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Non-invasive stroke volume assessment during cardiopulmonary exercise testing provides additional insight beyond O_2-pulse in hypertrophic cardiomyopathy

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In hypertrophic cardiomyopathy (HCM), cardiopulmonary exercise testing (CPET) is considered the gold standard for assessing exercise tolerance, with O_2-pulse commonly used as a surrogate for stroke volume (SV). However, because SV reduction can be masked by increased oxygen extraction, direct non-invasive measurement of SV is valuable. This study involved 102 HCM patients (mean age 53 ± 16 years, 78% male), predominantly with a non-obstructive phenotype (74%), who underwent CPET with SV measurement using Physioflow (PF). Abnormal O_2-pulse kinetics were observed in 12 patients, all confirmed by abnormal SV trends with PF. Additionally, PF identified another 28 patients with altered SV kinetics. Abnormal SV trends were associated with higher peak VE/VO_2 ratios (42.6 [37.4–47.5] vs. 38.0 [33.6–41.3]) and lower end-tidal CO_2 values (31.8 ± 4.9 vs. 34.3 ± 5.6 mmHg, $p < 0.05$). Patients with greater SV growth during the final 25% of exercise showed improved anaerobic threshold VO_2 (49.8 ± 12.3% vs. 43.9 ± 15.2% predicted peak VO_2), VO_2/work slope (10.2 ± 2.0 vs. 9.3 ± 1.3 mL/min/Watt), and peak $PetCO_2$ (34.5 ± 5.6 vs. 32.3 ± 5.2 mmHg), alongside a lower VE/VCO_2 slope (28.7 [24.9–31.0] vs. 31.3 [27.3–34.2], $p < 0.05$). Integrating PF and CPET may enhance the detection of abnormal SV kinetics, which are associated with reduced functional capacity in HCM patients.

Keywords Hypertrophic cardiomyopathy, Cardiopulmonary exercise testing, Physioflow, Stroke volume evaluation

Abbreviations

AF	Atrial fibrillation
AT	Anaerobic threshold
BMI	Body mass index

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BR	Breathing reserve
CI	Cardiac index
CO	Cardiac output
CPET	Cardiopulmonary exercise test
ECG	Electrocardiogram
FEV1	Forced expiratory volume in the 1st second
FVC	Functional vital capacity
HCM	Hypertrophic cardiomyopathy
HR	Heart rate
IQR	Interquartile range
LV	Left ventricle
LVEF	Left ventricle ejection fraction
LVOTO	Left ventricular outflow tract obstruction
MR	Mitral regurgitation
OHCM	Obstructive hypertrophic cardiomyopathy
P/LP	Pathogenic /likely-pathogenic
PF	Physioflow
$PetCO_2$	Pressure end-tidal CO_2
RER	Respiratory exchange ratio
SAM	Systolic anterior movement
SBP	Systolic blood pressure
SD	Standard deviation
SM-ICG	Signal morphology-based impedance cardiography
SV	Stroke volume
VCO_2	Carbon dioxide production
VE	Ventilation
VO_2	Oxygen uptake

Hypertrophic Cardiomyopathy (HCM) is a primary heart muscle disease, defined by a left ventricular (LV) thickness of 15 mm or greater at any site, without any cardiac or systemic disease that could explain such a degree of hypertrophy[1,2].

The reduction in exercise tolerance is a common occurrence in individuals diagnosed with HCM, largely associated to early onset of symptoms such as shortness of breath, fatigue, angina, or syncope. The aetiology of these symptoms is multifactorial, related to structural and functional factors such as LV outflow obstruction (LVOTO), diastolic dysfunction, mitral regurgitation (MR), myocardial ischemia and arrhythmias[3].

Maximal Cardiopulmonary Exercise Test (CPET) is the gold standard for assessing functional capacity in patients with HCM[4,5]. Recent studies demonstrated functional improvement in HCM patients using new drug medications, with oxygen uptake (VO_2) being a key measurement[6,7].

Monitoring stroke volume (SV), heart rate (HR), and Cardiac Output (CO) during physical activity are essential for accurately assessing the hemodynamic impairment during exercise[8]. On this regard the O_2-pulse (calculated as VO_2/HR) evaluation has been utilized as a non-invasive surrogate of SV[9–12]. As regards HCM, an abnormal temporal O_2-pulse response during exercise was suggested as a marker of inadequate increase in SV, regardless of LVOTO[13]. Nevertheless, the O_2–pulse only provides an indirect estimation of SV, because the arterio-venous oxygen content difference ($\Delta a\text{-}vCO_2$) is a component of the O_2-pulse according to the Fick equation[14]. On the other hand, the direct measurement of SV and CO is invasive, potentially dangerous in HCM patients who have a high arrhythmic burden and, finally, can per se impair exercise performance.

The PhysioFlow (PF) device enables non-invasive, real-time hemodynamic monitoring, based on thoracic electrical impedance analysis[15]. Since it does not require patient cooperation nor effects exercise activity, it can be easily integrated with a standard CPET.

Previous studies investigated the role of PF in several cardiovascular settings including cardiac rehabilitation and heart failure[16–20]. However, data are lacking in the specific setting of HCM[16,21].

For this reason, this study aimed to evaluate the cardiopulmonary response to exercise in a multicentric cohort of HCM patients by integrating the measurements provided by CPET and PF. By correlating the SV behaviour with CPET parameters, we aim to understand exercise hemodynamic pathophysiology in HCM better and, consequently, to suggest possible improvement in diagnostic and therapeutic strategies.

Methods

Study population

In this multicenter, prospective, non-interventional study, we enrolled patients with HCM who performed CPET with simultaneous PF evaluation between January 2021 and March 2024 at two Italian referral centers for cardiomyopathies, the Hospital of Hospital of Cattinara in Trieste and Centro Cardiologico Monzino IRCCS. HCM was defined as the presence of maximum LV thickness ≥ 15 mm measured at any site in the absence of any cardiac or systemic disease capable of producing such magnitude of hypertrophy. A lower cut-off (13 mm) was applied in family members of HCM in conjunction with a positive genetic test, the presence of LVOTO, or a distinctly abnormal electrocardiogram (ECG)[3]. Obstructive HCM (OHCM) was diagnosed in case of rest or stress-induced LVOTO with a peak gradient exceeding 30 mmHg[3].

In the current study, patients were enrolled independently of LVOTO if they were clinically stable (i.e. at least six months after hospitalization for heart failure, invasive procedures, and change in drug therapy) and able to perform a maximal CPET. All patients were treated with optimized medical therapy according to the available

guidelines at the time of the study[22,23]. For this reason, none of the patients was treated with myosin inhibitors (i.e. Mavacamten). The echocardiographic parameters were collected from the closest exam to the CPET. A positive genetic finding was considered in the case of "pathological" or "likely pathological" mutation correlated to HCM phenotype[24].

Informed consent was obtained from all participants following the institutional review board policy of the two enrolling centres. The study was approved as R1638/22 CCM 1750 by the local Ethic committee (Comitato Etico Territoriale Lombardia 2). This investigation complies with the Helsinki Declaration.

Cardiopulmonary exercise testing (CPET)

CPET was conducted using an individualized cycle-ergometer ramp protocol, as previously described[25]. The exercise ramp protocol was personalized to achieve peak exercise between 8 and 12 minutes[26]. In the absence of clinical events, CPET was symptom-limited and self-interrupted by patients regardless of the peak respiratory exchange ratio (RER) achieved. A "breath-by-breath" analysis of respiratory gases and ventilation was performed.

PeakVO_2 was defined as the highest measured VO_2 rate during the final 30 s of the CPET. The Hansen/Wasserman equation was used to calculate the percentage of predicted peakVO_2 (%VO_2 peak)[27]. Exercise periodic breathing was defined as cyclic fluctuations in ventilation based on commonly used diagnostic criteria. The anaerobic threshold (AT) was calculated by the V-Slope graphic analysis of carbon dioxide production (VCO_2) and VO_2, thereafter confirmed by examining ventilatory equivalents and end-tidal pressures of CO_2 and O_2. Premature AT was defined by values < 40% of the predicted % peakVO_2[28]. Peak O_2-pulse was determined as peakVO_2/HR.

The behaviour of the O_2-pulse during exertion was evaluated by visually inspecting the shape of the O_2-pulse/work relationship, as previously described by Mapelli et al.[8]. Specifically, patients were categorized according to 4 O_2-pulse patterns during exercise: continuously up-sloping, late flattening, early flattening and down-sloping; these latter two have been interpreted as a pathological behaviour in the trend of the O_2-pulse (Fig. 1A-B).

The ventilation-to-carbon dioxide output (VE/VCO_2) slope was calculated as the linear relationship between VE and VCO_2 from 1 min after the onset of loaded exercise until the end of the isocapnic buffering period as previously outlined[25]. Predicted maximal HR was defined using the Fox formula (220 – age). The percentage of predicted HR peak (%peakHR) was calculated as (peakHR / predicted maximal HR)*100[29].

The VyntusTM CPX Metabolic Cart (Vyaire Medical®) or QuarkTM CPET Metabolic Cart (COSMED®) were the equipment used in this study. CPET data were collected by re-analyzing the related reports by three expert independent operators (NB, TMC, NC), without being aware of the patients' status during reanalysis.

Transthoracic impedance cardiography

Patients' hemodynamic parameters were assessed with a non-invasive Signal Morphology-based Impedance Cardiograph (SM-ICG) with real-time monitoring. The PF device employed a six-electrode system (HTFS50PF, PF07 Q-Link, Manatec Biomedical, Macheren, France) to detect morphology-based impedance cardiography signals. These electrodes were placed on the patient's body and connected to an electronic unit that generates a high-frequency (66 kHz) and low-amplitude current (4.5 mA). This methodology is based on variations in thoracic impedance during cardiac ejection, producing a specific waveform to calculate SV non-invasively[15]. The High-Definition Impedance was computed to filter all the artifacts from the chest impedance that are not correlated with the heart cycle and the cardiac flow.

Before the application of the electrodes, the patients' skin was prepared with a mildly abrasive gel (NuPrep®). Electrodes were placed once the skin had dried[30]. Alternative configurations of the electrodes were used in case of wide QRS, paced rhythm or deep inverted T waves at electrocardiogram, as indicated in technical instructions. Rest signal calibration of 30 s was mandatory before starting the registration.

In all patients, PF parameters were measured at rest before the exercise testing and during the CPET. To assess the behavior of cardiac index (CI), and SV, both a qualitative and a computerized analysis were employed. The first one was evaluated by visually inspecting the curve, following the above-mentioned method for the O_2-pulse (see Fig. 1C-D). The CPETs (particularly the PF parameters) were independently evaluated by two operators with extensive experience in cardiopulmonary exercise testing. In case of any disagreement, a third expert was involved to reach a consensus. The second one allowed the evaluation of SV and CI kinetics calculating the slope of their curves in the last 25% of the exercise. All exams with poor mean signal quality (lower than 80%) were excluded as a proposal of the study.

Statistical analysis

Descriptive statistics for clinical and instrumental variables were presented as mean ± standard deviation (SD), median (interquartile range, IQR), or counts and percentages, depending on the data type. The T-test was applied for continuous variables with a normal distribution. For continuous variables that did not follow a Gaussian distribution, the Mann-Whitney U test was used. The Chi-square test was applied to categorical variables, with the Fisher's exact test used when appropriate.

The behavior of PF parameters was analyzed by determining the slope of the linear regression between each variable of interest (dependent variable) and exercise duration (independent variable). The SV slope was expressed in μL/s, while the CI slope was calculated in $cL/min/m^2/s$. The change in SV (ΔSV) was calculated as ΔSV = peakSV – restSV. All statistical analyses were conducted using IBM SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA).

Part of the data presented in this manuscript has previously been reported in abstract form as the summary of a presentation delivered at an international congress[31]; the present paper provides the complete dataset and full analyses.

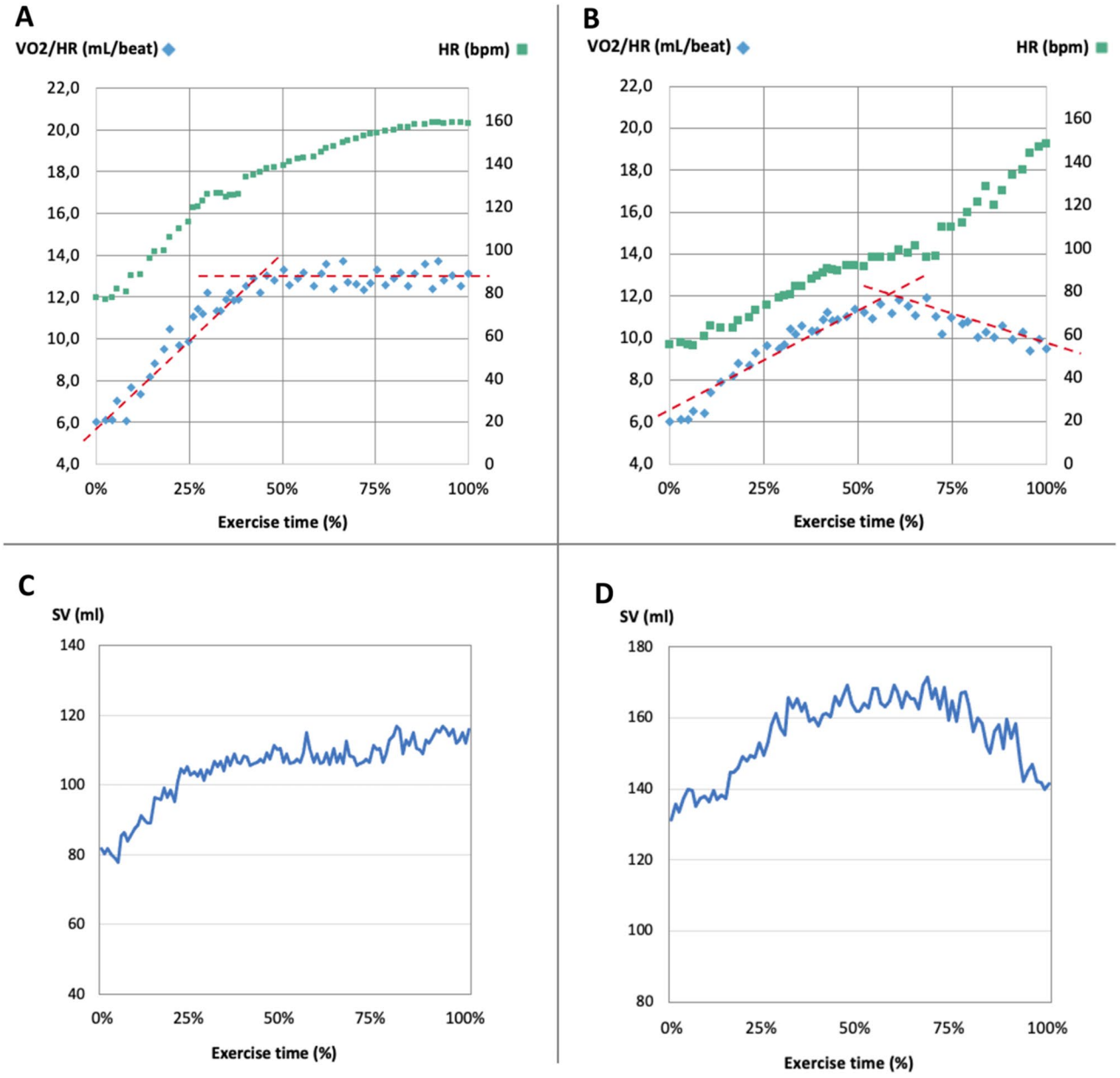

Fig. 1. Pathological O_2-pulse and stroke volume (SV) kinetics. The figure shows in the upper panels two abnormal O_2-pulse behaviors (Panel **A**: early flattening, Panel **B**: downsloping). In the lower panel similar exercise-induced behaviors are shown for SV evaluation with PF (Panel **C**: early flattening; Panel **D**: downsloping). SV: Stroke Volume; PF: Physioflow.

Results

Study population characteristics

The study cohort comprised 102 patients with HCM (mean age 53 ± 16 years; male 78%), with a prevalent phenotype of non-OHCM (74%). Approximately 10% of the originally enrolled patients were excluded from the study because they did not meet the minimum quality criteria for the Physioflow signal.

The main characteristics of the population are shown in Table 1. As regards patients with LVOTO ($n = 27$), 16% ($n = 16$) of the entire population presented obstruction at rest, while 11% ($n = 11$) during exercise or the Valsalva manoeuvre. Maximal LVOTO gradient was 54 [35–78] mmHg. All patients exhibited severe LV hypertrophy (median LV thickness 18 mm [15–21]), and 40% of the population presented a systolic anterior movement (SAM) at cardiac ultrasound. The global median LV ejection fraction (LVEF) was preserved (62% [58–68]), an LVEF < 50% was present in 6 patients (6%). Genetic testing was available in 49 over 102 (48%) cases and yielded positive results in 25 cases (51% of the tested population). Rhythm alterations, such as atrial fibrillation and previous pacemaker implantation, were identified in 7% of the cohort. A comparison of the patients with non-OHCM and OHCM (Table 1) revealed a significantly higher prevalence of SAM, previous

	Overall population ($n=102$)	no-LVOTO ($n=75$)	LVOTO ($n=27$)	p-value
Age (years)	53 ± 16	53 ± 15	53 ± 18	0.903
BMI (Kg/m^2)	26 ± 4	26 ± 4	27 ± 3	0.057
Male sex (n, %)	80 (78%)	59 (79%)	21 (78%)	0.923
LVEF (%)	62 [58–68]	62 [58–67]	62 [57–72]	0.206
LVEF < 50% (n, %)	6 (6%)	5 (7%)	1 (4%)	0.547
Maximal LV thickness (mm)	18 [15–21]	17 [15–21]	18 [16.5–20]	0.269
SAM (n, %)	41 (40%)	19 (25%)	22 (82%)	**< 0.001**
Moderate to severe MR (n, %)	18 (18%)	9 (12%)	9 (36%)	**0.007**
Sinus rhythm (n, %)	94 (93%)	69 (93%)	25 (93%)	0.909
AF or paced rhythm (n, %)	7 (7%)	5 (7%)	2 (7%)	0.909
Beta-blockers (n, %)	82 (81%)	58 (78%)	24 (89%)	0.232
Verapamil (n, %)	10 (10%)	4 (5%)	6 (22%)	**0.012**
Disopyramide (n°, %)	3 (3%)	0 (0%)	3 (11%)	**0.004**
Previous myectomy (n, %)	6 (6%)	2 (3%)	4 (15%)	**0.023**

Table 1. Clinical and demographic characteristics of the study population according to the presence of LVOTO (defined at gradient > 30 mmhg at rest or stress-induced). LVOTO: Left Ventricular Outflow Tract Obstruction; BMI: Body Mass Index; LVEF: Left Ventricular Ejection Fraction; LV: Left Ventricular; SAM: Systolic Anterior Motion; MR: Mitral Regurgitation; AF: Atrial Fibrillation.

septal myectomy surgery and moderate-to-severe MR in the group with LVOTO. Furthermore, the use of Verapamil or Disopyramide was more prevalent in patients with OHCM.

CPET and PF findings

Table 2; Fig. 2 show the main CPET and PF parameters in the entire population and according to LVOT presence/absence. In the whole population, we observed a mild reduction in functional capacity (%peakVO_2 82% [69–94]) with mild signs of chronotropic incompetence (peak HR = 81.3 ± 14.1% predicted value), and cardiogenic limitation and/or deconditioning (AT as %peakVO_2 = 46.9 ± 13.9%). No evidence of pulmonary vascular or ventilatory limitation was identified (VE/VCO_2 29.5 [26.0–33.2]; BR 36.3% [27.5–42]); peak $PetCO_2$ 33.4 ± 5.5 mmHg). The mean peak exercise O_2-pulse value was within the normal range (percentage of the predicted O_2-pulse value = 99.5 ± 22.7%). Nevertheless, abnormal O_2–pulse kinetics (qualitatively assessed) was observed in 12% of the patients with 3 cases showing an early plateau and 9 cases displaying a downsloping behaviour (Fig. 1, panel A-B). LVOT presence/absence did not influences O_2–pulse kinetics characteristics.

All patients exhibited normal values for SV and CI at rest (SV: 88 ml, [75–97]; CI: 3.0 ± 0.8 L/min/m^2). The difference between the SV value at rest and peak exercise (ΔSV) was 33 [22–47] ml. Abnormal SV kinetics was observed in 40 patients (39%), with 14 and 26 exhibiting an early plateau and downsloping, respectively (Fig. 1, panel C-D). No significant differences in PF derived parameters were found concerning the presence of LVOTO.

Comparison between normal vs. pathological O_2– pulse and SV behaviour

All patients with an abnormal O_2-pulse kinetic showed an abnormal SV trajectory. Conversely, PF identified 40 patients with abnormal SV trajectory, of those 28 had a normal O_2-pulse behaviour. Dividing the population based on O_2-pulse kinetics, we found that patients with a pathological O_2-pulse slope had a higher RER and peak VE/VO_2 and lower peak O_2-pulse value, VO_2/work slope value, and a lower SV slope in the last 25% of the exercise (Table 3). Notably, patients with pathological O_2-pulse slope exhibited a lower rest to peak ΔSV (14.6 ml [2.0–32.8] vs. 34.8 [25.5–49.9] $p = 0.003$).

Similarly, dividing the population into two groups based on SV kinetics (Table 3), among patients with abnormal SV kinetics, a lower ΔSV was confirmed, as well as a reduced SV slope growth in the last 25% of exercise (33 [12–72] µL/s vs. 46 [35–84 µL/s], $p = 0.003$). Patients with a pathological SV slope also exhibited worse pulmonary vascular efficiency in the last part of exercise, represented by higher VE/VO_2 ratio and lower $PetCO_2$ values at peak exercise.

The population was also subdivided according to the median value of the SV slope during the final 25% of exercise (Supplementary Table S1). Those with a SV slope above the median demonstrated superior functional capacity and cardiovascular performance, as suggested by a higher VO_2 at AT (49.8 ± 12.3% vs. 43.9 ± 15.2%, $p = 0.037$), a trend toward a higher peakVO_2 (22.2 mL/Kg/min [19.4–27.4] vs. 20.8 [15.6–26.6]; $p = 0.064$), and a significantly greater VO_2/work (10.2 ± 2.0 vs. 9.3 ± 1.3 ml/min/Watt, $p = 0.013$), and ΔSV (44.6 mL [33.2–66.4] vs. 26.9 [15.5–32.9]; p value (< 0.001). The group with a SV slope above the median value was also characterized by enhanced vascular pulmonary performance, as evidenced by a lower VE/VCO_2 slope (28.6 [24.9–31.0] vs. 31.4 [27.3–34.2]; $p = 0.003$) and higher peak exercise $PetCO_2$ (34.5 ± 5.6 mmHg vs. 32.3 ± 5.2; $p = 0.038$).

Discussion

This trial demonstrated for the first time that adding a non-invasive evaluation of SV with PF device, could help in better identify HCM patient with abnormal hemodynamic pattern during exercise. Moreover, lower SV values, as well as pathological SV kinetics during exercise, are both associated with worst key exercise parameters

	Overall population ($n=102$)	no LVOTO ($n=75$)	LVOTO ($n=27$)	p-value
Watt peak (Watts)	149 ± 61	155 ± 62	135 ± 54	0.146
RER peak	1.15 [1.10–1.22]	1.20 [1.10–1.20]	1.14 [1.10–1.20]	0.747
HR peak (bpm)	135 ± 27	138 ± 27	128 ± 26	0.104
%Peak-HR	81.3 ± 14.1	82.5 ± 14.6	77.9 ± 12.4	0.146
Peak VO_2 (ml/Kg/min)	22.1 [18.1–27.2]	22.5 [18.1–29.0]	20.5 [16.4–26.3]	0.363
Peak VO_2 (% of predicted valute)	82 [69–94]	82 [69–95]	83 [70–91]	0.603
Peak VE/VO_2	38.6 [34.2– 45.1]	38.2 [33.6–45.7]	40.0 [37.4–43.0]	0.258
Peak VE/VCO_2	33.9 [34.2–45.1]	32.7 [30.4–37.0]	34.4 [32.0–38.3]	0.184
%AT VO_2	46.9 ± 13.9	46.1 ± 8.4	47.2 ± 15.4	0.741
$\%O_2$-pulse peak	99.5 ± 22.7	98.3 ± 24.1	102.9 ± 18.3	0.373
Pathological O_2-pulse kinetic (n°, %)	12 (12%)	10 (13%)	2 (12%)	0.412
VO_2/Work slope (mL/min/Watt)	9.7 ± 1.8	9.7 ± 1.9	9.9 ± 1.5	0.469
VE/VCO_2 slope	29.5 [26–33.2]	29 [26–33]	31 [25.2–33.7]	0.614
Peak $PetCO_2$ (mmHg)	33.4 ± 5.5	33.4 ± 5.6	33.1 ± 38.6	0.800
Periodic Breathing (n°, %)	7 (6.9%)	5 (6.7%)	2 (7.4%)	0.896
PeakSV (ml)	119 [103–142]	115 [100–142]	122 [110–142]	0.406
DeltaSV (ml)	33 [22–47]	33 [22–46]	38 [16–54]	0.559
SV slope last 25% of exercise (μL/s)	44 [32–73]	40 [32–72]	47 [33–84]	0.159
Pathological SV kinetic (n°, %)	40 (39%)	28 (37%)	12 (44%)	0.516
Pathological CI kinetic (n°, %)	22 (22%)	15 (20%)	7 (26%)	0.521
CI rest (L/min/m2)	3.0 ± 0.8	3.0 ± 0.8	2.9 ± 0.8	0.508
CI peak (L/min/m2)	8.7 ± 2.9	8.7 ± 2.8	8.6 ± 3.3	0.865
Pathological CI slope (qualitative) (n°. %)	22 (22%)	15 (20%)	7 (26%)	0.521
CI Slope 25% before recovery (quantitative), (cL/min/m2/s)	0.9 [0.6–1.2]	0.9 [0.6–1.1]	0.9 [0.5–1.3]	0.952
SV rest (ml)	88 [75–97]	86 [74–93]	94 [75–107]	0.178

Table 2. Main CPET and PF parameters and their differences based on the presence of LVOTO. CPET: Cardiopulmonary Exercise Testing; PF: Physioflow; LVOTO: Left Ventricle Outflow Tract Obstruction; RER: Respiratory Exchange Ratio; HR: Heart Rate; %Peak HR: HR at peak exercise as % of the predicted; VO_2: Oxygen Uptake; AT: Anaerobic Threshold; VE/VCO_2: Minute ventilation-to-carbon dioxide output; $PetCO_2$: end-tidal CO_2; SV: Stroke volume; ΔSV: peakSV – restSV; CI: Cardiac Index.

measured by CPET during a maximal effort. Interestingly, PF evaluation is more sensitive than the one limited to metabolic data, being able to identify up to 28% more patients with impaired exercise hemodynamic (Fig. 3), compared to O_2-pulse kinetic by CPET analysis alone. This indicates a potential additional role of PF in HCM, even in mostly asymptomatic patients. Of note, none of the patients with normal SV behaviour exhibited abnormal O_2-pulse kinetics. Furthermore, in patients with abnormal SV kinetic, CO increase was lower (lower SV at peak and ΔSV) and this was accompanied by development of pulmonary vascular limitation at the end of exercise (as shown by peak exercise $PetCO_2$ and VE/VO_2 values and absence of VE/VCO_2 slope difference) at CPET (Table 3). This suggests how the inability to adequately increase CO during the effort may represent a key pathophysiological mechanism in HCM patients. Indeed, a flattening (i.e. incapacity to appropriately increase VO_2 to the level of exercise) of O_2-pulse and SV along the stages of graded exercise was demonstrated in our population. This flattening was previously shown to be associated with high-risk features and worse prognosis[32]. While a O_2-pulse slope flattening if often the result of multiple contributors to effort intolerance including blunted SV response, worse diastolic function, but also the incapacity of the exercising muscles to appropriately extract O_2 from the blood, the SV measurement gives us the opportunity to solve the Fick equation and to understand how much of the VO_2 deficit can be attributed to a peripheral extraction problem.

Our study cohort consisted of middle-aged participants with mild impairment in functional capacity and no signs of pulmonary vascular limitation (Table 1). As previously described, HCM is characterized by exercise-induced chronotropic incompetence[33,34]. In our population, peak HR was 81% of predicted value which was associated to a concomitant poor SV reserve (mean ΔSV was only 33 ml). Surprisingly, these alterations in CPET and PF occurred regardless of LVOTO presence (Table 2), as a confirmation of a previous study from our group[13]. This suggests that even mostly asymptomatic, NOHCM patients could present pathologic hemodynamic responses during maximal exercise. Moreover, the present findings emphasize, once again, the marginal role of the rest LVOT gradient in stratifying the functional limitation of HCM patients suggesting that it should not be the only therapeutic target to focus on. Our data also indicates that LVOT gradient at rest was also not related to reduced SV values, nor at rest neither during exercise despite the higher prevalence of moderate or severe MR in HOCM participants compared to non-obstructive counterparts.

Our data must be seen in the context of what is already known in the literature. Lele et al.[35] previously suggested that SV, invasively measured, could be the major determinant of peak exercise capacity in HCM. In a recent study by Erez et al.[36], the authors tried to correlate SV measurement obtained with stress echocardiography to

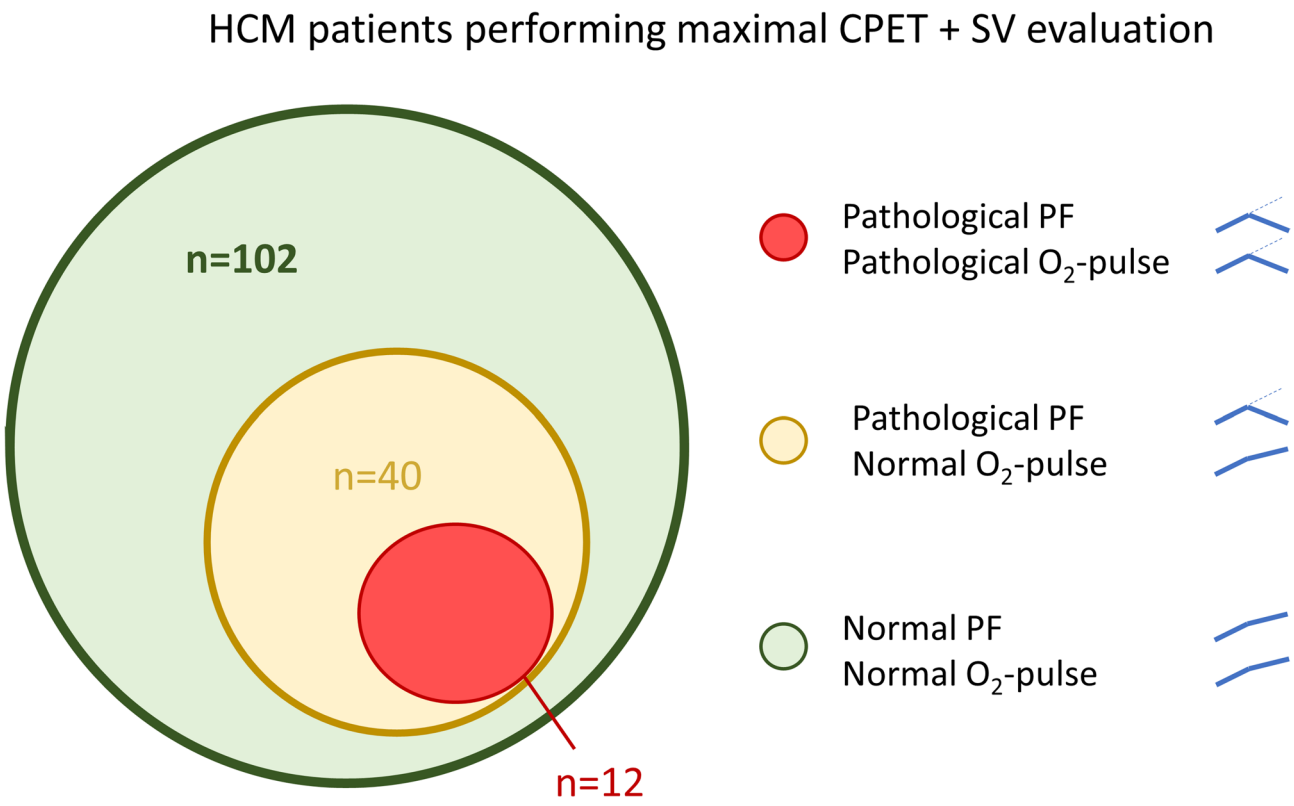

Fig. 2. The additional role of physioflow (PF) in detecting abnormal exercise-induced hemodynamic responses. This figure highlights the proportion of patients identified with abnormal hemodynamic responses using PF, in addition to those identified through O_2-pulse behavior alone. Abbreviations are explained in the Fig. 1 legend.

	Normal O_2-pulse kinetic ($n=90$)	Pathological O_2-pulse kinetic ($n=12$)	*p*-value	Normal SV kinetic ($n=62$)	Pathological SV kinetic ($n=40$)	*p*-value
% Peak-HR	80.1 ± 13.9	90.1 ± 12.7	0.021	80.3 ± 13.4	82.8 ± 15.2	0.378
Peak RER	1.14 [1.10–1.20]	1.24 [1.12–1.34]	**0.041**	1.14 [1.10–1.20]	1.17 [1.11–1.29]	0.063
PeakVO_2 (% of the predicted)	82.9 [68.8–94.0]	79.5 [70.7–90.8]	0.670	82.9 [69.8–95.0]	82.0 [64.5–92.0]	0.537
Peak O_2-pulse (mL/bpm)	13.7 ± 3.5	10.8 ± 4.7	**0.011**	13.7 ± 3.5	12.9 ± 4.1	0.341
Peak O_2-pulse (% of the predicted)	100.8 ± 22.8	90.2 ± 21.2	0.130	102.4 ± 19.7	95.1 ± 26.4	0.114
VO_2/Work slope (mL/min/Watt)	9.8 ± 1.8	9.2 ± 1.2	0.285	9.8 ± 1.7	9.7 ± 1.9	0.817
Pathological VO_2/Work Slope kinetic (n°, %)	0 (0%)	6 (50%)	**< 0.001**	0 (0%)	6 (15%)	**0.002**
VE/VCO_2 slope	29.4 [26.0–33.3]	30.5 [27.0–32.9]	0.571	29.4 [26.2–32.8]	29.8 [26.0–34.2]	0.375
Peak VE/VO_2	38.3 [33.8–43.1]	44.1 [38.0–49.6]	**0.029**	38.0 [33.6–41.3]	42.6 [37.4–47.5]	**0.004**
Peak VE/VCO_2	33.7 [30.6–37.3]	36.4 [30.9–42.4]	0.232	32.5 [30.2–37.1]	35.2 [31.3–39.1]	0.096
Peak $PetCO_2$ (mmHg)	33.6 ± 5.6	31.3 ± 4.2	0.162	34.3 ± 5.6	31.8 ± 4.9	**0.024**
Peak SV (mL)	119.3 [103.9–142.3]	110.3 [89.2–137.2]	0.375	123.6 [105.3–149.4]	112.7 [97.5–136.9]	0.082
ΔSV (mL)	34.8 [25.5–49.9]	14.6 [3.8–32.8]	**0.003**	37.0 [27.9–57.3]	29.3 [10.2–37.0]	**< 0.001**
SV slope last 25% of exercise (μL/s)	45 [33–74]	14 [6–49]	**0.019**	46 [35–84]	33 [12–72]	**0.003**
Pathological CI kinetic (n°, %)	17 (19%)	5 (42%)	0.072	1 (2%)	21 (53%)	**< 0.001**
Beta-blockers (n, %)	72 (80%)	10 (83%)	0.839	51 (82%)	31 (78%)	0.442
Verapamil (n, %)	10 (11%)	0 (0%)	0.221	5 (8%)	5 (12%)	0.479
Disopyramide (n, %)	3 (3%)	0 (0%)	0.518	1 (2%)	2 (5%)	0.331
Previous myectomy (n, %)	4 (4%)	2 (17%)	0.094	2 (3%)	4 (10%)	0.162

Table 3. Comparison between normal vs. pathological O_2-pulse slope and SV slope. SV: Stroke Volume; VO_2: Oxygen Uptake; VE/VCO_2: Minute ventilation-to-carbon dioxide output; VE/VO_2: Minute ventilation-to-Oxygen intake; $PetCO_2$: end-tidal CO_2; ΔSV: peak SV – rest SV.

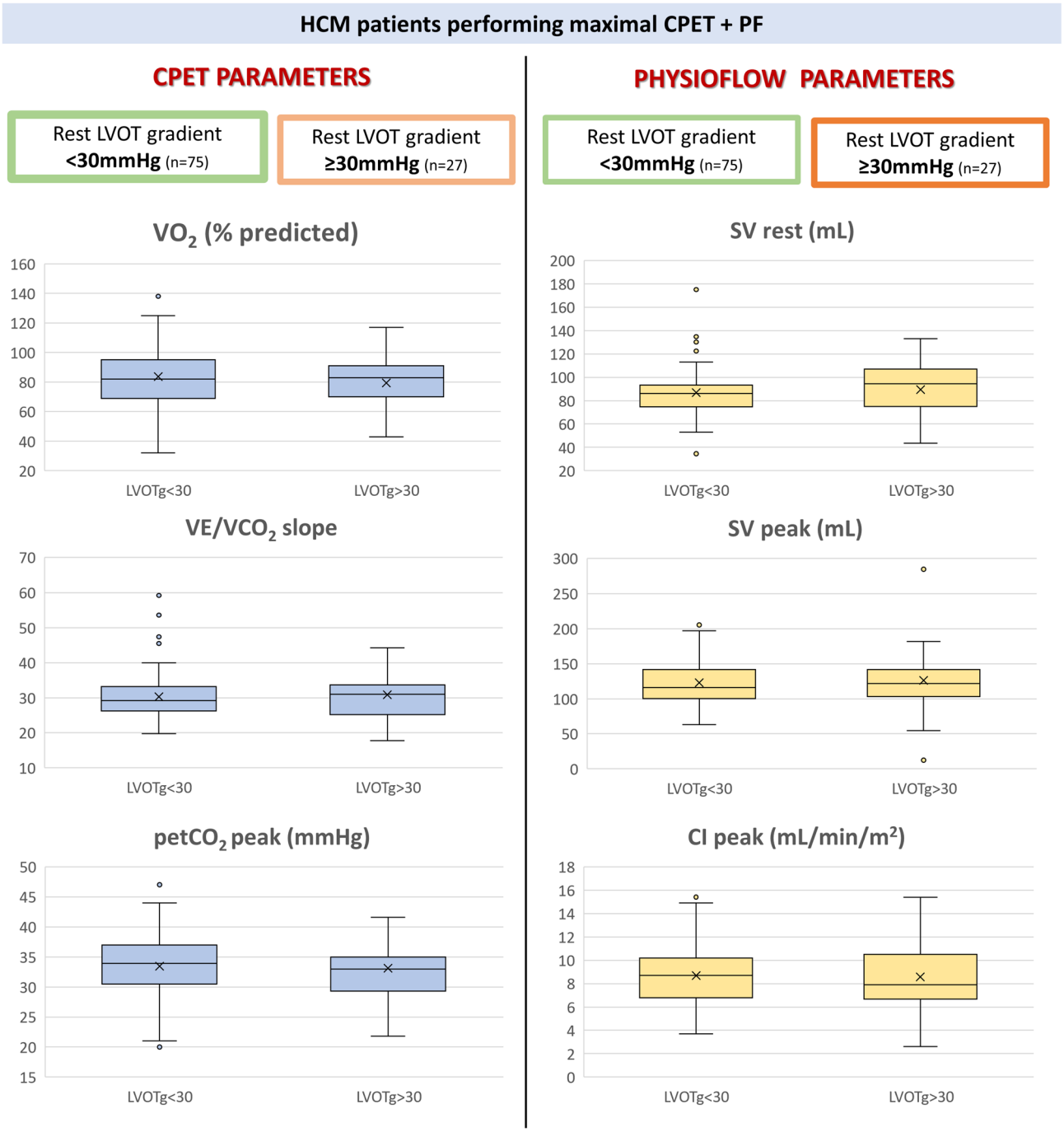

Fig. 3. Difference in cardiopulmonary exercise test (CPET) and Physioflow (PF) parameters by diagnosis of obstructive hypertrophic cardiomyopathy (HCM). The figure compares key CPET parameters (left panel) and PF parameters (right panel) based on the diagnosis of obstructive HCM, defined as stress-induced left ventricular outflow tract obstruction (LVOTO) with a peak gradient greater than 30 mmHg. No parameter showed statistical significance. CI: cardiac index; LVOT: left ventricular outflow tract; $petCO_2$: pressure end-tidal for carbon dioxide; VE/VCO_2 slope: relationship between ventilation (VE) and CO_2 production. For other abbreviations, see Fig. 1 legend.

functional capacity and demonstrated how O_2 intake limitation in HCM was affected by different and interacting factors, both central and peripheral (diastolic dysfunction, supraventricular tachyarrhythmias, peripheral O_2 extraction). Of note, their data indicate no significant differences between HOCM and NOHCM in terms of VO_2, SV, and VE/VCO_2. However, a previous trial with PF by Finocchiaro et al.[37] involving 156 HCM patients demonstrated how peak CI was the main determinant of exercise capacity, being strongly related to peak VO_2 but not to VE/VCO_2. Interestingly, the authors showed how the CI and CPET data were unrelated to HCM genotype and confirmed the prognostic role of peakVO_2 and VE/VCO_2 slope[38]. Indeed, while the prognostic

role of peakVO_2 and VE/VCO_2 slope is well known, only few data are available to suggest the impact of CI on survival in HCM patients and, mainly, refer to rest values[39].

Taken together, the data obtained implementing the PF tool during CPET suggested that an abnormal O_2-pulse corresponded with a pathological behaviour of the measured SV. However, SV increase may be low regardless of a normal O_2-pulse kinetics likely due to a compensatory high Δ a-vCO_2 during maximal effort.

Finally, we showed that the computerized analysis of the SV slope calculated in the last 25% of the exercise was feasible and the data were consistent with the qualitative assessment performed by expert operators. The evaluation of the SV curve trend in the last 25% of the exercise has furthermore allowed the characterization of patients based on their functional capacity and pulmonary vascular limitations. Moreover, it is possible to suggest, albeit not directly tested in the present analysis, that patients with abnormal O_2-pulse pattern have the worst hemodynamic pattern and, consequently the greater anaerobic activity at peak exercise as suggested by lowest ΔSV and SV slope the former and highest peak RER, the latter. This is, however, only a fascinating hypothesis at this stage.

Since PF does not require patient cooperation and can be performed at rest or during CPET without significantly affecting its duration or cost, it represents a practical addition to standard testing. The required training is comparable to that for other cardiac output estimation methods, with most effort focused on minimizing artifacts. Therefore, we believe PF can be widely integrated with CPET, when appropriate, to distinguish between cardiogenic limitations and deconditioning.

Further studies are needed to confirm our results and to put them in clinical practice together with outcome data. Future perspectives could explore the potential prognostic of these exercise-induced hemodynamic changes in HCM. At this regard, we recently conducted a prospective study aimed at classifying HCM patients based on SV, O_2-pulse, and HR during exercise (the "RoMa classification"[39]). Although these are not outcome data, the study clearly shows that chronotropic incompetence and reduced SV increase are key parameters correlating with important prognostic variables in cardiomyopathies, such as peak VO_2 and VE/VCO_2 slope. With the accumulation of follow-up events over time, it will be of great interest to correlate these exercise-derived hemodynamic data with actual patient prognosis. This represents a clinical need in the era of new drug treatment for HCM[6].

Study limitations

Our study has several limitations worth mentioning. The continuous assessment of CO through the PF method was not paired with a simultaneous echocardiographic evaluation. Therefore, the underlying pathophysiological rationale for the observed pathological trend in SV is based solely on a hypothesis rather than a specific identifiable functional alteration (e.g., exercise-induced MR, increased filling pressures, diastolic dysfunction). The PF method for estimating SV and CO may be affected by poor agreement with gold-standard methods, such as the Fick method or thermodilution, and it can over- or underestimate values in specific populations. The algorithm relies heavily on anthropometric data, which can reduce reliability in patients with extreme body characteristics or chronic conditions. Technical factors, including motion artifacts during exercise and electrode placement, may also affect signal quality. However, in our population the anthropometric characteristics were favourable (non-obese, relatively young patients), and in the hands of experienced operators, signal quality was consistently sufficient to ensure reliable assessment.

The visual assessment of the O_2-pulse kinetics is arbitrary and possibly operator-dependent. However, our results were reproducible when obtained by different operators. Furthermore, to reduce this potential source of bias, a computerized evaluation was employed to analyse the O_2-pulse and SV trend, which demonstrated consistency with the visual assessment. Indeed, we reported for the first time the use of a computerized assessment of SV changes in the last 25% of active exercise. Consequently, no normal value is available so that we grouped our population above and below the observed median value. According applying the visual inspection analysis, we might have included patients with normal SV increase in the low SV/time slope group. The functional capacity among the patients enrolled in the study was only slightly impaired as most patients exhibited minimal symptoms. Therefore, our findings may not be generalizable to more severe HCM patients. Despite the numerous listed limitations, it should be emphasized that, to our knowledge, this is the first and largest study to non-invasively investigate the hemodynamic response of the SV during exercise in patients with HCM, regardless of LVOTO. Further studies are needed to confirm our results and, more importantly, to correlate them with prognostic and therapeutic data.

Data availability

The datasets generated and/or analysed during the current study are available in the Zenodo repository (www.zenodo.org). Link: https://zenodo.org/records/14280845.

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References

1. Maron, B. J. Clinical course and management of hypertrophic cardiomyopathy. *N Engl. J. Med.* **379**, 655–668. https://doi.org/10.1056/NEJMra1710575 (2018).
2. Maron, B. J. & Maron, M. S. Hypertrophic cardiomyopathy. *Lancet* **381**, 242–255. https://doi.org/10.1016/S0140-6736(12)60397-3 (2013).
3. Maron, B. J. et al. Diagnosis and evaluation of hypertrophic cardiomyopathy: JACC State-of-the-Art review. *J. Am. Coll. Cardiol.* **79**, 372–389. https://doi.org/10.1016/j.jacc.2021.12.002 (2022).

4. Coats, C. J. et al. Cardiopulmonary exercise testing and prognosis in hypertrophic cardiomyopathy. *Circ. Heart Fail.* **8**, 1022–1031. https://doi.org/10.1161/CIRCHEARTFAILURE.114.002248 (2015).
5. Mikic, L. et al. The role of cardiopulmonary exercise testing in hypertrophic cardiomyopathy. *Med. (Kaunas).* **59** https://doi.org/10.3390/medicina59071296 (2023).
6. Olivotto, I. et al. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **396**, 759–769. https://doi.org/10.1016/S0140-6736(20)31792-X (2020).
7. Wheeler, M. T. et al. Effects of Mavacamten on measures of cardiopulmonary exercise testing beyond peak oxygen consumption: A secondary analysis of the EXPLORER-HCM randomized trial. *JAMA Cardiol.* **8**, 240–247. https://doi.org/10.1001/jamacardio.2022.5099 (2023).
8. Agostoni, P. et al. Deceived by the Fick principle: blood flow distribution in heart failure. *Eur. J. Prev. Cardiol.* https://doi.org/10.1093/eurjpc/zwae203 (2024).
9. Agostoni, P. G. et al. Non-invasive measurement of stroke volume during exercise in heart failure patients. *Clin. Sci. (Lond).* **98**, 545–551 (2000).
10. Belardinelli, R. et al. Exercise-induced myocardial ischaemia detected by cardiopulmonary exercise testing. *Eur. Heart J.* **24**, 1304–1313. https://doi.org/10.1016/s0195-668x(03)00210-0 (2003).
11. Munhoz, E. C. et al. Flattening of oxygen pulse during exercise May detect extensive myocardial ischemia. *Med. Sci. Sports Exerc.* **39**, 1221–1226. https://doi.org/10.1249/mss.0b013e3180601136 (2007).
12. Liu, W. et al. The value of cardiopulmonary exercise testing in predicting the severity of coronary artery disease. *J. Clin. Med.* **11** https://doi.org/10.3390/jcm11144170 (2022).
13. Mapelli, M. et al. Exercise oxygen pulse kinetics in patients with hypertrophic cardiomyopathy. *Heart* **108**, 1629–1636. https://doi.org/10.1136/heartjnl-2021-320569 (2022).
14. Mattavelli, I. et al. Beyond VO2: the complex cardiopulmonary exercise test. *Eur. J. Prev. Cardiol.* **30**, ii34–ii39. https://doi.org/10.1093/eurjpc/zwad154 (2023).
15. Richard, R. et al. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur. J. Appl. Physiol.* **85**, 202–207. https://doi.org/10.1007/s004210100458 (2001).
16. Greenberg, B. H., Hermann, D. D., Pranulis, M. F., Lazio, L. & Cloutier, D. Reproducibility of impedance cardiography hemodynamic measures in clinically stable heart failure patients. *Congest Heart Fail.* **6**, 74–80. https://doi.org/10.1111/j.1527-5299.2000.80140.x (2000).
17. Kurpaska, M., Krzesinski, P., Gielerak, G., Golebiewska, K. & Piotrowicz, K. Cardiopulmonary exercise testing and impedance cardiography in the assessment of exercise capacity of patients with coronary artery disease early after myocardial revascularization. *BMC Sports Sci. Med. Rehabil.* **14**, 134. https://doi.org/10.1186/s13102-022-00527-w (2022).
18. Taylor, K. et al. A comparison of cardiac output by thoracic impedance and direct Fick in children with congenital heart disease undergoing diagnostic cardiac catheterization. *J. Cardiothorac. Vasc Anesth.* **25**, 776–779. https://doi.org/10.1053/j.jvca.2011.05.002 (2011).
19. Gonzalez-Represas, A. & Mourot, L. Stroke volume and cardiac output measurement in cardiac patients during a rehabilitation program: comparison between tonometry, impedancemetry and echocardiography. *Int. J. Cardiovasc. Imaging.* **36**, 447–455. https://doi.org/10.1007/s10554-019-01738-y (2020).
20. Fukuda, T. et al. Cardiac output response to exercise in chronic cardiac failure patients. *Int. Heart J.* **53**, 293–298. https://doi.org/10.1536/ihj.53.293 (2012).
21. Charloux, A. et al. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the direct Fick method. *Eur. J. Appl. Physiol.* **82**, 313–320. https://doi.org/10.1007/s004210000226 (2000).
22. Writing Committee, M. et al. AHA/ACC/AMSSM/HRS/PACES/SCMR guideline for the management of hypertrophic cardiomyopathy: A report of the American Heart Association/American college of cardiology loint committee on clinical practice guidelines. *J. Am. Coll. Cardiol.* **83**, 2324–2405 https://doi.org/10.1016/j.jacc.2024.02.014 (2024).
23. Force, A. T. et al. m. ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the European society of cardiology (ESC). *Eur. Heart J.* **35**, 2733–2779 https://doi.org/10.1093/eurheartj/ehu284 (2014).
24. Marian, A. J. Molecular genetic basis of hypertrophic cardiomyopathy. *Circ. Res.* **128**, 1533–1553. https://doi.org/10.1161/CIRCRESAHA.121.318346 (2021).
25. Agostoni, P. & Dumitrescu, D. How to perform and report a cardiopulmonary exercise test in patients with chronic heart failure. *Int. J. Cardiol.* **288**, 107–113. https://doi.org/10.1016/j.ijcard.2019.04.053 (2019).
26. Agostoni, P. et al. Work-rate affects cardiopulmonary exercise test results in heart failure. *Eur. J. Heart Fail.* **7**, 498–504. https://doi.org/10.1016/j.ejheart.2004.06.007 (2005).
27. Wasserman, K. *Principles of Exercise Testing and Interpretation: Including Pathophysiology and Clinical Applications* (Wolters Kluwer Health/Lippincott Williams & Wilkins, 2012).
28. Salvioni, E. et al. Pick your threshold: A comparison among different methods of anaerobic threshold evaluation in heart failure prognostic assessment. *Chest* **162**, 1106–1115. https://doi.org/10.1016/j.chest.2022.05.039 (2022).
29. Magri, D. et al. Old and new equations for maximal heart rate prediction in patients with heart failure and reduced ejection fraction on beta-blockers treatment: results from the MECKI score data set. *Eur. J. Prev. Cardiol.* **29**, 1680–1688. https://doi.org/10.1093/eurjpc/zwac099 (2022).
30. Nasis, I. et al. Hemodynamic effects of high intensity interval training in COPD patients exhibiting exercise-induced dynamic hyperinflation. *Respir Physiol. Neurobiol.* **217**, 8–16. https://doi.org/10.1016/j.resp.2015.06.006 (2015).
31. Mapelli, M. et al. The adding value of non-invasive assessment of stroke volume during cardiopulmonary exercise testing in hypertrophic cardiomyopathy: beyond O2-pulse *Eur. J. Prevent. Cardiol.* **32**, Supplement_1 (2025).
32. Guazzi, M. et al. EACPR/AHA joint scientific Statement. Clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *Eur. Heart J.* **33**, 2917–2927. https://doi.org/10.1093/eurheartj/ehs221 (2012).
33. Efthimiadis, G. K. et al. Chronotropic incompetence and its relation to exercise intolerance in hypertrophic cardiomyopathy. *Int. J. Cardiol.* **153**, 179–184. https://doi.org/10.1016/j.ijcard.2010.08.026 (2011).
34. Magri, D. et al. Clinical and prognostic impact of chronotropic incompetence in patients with hypertrophic cardiomyopathy. *Int. J. Cardiol.* **271**, 125–131. https://doi.org/10.1016/j.ijcard.2018.04.019 (2018).
35. Lele, S. S. et al. Exercise capacity in hypertrophic cardiomyopathy. Role of stroke volume limitation, heart rate, and diastolic filling characteristics. *Circulation* **92**, 2886–2894. https://doi.org/10.1161/01.cir.92.10.2886 (1995).
36. Erez, Y. et al. Exercise limitation in hypertrophic cardiomyopathy: combined stress echocardiography and cardiopulmonary exercise test. *ESC Heart Fail.* **11**, 2287–2294. https://doi.org/10.1002/ehf2.14776 (2024).
37. Finocchiaro, G. et al. Cardiopulmonary responses and prognosis in hypertrophic cardiomyopathy: a potential role for comprehensive noninvasive hemodynamic assessment. *JACC Heart Fail.* **3**, 408–418. https://doi.org/10.1016/j.jchf.2014.11.011 (2015).
38. Magri, D. et al. Risk stratification in hypertrophic Cardiomyopathy. Insights from genetic analysis and cardiopulmonary exercise testing. *J. Clin. Med.* **9** https://doi.org/10.3390/jcm9061636 (2020).

39. Zhang, Y. et al. Cardiac index: A superior parameter of cardiac function than left ventricular ejection fraction in risk stratification of hypertrophic cardiomyopathy. *Heart Rhythm*. **20**, 958–967. https://doi.org/10.1016/j.hrthm.2023.02.028 (2023).

Author contributions

M. M., N. B., N. C.: Conceptualization, Investigation, Data curation, Writing—original draftE. S., N. C.: Data curation, Formal analysis, Writing—original draftT. M. C., (A) N., C. V., F. M. R., D. M., (B) P., I. M., S.C., F.P., (C) C., I. T., M. R., C. C. D., R. W., M. M., G. S.: Investigation, Writing—review & editingP. A.: Conceptualization, Investigation, Project administration; Supervision; Data curation, Writing—original draft.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The study was approved by the local ethics committee R1638/22 CCM 1750 (Comitato Etico Territoriale Lombardia 2).

Additional information

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