Use of Ventilatory Efficiency Slope as a Marker for Increased Mortality in Wild-Type Transthyretin Cardiac Amyloidosis

Adil Yunis, MD[d], Gheorge Doros, PhD[b], Ivan Luptak, MD, PhD[a,c,d], Lawreen H. Connors, PhD[e], and Flora Sam, MD[a,c,d,*]

Wild-type transthyretin amyloidosis (ATTRwt) results in an infiltrative cardiomyopathy often culminating in symptomatic heart failure. The use of cardiopulmonary exercise testing (CPET) in determining outcomes in ATTRwt cardiac amyloidosis is unknown. Given the emergence of novel therapies to treat transthyretin amyloidosis, we sought to investigate the utility of CPET on outcomes in patients with ATTRwt cardiomyopathy. Fifty-six patients, with biopsy and immunohistochemically proved ATTRwt, were enrolled between 2005 and 2015, as part of an NIH ATTRwt substudy at the Boston University Amyloidosis Center. Patients were prospectively studied, which included laboratory tests, electrocardiogram, echocardiography, in addition to CPET. In this cohort of ATTRwt patients who performed CPET were elderly, all were male, and predominantly white (69.9%). The overall median survival was 59.01 months (95% confidence interval [CI] 49.29 to 88.69). By multivariate analysis, C-reactive protein (CRP; hazard ratio [HR] 1.10 [1.03 to 1.18]), decreased sodium (HR 0.75 [0.58 to 0.97]), creatinine (HR 7.48 [2.44 to 22.98]) and VE/VCO_2 (HR 1.10 [1.05 to 1.16]) were significant risk factors for mortality ($p < 0.05$). Peak VO_2 was insignificant by both univariate and multivariate analyses. ATTRwt patients with $VE/VCO_2 > 40$ had a worse median survival of 38.54 months (95% CI 32.63 to 51.47) versus 88.69 months (95% CI 56.26 to 89.49) than patients with VE/VCO_2 slope ≤ 40. Receiver-operating characteristic curve showed that the combination of VE/VCO_2, CRP, sodium, and creatinine (Area under the ROC Curve [AUC], 0.89) predicted 1-year mortality in ATTRwt cardiac amyloidosis. In conclusion, increased VE/VCO_2, in combination with CRP, sodium, and creatinine, may identify patients at increased risk of death in ATTRwt cardiomyopathy. VE/VCO_2 might have a role in objectively assessing therapeutic response in ATTRwt cardiac amyloidosis. **(Am J Cardiol 2019;124:122−130)**

Wild-type transthyretin (TTR) amyloidosis (ATTRwt), previously referred to as "senile systemic amyloidosis," is characterized by misfolding and deposition of TTR amyloid protein predominantly in the heart, resulting in a progressive infiltrative cardiomyopathy and heart failure (HF).[1,2] Nervous system involvement may occur with carpal tunnel syndrome being the most common antecedent finding.[3] TTR amyloid deposition, once believed to be an incidental finding, was found in up to 25% of those >85 years in an autopsy study.[4] The median survival in ATTRwt cardiac amyloidosis is quite variable and ranges from 2.71 years[5] up to 6.1 years.[6] Until recently[7,8] there were limited therapeutic options for ATTR amyloidosis, but promising new drug therapies have emerged demonstrating improvement in survival and 6-minute walk test.[8] As such, there is a need to define parameters that may objectively monitor response to therapy and mortality in ATTR. Cardiopulmonary exercise testing (CPET) allows for the assessment of peak oxygen uptake, establishes causes of dyspnea on exertion, is used to assess functional capacity and prognosis for nonamyloid HF and cardiomyopathies,[9,10] but little is known about its utility in ATTRwt. Unlike a 6-minute walk test, there is neither learning[11] nor familiarization effect with CPET.[12] In the present study, we prospectively evaluated 56 patients with biopsy and immunohistochemically proved ATTRwt cardiac amyloid who underwent CPET, clinical assessment, and noninvasive studies, to identify risk factors of mortality.

Methods

Between 2005 and 2015, 121 patients with biopsy and immunohistochemically proved ATTRwt were prospectively

[a]Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston Medical Center, Boston, Massachusetts; [b]Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; [c]Cardiovascular Section, Boston University School of Medicine, Boston Medical Center, Boston, Massachusetts; [d]Evans Department of Medicine, Boston University School of Medicine, Boston Medical Center, Boston, Massachusetts; and [e]Alan and Sandra Gerry Amyloid Research Laboratory in the Amyloidosis Center, Boston University School of Medicine, Boston Medical Center, Boston, Massachusetts. Manuscript received January 21, 2019; revised manuscript received and accepted March 19, 2019.

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*Corresponding author: Tel: (617) 638-8072; fax: (617) 638-4066.
E-mail address: flora.sam@bmc.org (F. Sam).

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enrolled in a NIH funded study RO1AG031804[2] at the Amyloidosis Center at Boston University School of Medicine and Boston Medical Center. Of these, 56 patients with confirmed ATTRwt cardiac amyloidosis completed CPET on initial evaluation and were included in this analysis. The institutional review board approved the study and all subjects gave written informed consent. Clinical and laboratory evaluations including a medical history, physical examination, electrocardiogam, echocardiography, and CPET testing were performed at this initial visit to the Amyloidosis Center.

ATTRwt amyloidosis was confirmed by biopsy and immunohistochemical staining in all patients. The majority of tissue samples were obtained from endomyocardial biopsy (n = 46). ATTRwt amyloidosis was confirmed to a lesser extent in other tissues (fat pad, tenosynovium, small bowel, lung or bladder; n = 10). Age, in some cases Tc-99m pyrophosphate imaging, absence of a plasma cell dyscrasia, and importantly the exclusion of a mutant TTR allele (by genomic DNA sequencing or mass spectrometry) were all used to diagnose ATTRwt.

Patients were classified according to New York Heart Association functional class I to IV. Clinical course was monitored with regular follow-up at the Amyloidosis Center and if patients were unable to return to the Amyloidosis Center by telephone and/or by contacting referring physicians. The follow−up end point was all−cause death obtained from medical records or publicly available databases.

Two-dimensional echocardiography was performed using the GE VingMed Vivid FiVe Echocardiography System (GE Vingmed, Milwaukee, Wisconsin) with a 2.5-MHz phased-array transducer as described previously.[13] Echocardiogram was performed and analyzed in a blinded manner. Left ventricular (LV) ejection fraction (LVEF) was calculated using the modified Simpson's rule. Systolic and diastolic chamber dimensions and wall thickness measurements were obtained by 2D imaging according to the American Society of Echocardiography recommendations.[14] The standard cube formula was utilized to calculate LV mass.[14] Relative wall thickness was calculated as (2 × posterior wall thickness)/left ventricle end-diastolic diameter. The apical 4-chamber view was used to determine transmitral Doppler LV measurements and analyzed for diastolic filling indexes, including peak E- and A-wave velocities, the E/A ratio, and E-wave deceleration time. Myocardial velocity of the mitral annulus and derivation of E′ was determined using tissue Doppler imaging. An E′ velocity of <10 cm/s by tissue Doppler imaging suggested impaired diastolic function. A higher E/e′ index indicated elevated LV end-diastolic pressure.[15]

CPET was performed using a standard exercise treadmill with the Medgraphics CPX Ultima. Expired gas analyses were performed, with the highest values obtained during the final 30 seconds of exercise used as peak scores. Peak oxygen consumption (VO_2max), ventilatory equivalent for carbon dioxide (VE/VCO_2 slope), tidal volume, exercise duration, breathing reserve, and respiratory exchange ratio (RER) were calculated as described.[16]

Blood samples were collected at the initial visit to the Amyloidosis Center and included brain natriuretic peptide (BNP), cardiac troponin I, C-reactive protein (CRP), and erythrocyte sedimentation rate as part of routine laboratory testing.

Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as number of patients and percentages or median (interquartile range). Comparisons between the $VE/VC0_2$ slope >40 and ≤40 groups were evaluated by the Student's t test for continuous variables or the chi-square test for categorical variables. However, when comparing continuous baseline characteristics here, we report the Wilcoxon's test result whenever the Shapiro-Wilk test for normality is significant ($p < 0.05$). Univariate analyses were performed to identify variables associated with increased mortality from demographic, clinical, laboratory, electrocardiography, and echocardiographic variables. A p value ≤0.05 was considered statistically significant. Covariates were chosen based on biologic and statistical significance and entered into a multivariate Cox proportional hazards model. Backward elimination was then conducted with p value criteria for retention set at 0.20. As proposed by Heagerty and Zheng,[17] to define the predictive accuracy of the Cox regression models under considerations we use the time-dependent accuracy measures (sensitivity, specificity, and receiver-operating characteristic concepts). These measures, reported as t = months, provide measures to characterize the ability of these markers to distinguish cases from controls. The analyses were conducted using SAS software version 9.4 (SAS Institute Inc.) or R version 3.5.0 (R Core Team (2018). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/).

Results

Of the 121 patients with confirmed ATTRwt cardiac amyloidosis, 56 were able to exercise and underwent CPET, as part of this substudy, on initial enrollment at the Amyloidosis Center (Table 1). All subjects were male, predominantly white, and elderly with a mean age of 74.8 ± 6.2 years. The major associated co-morbidities were hypertension and atrial fibrillation/atrial flutter. Most patients had HF symptoms (>80% were New York Heart Association functional class ≥2). There was evidence of neurologic involvement, the most common of which was carpal tunnel syndrome, which preceded the diagnosis of ATTRwt cardiac amyloidosis. The majority of patients were on loop/thiazide diuretics (88%). Beta blocker and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use were present in 66% and 43% of these patients, respectively. Cardiac biomarkers were elevated with a median BNP of 401.5 pg/ml (interquartile range 242.8 to 704.3) and cardiac troponin I of 0.104 ng/ml (interquartile range 0.066 to 0.170). Creatinine, uric acid, and erythrocyte sedimentation rate levels were also elevated.

QRS duration was prolonged on electrocardiogram with evidence of intraventricular conduction delay (52%), right bundle branch block (18%), and left bundle branch block (2%). Echocardiography demonstrated structural cardiac changes as seen in Table 2. Mean LVEF was 50.2 ± 10.9%, with increased interventricular septal thickness (16.2 ± 2.9 mm), posterior wall thickness (15.98 ± 2.7 mm), LV mass (299.3 ± 72.9 g), and relative wall thickness (0.74 ± 0.2). Left atrial volume (50.1 ± 13.0 ml/m^2) was

Table 1
Baseline characteristics, in patients with ATTRwt cardiac amyloidosis

Variable	Value (n = 56)	Median (IQR range)	Normal values
Age (years)	74.8 ± 6.2	75.5 (71.0-79.0)	
Men	56 (100%)		
White	39 (69%)		
Black	2 (4%)		
Hispanic (non-black)	11 (20%)		
Other	4 (7%)		
Body mass index (kg/m^2)	28.9 ± 3.9	28.6 (25.3-31.2)	
Systolic blood pressure (mm Hg)	126.9 ± 16.2	125 (113.0-135.0)	
Diastolic blood pressure (mm Hg)	76.3 ± 8.0	74.0 (70.0-82.0)	
Pulse rate (bpm)	71.4 ± 11.5	69.0 (63.0-78.0)	
Coronary artery disease	15 (27%)		
Hypertension	27 (48%)		
Diabetes Mellitus	8 (14%)		
Atrial fibrillation/atrial flutter	38 (68%)		
Chronic kidney disease	7 (13%)		
Obstructive sleep apnea	10 (18%)		
Hypothyroidism	8 (14%)		
NYHA class	2.27 ± 0.75	2.0 (2.0-3.0)	
I	18%		
II	37%		
III	45%		
IV	0		
Electrocardiogram			
QRS duration (ms) on ECG	122.0 ± 32.0	116.0 (94.0-150.0)	
LBBB	1 (2%)		
RBBB	10 (18%)		
IVCD	29 (52%)		
First degree AVB	18 (32%)		
Neurologic involvement			
Peripheral	15 (27%)		
Autonomic	2 (4%)		
Carpal Tunnel	38 (68%)		
Laboratory data			
BNP (pg/ml)	467.5 ± 308.3	401.5 (242.8-704.3)	0-176
Troponin I (ng/ml)	0.131 ± 0.097	0.104 (0.066-0.170)	<0.033
ESR (mm/hour)	16.7 ± 12.6	14.0 (7.0-23.0)	0-20
C-reactive protein (mg/L)	4.2 ± 7.1	1.6 (0.8-4.8)	0-5.0
Hemoglobin (g/dl)	13.4 ± 1.7	13.6 (12.5-14.7)	13.5-17.5
Sodium (mmol/L)	139.3 ± 2.5	139.0 (138.0-141.0)	135-145
Creatinine (mg/dl)	1.3 ± 0.4	1.2 (1.0-1.5)	0.7-1.3
Uric acid (mg/dl)	7.7 ± 2.1	7.8 (6.0-9.1)	2.4-6.0
Medications			
Beta blocker	37 (66%)		
ACE-I/ARB	24 (43%)		
Loop/thiazide diuretics	49 (88%)		
Aldactone	6 (11%)		
Statin	32 (57%)		
Anticoagulants	36 (64%)		

ACE-I = angiotensin-converting enzyme-inhibitor; ARB = angiotensin receptor blocker; AVB = atrioventricular block; BNP = brain natriuretic peptide; ECG = electrocardiogram; ESR = erythrocyte sedimentation rate; IVCD = intraventricular conduction delay; LBBB = left bundle branch block; NYHA = New York Heart Association; RBBB = right bundle branch block.

Data are expressed as mean ± SD for continuous variables or numbers or percentage (%) for categorical variables and median (IQR, interquartile range).

Anticoagulants include warfarin and nonvitamin K antagonist oral anticoagulants.

significantly increased and there was evidence of severe diastolic dysfunction as demonstrated by the increased E/A (2.41 ± 1.20) and E/e′ (25.8 ± 11.9) ratios and decreased e′ mean (4.41 ± 1.4 cm/s). Pulmonary artery systolic pressures were also elevated (32.8 ± 15.2 mm Hg).

Because there are no normal values for CPET in this age group, ATTRwt patients were compared with a group of age-matched control subjects who had no cardiac amyloidosis and no coronary artery disease (CAD; Table 3). These subjects had been referred for atypical chest pain or were interested in participating in an exercise program (n = 10). Seventy percent were male and the average age was 75 ± 7.0 years. In the ATTRwt cohort, mean RER was 1.14 ± 0.12 suggesting adequate effort during CPET testing (16),

Table 2
Baseline echocardiographic measures at initial clinic visit in ATTRwt cardiac amyloidosis

Variable	Value	Median (IQR range)	Normal range
LV structure			
Ventricular septum (mm)	16.2 ± 2.9	16 (15-18)	<11
Posterior wall thickness (mm)	15.98 ± 2.7	16 (14-18)	6-11
Relative wall thickness	0.74 ± 0.20	0.72 (0.60-0.86)	0.22-0.42
LV mass index (g/m^2)	149.3 ± 34.5	148.7 (127.7-172.2)	<131 (male)
LV mass (g)	299.3 ± 72.9	294.3 (258-340)	67-162
LV size			
LV EDD (mm)	45.7 ± 14.9	43 (39-49)	<57
LV ESD (mm)	33.1 ± 6.6	32 (28-38)	21-40
Left atria volume (ml/m^2)	50.3 ± 13.0	49.3 (41.3-56.0)	34
Left atria (mm)	42.6 ± 4.9	43 (40-46.0)	
LV function			
LV EF (%)	50.2 ± 10.9	50 (40-60)	>50
E/A	2.41 ± 1.20	2.35 (1.5-3.2)	0.9*
e′ medial (cm/s)	3.69 ± 1.3	3.4 (3.0-4.6)	~7.5*
e′ lateral (cm/s)	5.14 ± 1.8	5.0 (4.0-6.0)	~10.5*
e′ mean (cm/s)	4.41 ± 1.4	4 (3.5-5.5)	~9.0*
E /e′	25.8 ± 11.9	21.5 (17-29)	<8
PA systolic pressure (mm Hg)	32.8 ± 15.2	36.5 (29-42)	15-25

EDD = end-diastolic diameter; EF = ejection fraction; ESD = end-systolic diameter; E/A = mitral inflow E/A ratio; e′ = early diastolic velocity; E/e′ = ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LV = left ventricular; PA = pulmonary artery.

Data are expressed as mean ± SD for continuous variables and mean (IQR, interquartile range).

* Age-specific normal reference ranges for age 65 to 75 years.

Other normal values per American Society of Echocardiography.[14]

but there was a trend to lower average exercise duration than control subjects (397.93 ± 173.35 vs 423.00 ± 82.19 ms; p = NS). In both groups, 70% of participants achieved ventilatory threshold. Peak VO_2 was reduced in both ATTR and control groups (13.49 ± 4.46 and 13.59 ± 1.26 ml/kg/min, respectively; p = NS), but VE/VCO_2 slope was significantly elevated in ATTRwt compared with controls (41.3 ± 9.7 vs 35.1 ± 3.7; p <0.05 vs controls).

In the ATTRwt cohort that was able to perform CPET, the overall median survival was 59.01 months (95% confidence interval [CI] 49.29 to 88.69). The median follow-up was 35.0 months with a minimum to maximum range of 10.8 to 89.5 months. The 1-, 3-, and 5-year survival were 96.4% (95% CI 0.86 to 0.99), 76.6% (95% CI 0.61 to 0.87), and 41.3% (95% CI 0.21 to 0.60), respectively. As expected survival was better than the group as a whole where the median survival was 46.69 months.[2]

Table 3
Cardiopulmonary exercise testing (CPET) in ATTRwt cardiac amyloidosis compared with age and gender-matched controls

CPET variables	ATTRwt	Controls
Peak VO_2 (ml/kg/min)	13.49 ± 4.46 *13.35 (9.63-16.33)	13.59 ± 1.26
VE/VCO_2 slope	41.30 ± 9.68 *40 (34-46)	35.14 ± 3.66
1 - Ventilatory reserve (VE/MVV) (%)	42.79 ± 16.18 *43.5 (32.75-55.0)	70.28 ± 4.27
RER	1.14 ± 0.12 *1.13 (1.07-1.2)	1.06 ± 0.02
Exercise duration (s)	397.93 ± 173.35 *360 (300-540)	433.00 ± 82.19
VT (ml/kg/min)	11.94 ± 4.45 *10.90 (8.8-15)	12.90 ± 0.93

MVV = maximum voluntary ventilation; RER = respiratory exchange ratio; VE = minute ventilation; VE/VCO_2 = ventilatory equivalent for carbon dioxide; VO_2 = oxygen uptake; VT = ventilatory threshold.

Data are expressed as mean ± SD for continuous variables and ∗median (IQR, interquartile range). Controls (70% male; age 75 ± 7 years; n = 10).

By univariate analyses (Table 4), the strongest risk factors for all-cause mortality were obstructive sleep apnea, diabetes, elevated creatinine, VE/VCO_2, BNP and CRP levels and lower A-velocity, systolic blood pressure, LVEF, and sodium levels (p <0.05). Multivariate analysis (Table 4), using a Cox proportional hazards regression model with backward elimination, was used to evaluate interactions among all the risk factors for overall mortality. Creatinine, VE/VCO_2, CRP, and sodium persisted as significant risk factors (p <0.05). The other univariate risk factors entered into the model were not retained by the final multivariate Cox proportional hazards model. Peak VO_2 was not significant for increased death by univariate and multivariable analyses.

To determine the contribution of single variables or the full model as a whole in predicting 1-year mortality, receiver-operating characteristic analysis was performed (Figure 1). The Area under the ROC Curve (AUC) values were comparable for CRP (AUC 0.67), sodium (AUC 0.63), creatinine (AUC 0.68), and VE/VCO_2 (AUC 0.68). However, the inclusive model using all 4 variables gave the greatest power, AUC of 0.89 in predicting 1-year mortality in ATTRwt cardiac amyloidosis in those who were able to exercise on initial evaluation.

The significant impact of VE/VCO_2 slope on mortality, both by univariate and multivariate analysis, prompted further subgroup analysis of VE/VCO_2 greater (≥) and less

Table 4
Univariate and multivariate analysis for predictors of mortality in ATTRwt cardiac amyloidosis who performed CPET

Variable	Univariate hazard ratio	p Value	Multivariate hazard ratio	p Value
Obstructive sleep apnea	3.81 (1.41-10.26)	0.0082		
Diabetes	3.20 (1.12-9.13)	0.0293		
BNP	1.00 (1.001-1.003)	0.0012		
A-velocity	0.95 (0.91-0.99)	0.0284		
Systolic blood pressure	0.95 (0.92-0.99)	0.0137		
LVEF (%)	0.95 (0.91-0.99)	0.0235		
peak VO_2	0.91 (0.83-1.01)	0.0662*		
Creatinine	4.18 (1.68-10.42)	0.0021	7.48 (2.44-22.98)	0.0004
VE/VCO_2 slope	1.06 (1.02-1.10)	0.0012	1.10 (1.05-1.16)	0.0003
C-reactive protein	1.07 (1.02-1.12)	0.0073	1.10 (1.03-1.18)	0.0031
Sodium	0.84 (0.71-0.99)	0.0350	0.75 (0.58-0.97)	0.0291

BNP = brain natriuretic peptide; LVEF = left ventricular ejection fraction; peak VO_2 = peak oxygen uptake; VE/VCO_2 = minute ventilation/carbon dioxide production.

A-velocity reflects the LA-LV pressure gradient during late diastole.

* p = NS.

than or equal (≤) to the median value 40. Baseline demographics and clinical characteristics were compared between the 2 groups (Table 5). ATTRwt patients with VE/VCO_2 slope >40 had lower body mass index, lower medial e′ by tissue Doppler (a measure of early diastolic mitral annular tissue velocity), higher circulating BNP and troponin I levels and lower exercise duration during CPET testing (p <0.05).

Kaplan-Meier survival estimates showed worse survival in $VE/VECO_2$ slope >40 versus $VE/VECO_2$ slope ≤40 over 8 years (p <0.01; Figure 2). Median survival was significantly worse in those with VE/VCO_2 slope >40 versus VE/VCO_2 slope ≤40 (38.54 months [95% CI 32.63 to 51.47] vs 88.69 months [95% CI 56.26 to 89.49]; p <0.01). The 1-, 3-, and 5-year cumulative survival in $VE/VECO_2$ slope >40 was 91.7% (95% CI 0.86 to 0.97), 64.1% (95% CI 0.53 to 0.76) and 24.9% (95% CI 0.13 to 0.37), respectively. Conversely, survival was better for those with a $VE/VECO_2$ slope ≤40 at those same time points: 100%, 84.7% (95% CI 0.77 to 0.92), and 56.5% (95% CI 0.39 to 0.73), respectively.

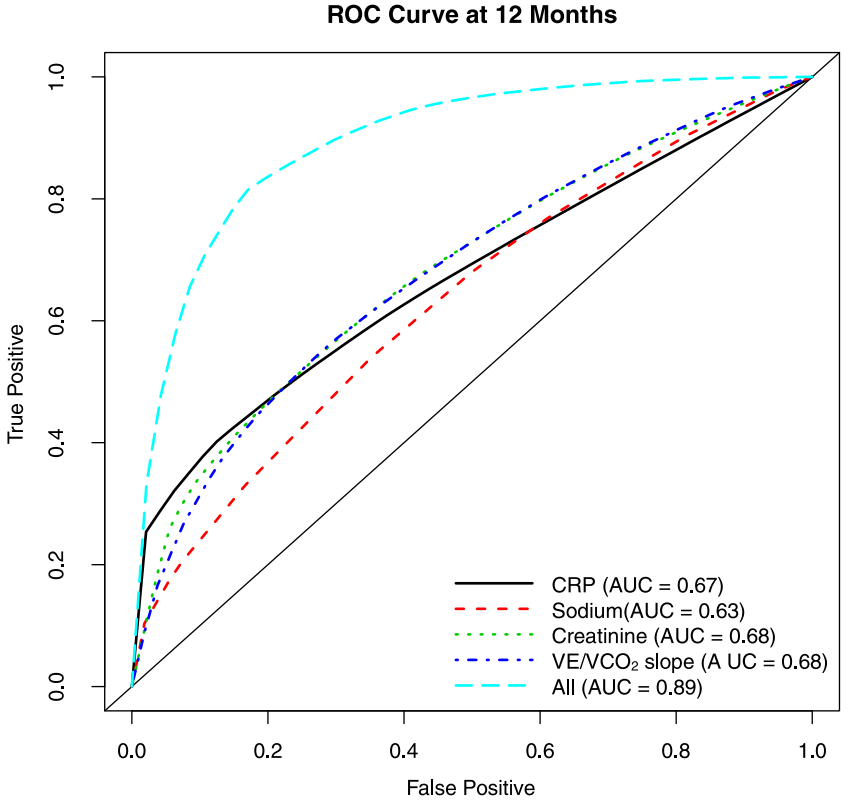

Figure 1. Receiver-operating characteristic (ROC) curves demonstrated that VE/VCO_2 slope, creatinine, sodium, and CRP are predictors of 1-year mortality in patients with ATTRwt cardiac amyloidosis.

Table 5
Clinical characteristics above and below the median for VE/VCO_2 slope

Variables	VE/VCO_2 slope >40 (n = 24)	VE/VCO_2 slope ≤40 (n = 31)	p Value
Age (years)	76.4 ± 3.9 †76 (73.5-79.5)	73.6 ± 7.4 †75 (67-79)	0.254
White	13 (54%)	26 (84%)	0.016*
Systolic blood pressure (mm Hg)	123.5 ± 18 †123 (110-127)	130 ± 14.5 †131 (120-139)	0.144
Body mass index (kg/m^2)	27.8 ± 3.7 †27.5 (24.9-29.9)	29.9 ± 3.9 †29.2 (27.2-33.1)	0.049
Hypertension	13 (54%)	14 (45%)	0.508
Diabetes mellitus	5 (21%)	3 (10%)	0.244
Coronary artery disease	5 (21%)	9 (29%)	0.488
Carpal tunnel	15 (63%)	23 (74%)	0.352
NYHA class	2.4 ± 0.7 †2.5 (2-3)	2.2 ± 0.8 †2.0 (1-3)	0.270
Echocardiography			
LVEF (%)	49.2 ± 11.8 †49.5 (42.5-60)	50.5 ± 10.2 †50 (40-60)	0.714
LV mass index (g/m^2)	149.6 ± 34.6 †153.3 (124-173.5)	150.1 ± 35.3 †147.1 (132.5-170)	0.962
RWT	0.79 ± 0.23 †0.82 (0.66-0.93)	0.69 ± 0.16 †0.69 (0.58-0.76)	0.062
E/A	2.63 ± 1.28 †2.49 (1.63-3.63)	2.24 ± 1.2 †2.12 (1.22-3)	0.327
Tissue Doppler e′ medial (cm/s)	3.28 ± 1.01 †3 (2.7-4)	3.99 ± 1.39 †3.8 (3-5)	0.044*
E/e′	26.85 ± 11.97 †24 (17.6-30)	25.06 ± 12.28 †20 (16.4-29)	0.607
PA systolic pressure (mm Hg)	34 ± 16.1 †36 (30-46)	31.4 ± 14.8 †36 (27-41.5)	0.506
Laboratory data			
BNP (pg/ml)	600.2 ± 325.9 †448 (364-777)	375.0 ± 257.9 †295 (188-469)	0.009*
Troponin I (ng/ml)	0.17 ± 0.11 †0.16 (0.12-0.2)	0.1 ± 0.08 †0.08 (0.05-0.12)	0.002*
C-reactive protein (mg/L)	3.68 ± 4.11 †3.1 (0.8-4.5)	4.69 ± 8.62 †1.45 (0.8-5.2)	0.918
Sodium (mmol/L)	139.1 ± 2.95 †139 (138-141)	139.6 ± 2.06 †140 (138-141)	0.904
Creatinine	1.44 ± 0.48 †1.37 (1.08-1.6)	1.25 ± 0.34 †1.17 (0.98-1.42)	0.116
Uric acid (mg/dl)	7.8 ± 2.1 †8.25 (6.35-9.3)	7.7 ± 2.1 †7.7 (6-9.1)	0.824
Exercise testing (CPET)			
Peak VO_2 (ml/kg/min)	12.2 ± 4.4 †10.85 (8.75-14.8)	14.4 ± 4.4 †14.8 (10.3-17)	0.048
Exercise duration (s)	328.2 ± 149.6 †335 (237-420)	445.1 ± 176.0 †420 (300-592)	0.013*
VT (ml/kg/min)	10.39 ± 5.1 †9.25 (7.6-12.3)	12.94 ± 3.78 †12.85 (9.8-16.2)	0.083
1 - Ventilatory reserve (VE/MVV) (%)	0.41 ± 0.18 †0.42 (0.32-0.55)	0.43 ± 0.15 †0.44 (0.34-0.56)	0.765
RER	1.12 ± 0.08 †1.13 (1.08-1.16)	1.16 ± 0.14 †1.13 (1.04-1.25)	0.615

BNP = brain natriuretic peptide; E/A = mitral inflow E/A ratio; e′ = early diastolic velocity; E/e′ = ratio between early mitral inflow velocity and mitral annular early diastolic velocity; EF = ejection fraction; LV = left ventricular; MVV = maximum voluntary ventilation; NYHA = New York Heart Association; PA = pulmonary artery; RER = respiratory exchange ratio; RWT = relative wall thickness; VE = minute ventilation; VE/VCO_2 = ventilatory equivalent for carbon dioxide; VO_2 = oxygen uptake; VT = ventilatory threshold.

Data are expressed as mean ± SD or median (range) for continuous variables or numbers (%) for categorical variables and †median (IQR, interquartile range).

* $p < 0.05$.

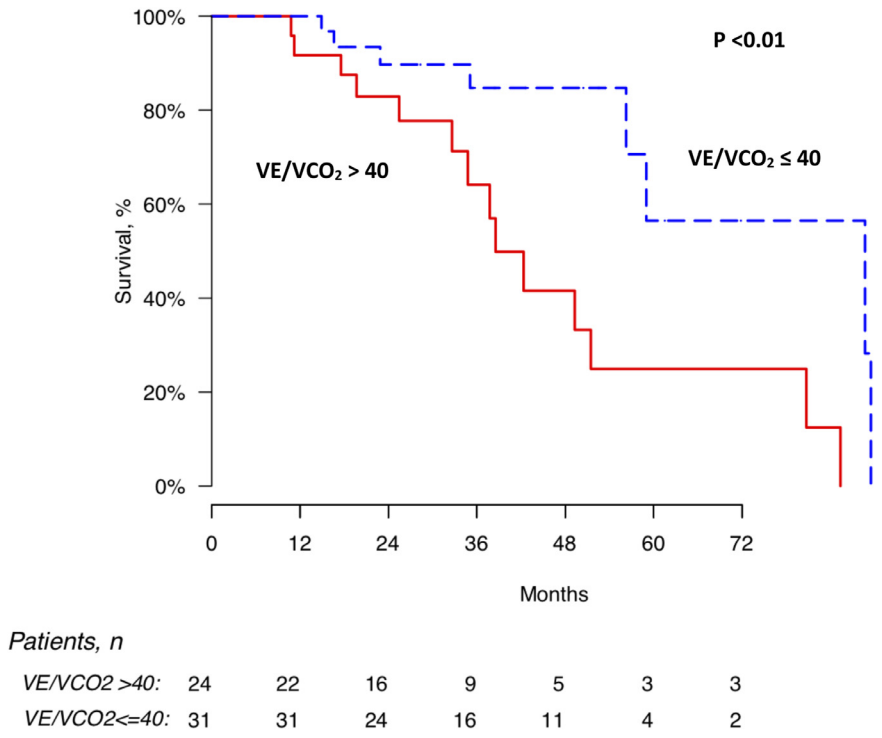

Figure 2. Kaplan-Meier survival curve for all-cause mortality in VE/VCO_2 >40 and ≤40 in ATTRwt cardiac amyloidosis. Survival was significantly worse in patients with VE/VCO_2 >40 than those with VE/VCO_2 ≤40 (p <0.01)

Discussion

In this subgroup study, the increased VE/VCO_2 slope was associated with more severe clinical disease and with increased mortality in ATTRwt cardiac amyloidosis. An inclusive model of increased VE/VCO_2 with elevated CRP, creatinine, and low sodium identified those ATTRwt patients at the highest risk of dying at 1 year. Importantly, peak VO_2 was not a risk factor for mortality by univariate or multivariable analysis in this cohort.

Peak VO_2 has long been used in the assessment of functional capacity and as a predictor of survival in HF with reduced ejection fraction.[18] This was not seen here and our findings demonstrate the limitations of the prognostic value of peak VO_2 in ATTRwt cardiomyopathy. It had been suggested that patients unable to achieve peak VO_2 might be due to the association of multiple co-morbidities, advanced age or performing a submaximal exercise test.[19] The first 2 are likely relevant to this ATTRwt cohort but 93% of patients in this exercising cohort were able to attain RER (median 1.13; range 1.07 to 1.20).

It is unclear why peak VO_2 does not have a prognostic value in ATTRwt cardiomyopathy. Peak VO_2 is a function of both cardiac output and arterial-venous oxygen difference. During exercise in normal healthy adults, peak VO_2 rises to meet increasing metabolic demand. However, energy expenditure and both its components of active and resting metabolic rate decrease with age,[20] leading to age as the strongest predictor of peak VO_2 .[21]

Patients with ATTRwt cardiac amyloidosis are believed to phenocopy patients with HF with preserved ejection fraction (HFpEF) with clinical HF, LV hypertrophy and diastolic dysfunction.[22] VO_2 and VE/VCO_2 slope both have a predictive outcome in HFpEF.[23] However, in a cohort of HFpEF patients where LVEF ≥50%, only peak VO_2, and not VE/VCO_2 slope was associated with all−cause mortality or cardiac transplant after adjusting for age, gender, and β−blockade therapy. They were followed for a median of 5.2 years[24] with all patients reaching RER, but an important difference in addition to being nonamyloid HF was that patients were younger (the mean age was 54 ± 14 years) and 45% were female. Conversely, Nadruz et al[23] showed in nonamyloid HF, that both VE/VCO_2 slope in addition to peak VO_2 had greater prognostic discrimination in HFpEF than HF with reduced ejection fraction. Other HFpEF studies, similar to our present study, showed that only VE/VCO_2 slope, and not peak VO_2, was associated with all−cause mortality and raised intrapulmonary pressures.[25]

In the present study, VE/VCO_2 persisted as a significant indicator of survival by both univariate and multivariate cox regression analysis in ATTRwt cardiac amyloidosis. Indeed, earlier studies, in patients with nonamyloid HF, showed that ventilatory efficiency was an independent prognostic marker.[26,27] As seen in Table 1, most of the ATTRwt patients had HF ("diastolic heart failure") as the mean LVEF was 50.2 ± 10.9%. This was similar to Guazzi et al[28] who showed in 284 patients with diastolic HF, VE/VCO_2, by multivariate analysis was a significant predictor of mortality.

The mean VE/VCO_2, in this study, was also much higher than the mean value in other studies in patients with nonamyloid diastolic HF[27] where those with a higher VE/VCO_2 had a greater mortality. Indeed, the mean VE/VCO_2 was significantly greater in the ATTRwt cohort compared with

age-matched controls (41.30 ± 9.68 vs 35.14 ± 3.66, respectively). This might be explained by the augmentation in the ventilatory response leading to elevations in VE/VCO_2, which are linked to reduced pulmonary perfusion, as demonstrated by elevations in pulmonary artery and capillary wedge pressures, as well as ventilation/perfusion mismatch with increased dead space.[19,29] Although hemodynamic measurements were not measured in our study, it is plausible that with the degree of diastolic dysfunction already evident on the baseline, resting echocardiography, these ATTRwt patients likely experienced significant elevations in filling pressures during exercise (as occurs with normal and other HF patients),[30] which resulted in the marked increase in VE/VCO_2 seen in these ATTRwt patients.

Finally, those patients with VE/VCO_2 >40 were severely impaired with evidence of poor exercise capacity, elevated cardiac biomarkers, and the tissue Doppler measurements of a lower e′ medial (demonstrating impaired LV longitudinal myocardial relaxation and diastolic dysfunction).

Until recently,[7,8] there have been limited therapeutic options for ATTR amyloidosis, with limitations associated with organ transplant and traditional guideline-directed HF therapies being relatively contraindicated. With the emergence of promising amyloid therapies, the need for reliable noninvasive, objective prognostic markers to assess disease progression, and response to therapy is increasingly needed. Further studies are warranted to determine the effectiveness of CRP, creatinine, sodium, and VE/VCO_2 in risk stratification and their ability to help guide treatment choices for ATTRwt cardiac amyloidosis.

This prospective cohort study has several limitations. First, the Amyloidosis Center at BUMC is a referral center for the diagnosis and treatment of amyloidosis and thus, a referral bias is possible in the selection of patients. Second, the predictive model is center-specific and in a small cohort. Like many ATTRwt studies, our study sample size was small due to the low disease prevalence and the ability to exercise. Further validation in other cohorts is needed. However, our study participants shared several baseline characteristics and echocardiography parameters with other small cohort studies, and is thus likely representative of the greater ATTRwt patient population. Third, about 30% of study participants did not attain a ventilatory threshold during CPET. The absence of ventilatory threshold is sometimes cited as reflecting a noncardiac limitation for exercise and restricts prognostication relative to the underlying cardiomyopathy. However, special consideration must be made for our unique study population. Only a minority of study participants had documented pulmonary or musculoskeletal disease that would prevent the attainment of ventilatory threshold. The absence of ventilatory threshold in some of our study participants is more likely related to their advanced age. It is also possible that ventilatory threshold[16,21] may not be detected in HF patients due to oscillatory ventilation and shorter exercise time.[19,21,29]

Thus, in conclusion, CPET testing may be used in ATTRwt cardiac amyloidosis to provide objective measures of prognosis and may assist in assessing outcomes. As such VE/VCO_2 is a useful predictor of mortality. Enhanced prognosis can be made when utilizing VE/VCO_2 slope in a model that includes Cr, sodium, and CRP in cardiac amyloidosis.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.03.035.

1. Falk RH. Diagnosis and management of the cardiac amyloidoses. *Circulation* 2005;112:2047–2060.
2. Connors LH, Sam F, Skinner M, Salinaro F, Sun F, Ruberg FL, Berk JL, Seldin DC. Heart failure resulting from age-related cardiac amyloid disease associated with wild-type transthyretin: a prospective, observational cohort study. *Circulation* 2016;133:282–290.
3. Grogan M, Scott CG, Kyle RA, Zeldenrust SR, Gertz MA, Lin G, Klarich KW, Miller WL, Maleszewski JJ, Dispenzieri A. Natural history of wild-type transthyretin cardiac amyloidosis and risk stratification using a novel staging system. *J Am Coll Cardiol* 2016;68:1014–1020.
4. Tanskanen M, Peuralinna T, Polvikoski T, Notkola IL, Sulkava R, Hardy J, Singleton A, Kiuru-Enari S, Paetau A, Tienari PJ, Myllykangas L. Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. *Ann Med* 2008;40:232–239.
5. Pinney JH, Whelan CJ, Petrie A, Dungu J, Banypersad SM, Sattianayagam P, Wechalekar A, Gibbs SD, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ. Senile systemic amyloidosis: clinical features at presentation and outcome. *J Am Heart Assoc* 2013;2:e000098.
6. Gonzalez-Lopez E, Gagliardi C, Dominguez F, Quarta CC, de Haro-Del Moral FJ, Milandri A, Salas C, Cinelli M, Cobo-Marcos M, Lorenzini M, Lara-Pezzi E, Foffi S, onso-Pulpon L, Rapezzi C, Garcia-Pavia P. Clinical characteristics of wild-type transthyretin cardiac amyloidosis: disproving myths. *Eur Heart J* 2017;38:1895–1904.
7. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, Tournev I, Schmidt HH, Coelho T, Berk JL, Lin KP, Vita G, Attarian S, Plante-Bordeneuve V, Mezei MM, Campistol JM, Buades J, Brannagan TH III, Kim BJ, Oh J, Parman Y, Sekijima Y, Hawkins PN, Solomon SD, Polydefkis M, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med* 2018;379:11–21.
8. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, Kristen AV, Grogan M, Witteles R, Damy T, Drachman BM, Shah SJ, Hanna M, Judge DP, Barsdorf AI, Huber P, Patterson TA, Riley S, Schumacher J, Stewart M, Sultan MB, Rapezzi C. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. *N Engl J Med* 2018;379:1007–1016.
9. O'Connor CM, Whellan DJ, Wojdyla D, Leifer E, Clare RM, Ellis SJ, Fine LJ, Fleg JL, Zannad F, Keteyian SJ, Kitzman DW, Kraus WE, Rendall D, Pina IL, Cooper LS, Fiuzat M, Lee KL. Factors related to morbidity and mortality in patients with chronic heart failure with systolic dysfunction: the HF-ACTION predictive risk score model. *Circ Heart Fail* 2012;5:63–71.
10. Gitt AK, Wasserman K, Kilkowski C, Kleemann T, Kilkowski A, Bangert M, Schneider S, Schwarz A, Senges J. Exercise anaerobic threshold and ventilatory efficiency identify heart failure patients for high risk of early death. *Circulation* 2002;106:3079–3084.
11. Wu G, Sanderson B, Bittner V. The 6-minute walk test: how important is the learning effect? *Am Heart J* 2003;146:129–133.
12. Bensimhon DR, Leifer ES, Ellis SJ, Fleg JL, Keteyian SJ, Pina IL, Kitzman DW, McKelvie RS, Kraus WE, Forman DE, Kao AJ, Whellan DJ, O'Connor CM, Russell SD. Reproducibility of peak oxygen uptake and other cardiopulmonary exercise testing parameters in patients with heart failure (from the heart failure and a controlled trial investigating outcomes of exercise training). *Am J Cardiol* 2008;102:712–717.
13. Biolo A, Ramamurthy S, Connors LH, O'Hara CJ, Meier-Ewert HK, Soo Hoo PT, Sawyer DB, Seldin DS, Sam F. Matrix metalloproteinases and their tissue inhibitors in cardiac amyloidosis: relationship to structural, functional myocardial changes and to light chain amyloid deposition. *Circ Heart Fail* 2008;1:249–257.

14. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2015;28:1–39.
15. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, Marino P, Oh JK, Popescu BA, Waggoner AD. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016;29:277–314.
16. Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ, Lavie CJ, Macko R, Mancini D, Milani RV. Clinician's guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation* 2010;122:191–225.
17. Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics* 2005;61:92–105.
18. Mancini DM, Eisen H, Kussmaul W, Mull R, Edmunds LH Jr, Wilson JR. Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. *Circulation* 1991;83:778–786.
19. Metra M, Dei CL, Panina G, Visioli O. Exercise hyperventilation chronic congestive heart failure, and its relation to functional capacity and hemodynamics. *Am J Cardiol* 1992;70:622–628.
20. Manini TM. Energy expenditure and aging. *Ageing Res Rev* 2010;9:1–11.
21. Forman DE, Clare R, Kitzman DW, Ellis SJ, Fleg JL, Chiara T, Fletcher G, Kraus WE. Relationship of age and exercise performance in patients with heart failure: the HF-ACTION study. *Am Heart J* 2009;158(4 Suppl):S6–S15.
22. Gonzalez-Lopez E, Gallego-Delgado M, Guzzo-Merello G, de Haro-Del Moral FJ, Cobo-Marcos M, Robles C, Bornstein B, Salas C, Lara-Pezzi E, Alonso-Pulpon L, Garcia-Pavia P. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. *Eur Heart J* 2015;36:2585–2594.
23. Nadruz W Jr, West E, Sengelov M, Santos M, Groarke JD, Forman DE, Claggett B, Skali H, Shah AM. Prognostic value of cardiopulmonary exercise testing in heart failure with reduced, midrange, and preserved ejection fraction. *J Am Heart Assoc* 2017;6. pii: e006000.
24. Shafiq A, Brawner CA, Aldred HA, Lewis B, Williams CT, Tita C, Schairer JR, Ehrman JK, Velez M, Selektor Y, Lanfear DE, Keteyian SJ. Prognostic value of cardiopulmonary exercise testing in heart failure with preserved ejection fraction. The Henry Ford HospITal CardioPulmonary EXercise Testing (FIT-CPX) project. *Am Heart J* 2016;174:167–172.
25. Klaassen SHC, Liu LCY, Hummel YM, Damman K, van der Meer P, Voors AA, Hoendermis ES, van Veldhuisen DJ. Clinical and hemodynamic correlates and prognostic value of VE/VCO_2 slope in patients with heart failure with preserved ejection fraction and pulmonary hypertension. *J Card Fail* 2017;23:777–782.
26. Arena R, Myers J, Aslam SS, Varughese EB, Peberdy MA. Peak VO_2 and VE/VCO_2 slope in patients with heart failure: a prognostic comparison. *Am Heart J* 2004;147:354–360.
27. Chua TP, Ponikowski P, Harrington D, Anker SD, Webb-Peploe K, Clark AL, Poole-Wilson PA, Coats AJ. Clinical correlates and prognostic significance of the ventilatory response to exercise in chronic heart failure. *J Am Coll Cardiol* 1997;29:1585–1590.
28. Guazzi M, Myers J, Arena R. Cardiopulmonary exercise testing in the clinical and prognostic assessment of diastolic heart failure. *J Am Coll Cardiol* 2005;46:1883–1890.
29. Sullivan MJ, Higginbotham MB, Cobb FR. Increased exercise ventilation in patients with chronic heart failure: intact ventilatory control despite hemodynamic and pulmonary abnormalities. *Circulation* 1988;77:552–559.
30. Dorfs S, Zeh W, Hochholzer W, Jander N, Kienzle RP, Pieske B, Neumann FJ. Pulmonary capillary wedge pressure during exercise and long-term mortality in patients with suspected heart failure with preserved ejection fraction. *Eur Heart J* 2014;35:3103–3112.