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Comprehensive Exercise Stress Echocardiography: a Pathophysiological Prism Dissecting the Spectrum of Hypertrophic Cardiomyopathy

Ph.D. Thesis

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Publications

Publications related to the thesis

1) **Pálinkás ED**, Re F, Peteiro J, Tesic M, Pálinkás A, Torres MAR, Dikic AD, Beleslin B, Van De Heyning CM, D'Alfonso MG, Mori F, Ciampi Q, de Castro Silva Pretto JL, Simova I, Nagy V, Boda K, Sepp R, Olivotto I, Pellikka PA, Picano E. Pulmonary congestion during Exercise stress Echocardiography in Hypertrophic Cardiomyopathy. Int J Cardiovasc Imaging. 2022 Dec;38(12):2593-2604. doi: 10.1007/s10554-022-02620-0. Epub 2022 Nov 2. PMID: 36322266; PMCID: PMC9708780.

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2) **Pálinkás E**, Nagy V, Varga A, Ágoston G, Kákonyi KM , Szűcsborus T, Somfay A, Badó A, Sepp R, Czakó L, Pálinkás A. A Látens Bal Kamra Kiáramlási Pálya Obstrukció Vizsgálata Kerékpáros Stressz Echokardiográfiával Hypertrophiás Cardiomyopathiás Betegeken. Medicina Thoracalis (Budapest). 2019 Feb;72: 3–11.

3) Ciampi Q, Olivotto I, Peteiro J, D'Alfonso MG, Mori F, Tassetti L, Milazzo A, Monserrat L, Fernandez X, Pálinkás A, **Pálinkás ED**, Sepp R, Re F, Cortigiani L, Tesic M, Djordjevic-Dikic A, Beleslin B, Losi M, Canciello G, Betocchi S, Lopes LR, Cruz I, Cotrim C, Torres MAR, Bellagamba CCA, Van De Heyning CM, Varga A, Ágoston G, Villari B, Lorenzoni V, Carpeggiani C, Picano E, The Stress Echo Study Group On Behalf Of The Italian Society Of Echocardiography And Cardiovascular Imaging Siecvi. Prognostic Value of Reduced Heart Rate Reserve during Exercise in Hypertrophic Cardiomyopathy. J Clin Med. 2021 Mar 24;10(7):1347. doi: 10.3390/jcm10071347. PMID: 33805111; PMCID: PMC8037369.

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1) Picano E, Ciampi Q, Cortigiani L, Arruda-Olson AM, Borguezan-Daros C, de Castro E Silva Pretto JL, Cocchia R, Bossone E, Merli E, Kane GC, Varga A, Agoston G, Scali MC, Morrone D, Simova I, Samardjieva M, Boshchenko A, Ryabova T, Vrublevsky A, Palinkas A, **Palinkas ED**, Sepp R, Torres MAR, Villarraga HR, Preradović TK, Citro R, Amor M, Mosto H, Salamè M, Leeson P, Mangia C, Gaibazzi N, Tuttolomondo D, Prota C, Peteiro J, Van De Heyning CM, D'Andrea A, Rigo F, Nikolic A, Ostojic M, Lowenstein J, Arbucci R, Haber DML, Merlo PM, Wierzbowska-Drabik K, Kasprzak JD, Haberka M, Camarozano AC, Ratanasit N, Mori F, D'Alfonso MG, Tassetti L, Milazzo A, Olivotto I, Marchi A, Rodriguez-Zanella H, Zagatina A, Padang R, Dekleva M, Djordievic-Dikic A, Boskovic N, Tesic M, Giga V, Beleslin B, Di Salvo G, Lorenzoni V, Cameli M, Mandoli GE, Bombardini T, Caso P, Celutkiene J, Barbieri A, Benfari G, Bartolacelli Y, Malagoli A, Bursi F, Mantovani F, Villari B, Russo A, De Nes M, Carpeggiani C, Monte I, Re F, Cotrim C, Bilardo G, Saad AK, Karuzas A, Matuliauskas D, Colonna P, Antonini-Canterin F, Pepi M, Pellikka PA, The Stress Echo Study Group Of The Italian Society Of Echocardiography And Cardiovascular Imaging Siecvi. Stress Echo 2030: The Novel ABCDE-(FGLPR) Protocol to Define the Future of Imaging. J Clin Med. 2021 Aug 17;10(16):3641. doi: 10.3390/jcm10163641. PMID: 34441937; PMCID: PMC8397117.

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Abbreviations

- CI – confidence interval
- EDV - end-diastolic volume
- EDV - end-diastolic volume
- EF - ejection fraction
- ESE - exercise stress echocardiography
- ESV - end-systolic volume
- HCM - hypertrophic cardiomyopathy
- HF - heart failure
- HR - hazard ratio
- HRR - heart rate reserve
- LA - left atrial
- LUS - lung ultrasound
- LV – left ventricular
- LVOT - left ventricular outflow tract
- LVOTG - left ventricular outflow tract gradient
- LVOTO - left ventricular outflow tract obstruction
- MR - mitral regurgitation
- OR - odds ratio
- SBP - systolic blood pressure
- SCD - Sudden cardiac death risk
- SPAP - systolic pulmonary arterial pressure
- SV - stroke volume
- WMSI - Wall motion score index

1. Introduction

Hypertrophic Cardiomyopathy (HCM)

HCM is the most common inheritable myocardial disease characterized by heterogeneous clinical expression, distinctive pathophysiologic features, and diverse natural history. It can manifest in all phases of life and affects approximately 1 out of 500 individuals worldwide (1). The diagnosis of HCM is based on a hypertrophied, nondilated left ventricle identified in the absence of another cardiac, systemic, metabolic, or syndromic disease capable of producing the magnitude of left ventricular (LV) hypertrophy. The threshold for LV hypertrophy in adults is a maximal end-diastolic wall thickness of ≥15 mm by any imaging modality. In first-degree relatives of patients with HCM or in patients with causative HCM genetic variant ≥13 mm of maximal wall thickness can be diagnostic (2). In most patients, HCM is associated with a normal life expectancy and a good quality of life, however, about 50% of patients experience symptoms related to effort or meals. In some patients, HCM progresses along specific disease pathways, marked by clinical events that alter the natural history of the disease and dictate targeted treatment strategies: approximately 25% develop atrial fibrillation, 15% progress toward LV dysfunction and heart failure (HF), including 5% ultimately developing end-stage disease. Moreover, the condition is associated with a 0.5–1 % annual risk of sudden cardiac death (3).

Pathophysiology

HCM has multifaceted pathogenesis made up of several interconnected abnormalities including abnormally increased LV function, dynamic intraventricular obstruction, coronary microvascular dysfunction and myocardial ischemia, diastolic dysfunction and restrictive physiology, functional mitral regurgitation (MR), tachyarrhythmias and altered cardiac autonomic nervous system balance (2). Intraventricular dynamic obstruction causes an increase in LV systolic pressure, which leads to a complex interplay of pathophysiological processes including prolongation of ventricular relaxation, elevation of LV diastolic pressure, MR, myocardial ischemia, and a decrease in cardiac output (4,5). Obstruction occurs most frequently in the LV outflow tract, but in 10% it can take place also at the midventricular level (6). Since it often contributes to exercise limitation and symptoms and is related to a poorer prognosis, dynamic obstruction in the LV outflow tract (LVOTO) is a well-known and distinctive hallmark of HCM with major clinical relevance. Numerous factors are known to contribute to dynamic LVOTO such as mitral leaflet elongation, hyper-contractile small or normal-sized LV, abnormally positioned papillary muscles or small LVOT dimensions (7,8). By convention, the

peak instantaneous gradient detected in the LV outflow tract gradient (LVOTG) is indicative of obstruction if reaches 30 mm Hg whilst rising above 50 mm Hg it becomes hemodynamically important and attributable to effort-related symptoms and means a threshold for invasive treatment (9). One-third of the patients demonstrate LVOTO at rest, one-third develop obstruction on effort (labile/latent obstruction) while one-third remain in the absence of obstruction under both conditions (10). Studies on large groups of HCM patients confirmed that a resting LVOTG exceeding 30 mm Hg is an independent predictor of death, progression of HF and stroke in HCM patients (11,12). However inconsistent evidence exists regarding the clinical significance of latent obstruction in HCM (13). Obstructive HCM had always a major focus due to peculiar symptoms and management considerations. Contrary to nonobstructive HCM, which has been much less known and studied. As a matter of fact, nonobstructive HCM comprises a notable proportion (30-40%) of all HCM cases, is frequently related to treatment refractory dyspnea and chest discomfort, and appears to carry the same risk for HCM-associated death as obstructive HCM (2,14,15). The predominant pathophysiological alteration responsible for symptoms in nonobstructive patients is thought to be diastolic dysfunction (1). Several elements can be mentioned that interfere with normal lusitropy and leads to enhanced LV stiffness in HCM. These include heart muscle hypertrophy, replacement fibrosis, inadequate microvascular coronary flow or myocardial disarray (1). These alterations in certain nonobstructive individuals lead to "hypokinetic-restrictive" end-stage HCM, which is characterised by a tiny, stiff LV, severe diastolic dysfunction and huge atria, and in spite of a modestly impaired or retained LV ejection fraction (EF), necessitates heart transplantation (1,3).

Clinical Indications of Exercise Stress Echocardiography (ESE) in HCM

In HCM patients who do not exhibit LVOTG $\geq$50 mm Hg during standard echocardiographic evaluation and are symptomatic, ESE should be performed to detect and quantify provocable LVOTO and mitral regurgitation (MR) (2,9). In asymptomatic patients without inducible LVOTO $\geq$50 mm Hg on standard transthoracic echocardiography, it can be beneficial to perform ESE, considering that it provides a comprehensive understanding on their individual pathophysiology, especially if the presence of LVOTO is relevant to lifestyle advice and decisions on medical treatment (9). Of note, regardless of symptomatic status, LVOT assessment during exercise should be performed in every HCM patient without resting obstruction (>50 mmHg) who has positive history of syncope (16). According to the recent European Society of Cardiology Guidelines on Sports Cardiology, all individuals with HCM

who wish to participate in sports activity should have the LVOTG assessed after light exercise (17).

Multiparametric ESE Approach in HCM

Despite that ESE examination plays a central role in the management of HCM patients, current HCM guidelines recognize LVOTO as the utmost parameter to look for during exercise stress (2,18). However, with this approach, the etiology of symptoms can be overlooked and the opportunity for individualized care can be missed. In fact, numerous other guidelines and consensus statements recommend the evaluation of other ESE parameters for HCM patients (19–21). Furthermore, several studies have pointed out lately other SE imaging variables that might aid in improving risk stratification for those with HCM (22–25). In the Stress echo 2020 (NCT03049995) international, multicenter, prospective, effectiveness study, a novel multiparametric stress echocardiography (SE) perspective was used for the first time. The study started in 2016 and more than 100 quality-controlled, high-volume SE labs adhered for its clinical, laboratory and imaging data collection (26,27). As a result, a new approach for functional testing was developed, verified, and widely used in coronary artery disease and other disorders, comprising HCM (27). The study finished in 2020 and the Stress echo 2030 (NCT05081115) study, with minor changes, followed its methodology towards the development of a perfect functional cardiology testing. The study protocol includes 5 basic steps, feasible in all patients with all stress modalities: A: regional wall motion abnormalities (RWMA), B: B-lines, C: LV contractile and preload reserve, D: Doppler coronary flow velocity reserve (CFVR) in the left anterior descending coronary artery and E: ECG-based assessment of heart rate reserve (HRR) (27,28). This procedure provides comprehensive information on the different vulnerabilities of HCM patients as it makes possible to uncover easily and systematically concealed myocardial ischemia (step A), pulmonary congestion (step B), preload reserve and contractile reserve impairment (step C), coronary microcirculatory dysfunction (step D) and cardiac autonomic dysfunction (step E) (27). Step G (LVOTG) and step F (flow of MR) are two additional steps that are crucial for the HCM-specific subproject of the study. Other steps providing further insight into HCM pathophysiology include step L (left atrium), step P (pulmonary pressures) and LV deformational imaging as part of step A (27).

Step A – Asynergy

Although angina is common complaint of HCM patients, RWMA rarely develop during stress. RWMA occur only in about 6-13% of patients, they have a complex and multifactorial pathophysiology and are usually not related to epicardial coronary artery disease (5,23,29–32).

RWMA are more frequently detected during physical than pharmacological stress and carry a substantial risk of future adverse events (23,30,33,34). Patients with peak exercise RWMA are characterized by poorer exercise capacity, lower rest and peak stress EF, lower rest and post-exercise LVOT gradients, and more extensive myocardial fibrosis on cardiac resonance imaging (29,30). In 2015, Peteiro et al. demonstrated that exercise LV wall motion abnormalities have an independent prognostic value in predicting follow-up events and their prognostic value is additive to the presence of extensive myocardial fibrosis (30). While a positive SE for RWMA is less useful in identifying coronary artery disease and leading ischemia-driven revascularization in HCM (due to the high rate of false positive responses, particularly in the presence of pronounced hypertrophy), a negative ESE may help rule out functionally relevant coronary artery disease (32,35). However, in patients with chest pain and positive SE for inducible RWMA, a non-invasive coronary computed angiography is indicated prior to referring the patient to ischemia-driven revascularization (36). RWMA are evaluated quantitatively by the wall motion score index (WMSI). To obtain WMSI, a four-point score needs to be calculated for every LV segment (1 = normal/hyperkinetic, 2 = hypokinetic, 3 = akinetic, 4 = dyskinetic) and then the sum of the segment scores has to be divided by the number of scored segments (37,38). Positivity for step A can be defined as higher WMSI at stress than rest, or at least 1 grade increment in at least 2 segments at peak ESE in a the 17-segment model of the LV (31,39).

Step B – B-lines

B-lines (also known as ultrasound lung comets) are a pre-clinical and pre-radiological sign of pulmonary congestion assessed with lung ultrasound (40). They are defined as hyperechoic, laser-like items, rising from the pleural line to the bottom of the screen moving synchronously with lung sliding without fading (41,42). B-lines can be assessed by lung ultrasound (LUS) during ESE and allow a quick, point-of-care method for measuring semi-quantitatively extravascular lung water, a phenomenon with well-proven diagnostic and prognostic relevance in a variety of heart diseases (28,39). Appearance of extravascular lung water during stress indicates an acute backward HF which may have different origins such as worsening diastolic dysfunction, acute functional mitral insufficiency, provocable ischemia or RWMA (43). In the Stress echo 2020 and Stress echo 2030 studies, ESE LUS acquisition is performed by the 4-site simplified scan: the same probe employed for the cardiac imaging, is placed at the third intercostal spaces on the anterior and lateral chest walls to assess B-lines at rest and peak (or immediately after) stress (26,27).

Step C – Contractile and Preload Reserve

It is particularly important to determine the contractile status in HCM, since the initially assessed normal/hyperdynamic systolic function may fail to increase during stress (44). Furthermore, the diastolic alterations and their relation to symptoms may not be revealed during resting echocardiography (22,45). With two-dimensional volumetric ESE, it is possible to uncover and identify the diverse hemodynamic phenotypes and acquire an integrated view of LV preload and contractile reserve (46). LV contractile reserve (LVCR) is derived from the ratio of stress and rest LV force, which in HCM, is defined by the following formula: (LVOTG + SBP)/end-systolic volume (ESV) (47). As a clear indicator of LV contractility, LV force differs from EF as it does not comprise end-diastolic volume (EDV) (28). Preload reserve, in turn, is evaluated through EDV, and defined as rest to stress increment in EDV (46). In the clinical era of novel HCM-specific precision medicines, the concept of utilising straightforward biomarkers of LV contractility and relaxation in everyday practice seems particularly appealing.

Step D – Doppler Coronary Flow Velocity Reserve

Step D is based on the color- and pulsed-wave Doppler evaluation of CFVR in the mid-distal left anterior descending coronary artery (48). CFVR is calculated by dividing hyperaemic and baseline peak diastolic coronary flow velocity and is considered a marker of microvascular function (5). Reduced CFVR ($\leq$2.0) during vasodilator stress (adenosine or dipyridamole) proved to be a powerful, independent predictor of unfavourable outcomes in several studies on HCM (34,49,50). The feasibility of vasodilator CFVR is >90 % in the general population, and may be higher in HCM since the detection and sampling of coronary flow is faster and easier because of the thick septum, the large coronary diameter, and the increased resting flow (37,51). CFVR can be assessed with great feasibility (>80%) during semi-supine ESE in unselected patients as well, however, it has not been extensively evaluated in patients with HCM yet (52).

Step E – Electrocardiography-based Heart Rate Reserve

Step E represents the simplest and perhaps the most important of exercise-related risk stratification parameter in HCM. It is simple, easily accessible and compared to other chronotropic indexes and can similarly be applied on patients with and without beta-blocker (BB) therapy (28). It is measured from the 12-lead ECG of the polygraph or from one-lead ECG present on the echocardiograph's monitor, by dividing peak stress heart rate by resting heart rate. HRR provides an insight in the cardiac autonomic function, which is known to be altered in HCM (2).

2. Aims

In the frame of the Stress echo 2020 (NCT03049995) international observational study, 3 investigations were carried out to evaluate the clinical value of ABCDE-related ESE parameters in patients with HCM, with the following goals:

1) to assess the clinical, anatomical and functional correlates of pulmonary congestion elicited by exercise in HCM
2) to evaluate latent LVOTO during semi-supine ESE
3) to determine the value of HRR in predicting prognosis in HCM

3. Methods

Study Population

Consecutive HCM patients were enrolled from the Stress echo 2020 study and the corresponding multicenter database built over the last 30 years. Diagnosis of HCM was based on the contemporary guidelines cautiously excluding HCM phenocopies. Each study required fulfilment of the following entry criteria: 1) diagnosis of HCM; 2) age >18 years; 3) no known coronary artery disease; 4) ability to perform ESE. The following exclusion criteria were applied: 1) technically poor acoustic window precluding sufficient imaging of the left ventricle (LV); 2) atrial fibrillation; 3) resting EF <40%, 5) HCM phenocopies of non-sarcomeric nature. Special exclusion criteria were applied for each studies: for studies with LUS assessment: comorbidities known to generate B-lines of extracardiac origin, e.g., pulmonary fibrosis, lung cancer or pneumonia and for the latent LVOTO study: resting LVOTG ≥30 mm Hg. The studies were conducted in accordance with the Declaration of Helsinki, and the protocols and informed consents were reviewed and approved by the institutional ethics committees as a part of the SE 2020 study. All subjects gave their informed consent for inclusion before they participated in the studies. Sudden cardiac death risk (SCD) was determined according to the European Society of Cardiology's HCM Risk-SCD formula.

Exercise Stress

All patients underwent symptom-limited dynamic echocardiographic examination according to the referring physician's indications as part of the routine workup. The exams were performed according to the recommended protocols with one of the following stresses: semi-supine bicycle (25 watts increments every 2 or 3 min), upright bicycle, treadmill exercise with modified Bruce protocol (19,20). Routinely used medications were administered as usual before and after the exam. Electrocardiogram and blood pressure were monitored continuously. Criteria for terminating the test were severe chest pain, diagnostic ST-segment shift, excessive blood pressure increase [systolic blood pressure (SBP) ≥240 mmHg, diastolic blood pressure ≥120 mm Hg], symptomatic hypotension with a sudden drop in blood pressure (>40 mmHg), limiting dyspnea, significant arrhythmias or limiting side effects.

Resting and Stress Echocardiography

All echocardiographic measurements were measured at rest and with stress by experienced cardiologists according to standard criteria of execution and interpretation recommended by the American Society of Echocardiography and the European Association of Cardiovascular

Imaging (53–55). Upstream to patient recruitment, each participant in Stress Echo 2020 study had passed the quality control procedures of reading examinations with inter-observer variability <10% in quantifying B-lines and estimating LV area by planimetric method. WMSI was calculated by applying the four-point score system ranging from 1 (normal) to 4 (dyskinetic) in a 17-segment model of the left ventricle (53,55). New RWMA were defined as an increase of at least one grade in at least two LV myocardial segments at peak stress. LV volumes were evaluated by the biplane Simpson method. LVOTG was the maximum instantaneous gradient measured by continuous-wave Doppler in the LVOT.

Hemodynamic measurements

LV force was defined by the following formula: (LVOTG + SBP)/end-systolic volume (ESV). LV contractile reserve was calculated by dividing the stress by rest LV force values. HRR was calculated as the peak/rest heart rate from 12-lead ECG. Stroke volume (SV) was calculated as end-diastolic volume (EDV)-ESV. Cardiac output was computed using the following formula: (EDV-ESV) x heart rate. Cardiac output and SV were normalized to body surface area to obtain SV index and cardiac index. Preload reserve impairment was defined as peak stress EDV < rest EDV (46). MR was evaluated with semi-quantitative method and graded as: none or trivial (0), mild (1), moderate (2), and severe (3) (20). Pulse pressure was assessed by the difference between SBP and diastolic blood pressure. Abnormal blood pressure response was defined as the fall of SBP by >20 mm Hg or a failure to increase the SBP by >20 mm Hg during exercise (9).

Lung ultrasound

The LUS acquisition was performed at rest and peak (or immediately after) stress with the 4-site simplified scan at the third intercostal space on the anterior and lateral hemithoraces, using the same probe employed for the cardiac scan. B-lines were defined as hyperechoic reverberation artifacts rising from the pleural line to the bottom of the screen moving synchronously with lung sliding without fading (56). After scanning the 4 chest sites, the cumulative B-line score was obtained by summing the number of detected B-lines at each site. B-lines were considered present if at least 2 B-lines could be detected.

Statistical analysis

Statistical Package for the Social Sciences (IBM SPSS Statistics, version 26), STATA (STATACorp LP, College Station, TX, USA, Release 14,) and R (version 3.6) were employed for analysis. Statistical significance was set at $p<0.05$.

Descriptive Statistics, Group Comparisons and Correlation analyses

Continuous variables were expressed as mean ± standard deviation or median and interquartile range, according to the variable's distribution. Categorical variables were reported as frequency and percentage. Data distribution was assessed graphically and with the Kolmogorov-Smirnov test. Student's independent t-test and Mann–Whitney U test were used to compare differences between continuous variables of two groups. One-sample rest-stress measurements were compared using paired t-test. One-way analysis of variance and Kruskal–Wallis tests were used to compare differences between continuous variables of multiple groups. When appropriate, post hoc comparisons were carried out using the Bonferroni correction. Categorical variables were compared using the Chi-squared test or Fisher's exact test. Spearman's correlation was used to assess the relationship between stress B-lines and functional parameters. Univariable and multivariable logistic regression analyses were performed to assess the baseline predictors of exercise B-lines and reduced HRR. Odds ratios (OR) with the corresponding 95% confidence intervals (CI) were indicated. The multivariable logistic regression was performed on all clinically and statistically relevant variables using stepwise logistic regression.

Outcome Assessment

Event-free survival was estimated using the Kaplan–Meier method and survival curves were compared by log-rank test. To identify clinical predictors of all-cause mortality, univariable and multivariable Cox regression analyses were utilized. All variables with $p<0.100$ at univariable analysis were considered for the inclusion in multivariable Cox proportional hazards model. The final multivariable models were obtained excluding just those variables causing collinearity evaluated using the variance inflation factor. None of the variables considered in the analysis violated the non-proportionality of hazard assumption according to the Schoenfeld test. Hazard ratios (HR) with the corresponding 95% confidence intervals (CI) were estimated. The incremental value of HRR was evaluated by comparing multivariable models with and without HRR using global X^2 value to evaluate the improvement of goodness-of-fit as well as continuous net reclassification index (NRI) to assess improvement in risk stratification.

4. Results

Pulmonary congestion

In a total of 128 HCM patients LUS and echocardiographic examinations were carried out applying semi-supine bicycle in 22%, upright in 42% and treadmill in 36%. Baseline clinical and demographic characteristics of the cohort are summarized in Table 1.

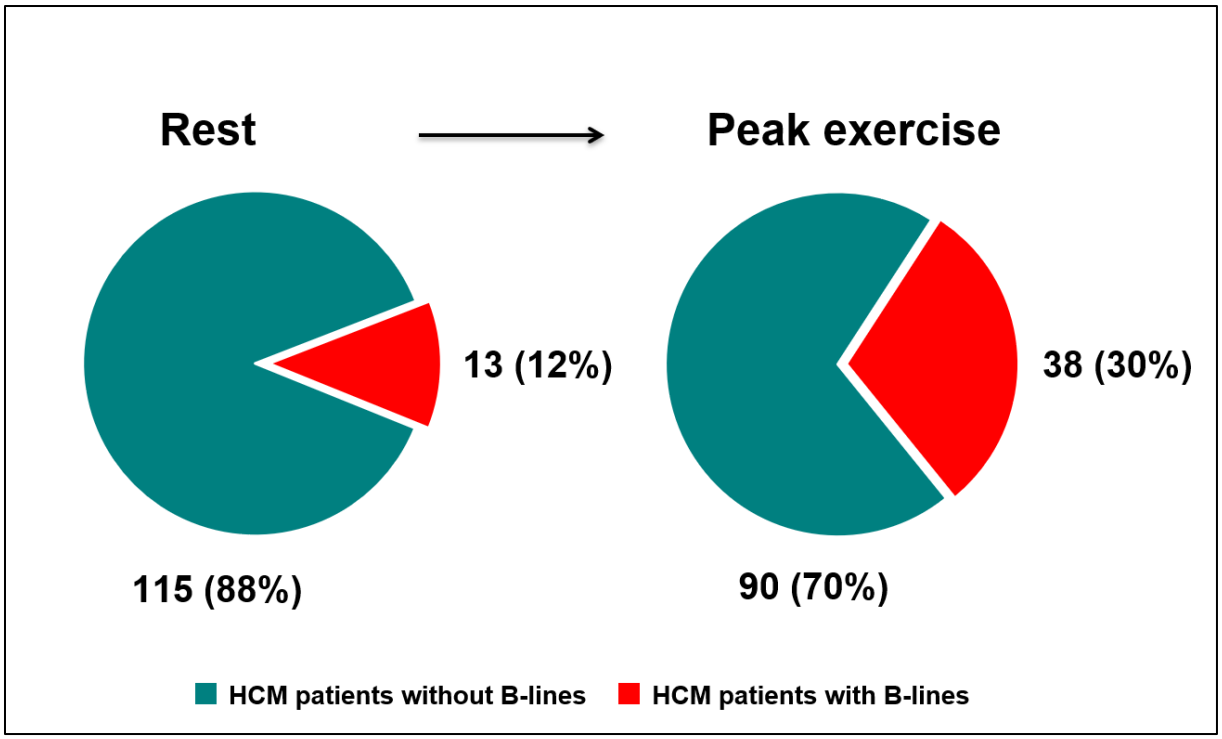
Table 1. *Baseline characteristics of the 128 hypertrophic cardiomyopathy patients undergoing exercise stress echocardiography with lung ultrasound.*

	All patients (n=128)	**HCM patients with stress B-lines (n=38)**	**HCM patients without stress B-lines (n=90)**	**p value**
Age (years)	50.3±15.4	53.0±17.3	49.2±14.5	0.200
Age at first diagnosis (years)	42.8±15.6	**48.4±17.8**	**40.9±14.5**	**0.031**
Male gender	85 (66%)	24 (63%)	61 (68%)	0.613
Body surface area (m^2)	1.9±0.2	1.9±0.2	1.9±0.2	0.401
SCD risk (%)	2.9±2.1	**4.1±3.2**	**2.6±1.5**	**0.039**
Syncope	8 (6%)	4 (11%)	4 (4%)	0.236
Coronary artery disease	4 (3%)	1 (3%)	3 (3%)	0.835
NYHA I-II	120 (94%)	35 (92%)	85 (94%)	0.694
Beta-blockers	92 (72%)	29 (76%)	63 (70%)	0.468
Diuretics	16 (13%)	7 (18%)	9 (10%)	0.188
LV max wall thickness (mm)	20.2±5.4	21.6±5.8	19.7±4.8	0.095
LVOT gradient ≥30 mm Hg	26 (21%)	11 (29%)	15 (17%)	0.122
LVOT gradient ≥50 mm Hg	18 (14%)	7 (18%)	11 (12%)	0.423

Data are expressed as mean value ±SD, median value with the corresponding first and third quartile or number (%) of patients. Abbreviations: HCM: hypertrophic cardiomyopathy; LV: left ventricular; LVOT: left ventricular outflow tract; NYHA: New York Heart Association; SCD: sudden cardiac death.

LUS was feasible in all subjects, with additional scanning and analysis time less than 1 minute each for rest and peak stress. B-lines were detected in 13 patients at rest and in 38 during stress (12% vs 31%, $p<0.0001$, Figure 1). B-lines were present both at rest and at peak stress in 13 patients (12%).

Figure 1. *The proportion of hypertrophic cardiomyopathy patients with B-lines at rest and peak stress. Abbreviation: HCM: hypertrophic cardiomyopathy.*



We divided the cohort into two groups according to the peak stress lung profiles: HCM patients with stress B-lines (congestive phenotype, with wet lungs, n=38) and without stress B-lines (non-congestive phenotype, with dry lungs, n=90). Exercise-time tended to be lower in patients with stress-induced B-lines (8.7±3.0 vs 10.8± 3.8 min, p=0.056). The reason for stopping the test was more frequently fatigue/exhaustion in patients with stress-induced B-lines (54% vs 32%, p=0.129). HCM patients with stress B-lines were older at first diagnosis and had higher SCD risk scores at the time of the evaluation compared to patients without stress B-lines (Table 1). Compared to patients without stress B-lines, those with exercise lung congestion showed a trend to higher prevalence of anamnestic syncope (11% vs 4%, p= 0.236), but similar NYHA class (Table 1). Regarding echocardiographic parameters, congestive patients had higher resting E/e' and SPAP, with similar MR grade and EDV at rest (Table 2). However, during stress beyond the more elevated stress E/e', SPAP, they also showed greater MR and smaller EDV, compared to patients with dry lungs (Table 2). Another important finding was that congestive patients showed twice more often a reduced preload response and an increase in MR in response to exercise (Table 2, Figure 3).

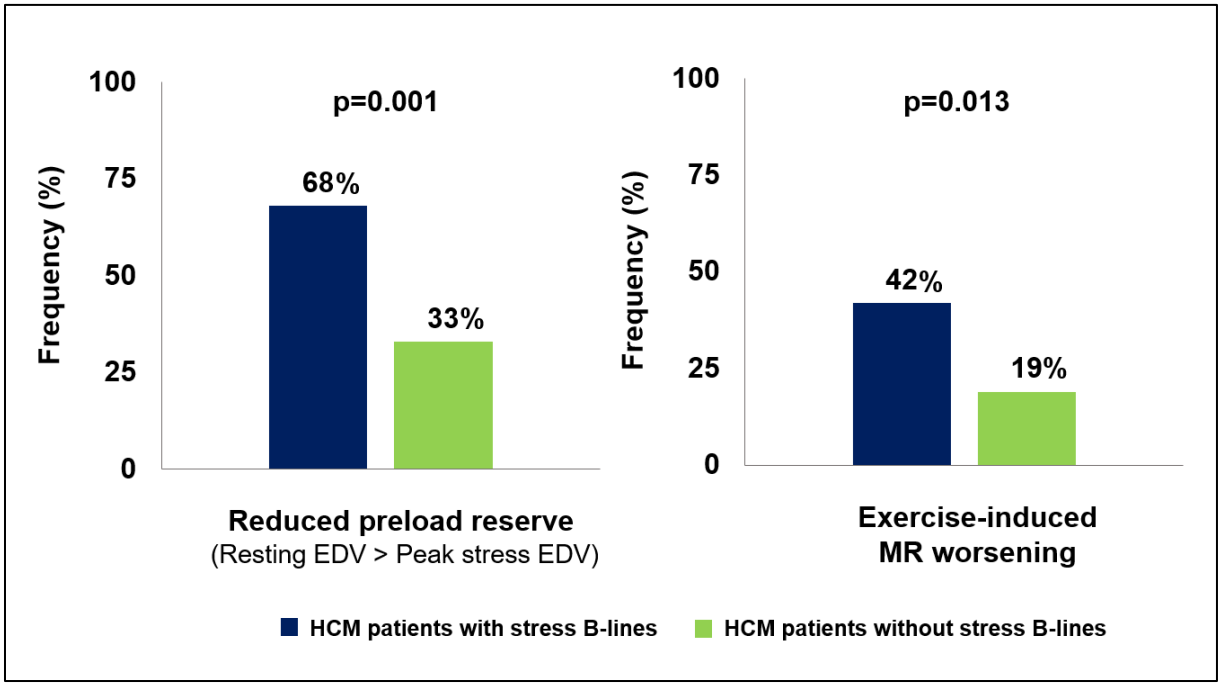
Table 2. *Rest and peak stress echocardiographic findings according to stress B-lines presence in the 128 hypertrophic cardiomyopathy patients.*

		All patients (n=128)	HCM patients with stress B-lines (n=38)	HCM patients without stress B-lines (n=90)	p value
LA diameter (mm)	rest	40.7±6.0	41.9±6.6	39.9±5.5	0.231
LA volume (ml)	rest	69.0±28.1	79.4±38.5	65.9±23.6	0.107
	stress	69.6±26.1	78.7±27.6	66.9±25.3	0.058
	Δ	-1.0 (-8.0; 6.0)	-3.0 (-12.0; 8.5)	-1.0 (-7.0; 4.0)	0.535
LAVi (ml/m^2)	rest	36.9±14.8	41.2±18.9	35.6±13.2	0.176
	stress	37.1±13.7	40.8±13.6	36.1±13.7	0.148
	Δ	-0.6 (-4.1; 3.1)	-2.0 (-6.0; 4.9)	-0.5 (-3.2; 1.8)	0.576
LV EDV (ml)	rest	95.8±32.0	92.2±4.0	97.3±31.2	0.424
	stress	97.2±32.0	**83.7±29.4**	**101.1±31.9**	**0.016**
	Δ	1.3±18.6	-4.8±18.8	3.1±18.3	0.064
Reduced preload response		56 (44%)	**26 (68%)**	**30 (33%)**	**0.001**
LV EDVi (ml/m^2)	rest	51.0±17.2	48.4±17.7	52.0±17.0	0.293
	stress	51.8±17.5	**43.4±14.1**	**54.2±17.7**	**0.006**
	Δ	0.8±10.5	-2.6±10.8	1.8±10.2	0.061
LV ESV (ml)	rest	33.3±14.5	31.4±14.1	34.0±14.7	0.358
	stress	30.6±16.7	27.8±13.3	31.4±17.6	0.340
	Δ	-2.7±13.4	-2.0±14.2	-3.0±13.2	0.751
LV EF (%)	rest	65.4±7.8	65.9±8.0	65.2±7.8	0.632
	stress	69.3±10.9	69.0±7.9	69.4±11.6	0.870
LVOT gradient (mm Hg)	rest	21.5±27.7	25.7±33.8	19.7±24.7	0.321
	stress	43.1±47.3	56.5±61.4	37.4±38.8	0.083
	Δ	21.6±30.7	30.7±42.7	17.7±23.1	0.084
LV force (mm Hg/ml)	rest	5.2±2.6	6.0±3.2	4.9±2.2	0.053
	stress	8.6±5.1	10.8±6.9	8.0±4.4	0.066
LVCR		1.7±0.7	1.8±0.8	1.7±0.6	0.591
MR (grade)	rest	1.0±0.8	1.0±0.8	1.0±0.8	0.963
	stress	1.2±0.9	1.5±1.0	1.1±0.8	0.057
	Δ	0.2±0.6	**0.6±0.8**	**0.1±0.5**	**0.008**
	any change	33 (26%)	**16 (42%)**	**17 (19%)**	**0.013**
SPAP (mm Hg)	**rest**	27.8±8.0	**33.0±9.6**	**26.6±7.2**	**0.002**
	stress	43.2±15.1	**55.2±18.2**	**40.2±12.6**	**<0.0001**
	Δ	15.4±12.9	**23.4±17.2**	**13.5±11.0**	**0.035**
TAPSE (mm)	rest	23.9±4.5	24.2±5.4	23.8±4.3	0.714
	stress	29.8±5.5	30.0±5.2	29.7±5.6	0.865
	Δ	5.8±4.9	5.6±5.4	5.9±4.8	0.854

E/e'	**rest**	11.5±4.7	**14.2±6.2**	**10.7±3.9**	**0.016**
	stress	13.5±5.7	**16.5±5.6**	**12.6±5.4**	**0.003**
	Δ	1.8±4.8	2.1±5.1	1.7±4.7	0.795
WMSI	rest	1.0±0.1	1.0±0.0	1.0±0.1	0.389
	stress	1.0±0.0	1.0±0.1	1.0±0.0	0.450
	Δ	0.0±0.1	0.0±0.1	0.0±0.1	0.199
LV GLS (n=67)	rest	-16.9±4.7	-17.1±4.9	-16.8±4.7	0.887

Data are expressed as mean value ±SD, median value with the corresponding first and third quartile or number (%) of patients. Abbreviations: Δ: delta (stress minus rest); E: early mitral inflow velocity; e': early diastolic mitral annular velocity; EDV: end-diastolic volume; EDVi: end-diastolic volume index; EF: ejection fraction; ESV: end-systolic volume; GLS: global longitudinal strain; HCM: hypertrophic cardiomyopathy; LA: left atrial; LAVi: left atrial volume index; LV: left ventricular; LVCR: left ventricular contractile reserve; LVOT: left ventricular outflow tract; MR: mitral regurgitation; SPAP: systolic pulmonary arterial pressure; TAPSE: tricuspid annular plane systolic excursion; WMSI: Wall motion score index.

***Figure 2**. Bar graphs showing the frequency of reduced preload reserve (defined as greater resting end-diastolic volume than peak stress end-diastolic volume) and exercise induced mitral regurgitation worsening in hypertrophic cardiomyopathy patients grouped by their stress lung profile. Abbreviations: EDV: end-diastolic volume; HCM: hypertrophic cardiomyopathy; MR: mitral regurgitation.*



Patients with wet lungs also showed lower baseline diastolic blood pressure, higher resting pulse pressure, blunted SBP increase, more abnormal blood pressure response and lower stress SV index and CI (Table 3).

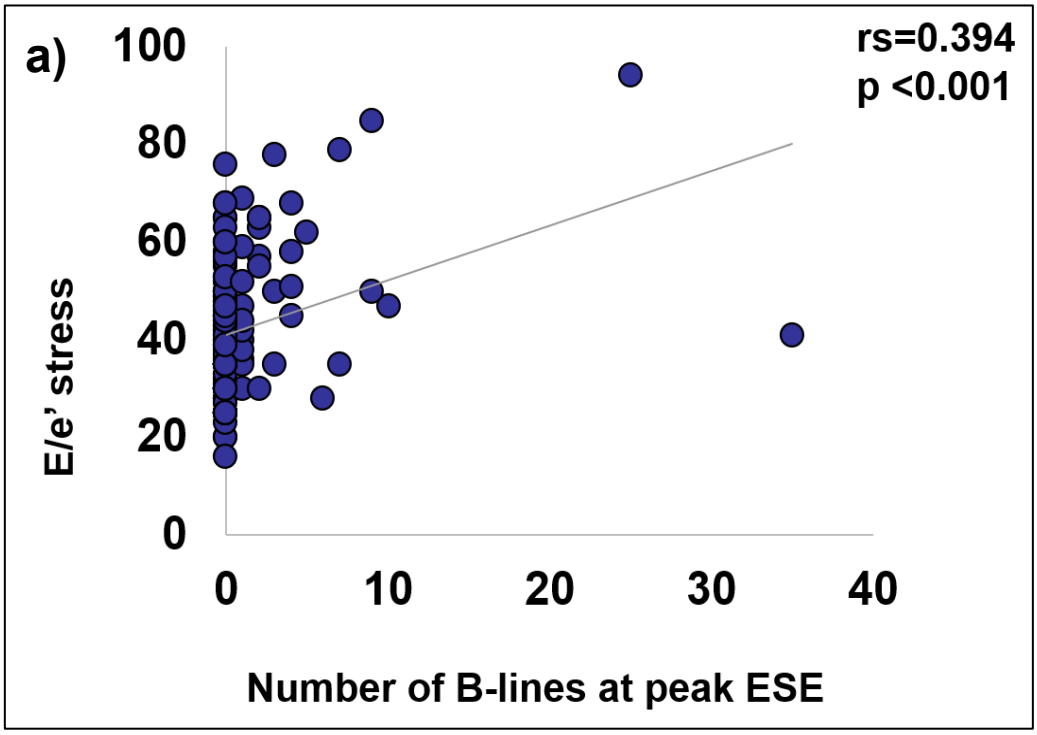
Table 3. Rest and stress hemodynamic findings according to stress B-lines presence in the 128 hypertrophic cardiomyopathy patients.

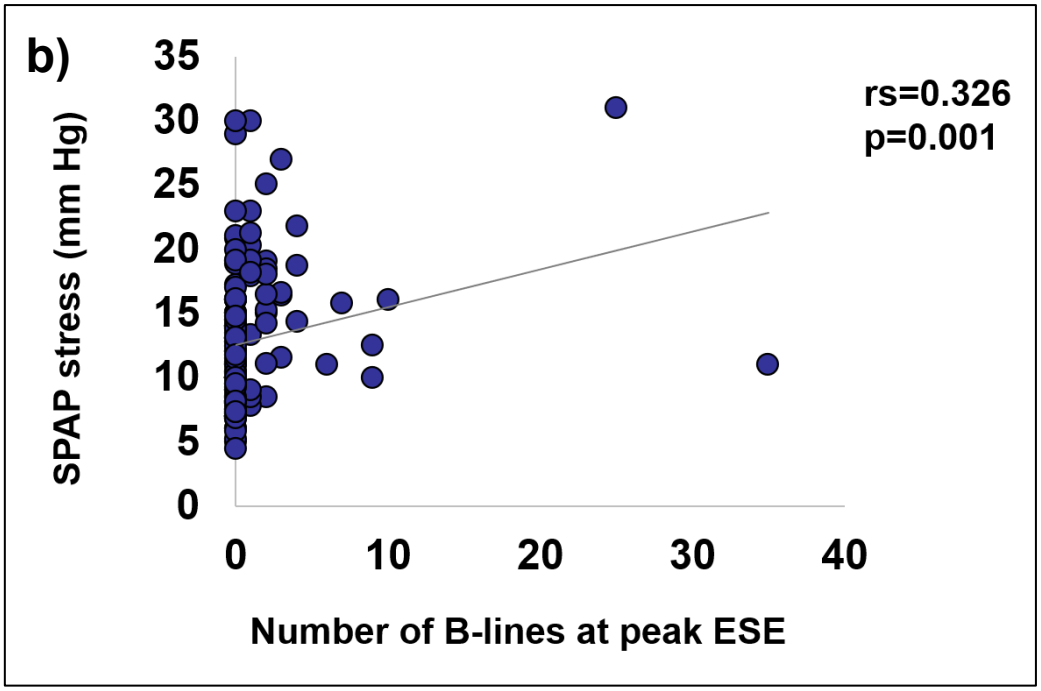
		All patients (n=128)	**HCM patients with stress B-lines (n=38)**	**HCM patients without stress B-lines (n=90)**	**p value**
SBP (mmHg)	rest	125.1±15.4	128.4±15.6	123.7±15.1	0.110
	stress	160.3±25.9	155.3±28.9	162.4±24.3	0.159
	Δ	35.2±27.4	**26.9±30.6**	**38.7±25.4**	**0.025**
DBP (mmHg)	**rest**	78.0±9.5	**73.6±6.8**	**79.0±9.7**	**0.025**
	stress	85.8±14.4	82.7±13.1	86.5±14.7	0.314
	Δ	7.8±13.7	9.1±14.9	7.5±13.4	0.640
Pulse pressure (mmHg)	rest	46.5±13.9	**52.7±15.5**	**45.0±13.2**	**0.029**
	stress	74.7±27.6	70.8±26.3	75.5±28.0	0.506
	Δ	28.2±28.3	18.1±26.1	30.5±28.4	0.085
Heart rate (beats/min)	rest	67.6±14.6	66.2±10.0	68.2±16.2	0.485
	stress	126.1±25.4	122.1±25.5	127.8±25.3	0.252
	Δ	58.5±24.7	55.9±25.7	59.6±24.4	0.445
HHR		1.9±0.4	1.9±0.5	1.9±0.4	0.576
SV index (ml/m^2)	rest	33.3±11.4	32.0±12.3	33.8±11.1	0.444
	stress	35.5±13.0	**29.1±10.6**	**37.3±13.1**	**0.005**
	Δ	2.2±9.7	**-1.7±7.4**	**3.3±10.0**	**0.023**
Cardiac index (ml/min/m^2)	rest	2212±835	2070±732	2271±871	0.220
	stress	4485±1797	**3474±1381**	**4778±1803**	**0.001**
	Δ	2275±1465	**1530±1179**	**2492±1475**	**0.003**
ABPR		32 (25%)	**18 (47%)**	**14 (16%)**	**<0.001**

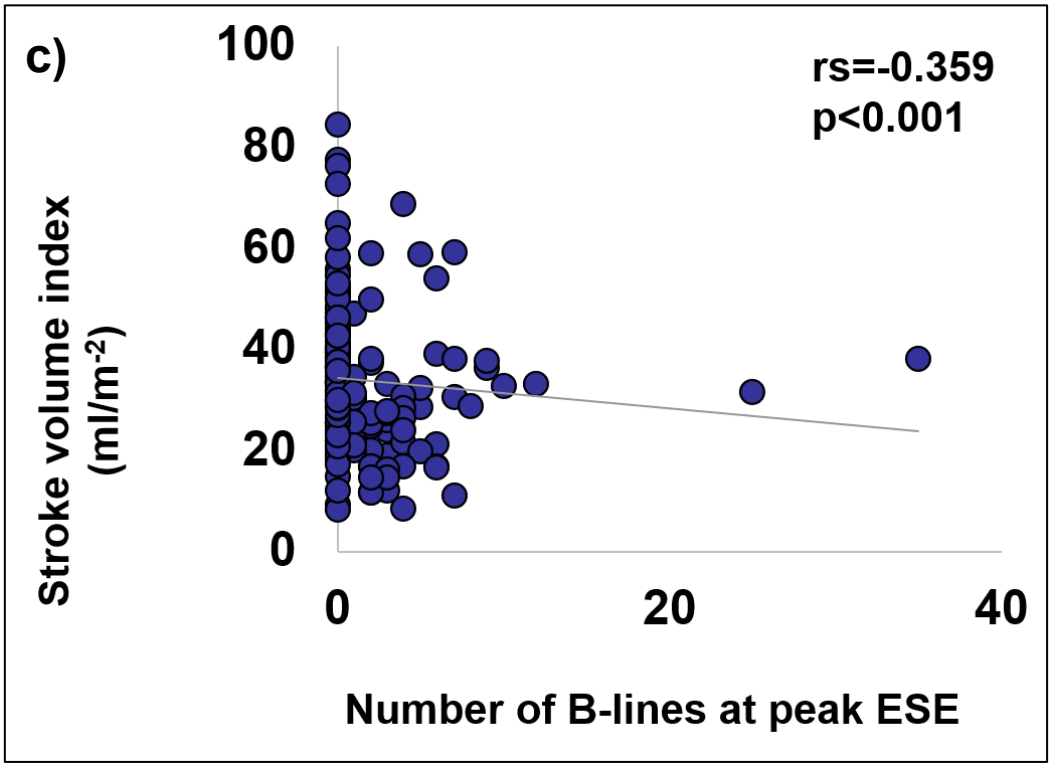
Data are expressed as mean value ±SD or number (%) of patients. Abbreviations: as in Table 1-2, ABPR: abnormal blood pressure response; DBP: diastolic blood pressure; HRR: heart rate reserve; SBP: systolic blood pressure SV: stroke volume.

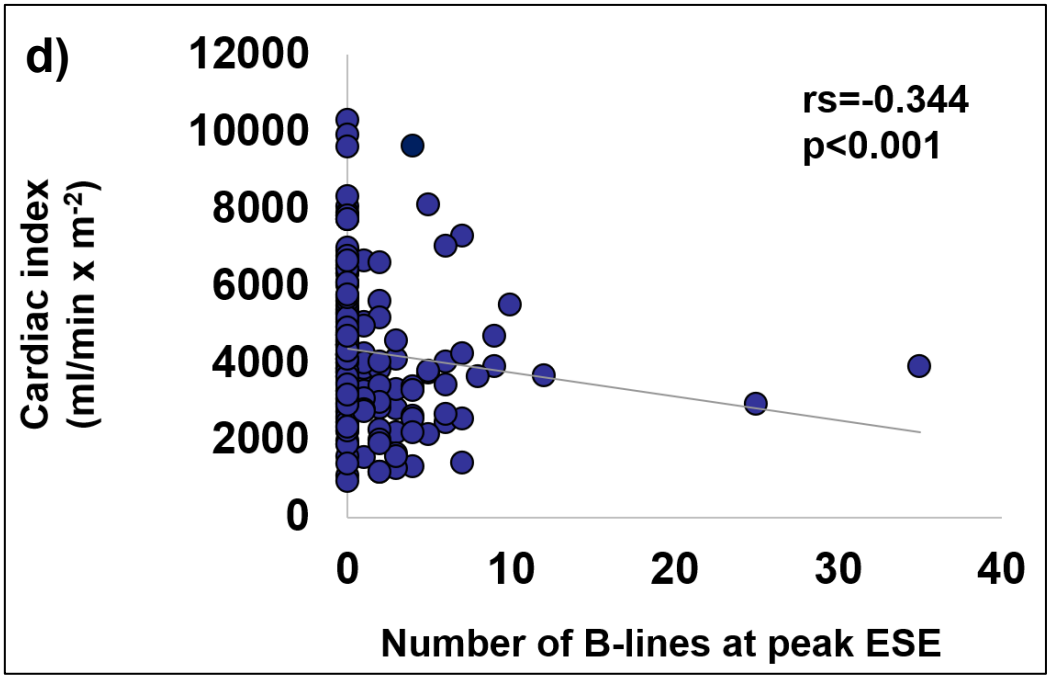
The individual number of stress B-lines showed moderate positive correlation with peak exercise E/e' (Figure 3, panel a) and SPAP (Figure 3, panel b) and inverse relationship with peak exercise SV index (Figure 3, panel c) and cardiac index (Figure 3, panel d).

Figure 3. *The correlation (Spearman's) between stress B-lines (x-axis) and (y-axis) stress E/e' (panel a), peak stress systolic pulmonary arterial pressure (panel b), peak exercise stroke volume index (panel c), and peak exercise cardiac index (panel d). Abbreviations: E: early mitral inflow velocity; e': early diastolic mitral annular velocity; ESE: exercise stress echocardiography; SPAP: systolic pulmonary arterial pressure.*




b)
SPAP stress (mm Hg)
35
30
25
20
15
10
5
0
0
10
20
30
40
Number of B-lines at peak ESE
rs=0.326
p=0.001


c)
Stroke volume index (ml/m-2)
100
80
60
40
20
0
0
20
40
Number of B-lines at peak ESE
rs=-0.359
p<0.001


d)
12000
10000
8000
6000
4000
2000
0
Cardiac index
(ml/min x m-2)
rs=-0.344
p<0.001
0
10
20
30
40
Number of B-lines at peak ESE

Multivariable logistic regression analysis revealed that among baseline parameters the number of B-lines and SPAP were independent predictors of B-lines with exercise (Table 4).

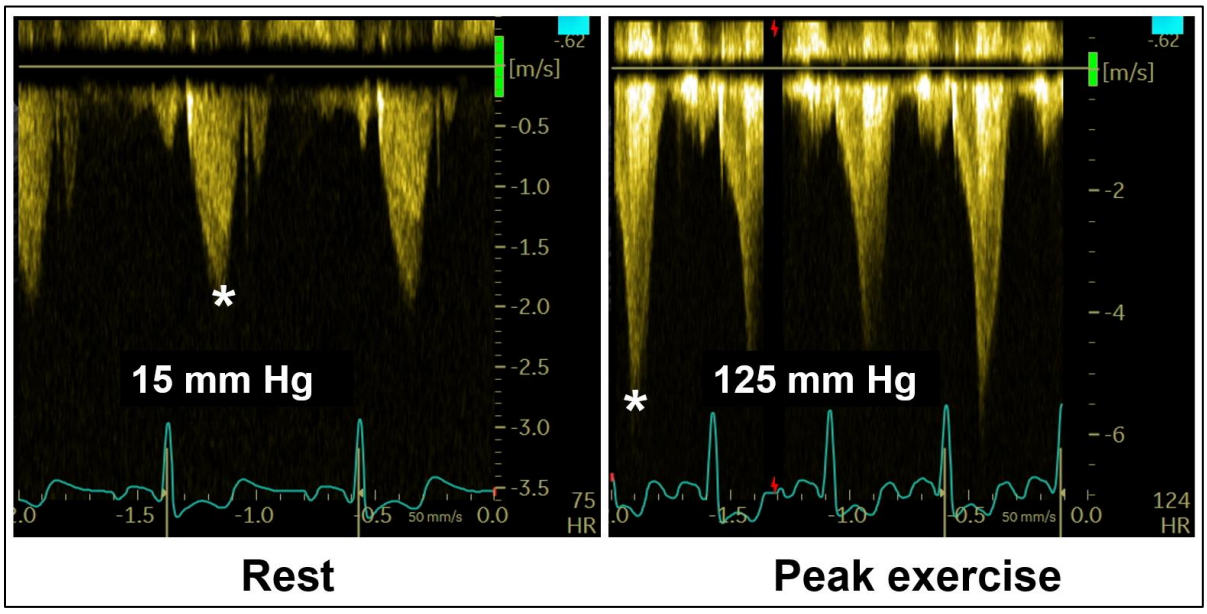
Table 4. *Baseline predictors of pulmonary congestion during exercise stress echocardiography in the 128 hypertrophic cardiomyopathy patients.*

	Univariable analysis			**Multivariable analysis**		
	OR	95% CI	p value	OR	95% CI	p value
Age at first diagnosis (years)	1.031	1.002-1.062	0.035			
SCD risk (%)	1.407	1.093-1.812	0.008			
B lines number rest	4.304	1.691-10.956	0.002	**4.282**	**1.120-16.364**	**0.033**
DBP (mmHg)	0.932	0.876-0.993	0.029			
Pulse pressure (mm Hg)	1.040	1.003-1.078	0.034			
LA volume (ml)	1.016	1.001-1.031	0.043			
E/e' rest	1.154	1.049-1.271	0.003			
LV force rest (mm Hg/ml)	1.183	1.019-1.373	0.027			
SPAP rest (mm Hg)	1.093	1.027-1.163	0.005	**1.070**	**1.000-1.144**	**0.048**
Abbreviations: as in Table 1-3., CI: confidence interval; OR: odds ratio.						

Latent LVOTO

After a comprehensive echocardiographic study, 47 Hungarian HCM patients [mean age=46±3 years, 29 males (62%)] without resting LVOTO underwent symptom limited semi-supine ESE. At baseline echocardiography, no LV RWMA were detected, the mean maximal LV septal thickness was 24.9±6.5 mm, and the average resting LVOTG was 9.8±8.2 mm Hg. At peak ESE, 4 (9%) patients terminated the exercise due to chest pain, while in the remaining 43 (91%) the reason for discontinuation was fatigue or shortness of breath. During ESE, heart rate and blood pressure values increased significantly compared to baseline values (heart rate: 66±12 beats/min vs 109±19 beats/min, $p<0.001$; SBP: 129±17 vs 154±27 mm Hg, $p<0.01$; DBP: 81±10 vs. 84±13 mm Hg, $p<0.05$). The double product increased significantly from baseline to peak exercise (8673±2318 mm Hg x [beats/min] vs 16694±4558 mm Hg x [beats/min], $p<0.001$). ABPR or any complications were not detected during ESE. LVOTG increased significantly from baseline to peak exercise (9.8±8.2 vs. 22.4±20.9 mmHg, $p<0.0001$). At peak exercise 11 (23%) out of the 47 patients developed LVOTO ≥30 mm Hg and were considered to have latent LVOTO. An example of latent LVOTO in HCM is shown in Figure 4. The remaining 36 (77%) had nonobstructive phenotype.

Figure 4. *An example of latent dynamic left ventricular outflow tract obstruction during semi-supine exercise echocardiography in a patient with hypertrophic cardiomyopathy. Continuous-wave Doppler recording of the left ventricular outflow tract reveals a late-peaking and concave-to-the-left Doppler spectrum with midsystolic flow acceleration peaking at 1.9 m/s at rest (left panel, asterisk) and at 5.6 m/s at peak exercise (right panel, asterisk), yielding a peak dynamic left ventricular outflow tract gradient of 15 mm Hg at rest (right panel) and 125 mm Hg at peak exercise (right panel).*



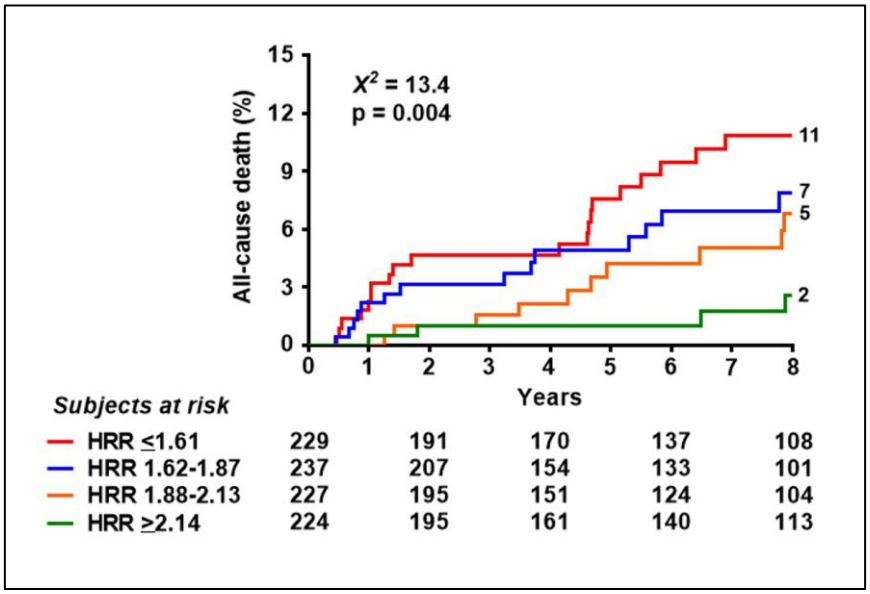
The magnitude of rest to peak LVOTG change was remarkably higher in patients with latent obstruction compared to nonobstructive patients (33.7±21.1 vs. 6.2±6.7 mm Hg $p<0.001$). Regarding demographic variables and exercise parameters no significant differences could be observed between the latent and nonobstructive HCM patients. Among resting echocardiographic parameters, LVOT diameter was significantly smaller (16.2±2.1 vs 22.4±2.8 mm, $p<0.01$) and resting LVOTG was more than two-times higher (17.8±6.8 vs 7.4±6.9, mm Hg, $p<0.01$) in latent obstructives compared to nonobstructives. In other echocardiographic parameters, no difference could be observed between latent and nonobstructive HCM patients.

HRR

Nine hundred seventeen prospectively enrolled consecutive HCM patients (age=49±15 years, 44% female) who underwent ESE at 11 centers were retrospectively analysed to assess the determinants and prognostic value of HRR in HCM. ESE modality was semi-supine bicycle in 51 (6%), upright bicycle in 476 (52%) and treadmill in 390 (42%). LVOTO ($\geq$50 mmHg) was present in 150 patients (16%) at rest and occurred in 281 (30%) at stress. In turn, RWMA were

present in 12 patients (1.3%) at rest and in 34 (3.7%) at peak stress, with new or worsening RWMA in 22 patients (2.4%). The mean HRR was 1.90±0.40. Four HRR groups were identified: 1) highest HRR quartile (HRR >2.13, 224 patients, 24%), 2) HRR 1.88–2.13 (227 patients, 25%), 3) HRR 1.62–1.87 (237 patients, 26%), 4) lowest HRR quartile (HRR≤1.61, reduced, 229 patients, 25%). Patients with the lowest quartile had more advanced age and higher NYHA functional class, were more often on therapy, had higher resting heart rate, more frequent LVOTO and moderate/severe mitral regurgitation at rest, poorer exercise capacity, lower peak heart rate, and more frequent LVOTO at stress. The independent determinants of reduced HRR were found to be resting heart rate (OR 1.027, CI 1.018–1.036, p<0.001), age (OR 1.021, CI 1.009–1.033, p<0.001), resting LVOTO (OR 1.504, CI 1.043–2.170, p=0.029) and peak exercise metabolic equivalents (OR 0.761, CI 0.708–0.817, p<0.001). During a median follow-up of 89 months (interquartile range: 36-145 months), 90 all-cause deaths occurred. The mortality rates from the highest to the lowest HRR quartiles were 5.7, 9.3, 12.4, and 15.5 for 1000 person months (p=0.004, Figure 5).

Figure 5. *Kaplan-Meier event-free survival curves for all-cause mortality stratified by HRR quartiles in hypertrophic cardiomyopathy patients. HRR: heart rate reserve.*



At multivariable analysis, reduced HRR (HR 2.354, CI 1.116–4.968, p=0.025) and exercise-induced new RWMA (HR 3.279, CI 1.441–7.461, p=0.004) independently predicted death, in addition to age (HR 1.064, CI 1.043–1.085 p<0.001) and LV maximal wall thickness (HR 1.081, CI 1.037–1.128, p<0.001). At incremental analysis, the global X^2 of the clinical model for the prediction of death increased from 64.5 to 68.1 (p<0.41) with the addition of HRR to RWMA, maximal wall thickness and age and risk reclassification also significantly improved with NRI:

0.24 (CI 0.030–0.442, p=0.025). Beta-blocker use was equally distributed in the 4 quartiles and was not a predictor of reduced HRR. ESE was performed with beta-blockers in 534 patients, and without beta-blockers in 383 patients. At univariable analysis beta-blocker use did not predict outcome (HR 1.264, CI 0.807–1.978, p=0.306) whereas the lowest quartile of HRR predicted survival in the subset studied off (n=524, HR 2.865, CI 1.353–6.067, p=0.006) or on (n=383, HR 4.777, CI 1.078–21.777, p=0.040) beta-blockers at the time of the ESE.

5. Discussion

Our investigation on exercise pulmonary congestion revealed that LUS during ESE is feasible and simple in HCM, with 100% success rate for B-lines and only a minimal increase in imaging time. We found B-lines in about 10% of HCM patients at rest and in about 30% during ESE. HCM patients presenting B-lines at stress were diagnosed later in life and had higher SCD risk scores. They showed higher pulse pressure at rest, with similar heart rate and cardiac output compared to patients without stress B-lines, suggestive of a stiff aorta contributing to abnormal ventricular arterial interactions during stress and eventually favouring myocardial fibrosis and dysfunction (57,58). Stress B-lines were associated with worse rest and stress diastolic function, greater rest and stress SPAP and larger increment in MR during stress. Furthermore, HCM patients with stress B-lines had lower cardiac index and cardiac index reserve at comparable heart rates and they exhibited more often abnormal blood pressure response to exercise, compared to those without stress B-lines. Hence, stress B-lines are relatively frequent findings in HCM patients, represent multiple mechanisms, are associated with signs of greater clinical and functional severity, and reflect hemodynamic vulnerability during exercise, mirrored by a reduced cardiac index reserve and prevalent abnormal blood pressure response. Notably, the development of stress B-lines could not be reliably foreseen by the baseline echocardiographic features of our patients, the best predictor was SPAP >28 mm Hg, with a positive predictive value of only 48% (95% CI 32-65%). Therefore, given its ease of implementation and utility, the systematic performance of lung scanning appears a valuable adjunct to ESE in HCM patients, even in the presence of baseline evidence of increased pulmonary pressures.

According to the results of our study on latent LVOTO, in almost a quarter of unselected Hungarian HCM patients without baseline LVOTO, latent LVOTO could be identified during ESE. In our study, regardless of their clinical symptoms, we examined all HCM patients without resting LVOTO. Based on our results, HCM patients with latent LVOTO have higher baseline LVOTG and smaller resting LVOT diameter compared to non-obstructive HCM patients.

In our HRR study, we revealed that a reduction of HRR is associated with worse survival in patients with HCM. Furthermore, we demonstrated that the prognostic value of HRR is independent of other established predictors such as age and LV maximal wall thickness. HRR outperformed LVOTG and exercise-induced hypotension for predicting survival and was independent and additive to RWMA. Notably, the prognostic value of HRR was observed in patients off and on beta-blockers at the time of testing. Not only high heart rate at rest but also poor exercise tolerance and low heart rate at peak exercise are associated with a reduced HRR, and both contribute to its capability to stratify outcome.

Exercise Methodology and Pathophysiology in HCM

Over the long history of HCM, many provocation strategies have been studied to reveal LVOTO but only a few of them have gained clinical significance due to the limitation of the various stressors and the lack of data. The most frequently applied provocative methods include simple standing, standing up from the squatting position, Valsalva strain, amyl nitrite inhalation, isoproterenol infusion and physical exercise in a fasted or postprandial state (2). The Valsalva manoeuvre is the most easily available at routine ambulatory settings, but it has a limited capacity to detect accurately LVOTO since it underestimates the presence and magnitude of provocable LVOTO (10,59,60). Exercise is the most physiologic form of all stressors and is preferred in every HCM patient who is capable of physical exercise (20). During exercise, complex hemodynamic, neuronal, and hormonal changes emerge which cannot be reproduced by any of the pharmaceutical agents. Exercise provocation lets us acquire information on the entire physiological chain that gets activated during exertion, including the central and peripheral nervous systems, skeletal muscle, lungs, myocardial, coronary circulation, peripheral blood circulation and mitochondrial oxygen use (37).

Pathophysiology of Pulmonary Congestion in HCM

The pathophysiology of heart failure and congestion in HCM is not completely understood yet (61). LV hypertrophy, ischemia and fibrosis lead to a stiff, non-compliant left chamber that restrains diastolic filling and elevates intracavitary end-diastolic pressures (1,4). When the left ventricle fails, blood accumulates in the left atrium and LA pressure and pulmonary capillary pressure similarly rises (62). When pulmonary capillary pressure elevates above a threshold, the imbalance in the Starling forces across the pulmonary capillary endothelial barrier results in an increased accumulation of extravascular lung water (63). Distinct factors beyond diastolic dysfunction that contribute to backward HF in HCM include LVOT obstruction, structural or functional mitral valve alterations, increased large artery stiffness or less commonly, abnormal

systolic function (64). Notably, baseline LVOTG was not a predictor of exercise pulmonary congestion in our study. There was a trend showing a higher LVOTG in the group of patients with stress B-lines but it was not significant. Although initially counterintuitive, this finding is consistent with clinical practice: only a minority of HCM patients with obstruction, even when severe, benefit from diuretics and many may worsen their symptoms due to preload reduction. Other factors seem to play a greater role than gradients, including the degree of MR at rest or during exercise and diastolic dysfunction.

Pathophysiology of LVOTG Changes and Methodological Concerns

LVOTG assessment by Doppler echocardiography can be performed in the standing, sitting, or semi-supine position (4). All positions require an instrument with modifiable workloads which permits echocardiographic assessment of exercise-induced hemodynamic changes. This can be obtained with treadmill, upright bicycle ergometer or a special semi-recumbent bicycle, with an additional left lateral tilting function (20). Each position has its unique hemodynamic and technical aspects which may become important if load-dependent parameters need to be assessed such as LVOTG. The advantage of treadmill equipment is its wide availability and the associated broad clinical experience with it (37). It is well-known that orthostatic position decreases venous return and hence facilitates the development of LVOTO. It is also noted, that exercising on treadmill triggers more the LVOTO than exercising on a bicycle in the semi-supine position (60). After the cessation of exercise, a sudden drop in the venous return occurs with a continuing sympathetic drive which is the main mechanism of the LVOTG increase in the early recovery phase (65). It is also recognized that remaining in the upright position following exercise results in a preload reduction and augments LVOTG more compared to the supine or semi-supine positions (20,60). However, it is quite difficult to execute imaging during treadmill exercise, so with treadmill testing, postexercise imaging is used in most instances by placing the patient immediately after exercise onto an imaging table in the left lateral decubitus position. Though, this is hardly representative of what happens at peak exercise in orthostatic position (37). Furthermore, positioning the patient from the standing to the lying position creates a great increase in venous return and so in preload, which consequently decreases the LVOT gradient. Additionally, in most cases, the patient does not lie down during routine physical activity. Therefore, the determination of the LVOTG in this position does not reflect properly the real-life circumstances. Because of the pronounced effect of the orthostatism and exercise on LVOTG, some laboratories recommend imaging in the upright position after treadmill exercise, however, this has not spread in general practice (66). The ability to collect

images throughout the entire exam is a major benefit of bicycle ESE (37). When using an upright bike, imaging is often restricted to the apical and subcostal views, while supine ergometers allow the use of all echocardiographic projections (37). It must be noted that in most patients, the supine cycling posture results in relatively shorter exercise duration, lower maximal workload and less of achieved maximal heart rates. This is mostly because leg fatigue appears earlier during the test (20). Furthermore, in the supine position, the EDV and mean arterial blood pressure are greater for a given level of stress. These variations result in higher wall stress, which in turn raises myocardial oxygen demand and filling pressures in comparison to an upright bicycle test (20). Due to the lack of evidence on different methods, both treadmill and bicycle ESE are accepted methods for LVOTO provocation in HCM (2).

Pathophysiology of Blunted HRR in HCM

Cardiac autonomic nervous system can be impaired in patients with HCM (67,68). HCM patients show reduced beta-receptors density and function, with an initial exaggerated response to sympathetic stimulation which may later progress to receptor desensitization (69–72). A blunted HRR can be therefore considered a marker of reduced sympathetic reserve often associated with higher baseline levels of sympathetic activity which can be detrimental in HCM for many reasons. Increased sympathetic activity and increased cardiac norepinephrine may increase myocardial cell growth, disarray and scarring, induce myocardial ischemia through alpha-adrenergic coronary constriction and increase the rate of spontaneous depolarizations in myocardial cells with resulting electrical instability (73).

Comparison with Previous Studies

Numerous investigations have shown the excellent feasibility, diagnostic and prognostic usefulness of B-lines assessment during stress echocardiography in different cardiovascular diseases (39,56,74). However, our report is the first in the literature focusing on HCM. We adopted the simplified 4-site scan technique which proved to be the best trade-off between accuracy and simplicity both at rest and especially after stress when imaging time is short and there are many parameters to scan (56). Prior studies have demonstrated that the number of stress B-lines is tightly related to E/e' and MR development during ESE in patients with HF, consistent with our findings in HCM (56,74). In addition, we observed that in HCM, stress B-lines were associated with lower EDV and CI reserve during stress. The findings of our study are in line with those of Lele et al., who evaluated 79 HCM outpatients in a hemodynamic study with radionuclide ventriculography and expiratory gas analysis during symptom-limited exercise stress (75). They found that the ability to increase LV EDV is a principal factor in

stroke volume and cardiac output augmentation during dynamic exercise in HCM (75). The advantage of ESE is that it provides a one-stop shop view of all these interconnected variables, including pulmonary congestion, preload reserve, dynamic intraventricular gradients and MR, both at rest and during stress.

Our results on latent LVOTO match those observed in earlier international studies, in which the prevalence of latent obstruction HCM ranged from 24% to 66% (10,12,76–81). The relatively wide range of latent LVOTO prevalence can be explained by the different patient selection criteria and definitions of LVOTO and ESE protocols. Several experts have recently drawn attention to the need for a standardized ESE protocol in HCM (82). In our study, regardless of their symptoms, we subjected all HCM patients without resting LVOTO to ESE, while in some studies ESE was performed only in symptomatic patients (77). Like most papers examining HCM patients with dynamic provocation, we defined LVOTO as the peak LVOTG at maximum effort while others considered the greatest LVOTG occurring at any point during or immediately after stress (76,82). Resting LVOTO is a recognized unfavourable prognostic predictor of HF and cardiovascular death in HCM and in the past ten years, has been the subject of hundreds of papers (2). Furthermore, it is also recognized that HCM patients with resting LVOTO have a much poorer prognosis than those without LVOTO (10–12). On the contrary, the natural history and clinical presentation of latent obstructive HCM, have been less documented (13). In 2014, Finocchiaro et al. evaluated 267 HCM patients undergoing treadmill ESE with ongoing medical therapy. In their cohort, latent obstruction was present in 24% of the cases and was associated with higher LV wall thickness and larger LA size compared to nonobstructive patients (79). Though, this finding was not confirmed in greater sample sizes later in the study of Lu et al. (81). Our results do not match their observations since in our cohort, a significant difference emerged among nonobstructive and latent obstructive patients regarding resting LVOT diameter and LVOTG. Increased LVOTG during exercise may be attributable to effort-related symptoms in patients with HCM, whereas the relation between provoked LV gradients and clinical outcomes remains uncertain (13). Inducible LVOT obstruction was associated with worse outcomes in 5 studies whereas it showed no association with prognosis in other 6 (13). In the most recent, Lu and coworkers examined 705 HCM patients (91% with treadmill ESE) and, surprisingly, found that patients with obstruction only on provocation had the lowest event rates and the best event-free survival for composite cardiovascular outcome compared to nonobstructive and rest obstructive patients (81). However, the screening of patients with latent obstructive HCM is still crucial since surgical or catheter LV septal reduction therapy clearly improves the long-term prognosis of this group

(79,83). Our results on latent LVOTO could aid in a more accurate assessment of Hungarian HCM patients and better decision-making on their clinical course.

Our investigation was the first that evaluated HRR as peak/rest heart rate in HCM. Previous studies assessing chronotropic response during exercise in HCM used slightly different formulas such as the percentage of age-predicted peak heart rate with different cutoff values for chronotropic index in patients on (<62%) or off beta-blockers (<80%) (84,85). Despite different definitions, all available data point to a link between a blunted chronotropic response and a greater functional or structural impairment as well as increased risk for adverse events (84–86). Efthmiadis et al. evaluated 68 patients and found that all 5 patients with adverse events showed severe chronotropic incompetence (84). Luo et al. found in 273 HCM patients that an impaired chronotropic response was associated with a trend toward a higher frequency of ventricular tachycardia/fibrillation and death compared to those with normal chronotropic response (85). In another large, retrospective, multicentre study of 681 consecutive HCM cohort with a median follow-up of 4.2 years, Magri showed that HCM patients with chronotropic incompetence have higher mortality (86). Our results suggest that HRR is a simply definable, easily accessible parameter which is also able to identify HCM patients at risk for future cardiac events. In HCM patients, the risk associated with HRR is best described as a continuum with shades of grey rather than with a binary, black-or-white cut-off. Our results corroborate previous pieces of evidence with the strengths of large sample size (917 patients), long follow-up (median 89 months) which allowed us to analyse death as the only significant endpoint, and the addition of SE which allowed us to evaluate stress imaging parameters of established prognostic value recognized by guidelines such as LVOTO and RWMA. Notably, exercise-induced hypotension was not a predictor of death, in keeping with recent evidences (87).

Clinical Implications

Given the necessity for repeated follow-up exams, resting and ESE are particularly appealing for functional evaluation and risk stratification in HCM patients (2,9). In addition, comprehensive and primary HCM centers mostly have the equipment and expertise needed for ESE (2).

Clinical signs of pulmonary congestion such as pulmonary crackles on chest auscultation have substantial intra- and interobserver variability and are only loosely related to lung water accumulation (88). B-lines are also obtainable with pocket size instruments after a limited training and may guide an effective decongestion therapy with symptomatic and prognostic benefit, as it has been shown by randomized trials based on resting lung ultrasound

in other clinical settings such as heart failure (42). Aggressive diuretic therapy can worsen symptoms related to LVOT obstruction by causing exaggerated decrease in preload and should be avoided in HCM. Conversely, with the clinical evidence of congestion, cautious use of low-dose diuretics can provide symptom relief and can be reasonable to apply also in patients with LVOT obstruction (2,9).

In addition to the echocardiographic data acquired during ESE, HRR is a basic, imaging-independent, and easily accessible measure that has not been included yet in scientific guidelines on HCM. Since HRR can be obtained from a single ECG lead, it may be incorporated into routine methodology without additional equipment or expertise.

Study Limitations

We combined data from bicycle and treadmill ESE which have different hemodynamic effects and could influence cardiac volume changes and stress B-lines to some extent. Dynamic gradients are more obvious in orthostatic position, and treadmill increases EDV of the left ventricle more than semi-supine exercise in healthy subjects (55). Semi-supine exercise increases pulmonary artery wedge pressure more than upright exercise (89). Supine bicycle increases blood pressure more and heart rate less than the treadmill, but the double product is similar (90). The observational study design did not interfere with the individual choice of the referring physician, which is a matter of personal experience, awareness of the individual patient indications and local practice. Data were obtained from different laboratories without core lab reading, but all readers underwent quality control before patient recruitment and had established experience as referral centers for HCM (26). Transthoracic 2-dimensional echocardiography has recognized limitations in estimating absolute LV volumes in HCM but it remains the recommended first-line technique (32,91). In our study on exercise B-lines, relative volumetric changes of EDV from rest to stress provided more information than absolute values. In assessing relative changes, most sources of inaccuracy average out and each patient acts as their control during stress. HRR was not contemplated in the risk stratification strategy of initial protocols, but rest and peak heart rate are an obligatory part of the minimum data of stress echo methodology since the beginning, and this allowed to retrieve the data, although with no information on heart rate recovery which may provide an index of parasympathetic activity (85). We analyzed only all-cause death which is the strongest and the most reliable of all possible outcome measures (92). Genetic testing was not systematically performed, and it would not have been feasible since the recruitment window started in 1984. The dose and type

of beta-blockers, calcium antagonists and other drugs were not available. However, the prognostic value of HRR was documented in populations both on and off beta-blockers.

6. Conclusion

Albeit stress echocardiography is included in current guidelines for the management of HCM, mostly it is considered only a tool to evaluate peak LVOT gradients. However, ESE is a powerful multi-purpose tool with far-reaching clinical implications also in non-obstructive patients and provides much broader information for clinical practice.

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Attachments

1. Attachment: List of publications that form the basis of the dissertation

2. Attachment: Co-author certification

3. Attachment: Copy of the publications that form the basis of the dissertation

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