IL and BS-AS patients (IA-IL = 4.6 (SD:0.94), BS-AS = 5.0 (SD:1.06) μg; $P = 0.79$). CD_{25} predicted all-cause mortality (hazard ratio 1.48, 95% confidence interval 1.32–1.67; $P < 0.0001$) after adjusting for age/heart rate. In conclusion, patients with ischemic HF show abnormal biomechanical adaptation to volume displacement and compensatory sympathetic overdrive. The association of reduced β-adrenergic sensitivity and sympathetic denervation in such patients warrants for careful therapeutic choices.

NEW & NOTEWORTHY The adaptation to volume displacement induced by tilt test was assessed in patients with heart failure and previous inferoapical/inferolateral or basal/apical septal myocardial infarction. The responsiveness of cardiac muscle to sympathetic nervous system stimulation predicts the mortality in patients with ischemic heart failure and may represent a useful tool for clinicians in the general assessment of this kind of patients.

autonomic nervous system; biomechanical adaptation; head-up tilt testing; ischemic heart failure; VEST

INTRODUCTION

Little is known about the relationship between hemodynamic changes in response to orthostatic volume displacement in vivo β-adrenergic receptor sensitivity and clinical outcomes in patients with ischemic heart failure (IHF) (5, 33).

Postural transition from supine to orthostatic position displaces ~700 mL of blood (11). Autonomic control of cardiac chronotropy/inotropy and vasomotor tone avoid pooling of blood in the lower limbs by ensuring venous return. Carotid baroreceptor reflexes and activation of cardiac mechanoreceptors are the main determinants of cardiovascular system adaptation to blood volume displacement (13). Short-term cardiac and systemic circulatory adaptation to the upright position consists in rapid (within the first 30 s) changes in blood pressure (BP) and heart rate (HR), followed by stabilization (after 1–2 min). These phenomena are regulated by the autonomic nervous system (13, 17). Myocardial infarction (MI) can abolish or strongly disrupt such autonomic system-mediated responses affecting heart performance (6, 30).

The site of myocardial injury may affect autonomic cardiac performance (4, 25, 34). In fact, acute inferoposterior MI usually results in bradycardia and hypotension, whereas anterior MI can induce tachycardia and hypertension (23).

In heart failure patients, alterations of myocardial autonomic control are observed. Heart failure is characterized by an

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activation of the sympathetic nervous system and by a withdrawal of parasympathetic tone (14). The combination of heart failure and MI is further detrimental for heart and vessel autonomic control (9, 18). Such alterations can be assessed by studying hemodynamic and neural adaptation in response to orthostatic volume displacement in patients with IHF. In addition, HR variability provides a noninvasive measure of autonomic effects on the heart. The "chronotropic 25-dose isoproterenol infusion test" (CD_{25}; i.e., the dose of isoproterenol required to increase HR by 25 beats/min beats/min from baseline) measures the in vivo β-adrenergic function of the heart. Lower CD_{25} values indicate higher receptor sensitivity. Furthermore, the ambulatory ventricular function monitoring device (VEST; Capintec, Inc., Florham Park, NJ) allows reliable, continuous, dynamic, noninvasive assessment of left ventricular function (2, 31). This approach may provide insights into ventricular performance on a sequential basis through noninvasive detection of the adaptation of ventricular function to volume displacement.

The aims of this prospective study were *1*) to evaluate whether the site of MI affects the dynamics of adaptation of left ventricular function to blood volume displacement during head-up tilt test in patients with mild-to-moderate heart failure [New York Heart Association (NYHA) functional class II-III, *2*) to assess the sympathovagal balance during the tilt test, *3*) to investigate the relationship between the site of MI and β-adrenergic sensitivity through CD_{25}, and *4*) to determine the prognostic value of biomechanical and neuroautonomic adaptation to acute blood volume displacement in patients with IHF.

METHODS

Study Population

A total of 522 patients with IHF were screened from January 2012 to January 2015.

Exclusion criteria were the following: non-IHF, preserved left ventricle ejection fraction (LVEF), permanent atrial fibrillation, ventricular ectopic beats $\geq$600/24 h, presence of a pacemaker or implantable cardioverter defibrillator, refusal to participate in the study, chronic lung disease or valvular heart disease, diabetes, neuromuscular disorders, peripheral vascular disease, and thyroid dysfunction.

Fifty-four patients with stable IHF were finally included.

Patients were selected only if there was no evidence of exercise-induced myocardial ischemia, arrhythmias, or claudication. To avoid the potential interference of transient myocardial ischemia during orthostatic volume displacement, we studied only those patients with previous transmural MI and fixed perfusion defects on stress-myocardial scintigraphy (1, 2). Diagnosis of heart failure was made in agreement with international guidelines (21).

This study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and the local Ethics Committee, and the Institutional Review Board (IRCCS Maugeri) approved the study. All procedures were in accordance with the ethical standards of institutional and national research. Informed consent was obtained from all individual participants included in the study.

Experimental Protocol

Patients were divided into two groups based on the anatomic location of the akinetic and diskinetic segments: the inferoapical and inferolateral akinetic and diskinetic (IA-IL) group and the basal septal and apical septal akinetic and diskinetic (BS-AS) group. Two patients had akinesia of both posterolateral and inferolateral segments and were included in the IA-IL group. Indeed, the anatomic location of the cardiac chamber wall dysmotility was the leading criterion for differentiating the two populations.

Briefly, all patients were clinically stable, physically inactive, and in sinus rhythm. Their pharmacological treatments were optimized to the maximum tolerated dose of β-blockers, angiotensin-converting enzyme (ACE) inhibitors/sartans, aspirin or clopidogrel, and statins. In particular, patients had been on a stable pharmacological regimen for at least 3 mo before admittance into the ward. Therefore, all of the patients were on optimal medical therapy at time of admission (Table 1). After the admission, all patients underwent clinical and anthropometric evaluations and standard echocardiography. β-Blockers, β-stimulants, and/or digoxin were discontinued for 3 days after admission, as per study protocol. On day *4* from admission, patients underwent a baroreflex sensitivity (BRS) test, VEST, tilt-up test, and plasma catecholamine measurement. Isoproterenol test with CD_{25} was performed on day *7* from admission. After the isoproterenol test with CD_{25} determination (i.e., day *8* after admission), all of the treatments were resumed and progressively titrated-up to the prestudy individual maximal tolerated dose in accordance with international guidelines (21). Figure 1 summarizes the study design. All patients underwent follow-up outpatient visits twice a year for 3 yr.

Ambulatory ventricular function monitoring. Patients avoided strenuous physical activity, alcohol, caffeine, and smoking for at least 24 h before the start of the study (8:00 AM). With the subject in a comfortable sitting position and a constant room temperature of 22°C, in vivo labeling of red blood cells was performed with 555 mBq (15 mCi) $^{0.99m}$Tc. The VEST detector was positioned under gamma camera control, as described previously (1, 2). A 2-min static gamma camera image was obtained to confirm the adequacy of the detector position.

Radionuclide angiography was analyzed with standard commercial software (GE Healthcare Medi-Physics, Inc., Arlington Heights IL). LVEF was computed from the raw time-activity curve, and peak filling rate was calculated after Fourier expansion with four expansions. The mean count rate (decay corrected) of the entire study was displayed if the curve had $<$10% deviation from a straight line, the VEST study was considered adequate. Radionuclide and electrocardiogram (ECG) data were analyzed beat by beat and summed at 30-s intervals. Excellent agreement was found between VEST and radionuclide angiography in measuring LVEF and peak filling rate (PFR).

LVEF was computed as stroke counts divided by background-corrected end-diastolic counts. The background value was determined by matching the initial resting VEST LVEF value to that from the gamma camera. This value was used throughout the remainder of each subject's VEST data analysis. PFR was obtained from the Fourier curve and computed as the inflection point after the end of systole, at which the second derivative shifts from positive to negative. The accuracy and reproducibility of this technique has been validated (1, 2). Values obtained at baseline, 30 s, and 1, 3, 8, and 10 min throughout the tilting were used.

Tilt-up test. After 30-min basal recording with VEST, the patient was placed on an electrically driven tilt table (with a mattress and footrest) and connected to a conventional bedside monitor (SMK 250 PPG Hellige, GE Healthcare Medi-Physics, Inc.; Arlington Heights IL) providing a two-lead electrocardiographic signal and a respiratory signal (1, 2). A noninvasive finger BP signal based on the Penaz method (Finapres 2300; Ohmeda, Enschede, The Netherlands) was recorded.

A polyethylene catheter was inserted into the antecubital vein to obtain blood samples to determine norepinephrine and epinephrine plasma levels in the supine position and after 3 min in the standing position. ECG, respiratory waveforms, and BP were recorded continuously on a personal computer during 20 min at rest and 10 min after assumption of the upright position (70°).

RR, systolic, and diastolic variability. Analysis of spontaneous fluctuations in RR interval and systolic (SAP) and diastolic (DAP) arterial pressures provided indexes of autonomic neural activity during changes in posture (35). This variability was quantified as the standard deviation (SD) and the power of spectral components in

Table 1. *Clinical characteristics and baseline values of patients with heart failure with reduced ejection fraction (n = 54)*

	Basal Septal and Apical Septal Segments	Inferoapical and Inferolateral Segments
Number of patients, *n*	30	24
Sex, M/F, *n*	26/4	22/2
Age, yr	62 (11)	58 (7)
Weight, kg	81 (13)	80 (9)
NYHA class II, *n*	15	10
NYHA class III, *n*	15	14
Inferior myocardial infarction, *n*	8	14*
Anterior myocardial infarction, *n*	16	16
History of hypertension, %	47	46
Diabetes mellitus, %	20	21
Heart rate, beats/min	81 (10)	88 (9)†
SAP, mmHg	123 (8)	124 (11
Hemoglobin, g/dL	13.8 (1.97)	12.7 (1.94)†
Creatinine, mg/dL	1.35 (0.62)	1.33 (0.96)
Sodium, mmol/L	141 (6.7)	142 (5.1)
Potassium, mmol/L	4.55 (0.77)	4.3 (0.65)
Uric acid, mg/dL	7.08 (2.98)	6.29 (2.7)
NT-proBNP, pg/mL	747 (211)	717 (195)
$\dot{V}O_{2max}$ VO_{2max}, mL·kg^{-1}·min^{-1}	16.03 (3.8)	15.3 (3.47)
KCCQ score (overall), pts	75 (11)	74 (10.6)
Therapies, %		
ACE-I/ARB	100	100
β-Blocker	97	96
Aldosterone antagonist	83	81
Digoxin	11	10
Diuretics	5	7
Statin	84	82
Warfarin	4	4
Nitrates	18	4
Aspirin	26	20
Echocardiography measurements		
LV end-diastolic dimension, cm	6.6 (0.48)	6.42 (0.33)
LV end-systolic dimension, cm	5.4 (0.56)	5.3 (0.48)
Posterior wall thickness at end diastole, cm	1.49 (0.3)	1.48 (0.33)
Septal thickness at end diastole, cm	1.43 (0.23)	1.39 (0.26)
LV end-diastolic volume, mL	224.2 (37.6)	211.7 (25.3)
LV end-systolic volume, mL	148.6 (24.1)	145.5 (22)
LV ejection fraction, %	33.5 (7)	31.3 (7.2)
Left atrial anteroposterior dimension, cm	4.9 (0.6)	5.1 (0.7)

Data are presented as means (SD) or percentages, where appropriate. ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; F, female; KCCQ, Kansas City Cardiomiopathy Questionnaire; M, male; NYHA, New York Heart Association functional class; LV, left ventricular; NT-proBNP, NH_2-terminal pro-B-type natriuretic peptide; SAP, systolic arterial pressure; $\dot{V}O_{2max}$, peak O_2 consumption. *$\chi^2 = 17.82$; $P < 0.001$; †$P < 0.05$.

the low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) bands. To estimate the relative predominance of sympathetic and parasympathetic activity, the sinoatrial node normalized powers were computed as the percentage of LF and HF components in relation to overall variability.

Baroreflex sensitivity. Computation of baroreflex sensitivity (BRS) was performed using the whole-band average (WBA) method (27). RR and SAP time series were plotted together, and the widest subrecord free from artifacts (minimum length 3 min) was selected (27). They were resampled at 2 Hz by cubic spline interpolation, and the transfer function was estimated via nonparametric cross-spectral analysis [weighted covariance method, 0.03 Hz bandwidth Parzen window (26)]. BRS was computed by averaging the modulus of the transfer function in the LF band (26, 27).

Isoproterenol test. All patients underwent an isoproterenol stimulation test on day 3 after the tilt test. An ECG monitor was used to record HR. After a 30-min rest and basal HR registration, an intravenous low-dose bolus (0.1 μg) of isoproterenol was administered. Incremental doses (0.25, 0.5, 1.0, 2.0, and 4.0 μg) were administered every 5 min until a 25-beats/min increase in HR or a total dose of 4.0 μg was reached. CD_{25} was calculated as in previous studies (8, 24). Briefly, for each patient, a dose-response curve (change in HR after isoproterenol infusion) was obtained. After log transformation of the dose (base 10), a linear fit was obtained, and the slope (m) and intercept (q) were computed. Finally, CD_{25} was obtained as follows: $CD_{25} = 10^{(25 - q)/m}$.

Plasma catecholamine evaluation. Plasma catecholamine assay was performed with reversed-phase high-performance liquid chromatography (Waters Corp., Milford, MA). Quantitative analysis was achieved through electrochemical detection. The detection limit of the assay was 10 pg. Intra- and interassay variation coefficients for norepinephrine were 5.2 and 11.2%, respectively.

Follow-up

Follow-up lasted 36 mo. Physicians phoned patients at 6, 12, 24, and 36 mo after hospital discharge to assess their clinical condition.

Death certificates and hospital records were checked if patients and/or relatives were not available. All available data were reviewed by two investigators (D. Acanfora, M. M. Ciccone) to define the cause of death. If consensus could not be reached, the opinion of the senior investigator (R. A. Incalzi) prevailed.

Statistical Analysis

A Shapiro-Wilks test was applied to all variables analyzed to check for normality. Variables that were not normally distributed were transformed to normalize them, if feasible. The absolute powers of the

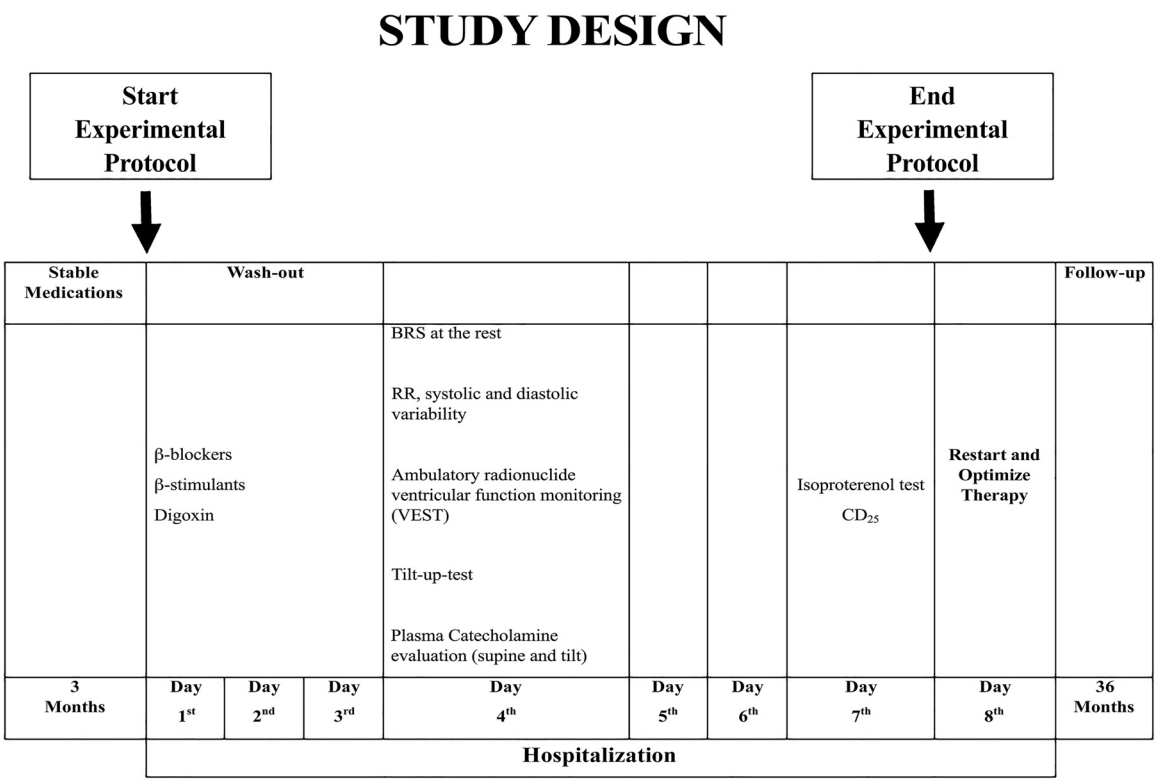

Fig. 1. Study design.

spectral components were presented as natural logarithms (ln ms^2 or ln $mmHg^2$) to minimize skewness. Data are presented as mean ± SD. Between-group comparisons were made by unpaired *t* test, Mann-Whitney *U* test, or χ^2 analysis as appropriate. Within-group comparisons were made by paired *t* test or Wilcoxon signed rank test as appropriate.

Differences in cardiac hemodynamics during the tilt-up test were examined using two-factor repeated-measures analysis of variance (ANOVA), with the site of the previous MI (BA-AS and IA-IL) as the between factor and time (baseline, 30 s, and 1, 3, 8, and 10 min) as the repeated, within factor. *Post hoc* analyses (Tukey-Kramer adjustment) were used to compare values at each observation time versus baseline within each group of patients and to compare values between groups at each time.

Univariate and multivariate Cox regression models were used to investigate the association of selected variables with death. We developed a prognostic model considering all variables in the study as potential predictors. To avoid overfitting, we allowed for a number of predictors in the final multivariable model equal to three (i.e., number of events/10). Variable selection was carried out using the best subset selection method. Briefly, Cox models were fitted using all possible subsets of clinical predictors, and the best model was identified as the one providing the highest χ^2.

Survival curves were estimated by the Kaplan-Meier method and compared using the log rank test.

All hypothesis tests were tested using a significance level of 0.05. All *P* values were two sided. Statistical analysis was carried out using SPSS Statistical Package Base System for Windows version 13.0 (SPSS Inc., Chicago, IL, 2004).

RESULTS

Table 1 shows the main characteristics of the two groups. All 54 patients completed 10 min of head-up tilt test without symptoms. There were no differences in baseline HR, SAP, or DAP. The echocardiographic measurements at baseline were reported. Figure 2 shows the variation in HR and BP during the tilt-up test.

Global results of repeated-measures ANOVA are shown in Table 2.

HR steadily increased during the first minute of the tilt-up test in both groups and decreased in later phases, with a more pronounced decrease in the IA-IL group. Repeated-measures ANOVA revealed significant group × time interaction ($P < 0.001$), indicating that the two groups had a different response across time and time effect ($P < 0.001$). However, *post hoc* analysis carried out to compare values in the BS-AS group versus IA-IL group at each observation time failed to reveal statistically significant differences, likely due to the adjustment for multiple comparisons.

The BS-AS group showed a slight reduction in SAP during the initial phase of the test, not observed in the IA-IL group. *Post hoc* analysis indicated no differences between groups in term of SAP and DAP.

The effects of volume displacement on cardiac hemodynamics during the tilt-up test are shown in Fig. 3. The evaluations of the cardiac performances were derived from VEST analysis. Repeated-measures ANOVA revealed that the group × time interaction was significant for end-diastolic volume (EDV; $P = 0.04$). EDV decreased more in IA-IL than in BA-AS patients during the first 5 min, as confirmed by *post hoc* analysis. The time trend of end-systolic volume (ESV) in the two groups was similar, with no significant differences between values for all observations.

Considering stroke volume (SV), cardiac output (CO), LVEF, and PFR, the time course was similar in both groups of patients.

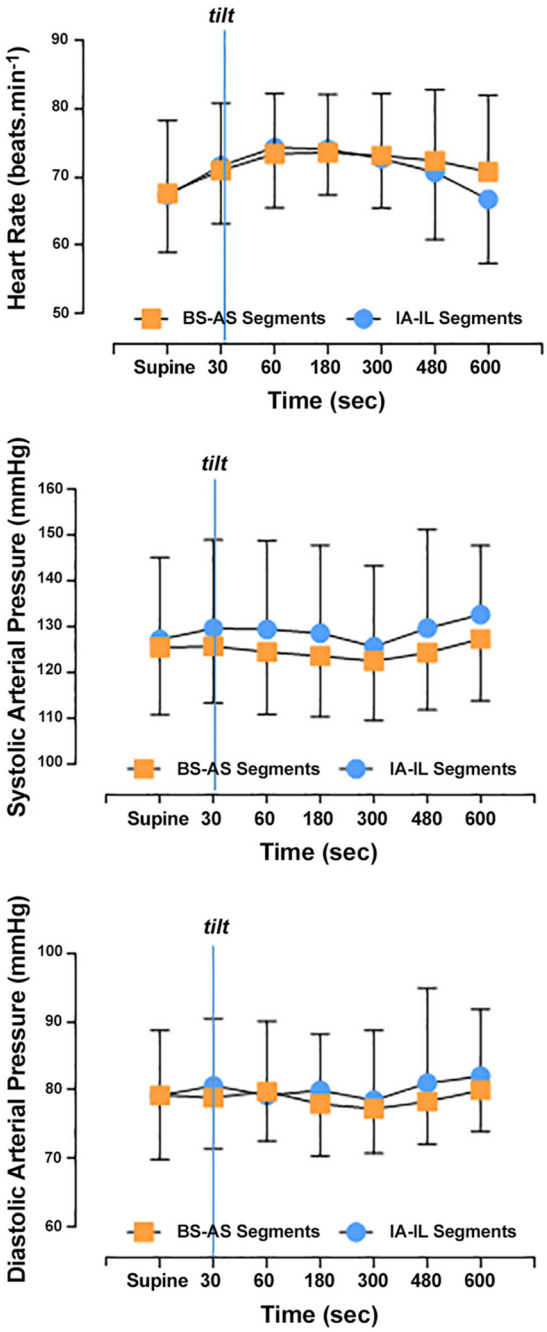

Fig. 2. Graphs of continuous heart rate (HR) and systolic (SAP) and diastolic (DAP) arterial pressure monitoring during head-up tilt test in patients with heart failure with akinesia-dyskinesia of the basal septal and apical or septal segments (BS-AS) or inferoapical and inferolateral segments (IA-IL). Statistical analyses were performed by repeated-measures ANOVA and Tukey-Kramer post hoc tests.

ANOVA revealed a significant group effect for all of the four variables, indicating that grouping all observation times generates a global difference in these variables between BA-AS and IA-IL groups.

The BS-AS group had higher values of SV, CO, LVEF, and PFR compared with the IA-IL group at all times, including baseline (Fig. 3).

Table 3 shows the variations in neurohumoral activity, RR interval, SAP, and DAP between baseline and the peak of the tilt-up test.

Both norepinephrine and epinephrine plasma concentrations increased when passing from supine to standing position (Table 3) in both groups ($P < 0.001$).

Table 3 also shows the representative HR and SAP spectral measurement for BS-AS and IA-IL patients in both supine and standing positions. No variations were observed for SD of RR intervals in standing versus supine. Nevertheless, SD of RR was reduced in BS-AS compared with IA-IL patients [supine: BS-AS = 22.8 (SD: 1.54) ms vs. IA-IL = 28.1 (SD: 1.35) ms, $P = 0.011$; standing: BS-AS = 19.9 (SD: 1.11) ms vs. IA-IL = 24.5 (SD: 0.9) ms, $P = 0.025$].

HR variability was reduced in both groups during the tilt test. LF and HF components were present in the spectra of RR interval variability. Respiratory activity had a significant relationship with HF component RR variability. When LF was expressed in absolute units, no significant difference was observed. Conversely, HF in absolute units was significantly reduced during the head-up tilt test in IA-IL patients. A significant difference in LF and HF components was found between the supine position and head-up tilt test in IA-IL patients when expressed in normalized units. At variance, the HF component showed no differences between supine position and head-up tilt test in both absolute and normalized units in BS-AS patients (Table 3).

During the tilt-up test, the normalized LF band increased in IA-IL patients and remained stable in BS-AS patients. The normalized high-frequency band remained unchanged in BS-AS patients, whereas IA-IL patients showed a significant reduction (Table 3). This finding was associated with a significant increase in the LF-to-HF ratio in IA-IL patients. Conversely, the LF-to-HF ratio in BS-AS patients decreased during postural tilt (Fig. 4). No difference was observed in baroreflex sensitivity between IA-IL and BS-AS patients [4.03 (SD: 0.56) vs. 3.7 (SD: 0.49); $P = 0.67$].

CD_{25} was similar in IA-IL and BS-AS patients [IA-IL = 4.6 (SD: 4.6) μg and BS-AS = 5.0 (SD: 5.8) μg, $P = 0.79$].

Median follow-up duration was 32 mo (lower quartile-upper quartile: 11–36 mo). Twelve patients underwent device implantation: eight cardiac resynchronization therapy, and four implantable cardioverter defibrillator. Twenty-seven patients died (50%). The causes of death were progressive heart failure

Table 2. *Global results of repeated-measures ANOVA*

	Group Effect		Time Effect		Interaction (Group × Time)	
	F	*P* value	F	*P* value	F	*P* value
HR	0.07	0.80	31.2	<0.001	3.9	<0.001
SAP	1.0	0.31	4.2	0.009	0.6	0.60
DAP	0.3	0.55	2.5	0.048	1.0	0.43
EDV	4.1	0.047	18.5	<0.001	3.1	0.043
ESV	0.3	0.61	8.5	<0.001	0.43	0.67
SV	10.8	0.002	8.3	<0.001	1.1	0.35
CO	12.1	0.001	5.7	0.001	1.4	0.26
LVEF	6.6	0.013	4.2	<0.001	1.0	0.43
PFR	10.6	0.002	1.5	0.18	1.2	0.29

HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; EDV, end diastolic volume; ESV, end systolic volume; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; PFR, peak filling rate.

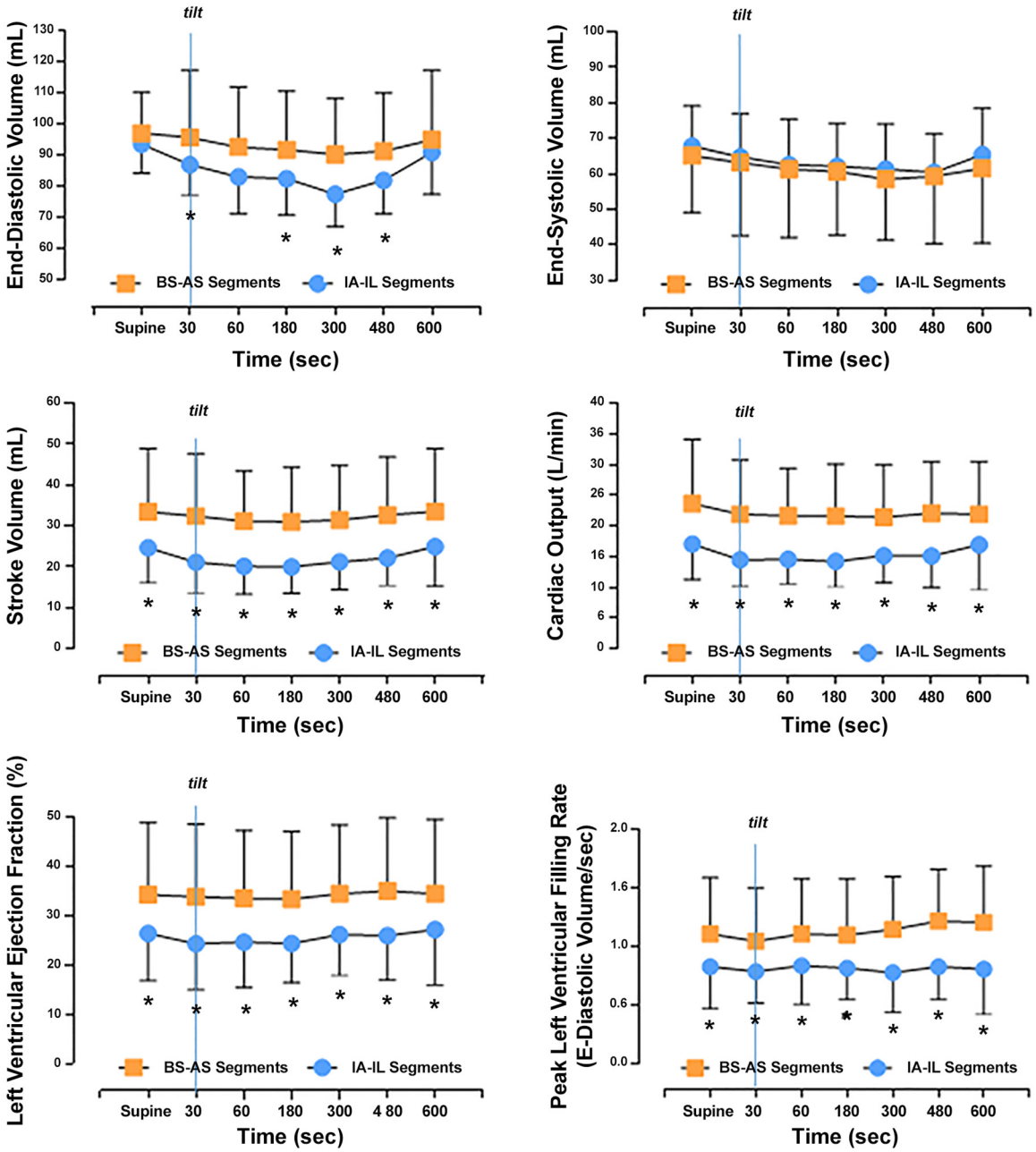

Fig. 3. Graphs of continuous ambulatory ventricular function monitoring (VEST) of end-diastolic volume, end-systolic volume, stroke volume, cardiac output, left ventricular ejection fraction, and peak left ventricular filling rate variation during head-up tilt test in patients with heart failure with akinesia-dyskinesia of the basal septal and apical or septal segments (BS-AS) or inferoapical and inferolateral segments (IA-IL). Statistical analyses were performed by repeated-measures ANOVA and Tukey-Kramer post hoc tests.

with multiorgan dysfunction (n = 18) and sudden cardiac death (n = 9). Among the deaths, 13 patients were classified as NYHA class II and 14 as NYHA class III.

Neither biomechanical nor neuroautonomic indexes of cardiovascular adaptation to volume displacement could predict survival, except for CD_{25}.

At univariate Cox analysis, the CD_{25} was predictive for all-cause mortality, with a 45% increase in risk for each unit increase in value [hazard ratio 1.45 (95% CI 1.30–1.62), $P <$ 0.0001]. CD_{25} also remained a significant predictor of mortality after adjustment for age and supine HR, the variables selected using the best subset selection method for three predictors (Table 4).

CD_{25} dichotomized (threshold = 2.5) was a strong predictor of mortality (log-rank = 42.06, $P < 0.0001$), as shown by the Kaplan-Meier curves in Fig. 5. As threshold for CD_{25} dichotomization, we used the median value, since carrying out procedures to select an optimal threshold might provide overoptimistic results given the relatively small sample size.

Table 3. *Neurohumoral and RR, systolic, and diastolic pressure variability effects of infarct size and tilting*

	Basal Septal and Apical Septal Segments			Inferoapical and Inferolateral Segments		
	Supine	Tilting	*P* value supine vs. tilting	Supine	Tilting	*P* value supine vs. tilting
Norepinephrine, pg/mL	533.9 (33.3)	768.8 (47.9)	<0.001	499.5 (28.8)	719.3 (41.5)	<0.001
Epinephrine, pg/mL	118.8 (8.9)	191.7 (10.2)	<0.001	125.7 (9.8)	193.7 (9.6)	<0.001
RR variability SD, ms	22.8 (1.54)	19.9 (1.11)	0.12	28.1 (1.35)*	24.5 (0.9)**	0.02
Total power, ln ms^2	6.27 (0.22)	5.81 (0.24)	0.02	6.67 (0.25)	6.12 (0.23)†	0.01
Low frequency, ln ms^2	4.41 (0.26)	4.30 (0.26)	0.44	5.29 (0.29)*	5.17 (0.17)‡	0.28
%	45.7 (5.1)	45.3 (4.3)	0.58	47.6 (4.2)	65.8 (2.5)**	<0.001
Hz	0.09 (0.0)	0.13 (0.03)	0.09	0.09 (0.0)	0.08 (0.0)*	0.09
High frequency, ln ms^2	4.13 (0.26)	4.19 (0.21)	0.80	5.11 (0.21)‡	4.06 (0.22)	<0.001
%	33.4 (4.03)	36.6 (2.62)	0.30	42.44 (4.11)	24.33 (2.5)**	<0.001
Hz	0.28 (0.01)	0.27 (0.01)	0.49	0.26 (0.01)	0.27 (0.01)	0.14
Systolic pressure variability, SD mmHg	5.75 (0.39)	6.38 (0.43)	0.09	5.97 (0.5)	7.32 (0.59)	0.05
Low frequency, ln $mmHg^2$	1.39 (0.28)	2.08 (0.24)	0.11	1.7 (0.21)	3.02 (0.28)*	<0.001
High frequency, ln $mmHg^2$	1.19 (0.28)	2.08 (0.24)	0.02	1.68 (0.25)	2.23 (0.2)	0.01
Diastolic pressure variability, SD mmHg	3.85 (0.34)	3.95 (0.32)	0.82	3.53 (0.34)	4.22 (0.43)	0.30
Low frequency, ln $mmHg^2$	1.06 (0.19)	1.35 (0.18)	0.22	1.62 (0.25)	1.94 (0.21)	0.31
High frequency, ln $mmHg^2$	0.61 (0.13)	0.9 (0.18)	0.13	0.87 (0.16)	1.02 (0.15)	0.42

Data are presented as means (SD). *$P < 0.05$, **$P < 0.005$, †$P < 0.001$; ‡$P < 0.01$ vs. basal septal and apical septal segments.

DISCUSSION

Patients with IHF showed differences in biomechanical and neuroanatomic responses to tilting depending on the site and severity of injury. Compared with BS-AS patients, IA-IL patients showed reduced EDV. These site-specific biomechanical responses were associated with a reduction in parasympathetic activity in IA-IL patients (decreased HF component and increased LF/HF ratio). Baroreflex sensitivity and the β-receptor sensitivity tests did not distinguish IA-IL from BS-AS patients. The decrease in preload (i.e., EDV) can likely activate the sympathetic nervous system as a compensatory mechanism and can account for the adaptation to volume displacement.

Patients in the IA-IL group revealed a decrease in EDV greater than BS-AS patients when moving from supine to upright position (Fig. 3). Nevertheless, IA-IL patients also showed decreased SV, CO, and LVEF. The reduction in SV, CO, and LVEF was also associated with the reduction in parasympathetic activity (Table 3). In contrast, Sobic-Saranovic et al. (29) found an increase in EDV and ESV and a reduction in LVEF more evident in patients with anterior MI than in patients with inferior MI. Our results were in line those of with Kiris et al. (20), who demonstrated a more pronounced decrease in LVEF in patients with anterior MI compared with patients with no previous MI or with posterior/inferior MI. The level of parasympathetic activity in IA-IL patients is also controversial. Flapan et al. (12) noted a significant reduction in cardiac parasympathetic activity in patients with anterior MI compared with patients with inferior MI. Tobaldini et al. (32) found similar results in patients with acute MI who had undergone primary percutaneous coronary angioplasty.

Indeed, MI can increase plasma concentrations of both norepinephrine and epinephrine. Fast occlusion and MI extension, rather than the site of the lesion, appear to be the main determinants of the release of these molecules (19). The apparent disagreement with our results may be due to an incomplete understanding of the mechanisms involved in cardiac nervous system damage after MI (28). IA-IL patients usually have greater residual myocardial mass than BS-AS patients. Indeed, patients surviving an inferior MI usually perform better than those surviving an anterior MI, as reflected by hemodynamic variables and exercise performance (7). Our IA-IL patients

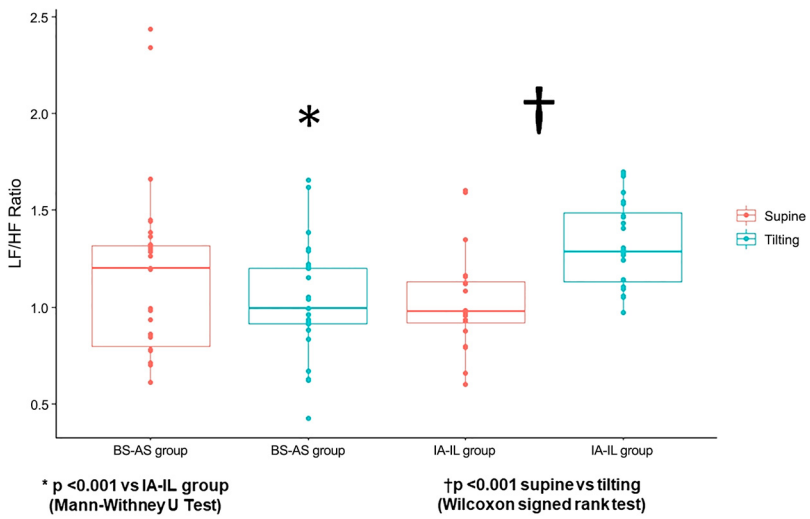

Fig. 4. Dot-plot graphs comparing baroreflex response to acute volume displacement during head-up tilt test in patients with heart failure with akinesia-dyskinesia of the basal septal and apical or septal segments (BS-AS) or inferoapical and inferolateral segments (IA-IL). LF/HF ratio, low frequency-to-high frequency ratio. The analysis had been performed by means of R (2013) (http://www.R-project.org/).

Table 4. *Adjusted risks for all-cause death (multivariate Cox model) for CD_{25}, age, and supine HR*

Parameter	χ^2 Statistic	*P* Value	Hazard Ratio (95% CI)
CD_{25}, μg	43.12	<0.0001	1.48 (1.32–1.67)
Age, yr	0.17	0.68	0.99 (0.95–1.04)
Supine HR, beats/min	1.82	0.18	0.92 (1.11–1.02)

CD_{25}, chronotropic 25-dose isoproterenol infusion test; CI, confidence interval; HR, heart rate.

showed baseline LVEF slightly lower than that of BS-AS patients (Fig. 3), and the lower the baseline contractile reserve may explain the worse adaptation to stressing conditions. This result likely explains the difference in tilt adaptation between the IA-IL and BS-AS groups. On the other hand, the change in sympathetic to parasympathetic balance in favor of the sympathetic drive in IA-IL patients (as suggested by the differences in LF/HF ratio; Fig. 4) seems to be the predictable consequence of the mechanical disadvantage eliciting the sympathetic overdrive. Nevertheless, no further data are available to support this statement. The lack of difference in baroreceptor sensitivity and CD_{25} between IA-IL and BA-BS groups rules out the possibility that factors other than mechanical ones would account for group-specific responses. Thus, cardiac adaptation to volume displacement largely depends on biomechanical reserve resulting from contractile reserve and neurohumoral reflex responses.

Interestingly, β-blockers might impair the adaptation to volume displacement in selected individuals by reducing the compensative sympathetic overdrive. Although β-blockers improve cardiac biomechanics after acute MI (15), evidence for negative effects of β-blockers in some categories of MI appears to corroborate our hypothesis (10).

Our study shows that myocardial β-adrenergic desensitization, assessed through CD_{25}, has major prognostic implications in IHF with depressed systolic function. The overall myocardial β-adrenergic desensitization was biologically relevant, as identified in our sample and confirmed in a multivariable analysis including traditional indexes of HR variability that reflect the autonomic regulation and biomechanical indexes. CD_{25} was demonstrated to implement the prognostic performance of left ventricular indexes in heart failure. Thus, myocardial β-adrenergic desensitization qualifies as the key index of myocardial dysfunction or, at least, as the one summarizing the prognostic implications of heart failure. This consideration supports the current view of heart failure as a systemic disease largely involving neuroautonomic tone and the recommendation of β-blockers as key long-term therapy. In contrast to previous studies, we rated β-adrenergic myocardial desensitization using a physiological method (CD_{25}), which guarantees a "valid" result free from confounders that tend to negatively affect methods indirectly rating the autonomic balance, such as the assessment of HR variability (8). However, CD_{25} is not suitable for routine use. As a diagnostic method it is not risk free, and it would burden the diagnostic path of heart failure patients (16). Thus, the value of the present findings lies in the demonstration of the prognostic role of β-adrenergic myocardial desensitization rather than in a proposal to enrich the diagnostic toolbox for patients with heart failure.

Although larger studies are needed, CD_{25} might be the target of heart failure therapy, with the objective of decreasing its value. Following CD_{25} evolution might also disclose different evolutions of myocardial β-adrenergic desensitization and allow for the identification of inherent correlates and selected therapeutic needs. Thus, the results of this study may lead to innovative clinical research into heart failure.

The association of reduced β-adrenergic sensitivity and sympathetic denervation (16) in patients with heart failure requires a careful choice of therapeutic strategy (3, 22).

Limitations

This study included only a small number of patients due to the selective criteria chosen to reduce biases. Indeed, a further limitation is related to the use of HR variability in patients with heart failure. HR variability can be negatively influenced by the presence of heart failure and be affected by respiratory variations. Indeed, our population was a homogenous one: all patients suffered from severe heart failure. Respiratory conditions were also standardized to reduce the influence of their variations. β-Blockers qualify as lifesaving drugs in heart failure with reduced ejection fraction, and thus, their indication is out

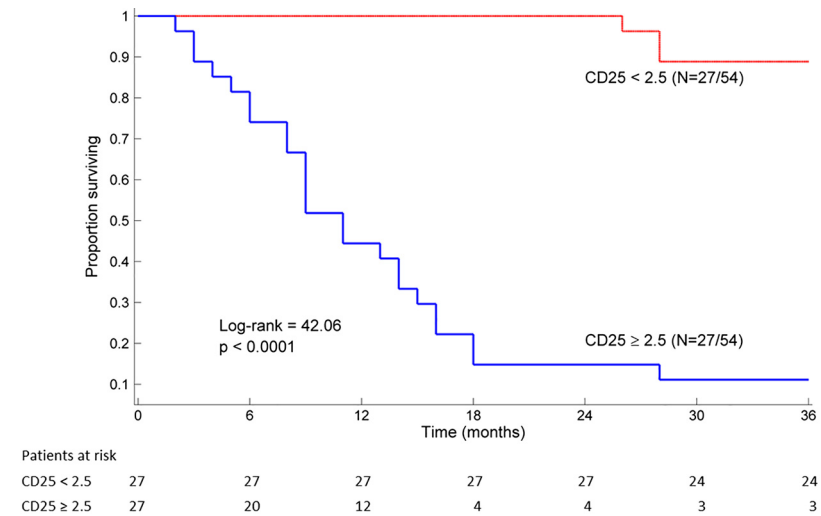

Fig. 5. Kaplan–Meyer survival curve for chronotropic 25-dose isoproterenol infusion test (CD_{25}).

of doubt. However, the 7-day interruption was harmless, and no adverse events were recorded within this period. Finally, the mortality rate of our patients was higher than expected on the basis of their NYHA class. Unfortunately, for most of them we could only rely on the death certificates. Thus, we cannot exclude that heart failure-unrelated conditions or poor adherence to the therapy contributed to such a high mortality.

Conclusions

Biomechanical adaptation to volume displacement worsens, whereas the sympathetic drive increases in relation to baseline LVEF in patients with heart failure due to MI. The CD25 test predicts mortality in patients with ischemic heart failure.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. conceived and designed research; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. performed experiments; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. analyzed data; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. interpreted results of experiments; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. prepared figures; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. drafted manuscript; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. edited and revised manuscript; D.A., M.N., R.A., G.C., M.C., P.S., T.L., L.C., R.M., C.P. and V.P. approved final version of manuscript.

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