CARDIOPULMONARY EXERCISE TESTING IN TRANSTHYRETIN AMYLOIDOSIS:

A NOVEL APPROACH TO DEFINE FUNCTIONAL PHENOTYPES & PROGNOSIS

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Key points

Question: What is the spectrum of functional phenotypes in transthyretin amyloidosis and how do they correlate with amyloid burden?

Findings: Prospective study of 506 patients assessing functional capacity using cardiopulmonary exercise test (CPET). Peak VO_2, O_2 pulse and ventilatory efficiency were impaired in cardiac phenotypes, whilst chronotropic incompetence and exercise oscillatory ventilation were highly prevalent across all phenotypes. Worsening amyloid burden correlated with decline in multiple CPET parameters, with peak VO_2 and peak systolic blood pressure independently associated with prognosis.

Meanings: CPET shown to characterise distinct patterns of functional impairment across the spectrum of amyloid infiltration predicting outcomes.

Abstract

Importance. Cardiopulmonary exercise testing (CPET) has an established role in the assessment of patients with heart failure (HF). However, data are lacking in patients with transthyretin (ATTR) amyloidosis.

Objective. Using CPET to characterise the spectrum of functional phenotypes in patients with ATTR amyloidosis, how they correlate with the cardiac amyloid burden and the association between CPET parameters and prognosis.

Design, setting and participants. This single-centre study evaluated patients diagnosed with ATTR amyloidosis (May 2019 to September 2022) who underwent CPET at the National Amyloidosis Centre.

Main outcomes and measures. Comparison of CPET parameters across disease phenotypes (ATTR with cardiomyopathy (ATTR-CM), polyneuropathy (ATTR-PN) or both (ATTR-mixed)), differences in CPET parameters based on degree of amyloid infiltration (as measured by cardiovascular magnetic resonance (CMR) with extracellular volume (ECV) mapping) and association between CPET parameters with prognosis.

Results. The study comprised 506 patients with ATTR amyloidosis (mean age 73.5 years [SD 10.2]; 457 males [90.3%]). Mean follow-up period was 22.4 months [SD 11.6]. Impairment in functional capacity was highly prevalent. Functional impairment in ATTR-CM and ATTR-mixed phenotypes (Peak VO_2 14.5ml/kg/min [SD 4.3] and 15.7ml/kg/min [SD 6.2] respectively) was observed alongside impairment in the O_2 pulse, with ventilatory efficiency highest in ATTR-CM (VE/VCO_2 slope 38.1 [SD 8.6]). Chronotropic incompetence and exercise oscillatory ventilation (EOV) were highly prevalent across all phenotypes, with both the prevalence and severity being higher than in HF from different aetiologies. Worsening of amyloid burden on CMR correlated with decline in multiple CPET parameters, although chronotropic response and EOV remained abnormal irrespective of amyloid burden. On

multivariable Cox regression analysis, peak VO_2 and peak SBP were independently associated with prognosis (peak VO_2: hazard ratio 0.89 (95% CI 0.81 to 0.99, p=0.030; peak SBP: hazard ratio 0.98, 95% CI 0.97 to 0.99, p<0.001).

Conclusion. ATTR amyloidosis is characterised by distinct patterns of functional impairment between all disease phenotypes. A high prevalence of chronotropic incompetence, EOV and ventilatory inefficiency was a characteristic feature of this population. CPET parameters correlated with amyloid burden by CMR, with peak VO_2 and SBP shown to be independent predictors of mortality.

Introduction

Transthyretin amyloidosis (ATTR amyloidosis) is a multi-system, life-threatening disease resulting from the deposition of transthyretin (TTR) amyloid fibrils(1–3). There are two distinct forms: hereditary or variant (ATTRv) amyloidosis, caused by pathogenic TTR mutations, and wild-type (ATTRwt) amyloidosis, where TTR accumulates in its wild-type form(2,3). The phenotypic presentation of ATTRv amyloidosis can be predominantly neurologic (ATTR-PN), predominantly cardiac (ATTR-CM), or a mix of both (ATTR-mixed)(2,4), whilst ATTRwt presents almost exclusively with a cardiomyopathic phenotype.

Impaired physical performance is a hallmark of ATTR amyloidosis, with the 6-minute walk test (6MWT) being increasingly used as primary and secondary endpoints in recent clinical trials(4,5). However, 6MWT provides a simple estimation of exercise tolerance, without insights into the functional phenotype. Cardiopulmonary exercise testing (CPET) is the gold-standard for the evaluation of putative mechanisms that underlie exercise intolerance in heart failure (HF)(6), and CPET-derived indices of cardiovascular and respiratory limitation have repeatedly emerged as strong predictors of mortality(7–10).

Recent studies of CPET in amyloidosis have highlighted the clinical and prognostic significance of peak oxygen consumption and ventilatory efficiency(11–19). However, many of these studies are limited by small patient numbers(11,13,15,17), whilst others have analysed cardiac amyloidosis of different aetiologies collectively(11–13). Furthermore, many studies have only reported on a limited number of CPET variables(11,13,14,16), such that the comprehensive metabolic, ventilatory and cardiovascular indices that can be measured by CPET remain largely unexplored.

Our study used CPET in a large cohort of patients with the objective to: [1] characterise the spectrum of functional capacity in ATTR amyloidosis, [2] characterise functional decline with respect to amyloidotic burden, [3] and to assess prognosis.

Methods

Patients referred to the National Amyloidosis Centre (NAC), United Kingdom (May 2019 to September 2022) in whom ATTR amyloidosis was confirmed were invited to participate in a prospective protocolized clinical follow-up programme comprising serial clinical assessments and systemic evaluation of cardiac parameters. We included in this study all patients who underwent CPET assessment as part of the clinical assessment. Disease phenotype was classified as either cardiomyopathy (ATTR-CM with no evidence of polyneuropathy), polyneuropathy (ATTR-PN with no evidence of cardiomyopathy) or mixed phenotype (ATTR-mixed: with features of both ATTR-CM and ATTR-PN), in accordance with validated diagnostic criteria for ATTR-CM(20) and recommended diagnostic algorithms for ATTR-PN(21). Histological proof was reserved in cases of diagnostic uncertainty. TTR gene sequencing was performed in all patients and biomarker staging determined based on current guidelines(22). Patients were managed in accordance with the Declaration of Helsinki and provided informed written consent for retrospective analysis and publication of their data with approval from the Royal Free Hospital ethics committee (ref: 06/Q0501/42).

All subjects underwent a symptom-limited CPET with data acquired using a metabolic cart (Ultima™ CardioO2®, MGC Diagnostics®, Saint Paul, MN, USA) and tiltable cycle ergometer(23). Habitual therapy was maintained. Lung spirometry was performed before the test, according with current ERS requirements(24,25). With the patient in a left semi-recumbent position, an incremental ramp protocol of 10 watts per minute was used to obtain a standard of exercise(12,26). Peak VO_2 and respiratory exchange ratio (RER) were defined as the highest 10-second averaged sample obtained during the final 20 seconds of exercise. Weight-adjusted Wasserman-Hansen equations were used to define percentage predicted peak VO_2. VE/VCO_2 slope was calculated as the regression line of VCO_2 measurements plotted against minute ventilation (VE) values, excluding the earliest and latest segments(27). Exercise oscillatory

ventilation (EOV) was defined as the presence of cyclic fluctuations in VE that endure for at least 60% of the exercise test at an amplitude of 15% or more of the average resting value(28) and characterised dichotomously as being either present or absent. Maximal Ventilatory Capacity (MVV) was estimated as FEV1 x 40. Breathing reserve was defined as (MVV - VE_{peak}) / MVV, where VE_{peak} is the minute ventilation at peak. Maximum predicted heart rate (HR) was defined as 220 – age. To explore the chronotropic response taking into account resting HR, a chronotropic index was calculated as: (Peak HR – resting HR)/ [(220 – age) – resting HR)](29). Heart rate reserve (HRR) was defined as peak HR – HR at 1 minute into recovery. Other CPET variables were measured according with current recommendations(28).

Echocardiography was performed using a GE machine (5S probe) and analysed offline using EchoPAC software by qualified operators, blinded to disease phenotype, and in accordance to current guidelines (eMethods)(30). Patients with no contraindications underwent cardiovascular magnetic resonance (CMR) at 1.5T (Magnetom Aera, Siemens Healthcare, Erlangen, Germany) comprising localisers, cine imaging, pre- and post-contrast T1 mapping to quantify extra-cellular volume (ECV) as a measure of cardiac amyloid burden (eMethods)(31).

Statistical Methods

Numerical variables are summarized by mean (SD) or median (IQR) where appropriate. A Kruskal-Wallis test was used to compare the distributions of each variable between subgroups and, if significant, was followed by a Bonferroni-corrected pairwise comparison to establish where differences lay. Categorical variables are summarized by frequencies (percentages) and groups compared using the Chi-square test. Standardized box plots, scatterplots and radar plots were obtained to compare functional phenotypes according with clinical presentations and degree of left ventricular ejection fraction (LVEF). A linear regression model was used to identify clinical, biomarkers and echocardiographic variables that were associated with peak

VO_2. The association between amyloid burden and CPET parameters was explored using myocardial ECV brackets as follows: <30.0%, 30.0-39.9%, 40.0-49.9%, 50.0-59.9%, 60.0-69.9%, >70.0%(6,32).

Mortality data was obtained via the UK Office of National Statistics. Survival was evaluated with Cox proportional hazards regression analysis and presented as hazard ratios, Kaplan-Meier survival graphs and restricted cubic spline curves. The proportional hazards assumption was checked and confirmed. Univariable and multivariable analyses were used to determine which covariates were independent predictors of mortality, with variables selected a priori based on clinical relevance (eMethods). Time-dependent receiver operating characteristic (ROC) curves were used to determine cut-off values for significant parameters for Kaplan-Meier Survival curves. All data were analysed using IBM SPSS Statistics Version 29 (IBM). Figures were constructed using Stata (StataCorp 2021, Stata Statistical Software Release 17, StataCorp LLC, College Station, TX). P-values were two-sided with a significance level of <0.05.

Results

Characteristics of the entire cohort

In total, 506 patients with ATTR amyloidosis were studied: 394 patients had ATTR-CM (mean age 76.7 years, [SD 6.6], 95.7% males), 92 had ATTR-mixed phenotype (65.3 years, [SD 9.5], 75% males) and 20 had ATTR-PN (48.3 years, [SD 14.1], 55% males). Baseline characteristics and echocardiography of each clinical presentation are shown in the supplement (eTable 1). In terms of genotypes: 332 (65.6%) patients were ATTRwt; 60 (11.9%) were V122I-associated ATTRv, 58 (11.5%) were T60A-associated ATTRv and 56 (11.1%) were non-V122I non-T60A-associated ATTRv (24 with V30M, 7 with S77Y, 4 with G47V, 2 with A1775, A97S, E42D, F33V, H90D and 1 with E54L, E89K, E89Q, G53A, I107V, I68L, I84S, R34G, S23N, S50R mutations). Sixty-one percent of patients underwent CPET when naïve to any clinical trial enrolment or disease-modifying treatment; a further 20% underwent CPET within 6 weeks of enrolment into a clinical trial or commencing disease-modifying therapy.

Comparison of CPET parameters according with clinical presentations (Table 1 and Figure 1)

Peak VO_2, percentage predicted peak VO_2 and peak workload in the ATTR-CM (14.5ml/kg/min [SD 4.3], 68.5% [SD 18.6], 72.3 watts [SD 26.2]) and ATTR-mixed (15.7ml/kg/min [SD 6.2], 64.8% [SD 24.2], 73.7 watts [SD 32.5]) patients were significantly lower compared than the ATTR-PN patients (22.4ml/kg/min [SD 8.1], 79.2% [SD 16.7] 112.8 watts [SD 42.9]). Similarly, percentage predicted peak O_2 pulse was preserved only in ATTR-PN (103.5% [SD 19.5]). Percentage predicted peak HR and chronotropic index was abnormal in all subgroups (ATTR-CM: 79.9% [SD 16.7], 0.60 [SD 0.32]; ATTR-mixed: 70.9% [SD 14.9], 0.43 [SD 0.27]; ATTR-PN: 76.4% [SD 9.5], 0.55 [SD 0.17]). HRR was mostly reduced in ATTR-PN and ATTR-mixed patients. VE/VCO_2 slope was significantly higher on average in ATTR-CM (38.1 [SD 8.6]) compared to ATTR-mixed (34.0 [SD 9.7]) and ATTR-PN (28.7

[SD 2.8]) cohorts. Distribution of both Weber Class of peak VO_2 and Ventilatory Class of VE/VCO_2 was more favourable in the ATTR-PN cohort compared to the ATTR-CM and ATTR-mixed cohorts (eFigure 1). A high prevalence of EOV was observed in all phenotypes (64.4% ATTR-CM; 63% ATTR-mixed; 75% ATTR-PN).

Linear regression analysis showed that age, eGFR, NT-proBNP, LVEF and E/e' were significant predictors for peak VO_2 (eTable 2). Comparison of CPET parameters between three common genotypes of ATTR (wild-type, V122I-associated ATTRv, and T60A-associated ATTRv) and by cardiac rhythm (sinus rhythm, atrial arrhythmia, and paced rhythm) can be seen in eTable 3 and eTable 4 respectively.

Relationship between amyloid burden and CPET parameters

In total, 326 (64.4%) patients underwent CMR with ECV mapping and divided into groups based on myocardial ECV (Figure 2) as a measure of amyloid infiltration. Percentage predicted peak VO_2 was reduced in all patients, but the degree of reduction was progressively more severe with increasing amyloid infiltration. Percentage predicted O_2 pulse was preserved in patients with either no or mild-to-moderate amyloid infiltration but was significantly reduced in those with severe infiltration. Ventilatory efficiency was preserved in patients with no cardiac infiltration, was reduced even in mild infiltration, and became progressively more severe with increasing infiltration. Percentage predicted peak HR was impaired in all patients, irrespective of the degree of cardiac infiltration. HRR was frequently impaired, and unrelated to the degree of cardiac infiltration. EOV was highly prevalent in all patients with a trend to be higher in patients with less severe degree of cardiac infiltration. The data is also presented as scatterplots with equations of linear correlation (eFigure 2).

Patients with ATTR-CM or mixed phenotype who underwent CMR (n=311) were dichotomised based on LVEF and shown in eFigures 3, 4 and 5. In subjects with LVEF >55% (n=107), abnormal values of percentage predicted peak VO_2 was observed in patients with

ECV values above 40%. Percentage predicted peak HR was reduced across the spectrum of ECV values above 40%, whereas percentage predicted peak O_2 pulse was normal (>85% of predicted) across the range of ECV values. Ventilatory efficiency was impaired in all patients with ECV above 40%, with the degree of impairment being progressively more severe with increasing ECV. EOV was highly prevalent in all patients. HRR was normal across the spectrum of ECV.

In subjects with LVEF <55% (n=204), abnormal values of percentage predicted peak VO_2 were observed in all patients with a trend for lower values with progressively higher degree of amyloid infiltration. Percentage predicted peak O_2 pulse was reduced (<85% of predicted) across the range of ECV values and those with a higher degree of amyloid infiltration displayed a more severe reduction. Percentage predicted peak HR was mildly reduced across the spectrum of ECV. Ventilatory efficiency was impaired in all patients, even for mild degrees of infiltration. EOV was highly prevalent in all patients. HRR was normal across the spectrum of ECV.

CPET parameters and prognosis

At follow-up (mean 22.4 months [SD 11.6]), 72 (14.2%) patients had died. Univariable cox regression analysis demonstrates all CPET variables were predictive of mortality except for EOV and peak RER (eTable 5). The multivariate model (table 2) demonstrates that peak VO_2, whether expressed as the absolute value (hazard ratio 0.89, 95% confidence internal (CI) 0.81 to 0.99; p=0.030) or as percentage predicted (hazard ratio 0.97; 95%CI 0.95 to 0.99; p=0.010), and peak SBP (hazard ratio 0.98, 95%CI 0.97 to 0.99, p<0.001) were independently associated with patient survival in the overall population. The same analysis with standardised hazard ratios is presented in the supplement (eTable 6). Kaplan-Meier survival curves and restricted cubic spline curves for peak VO_2 and peak SBP are shown in Figure 3. Separate multivariable

models were repeated to include the use of (1) rate-limiting medication and (2) disease-modifying therapies or enrolment in a clinical trial, and both show similar results (eTable 7a and 7b). Kaplan-Meier survival curves dividing patients by Weber Classification (peak VO_2) or Ventilatory Class (VE/VCO_2) are shown in the supplement (eFigure 6). Time-dependant Receiver Operating Curves gave cut off-values for peak VO_2 (14ml/kg/min) and peak SBP (145mmHg) and Kaplan-Meier survival curves dichotomising patients using each cut-off value are shown in the supplement (eFigure 7).

Discussion

This is the first study utilising CPET to characterise the functional capacity of a large cohort of patients with ATTR amyloidosis. The main findings are: [1] Impairment of functional capacity was highly prevalent in all patients, with each disease phenotype demonstrating distinctive patterns of functional impairment, [2] chronotropic incompetence, EOV and ventilatory inefficiency were highly prevalent in all disease phenotypes, with the prevalence being higher than in HF from different aetiologies; and [3] peak VO_2 and peak SBP emerged as independent predictors of prognosis, after adjusting for known predictors.

Functional capacity was invariably reduced in patients with ATTR amyloidosis, and the mechanisms contributing to functional impairment were observed to be different across each clinical phenotype. Patients with ATTR-PN showed the greatest preservation in exercise tolerance and ventilatory efficiency, with chronotropic incompetence observed as the main mechanism underlying the reduction in functional capacity. The preservation of O_2 pulse augmentation during exercise in ATTR-PN represents the ability for patients with no cardiac involvement to adequately increase their stroke volume when metabolic demand is increased. In contrast, subjects with ATTR-CM and ATTR-mixed phenotypes showed a variety of mechanisms contributing to functional impairment with reduced O_2 pulse, chronotropic incompetence and ventilatory inefficiency being present in most patients, with typically a more severe phenotype in ATTR-CM compared to ATTR-mixed disease. Differences in peak VO_2 between each clinical phenotype are likely to relate to the disease stage in terms of presence and severity of cardiac involvement. Baseline characteristics (eTable 1) show many differences between each group, with ATTR-CM patients having the most advance disease in terms of cardiac involvement and the lowest peak VO_2.

Linear regression analysis demonstrated that age, eGFR, NT-proBNP, LVEF and E/e' all significantly predicted peak VO_2, highlighting the complex nature of underlying pathophysiological mechanisms contributing to functional impairment in ATTR amyloidosis. Subgroup analysis of genotypes (eTable 3) demonstrated the greatest degree of functional impairment in patients with V122I-associated ATTRv, mediated by low peak VO_2 and O_2 pulse suggesting a predominant cardiomyopathic phenotype, whilst functional impairment in T60A-associated ATTRv was mediated by chronotropic incompetence, more akin to ATTR-mixed and in keeping with a milder degree of cardiac involvement. Subgroup analysis of cardiac rhythm (eTable 4) highlights the high prevalence of arrhythmia in ATTR disease and that remaining in sinus rhythm is associated with the most favourable functional phenotype compared to those with atrial arrhythmia or paced rhythm.

Cardiac amyloid infiltration is known to cause LV stiffening and reduced compliance, leading to restrictive physiology(33). Our study shows that during exercise, cardiac involvement limits the normal physiological increase in stroke volume. Categorising patients based on the degree of cardiac amyloid infiltration on CMR demonstrates two distinctive phenotypes based on LVEF. When LVEF is reduced, impairment in both peak O_2 pulse (inotropic reserve) and chronotropic incompetence are both important contributing mechanisms in driving functional impairment. As amyloid burden increases, the progressive decrease in peak O_2 pulse with no comparable trend in chronotropic response indicates that impaired inotropic reserve is the predominant mechanism for functional impairment. In contrast, when LVEF is preserved, the progressive decrease in chronotropic incompetence suggests this as the predominant mechanism for functional limitation, whilst the preservation of peak O_2 pulse indicates that inotropic reserve is maintained in these patients.

Despite the presence of important differences between each clinical phenotype as outlined above, we also observed abnormalities in CPET that were consistent across all ATTR disease

phenotypes, with distinguishing features compared to HF from non-amyloid aetiologies. Firstly, chronotropic incompetence was highly prevalent in all phenotypes and was independent from the degree of cardiac amyloid infiltration (as measured by ECV) and beta-blocker use, most likely reflecting the high prevalence of cardiovascular autonomic dysfunction that characterizes this population(34). In studies of HF with reduced ejection fraction (HFrEF), the prevalence of chronotropic incompetence has been reported as widely ranging between 23-72%, although was less prevalent in those with preserved EF (30-50%)(35,36). In patients with ATTR-PN and ATTR-mixed phenotypes, abnormal heart rate reserve was also observed post-exercise, possibly reflecting a more severe degree of autonomic dysfunction manifesting with parasympathetic imbalance(37).

Secondly, abnormal ventilatory efficiency emerged as a distinctive feature in ATTR-CM and ATTR-mixed phenotypes. Despite worsening ventilatory inefficiency being observed with increasing cardiac amyloid burden, both the prevalence and severity of ventilatory inefficiency were higher than reported in patients with HF from different aetiologies(9,38–40). This may suggest additional disease-specific mechanisms contributing to ventilatory inefficiency, such as primary amyloid infiltration of the alveolar-capillary membrane (ACM)(41,42) and compensatory mechanisms against abnormal vascular autonomic modulation and baroreflex function(43). Impaired diffusion of CO_2 across the ACM secondary to lung amyloid infiltration(44) could explain the higher degree of ventilatory inefficiency observed in this population.

Thirdly, the prevalence of EOV was extremely high in all ATTR patients and similar across each phenotype, compared to a much lower reported prevalence of between 12-58% in HFrEF(45). The prevalence of EOV was not associated with the degree of cardiac involvement, such that autonomic dysfunction, compensatory hyperventilation and lung amyloid deposition may be important contributing mechanisms.

All CPET parameters, except EOV and peak RER, were associated with prognosis, demonstrating the important value of metabolic stress testing in predicting outcomes in ATTR amyloidosis. Peak VO_2 and peak SBP were the only variables independently associated with survival in multivariable analysis, after adjusting for other CPET parameters, biomarkers and treatment. This finding is consistent with Fick's equation which suggests that peak VO_2 is highly dependent on physiological mechanisms including chronotropic and inotropic reserve, both of which were significantly impaired in the ATTR population, and correlates with existing literature in favour of peak VO_2 being independently prognostic(11,12,17). Similarly, the inability to adequately increase BP during exercise is a known marker of adverse prognosis in advanced HF(46), reflecting the loss of cardiac inotropic reserve and sympathetic-mediated vascular tone adaptation. Notably, we observe only a limited increase in mean SBP of less than 20mmHg at peak exercise. Unlike HF from other aetiologies, ventilatory efficiency was not an independent predictor in ATTR patients which likely reflects the different pathogenic processes that contribute to the increase in the VE/VCO_2 slope, driven not only by HF-related mechanisms but also direct lung amyloid infiltration.

Limitations

ATTR amyloidosis is a multi-systems disease with lung mechanisms potentially affecting the exercise tolerance. Our study was limited using only simple spirometry, such that measurement of alveolar gas diffusion would be required to explore the hypothesis of possible lung amyloid infiltration. Additional pathways, linked to peripheral, mitochondrial, and neuropathic function are not fully explored by simple CPET, requiring combined and invasive approaches. Patients with ATTR-PN are under-represented in this study, limiting the ability to explore the correlation between the functional capacity and the severity of the functional phenotype. Whilst beta-blockers use was not associated with the presence or degree of chronotropic incompetence, future prospective studies assessing the direct impact of beta-blockers on functional capacity are missing. Finally, the prognostic significance of CPET has been demonstrated for all-cause mortality as the outcome. Association of CPET variables with HF hospitalization could provide additional and clinically relevant information.

Conclusions

ATTR amyloidosis is characterised by distinct patterns of functional impairment across all disease phenotypes, attributable to multiple physiological mechanisms. A high prevalence of chronotropic incompetence, EOV and ventilatory inefficiency were characteristic features of this population. Multiple CPET parameters correlated with amyloid burden by CMR and using multivariable analysis, peak VO_2 and peak SBP were demonstrated to be independently associated with mortality, after adjusting for known predictors.

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Access to data and data analysis

Rishi Patel and Francesco Bandera had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest discolsures

Professor Gillmore reported consulting income from Alnylam, ATTRalus, Ionis, Intellia, AstraZenica and Pfizer. Professor Fontana reported consulting income from Alnylam, AstraZeneca, ATTRalus, Caelum/Alexion, Eidos/BridgeBio, Intellia, Ionis, Novo Nordisk, and Pfizer. No other disclosures were reported.

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Figure 1: Distribution of CPET parameters according with ATTR clinical presentations.

Comparison between disease phenotypes presented as Tukey's box plots (left panel; within the box, central line indicates the median value, extremities the 25th and 75th percentiles; out of the box, lines extend to the minimum and maximum values) and radar plots (right panel). CM – cardiomyopathy, EOV – exercise oscillatory ventilation, HR – heart rate, HRR – heart rate response, PN – polyneuropathy, VE/VCO_2 – ventilatory efficiency

Figure 2: Distribution of CPET variables according with myocardial ECV in ATTR.

Comparison between disease phenotypes presented as Tukey's box plots for each ECV range (within the box, central line indicates the median value, extremities the 25th and 75th percentiles; out of the box, lines extend to the minimum and maximum values). ECV – extracellular volume, EOV – exercise oscillatory ventilation, HR – heart rate, HRR – heart rate response, VE/VCO_2 – ventilatory efficiency

Figure 3 – Overall survival in patients with ATTR based on CPET parameters – peak VO_2 and peak systolic blood pressure (SBP)

Top-left panel: Kaplan-Meier Survival Curves according to peak VO_2 (divided into 2ml/kg/min cut-offs); Bottom-left panel: Kaplan-Meier Survival Curves according to peak systolic blood pressure (divided into 20mmHg cut-offs); top-right panel: restricted cubic spline curves for overall survival according to peak VO_2; Bottom-right panel: restricted cubic spline curves for overall survival according to peak SBP.

Table 1 – CPET findings according to the clinical phenotype: ATTR cardiomyopathy, ATTR mixed phenotype and ATTR polyneuropathy

Variable		ATTR-CM (n=394)	ATTR-Mixed (n=92)	ATTR-PN (n=20)	P-value
Baseline lung function					
FEV1 (% predicted)		80.6 (17.1)	77.5 (18.0)	95.8 (11.5)***	**0.034**
FVC (% predicted)		86.2 (20.0)	83.1 (20.6)**	86.8 (8.8)	**0.029**
FEV1/FVC ratio		80.6 (10.0)*	83.3 (11.3)	88.6 (8.6)***	**<0.001**
Metabolic					
Peak Work (watts)		72.3 (26.2)	73.7 (32.5)**	112.8 (42.9)***	**<0.001**
VO_2 (ml/kg/min)	AT	11.7 (3.2)	12.6 (4.4)**	15.9 (4.6)***	**<0.001**
	Peak ex	14.5 (4.3)	15.7 (6.2)**	22.4 (8.1)***	**<0.001**
	% Predicted	68.5 (18.6)	64.8 (24.2)**	79.2 (16.7)***	**0.001**
RER	Peak ex	1.09 (0.11)	1.10 (0.12)	1.15 (0.15)	0.173
Ventilatory					
VE/VCO_2 Slope		38.1 (8.6)*	34.0 (9.7)**	28.7 (2.8)***	**<0.001**
VE (L/min)	Rest	12.60 (3.28)	12.07 (3.11)	12.61 (3.19)	0.372
	Peak ex	50.34 (15.25)	47.25 (18.20)**	62.34 (23.88)	**0.007**
VT (L)	Rest	0.66 (0.18)	0.62 (0.15)	0.63 (0.13)	0.203
	Peak ex	1.55 (0.43)	1.45 (0.48)**	1.78 (0.52)	**0.010**
RR (breaths per min)	Rest	20 (5.9)	20 (5.4)	21 (6.0)	0.974
	Peak ex	33 (7.1)	33 (8.4)	35 (8.8)	0.509
Breathing reserve (%)		46.2 (13.5)	50.1 (17.1)	50.7 (13.7)	0.066
EOV, n (%)		253 (64.4%)	58 (63%)	15 (75%)	0.591
$P_{ET}CO_2$ (mmHg)	Rest	31.0 (7.0)*	33.4 (5.4)	34.7 (3.8)***	**<0.001**
	Peak ex	32.1 (8.3)*	36.2 (10.2)**	38.9 (3.9)***	**<0.001**
Cardiovascular					
HR (bpm)	Rest	72 (12.8)*	76 (12.6)	79 (12.1)	**0.002**
	AT	103 (19.7)	99 (19.6)**	114 (16.3)***	**0.016**
	Peak ex	115 (22.7)	111 (25.7)**	131 (22.3)***	**0.004**
	% Predicted	79.9 (16.7)*	70.9 (14.9)	76.4 (9.5)	**<0.001**

Chronotropic Index		0.60 (0.32)*	0.43 (0.27)	0.55 (0.17)	**<0.001**
HRR at 1 minute recovery (bpm)		15 (16.2)*	11 (17.3)	12 (8.6)	**<0.001**
VO_2/Work Slope (ml/min/watts)		10.3 (4.8)	10.0 (1.9)	10.7 (1.4)	0.101
VO_2/HR (ml/min/bpm)	Rest	4.2 (1.2)	4.1 (1.4)	4.6 (1.5)	0.157
	Peak ex	10.1 (2.9)	10.8 (3.9)**	13.0 (4.1)***	**0.003**
	% predicted	82.1 (23.0)	82.1 (33.4)**	103.5 (19.5)***	**<0.001**
SBP (mmHg)	Rest	136 (20.7)	136 (17.1)	130 (13.1)	0.337
	Peak ex	155 (28.3)	153 (33.3)**	179 (25.2)***	**<0.001**
DBP (mmHg)	Rest	79 (13.8)	79 (10.3)	80 (6.3)	0.728
	Peak ex	86 (17.4)	82 (17.3)**	98 (23.3)***	**0.003**

Bonferroni-corrected p-values from pairwise comparison: *p<0.05 for Cardiomyopathic vs. Mixed; **p<0.05 for Polyneuropathic vs. Mixed; ***p<0.05 for Cardiomyopathic vs. Polyneuropathic. Statistical significance is represented by p-values <0.05. Data are presented as means (SD), except for Weber Class, Ventilatory Class and EOV which are presented as number (%). AT – anaerobic threshold, DBP – diastolic blood pressure, EOV – exercise oscillatory ventilation, FEV1 – forced expiratory volume in 1 second, FVC – forced vital capacity, HR – heart rate, HRR – heart rate reserve, $P_{ET}CO_2$ – end tidal carbon dioxide, RR – respiratory rate, RER – respiratory exchange ratio, SBP – systolic blood pressure, VE – minute ventilation, VCO_2 – volume of carbon dioxide expired, VO_2 – oxygen consumption, VT – tidal volume

Table 2 – Multivariate Cox regression analysis of risk of death in patients with ATTR

	Peak VO_2 model		% predicted peak VO_2 model	
	Hazard Ratio (95%)	**P-value**	**Hazard Ratio (95%)**	**P-value**
Peak VO_2 (ml/kg/min)	0.89 (0.81 to 0.99)	**0.030**	-	-
% predicted peak VO_2	-	-	0.97 (0.95 to 0.99)	**0.010**
VE/VCO_2 Slope	0.99 (0.97 to 1.02)	0.593	0.99 (0.97 to 1.02)	0.536
Chronotropic Index	0.85 (0.35 to 2.03)	0.705	0.91 (0.37 to 2.23)	0.834
Peak VO_2/HR (ml/min/bpm)	0.90 (0.78 to 1.02)	0.100	0.89 (0.78 to 1.01)	0.077
Peak SBP (mmHg)	0.98 (0.97 to 0.99)	**<0.001**	0.98 (0.97 to 0.99)	**<0.001**
VO_2/Work slope (ml/min/watts)	0.98 (0.86 to 1.13)	0.796	1.00 (0.87 to 1.16)	0.970
Biomarker Stage				
- **1 vs 2**	2.29 (1.34 to 3.93)	**0.002**	2.59 (1.50 to 4.48)	**<0.001**
- **1 vs 3**	3.78 (1.65 to 8.66)	**0.002**	4.42 (1.92 to 10.16)	**<0.001**

Statistical significance is represented by p-values <0.05. Data are presented as Hazard Ratios and 95% confidence intervals. The Hazard Ratio represents the comparison of the risk of mortality for a unit increase in the variable of interest. GLS – global longitudinal strain, HR – heart rate, SBP – systolic blood pressure, VE – ventilatory efficiency, VCO_2 – volume of carbon dioxide expired, VO_2 – oxygen consumption.