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ORIGINAL PAPER

MRI-derived wall thickness heterogeneity in hypertrophic cardiomyopathy and in carriers of sarcomeric gene mutations

Giovanni Donato Aquaro [1*†], Roberto Licordari[2†], Giancarlo Todiere[3†], Umberto Ianni [4], Carmelo De Gori[3], Marco Merlo [5], Andrea Barison[3], Antonio De Luca[5], Crysanthos Grigoratos[3], Francesca Raimondi[6], Lamia Ait-Ali[3], Pierluigi Festa[3], Francesco Negri[5], Francesco Bianco[7], Fabrizio Ricci[8], Nicoletta Botto[3], Lapo Taddei[9], Mattia Alberti[1], Andrea Italiano[1], Luna Gargani[1], Lorenzo Faggioni [9], Dania Cioni[1], Riccardo Lencioni[1], Michele Emdin[3], Emanuele Neri[9], Gianfranco Sinagra[5], Raffaele De Caterina [1], and Gianluca Di Bella [2]

[1]Department of Surgical, Medical and Molecular Pathology and of Critical Area, University of Pisa, Via Savi, 10, Pisa 56126, Italy; [2]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; [3]Cardiothoracic Department, Fondazione Toscana G. Monasterio, Pisa, Italy; [4]Cardiology Department, Madonna del Soccorso Hospital, San Benedetto del Tronto, Italy; [5]Cardio-thoraco-vascular Department, University of Trieste, Trieste, Italy; [6]Cardiology Unit 2—Congenital Heart Disease in Children and Adults, Papa Giovanni XXIII Hospital, Bergamo, Italy; [7]Department of Cardiovascular Science, Ospedali Riuniti (University Hospital), Ancona, Italy; [8]Department of Neuroscience, Imaging and Clinical Science, University of Chieti, Chieti, Italy; and [9]Department of Translational Research and of New Technology, University of Pisa, Pisa, Italy

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Aims Hypertrophic cardiomyopathy (HCM) shows variable left ventricular hypertrophy, yet current diagnostic criteria rely on absolute wall thickness (WT) cut-offs that may miss early or subtle phenotypes, particularly in sarcomere mutation carriers.

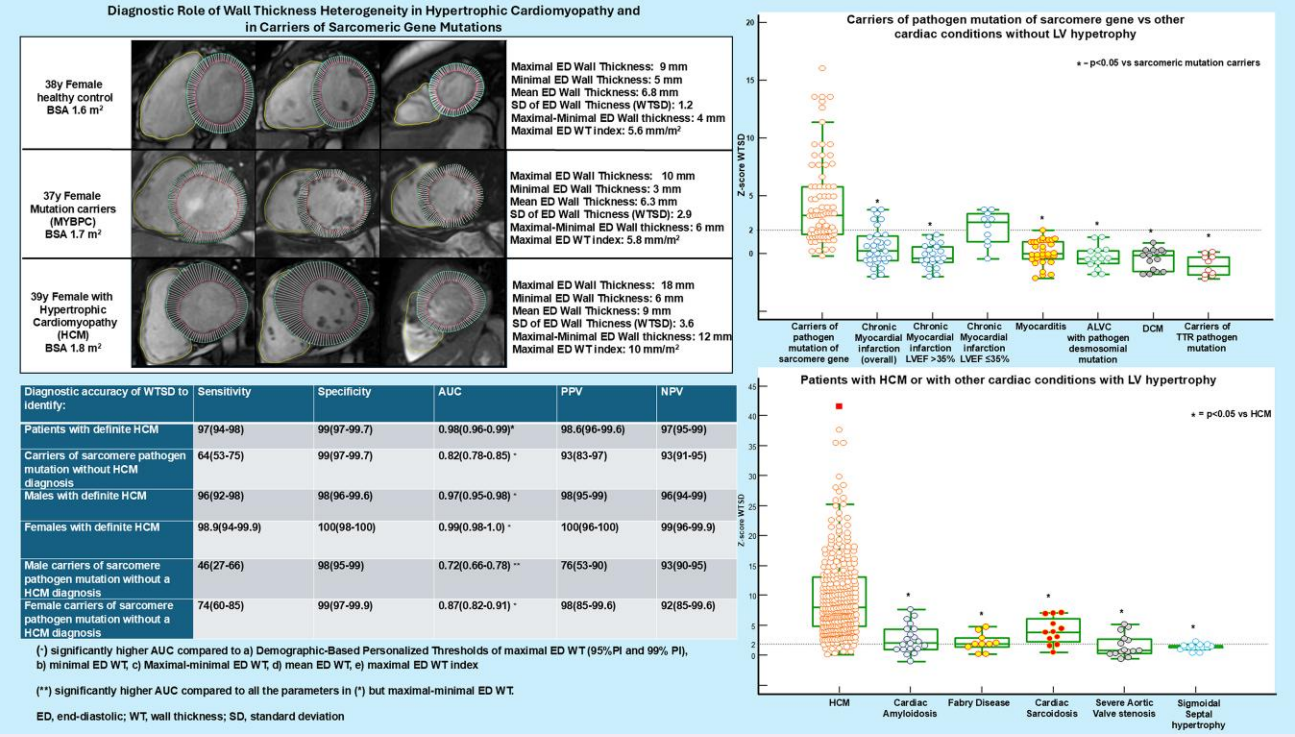
Objectives To determine whether wall thickness standard deviation (WTSD), reflecting WT heterogeneity, improves identification of HCM and mutation carriers.

Methods and results Cardiac magnetic resonance was performed in 382 healthy controls, 297 patients with HCM, 82 sarcomere mutation carriers without overt hypertrophy, and 180 patients with other cardiac conditions (75 with and 105 without LV hypertrophy). End-diastolic WT was measured in 16 myocardial segments, and WTSD was computed. Diagnostic performance was compared with other WT-derived parameters using age- and sex-specific thresholds. WTSD was higher in HCM (4.3 ± 1.1 mm) and mutation carriers (2.3 ± 0.3 mm) than in controls (1.3 ± 0.3 mm; $P < 0.0001$). WTSD identified 97% of HCM and 64% of carriers with 99% specificity. In females, WTSD achieved 98.9% sensitivity and 100% specificity for HCM and detected 74% of carriers. WTSD outperformed demographic-based thresholds and BSA-indexed maximal WT in all subgroups. Mutation carriers showed heterogeneous remodelling with both hypertrophic and thinned segments despite normal absolute WT. WTSD was significantly higher in HCM than in all cardiac conditions with LV hypertrophy and in mutation carriers vs. non-hypertrophic conditions, except post-ischaemic severe LV dysfunction.

Conclusion WTSD is a robust imaging biomarker that detects both overt and early sarcomeric HCM with high accuracy. Incorporating WT heterogeneity into diagnostic algorithms may enhance early identification, especially in women and mutation carriers.

* Corresponding author. E-mail: giovanni.aquaro@unipi.it
† GDA, RL and GT equally contributed to this study.

Graphical Abstract



Diagnostic accuracy of WTSD to identify:	Sensitivity	Specificity	AUC	PPV	NPV
Patients with definite HCM	97(94-98)	99(97-99.7)	0.98(0.96-0.99)*	98.6(96-99.6)	97(95-99)
Carriers of sarcomere pathogen mutation without HCM diagnosis	64(53-75)	99(97-99.7)	0.82(0.78-0.85) *	93(83-97)	93(91-95)
Males with definite HCM	96(92-98)	98(96-99.6)	0.97(0.95-0.98) *	98(95-99)	96(94-99)
Females with definite HCM	98.9(94-99.9)	100(98-100)	0.99(0.98-1.0) *	100(96-100)	99(96-99.9)
Male carriers of sarcomere pathogen mutation without a HCM diagnosis	46(27-66)	98(95-99)	0.72(0.66-0.78) **	76(53-90)	93(90-95)
Female carriers of sarcomere pathogen mutation without a HCM diagnosis	74(60-85)	99(97-99.9)	0.87(0.82-0.91) *	98(85-99.6)	92(85-99.6)

(·) significantly higher AUC compared to a) Demographic-Based Personalized Thresholds of maximal ED WT (95%PI and 99% PI), b) minimal ED WT, c) Maximal-minimal ED WT, d) mean ED WT, e) maximal ED WT index

(**) significantly higher AUC compared to all the parameters in (*) but maximal-minimal ED WT.

ED, end-diastolic; WT, wall thickness; SD, standard deviation

In the upper left panel, examples of image analysis in a healthy control, a mutation carrier, and a patient with HCM are reported. A panel shows three short-axis images from a 38-year-old female healthy control with a body surface area (BSA) of 1.6 m^2. B panel displays images from a 37-year-old female mutation carrier (pathogenic MYBPC3 mutation) with a BSA of 1.7 m^2. C panel shows images from a 29-year-old female with a definitive diagnosis of HCM. Notably, the standard deviation of wall thickness (WTSD) was more than twice as high in the mutation carrier than in the healthy control. **In the left part of the figure, box-and-whiskers plot comparing the Z-score of WTSD in different cardiac conditions.** As evident in the upper graph, carriers of pathogen mutation of sarcomeric gene had greater Z-score of WTSD than other cardiac conditions without hypertrophy, with the exception of severe post-ischaemic dysfunction (with LV EF ≤35%) (DCM, dilated cardiomyopathy, ALVD, arrhythmogenic left ventricular cardiomyopathy, TTR, transthyretin). The lower graph shows that HCM had greater Z-scores of WTSD compared to all cardiac conditions with LV hypertrophy. **In the lower part the summary table reporting the diagnostic accuracy of WTSD across the different patient groups analysed and in the overall population is reported**

Keywords hypertrophic cardiomyopathy • left ventricular thickness • wall thickness heterogeneity • sarcomeric gene mutation • cardiac magnetic resonance

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease and a leading cause of sudden cardiac death in young individuals.[1] It is characterized by increased left ventricular wall thickness unexplained by systemic, metabolic, or cardiac conditions.[2] Although HCM is usually caused by autosomal dominant sarcomeric mutations, incomplete penetrance and variable expressivity lead to a wide clinical spectrum ranging from asymptomatic forms to heart failure and sudden death.[3]

In adults, diagnosis relies on an end-diastolic maximal wall thickness (WT) ≥ 15 mm, or ≥13 mm in the presence of a family history or pathogenic mutation.[2] These thresholds overlook several factors: most HCM cases show asymmetric hypertrophy with multiple morphological patterns,[4] and normal WT values vary by sex, ethnicity, and body size.[5,6] Women, in particular, often present later and with worse outcomes, possibly because current thresholds are too high.[7,8] To address this, demographic-adjusted thresholds and WT indexed to body surface area have been proposed.[9–11]

Another hallmark of HCM is wall thickness heterogeneity, where hypertrophic and thinned segments coexist. We recently demonstrated on post-mortem cardiac magnetic resonance (CMR) that the standard deviation of wall thickness (WTSD) was significantly higher in HCM than in controls or in patients with secondary hypertrophy.[12]The aim of the present study is (i) to identify the normal range of the WTSD in a population of healthy subjects and (ii) to assess whether WTSD, as a marker of wall thickness heterogeneity, may have a role in the diagnosis

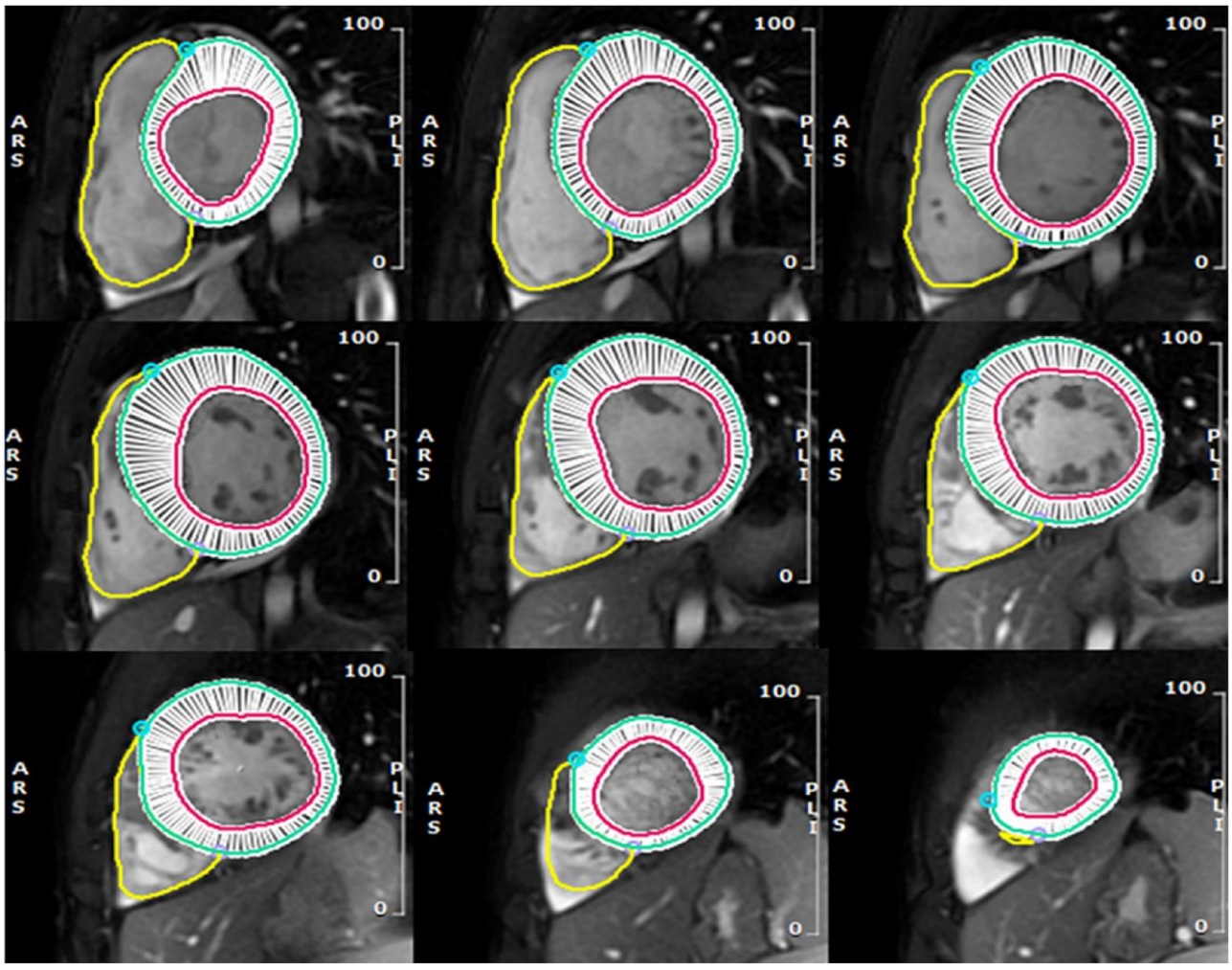


Figure 1 **Example of wall thickness measurement using CMR**. End-diastolic short-axis cineSSFP were analysed by tracing endocardial and epicardial contours, and 100 chords were automatically drawn orthogonally to the myocardial wall (white lines). For each segment, maximal wall thickness, minimal wall thickness, mean wall thickness, and WTSD were calculated from these chords. Inserts represent sequential slices from the same patient, showing progression from basal to apical segments.

of HCM by evaluating a cohort of patients with a definite diagnosis of HCM and a group of patients with a pathogenic mutation in sarcomeric genes but without a diagnosis of HCM (mutation carriers).

Methods

All participants were prospectively recruited from collaborating centres. Healthy controls were enrolled as volunteers fulfilling strict inclusion criteria (see below) and were not drawn from pre-existing cohorts. Patients with HCM and sarcomere mutation carriers were recruited during their routine clinical evaluations at the participating institutions, including Pisa, Massa, Messina, Trieste, Paris Necker Hospital, Palermo, and Chieti.

Study population: healthy controls

A population of 400 consecutive healthy controls was enrolled using the following inclusion criteria: age ≥18 years, no family history of cardiac disease, normal electrocardiogram, no history of cardiac diseases or symptoms, no cardiovascular risk factors (hypertension, dyslipidaemia, diabetes, obesity, smoking, family history), no known systemic diseases, and no contraindications to CMR. Healthy controls were divided by sex and age class (<35 years, ≥35–<50 years, ≥50–<65 years, ≥65 years). Four were excluded for suboptimal image quality and 14 for minor abnormal tissue or morphological findings at CMR.

Study population: HCM/mutation carriers and patients with other cardiac conditions

A population of 300 consecutive patients aged ≥18 years with guideline-based HCM diagnosis was enrolled: defined as end-diastolic maximal WT ≥15 mm unexplained by loading conditions (with or without known pathogenic sarcomere mutation) or WT 13–15 mm with a pathogenic sarcomere mutation (*Figure 1*). Alternative causes of LV hypertrophy were systematically excluded. Each subject underwent comprehensive clinical evaluation,

including family history, laboratory screening, and multimodality imaging, to rule out infiltrative, metabolic, or storage diseases.

A group of 82 mutation carriers aged ≥18 years with maximal WT <13 mm, known pathogenic sarcomere mutation, and family history of HCM were enrolled, excluding those with other cardiac or systemic diseases, cardiovascular risk factors, or CMR contraindications. Mutation carriers were selected among relatives of patients with sarcomeric HCM who had a positive genetic test. If cardiac MRI showed a wall thickness ≥13 mm, the patient was reassigned to the HCM group with a definitive diagnosis.

A further 180 patients with other cardiac conditions were included (see Supplementary data online, Data): 105 without LV hypertrophy (32 myocarditis, 32 prior myocardial infarction, 16 arrhythmogenic LV disease, 15 DCM, 10 ATTR mutation) and 75 with LV hypertrophy (15 aortic stenosis, 23 amyloidosis, 10 Fabry Disease, 12 sarcoidosis, 15 sigmoid septal hypertrophy).

Three HCM patients were excluded for poor image quality (two with PVCs, one with atrial fibrillation). Race/ethnicity was not routinely collected; all participants were recruited in Italian centres, thus predominantly Caucasian.

The study was approved by the local ethics committee, and all participants provided written informed consent. All underwent clinical, ECG, and echocardiographic evaluation at the time of CMR.

CMR acquisition protocol and post-processing

CMR scan was performed using different 1.5 Tesla machines with dedicated cardiac coil. CMR acquisition protocol and post-processing methods are described in details, in Supplementary data online, data.

All CMR studies were analysed offline by three experienced CMR readers with level III EACVI accreditation for CMR, blinded to all clinical data using a commercially available research software package (CVI42, Circle Cardiovascular Imaging Inc., Calgary, Canada). Briefly, LV end-diastolic WT of each of the 16 conventional AHA/ACC/ESC myocardial segment was automatically measured by using the endocardial and epicardial contours of short axis slices[13] (*Figure 1*). The ED maximal WT, the minimal WT, the mean WT and the standard deviation (WTSD) were obtained for each myocardial segment.

Statistical analysis

Values are expressed as mean ± standard deviation or as median (25th–75th percentile) for normally and non-normally distributed variables, respectively. Normality was assessed using the Kolmogorov–Smirnov test and homogeneity of variances with Levene's test. When variances were unequal, Welch's *t*-test (two groups) or Welch's ANOVA with Games–Howell post-hoc test (≥3 groups) were applied; otherwise, Kruskal–Wallis with Dunn–Holm post-hoc was used for non-normal data.

All parameters derived from end-diastolic WT in healthy controls, except maximal–minimal WT difference, were normally distributed across age and sex groups. Therefore, normality thresholds were defined as mean + 2 SD, whereas the 95th percentile was used for maximal–minimal WT difference. WTSD Z-scores were computed for each patient using age- and sex-specific mean and SD of controls to allow cross-group comparison.

Receiver operating characteristic (ROC) analysis evaluated the ability of WTSD Z-score to discriminate HCM and mutation carriers from controls and other cardiac conditions. Categorical variables were analysed using chi-square or Fisher's exact test. Sensitivity, specificity, positive and negative predictive values (PPV, NPV), area under the curve (AUC), and diagnostic accuracy were calculated for all wall-thickness–derived parameters. AUC comparison tested differences in diagnostic performance between parameters.

In a representative subsample of 50 subjects (including healthy controls and HCM patients), WTSD was re-measured to assess intra- and inter-observer reproducibility. For intra-observer variability, the same reader repeated WTSD measurements; for inter-observer variability, a second independent reader repeated the analysis. Agreement was quantified using the intraclass correlation coefficient (ICC).

Results

Population

The final study population included 382 healthy controls (216 males, 51 ± 15 years old), 297 patients with guideline-based diagnosis of HCM, 82 carriers of sarcomere pathogen mutations with a family history of HCM (characteristics in *Table 1*) and 180 patients with other cardiac conditions (75 with and 105 without LV hypertrophy, characteristics in *Table 2*)

Data from *Table 1* suggest that both HCM patients and mutation carriers are characterized by greater heterogeneity in WT, as the WTSD was significantly higher in both groups, 4.3 ± 1.1 and 2.3 ± 0.3 respectively, compared to healthy controls (WTSD 1.3 ± 0.3, $P < 0.0001$ for both comparisons). No difference of WTSD was found between obstructive and non-obstructive HCM (4.8 ± 2, vs. 4.5 ± 1.9, $P = 0.53$). Among HCM patients, those with LGE exhibited higher WTSD compared with those without (mean 4.8 ± 1.8 vs. 3.6 ± 1.2, $P = 0.01$). No significant differences were found among the most frequent mutation gene: the median WTSD z-score was 5.9 (25th–75th 4–10) for MYBPC3, 7.0 (25th–75th 4–11) for MYH7, and 7.1 (25th–75th 4–12) for TNNI3. WT heterogeneity is also manifest as the significantly greater difference maximal-minimal WT observed in HCM patients and mutation carriers compared to healthy controls ($P < 0.0001$ in both comparisons). Interestingly, in mutation carriers, who, by definition, did not have myocardial segments with WT ≥ 13 mm, this heterogeneity is associated with the presence of segments with reduced wall thickness: both the minimal WT ($P < 0.0001$) and the mean WT ($P = 0.001$) were significantly lower in mutation carriers compared to healthy controls. Myocardial crypts were found in 22 (27%) and myocardial bridge of left anterior descending artery in 15 (18%) mutation carriers. Twenty-three mutation carriers had ECG abnormalities (28%). WTSD was not significantly different between patients with and without ECG abnormalities (respectively 2.4 ± 0.4 vs. 2.3 ± 0.3, $P = 0.22$).

Parameters derived from ED WT measured in healthy controls are shown in *Table 3*. The thresholds of normality of each parameter was obtained, as explained in statistical analysis section, from this population of healthy controls divided for sex and class of age and are reported in *Table 4*. WT parameters in HCM and sarcomeric mutation carriers divided for sex and age-class are reported in Supplementary data online, *Table S1*.

Wall-thickness derived parameters in patients with definite HCM diagnosis and in mutation carriers against healthy controls

Table 5 shows the evaluation of the diagnostic performance of wall thickness-derived parameters in identifying HCM patients and carriers of pathogenic sarcomeric mutations compared to healthy controls, using the corresponding age- and sex-specific reference cut-offs provided in *Table 3*.

All patients with an HCM diagnosis based on current guidelines exhibited, for definition, either a maximal ED WT

Table 1 Characteristics of the study populations

Variables		Healthy controls	HCM	Carriers of pathogen sarcomeric mutation	Healthy. Vs HCM *P* value	Healthy. Vs Mutation carriers *P* value
n.		382	297	82		
Age (years)	mean ± SD	49(34–61)	52(32–69)	45(28–56)	0.24	0.03
Males	*n* (%)	216 (56%)	205(51%)	28(34%)	0.41	<0.0001
Systemic Hypertension	*n* (%)	0(0%)	5	0	0.02	–
Dyslipidaemia	*n* (%)	0(0%)	1	0	0.29	–
Diabetes	*n* (%)	0(0%)	1	0	0.29	–
Smoking	*n* (%)	0(0%)	19	5(6%)	0.29	–
Sarcomere-positive pathogenic variants	*n* (%)	0(0%)	164(55%)	82(100%)	<0.0001	<0.0001
Sarcomere-positive like-pathogenic variants	*n* (%)	0(0%)	62(21%)	0(0%)	<0.0001	–
Genetic analysis not performed	*n* (%)	382 (100%)	71(24%)	0(0%)	<0.0001	<0.0001
Mutation gene:						
MYBPC3	*n* (%)	0(0%)	110(37%)	45(55%)	–	–
MYH7	*n* (%)	0(0%)	65(22%)	20(24%)	–	–
MYH6	*n* (%)	0(0%)	1(0.3%)	1(1.2%)	–	–
TNNI3	*n* (%)	0(0%)	36(12%)	13(16%)	–	–
MYL2	*n* (%)	0(0%)	10(3%)	3(3.8%)	–	–
MYL3	*n* (%)	0(0%)	1(0.3%)	–	–	–
MYBPC3/MYH7	*n* (%)	0(0%)	3(0.9%)	–	–	–
Family history of HCM	*n* (%)	0	156(53%)	82(100%)	<0.0001	
ED maximal WT (mm)	mean ± SD	9.8 ± 1.6	18 ± 3	10.2 ± 1.6	<0.0001	0.04
ED maximal WT < 13 mm	*n* (%)	382 (100%)	0	82 (100%)	<0.0001	–
ED maximal WT ≥ 13 & < 15 mm	*n* (%)	0	41(14%)	0	<0.0001	–
ED maximal WT ≥ 15 mm	*n* (%)	0	256(86%)	0	<0.0001	–
ED mean WT (mm)	mean ± SD	7.1 ± 1.2	8.2 ± 2	5.7 ± 1.2	<0.0001	0.001
ED minimal WT (mm)	mean ± SD	4.9 ± 1.3	4.9 ± 1.4	3.8 ± 1	0.85	<0.0001
ED maximal-minimal WT (mm)	median (IQR)	4.8(4–6)	13(10–15)	6.5(5.6–7.6)	<0.0001	<0.0001
WTSD		1.3 ± 0.3	4.3 ± 1.1	2.3 ± 0.3	<0.0001	<0.0001
Maximal WT indexed by BSA	mm/m^2	5.1 ± 1	9.8 ± 2	6.0 ± 1.1	<0.0001	<0.0001
LV mass index	g/m^2	69 ± 13	93 ± 18	64 ± 11	<0.0001	0.17
LV EDVi (ml)	mL/m^2	75 ± 12	68 ± 11	71 ± 16	0.002	0.25
LV ESVi (ml)	mL/m^2	25(19–32)	21(15–29)	21(19–32)	0.0007	0.009
LV EF	%	66(62–71)	72(63–76)	70(62–71)	0.004	0.03
RV EDVi	mL/m^2	76(65–91)	66(58–77)	67(65–71)	<0.0001	0.03
RV EF	%	63(58–68)	64(58–71)	65(64–75)	0.14	0.08
LV atrium (cm2)	cm^2	11(10–13)	13(11–16)	11(10–13)	<0.0001	0.6
LV outflow obstruction	*n* (%)	0	37(12)	0	<0.0001	–
Septal hypertrophy	*n* (%)	0	220(74%)	0	<0.0001	–
Apical hypertrophy	*n* (%)	0	45(15%)	0	<0.0001	–
Diffuse hypertrophy	*n* (%)	0	29(10%)	0	<0.0001	–
Inferior/inferolateral hypertrophy	*n* (%)	0	3(1%)	0	<0.0001	–
Apical aneurysm	*n*(%)	0	9(3%)	0	0.015	–
Late Gadolinium Enhancement	*n* (%)	0(0%)	212(71%)	0	<0.0001	–

HCM, Hypertrophic cardiomyopathy; ED, end-diastolic; WT, wall thickness; WTSD, standard deviation of the wall thickness; BSA, body surface area; LV, left ventricle; EDVi, End-diastolic volume index; ESVi, end-systolic volume index; EF, ejection fraction

Table 2 Wall thickness parameters in cardiac conditions with and without LV hypertrophy

	Age (y)	Males, *n*(%)	Maximal WT (mm)	Maximal WT indexed by BSA (mm/m^2)	Minimal WT (mm)	Maximal-minimal WT (mm)	Mean WT (mm)	WTSD	N. of pts with maximal WT > Demographic-Based Personalized Thresholds of maximal WT (95%PI)
Conditions without LV hypertrophy									
Myocardial infarction	63 ± 12	27(84%)	11 ± 2	5.4 ± 0.9	5 ± 1*	5.7 ± 1.5	7.6 ± 1.2*	1.62 ± 0.4#,*	1(3%)
TTR mutation carriers	39 ± 13	3(30%)	9 ± 2	5 ± 1*	5 ± 1*	3.6 ± 1.2*	7.1 ± 1.5	1.02 ± 0.4#,*	0
Myocarditis	42 ± 16	19(59%)	11 ± 2	5.5 ± 0.8	5.5 ± 1*	4.9 ± 1.1*	7.5 ± 1.3*	1.3 ± 0.3#,*	4(12.5%)
DCM	62 ± 18	4(27%)	10 ± 2	5.8 ± 1	6 ± 1.5*	4.1 ± 1*	7.8 ± 1.5*	1.2 ± 0.33#,*	2(13%)
ALVC	39 ± 18	11(69%)	9 ± 1	4.8 ± 0.7*	3 ± 1*	7 ± 2	5.7 ± 0.8	1.16 ± 0.23#,*	0
Conditions with LV hypertrophy									
Sigmoid septum	63 ± 14	12(80%)	14 ± 1#	7.2 ± 1	6 ± 0.9	7.5 ± 1.6#	8.4 ± 1.4	1.94 ± 0.17#	5(33%)
Aortic valve stenosis	75 ± 4	10(66%)	15 ± 3	8.1 ± 1.6	8.4 ± 1.2#	7 ± 2#	11.4 ± 2#	2.96 ± 0.7#	9(60%)
Fabry disease	53 ± 8	6(60%)	14 ± 1#	5.9 ± 1.1	6.3 ± 1.6	7.3 ± 1.6#	8.2 ± 1.8	1.83 ± 0.34#	8(80%)
Cardiac amyloidosis	67 ± 7	16(70%)	16 ± 4	8.3 ± 1.8	7.6 ± 1.2#	8.6 ± 3#	11.2 ± 1.9#	2.53 ± 0.98#	10(44%)
Cardiac sarcoidosis	48 ± 7	9(75%)	15 ± 2	8 ± 1.2	6 ± 1	9 ± 2#	9.3 ± 1.2	2.4 ± 0.45#	9(75%)

WT, wall thickness; WTSD standard deviation of WT; LV, left ventricle; TTR, transthyretin; DCM, dilated cardiomyopathy; ALVC, left dominant arrhythmogenic cardiomyopathy with pathogen mutation of desmosomial gene.
*Significant difference vs. carriers of pathogen mutation of sarcomere gene.
#Significant difference vs. HCM; Significant difference for $P < 0.05$.

Table 3 Wall thickness parameters in healthy controls divided by age class

Variable	Age class, years				
	<35	≥35 &<50	≥50 & < 65	≥65	*P* value
Males					
ED maximal WT	8.2 ± 1.7	9.3 ± 1.1	9.3 ± 1.2	9 ± 1.4	**<0.0001**
ED minimal WT	4.7 ± 1.7	5.4 ± 1.1	5.5 ± 0.9	5.7 ± 0.8	**<0.0001**
Maximal-minimal WT	4.7(3.7–5.4)	5(4.1–5.6)	5.3(3.9–5.7)	4.9(4.2–6.6)	**0.048**
ED mean WT	6.9 ± 1.5	7.8 ± 1.0	7.7 ± 1.1	7.5 ± 1.2	**<0.0001**
ED WTSD	1.18 ± 0.23	1.31 ± 0.25	1.51 ± 0.39	1.51 ± 0.4	**<0.0001**
ED maximal WT index*	5.1 ± 0.6	5.0 ± 0.5	5.4 ± 0.8	5.7 ± 0.79	**<0.0001**
Max apex/base ratio	0.66 ± 0.17	0.68 ± 0.12	0.63 ± 0.16	0.73 ± 0.11	0.08
Apical max WT index	3.5 ± 0.6	3.5 ± 0.7	3.6 ± 0.8	3.7 ± 1	0.12
Females					
ED maximal WT	7.9 ± 0.8	8.8 ± 1	9.2 ± 0.8	9.1 ± 0.9	**<0.0001**
ED minimal WT	3.9 ± 0.74	4.3 ± 0.9	4.2 ± 1.1	5.3 ± 1.02	**<0.0001**
Maximal-minimal WT	4.0(3.3–4.4)	4.8(3.8–5.4)	4.9(4.3–6.8)	4.6(3.5–5.1)	**<0.0001**
ED mean WT	5.6 ± 0.79	6.4 ± 0.91	6.7 ± 1.01	6.6 ± 1.4	**0.002**
ED WTSD	0.99 ± 0.18	1.19 ± 0.21	1.33 ± 0.27	1.29 ± 0.27	**<0.0001**
ED maximal WT index*	4.8 ± 0.33	5.3 ± 0.91	5.3 ± 0.69	5.5 ± 0.79	**<0.0001**
Max apex/base ratio	0.63 ± 0.1	0.66 ± 0.12	0.62 ± 0.12	0.76 ± 0.11	0.08
Apical max WT index	3.2 ± 0.7	3.3 ± 0.9	3.5 ± 0.8	3.8 ± 0.9	0.18

ED, end-diastolic, WT wall thickness, SD standard deviation.
*ED maximal WT indexed for body surface area.

Table 4 Normality range of end-diastolic wall thickness parameters

	Age class (years)			
	<35	≥35 &<50	≥50 & < 65	≥65
Males				
ED maximal WT	<12	<12	<12	<12
ED minimal WT (mean +2SD)	≤8	≤8	≤7	≤7
Maximal-minimal WT (95°)	≤6	≤6	≤8	≤8
ED mean WT (mean +2SD)	≤10	≤10	≤10	≤10
ED WTSD (mean +2SD)	≤1.63	≤1.79	≤2.28	≤2.3
ED maximal WT index (mean +2SD)	≤6.3	≤6	≤7	≤7.3
Max apex/base ratio (mean +2SD)	≤1	≤0.92	≤0.95	≤0.95
Apical max WT index (mean +2SD)	≤4.7	≤4.9	≤5.2	≤5.7
Females				
ED maximal WT	<10	<11	<11	<11
ED minimal WT (mean +2SD)	≤5	≤6	≤6	≤7
Maximal-minimal WT (95°)	≤6	≤6	≤7	≤7
ED mean WT (mean +2SD)	≤7	≤8	≤9	≤9
ED WTSD (mean +2SD)	≤1.36	≤1.62	≤1.86	≤1.83
ED maximal WT index (mean +2SD)	≤5.5	≤7.1	≤6.8	≤7.1
Max apex/base ratio (mean +2SD)	≤0.83	≤0.9	≤0.86	≤0.94
Apical max WT index (mean +2SD)	≤4.6	≤5.1	≤5.1	≤5.6

ED, end-diastolic, WT wall thickness, SD standard deviation.

Table 5 Diagnostic accuracy of wall thickness derived parameters in the whole population of HCM and mutation carriers

Patients with guideline-based diagnosis of HCM vs. controls:	Sensitivity	Specificity	AUC	PPV	NPV	AUC comparison vs. WTSD (*P* value)
Demographic-based personalized thresholds of maximal WT (95%PI)*	84(79–87)	99.4(98–99.9)	0.91(0.89–0.94)	99.2(97–99.8)	87(85–90)	<0.0001
Demographic-based Personalized Thresholds of maximal WT (99.7%PI)*	73(67–78)	99.7(98–99.9)	0.86(0.83–0.89)	99.5(97–99.9)	82(80–85)	<0.0001
WTSD	97(94–98)	99(97–99.7)	0.98(0.96–0.99)	98.6(96–99,6)	97(95–99)	—
ED minimal WT	6(4–10)	97(94–98)	0.51(0.47–0.55)	59(42–74)	56(50–57)	<0.0001
Maximal-minimal WT	88(84–91)	90(86–92)	0.89(0.86–0.91)	89(86–92)	88(85–91)	<0.0001
ED mean WT	41(36–47)	97(94–98)	0.69(0.66–0.73)	91(85–95)	67(66–70)	<0.0001
ED maximal WT index	97(95–99)	89.5(86–92)	0.93(0.91–0.95)	87(84–91)	97(95–98)	0.0001
Maximal apex/base ratio	16(9–21)	99(96–99.9)	0.57(0.52–0.62)	95(71–99)	58(52–55)	<0.0001
Apical maximal WT index	15(11–21)	99(98–99.9)	0.59(0.53–0.61)	96(84–99)	63(59–61)	<0.0001
Carriers of sarcomere pathogen mutation without HCM diagnosis vs. controls:	**Sensitivity**	**Specificity**	**AUC**	**PPV**	**NPV**	**AUC comparison vs. WTSD (*P* value)**
Demographic-based Personalized Thresholds of maximal WT (95%PI)*	11(5–20)	99.4(98–99.9)	0.55(0.51–0.60)	82(50–95)	84(83–85)	<0.0001
Demographic-based Personalized Thresholds of maximal WT (99.7%PI)*	4(1–10)	99.5(98–99.9)	0.52(0.47–0.56)	60(20–90)	83(82–83)	<0.0001
WTSD	64(53–75)	99(97–99.7)	0.82(0.78–0.85)	93(83–97)	93(91–95)	—
ED minimal WT	1(0–7)	97(94–98)	0.49(0.44–0.54)	7(1–36)	82(81–83)	<0.0001
Maximal-minimal WT	56(44–67)	90(86–92)	0.72(0.68–0.77)	53(45–62)	90(88–92)	0.0006
ED mean WT	5(1–12)	97(94–98)	0.51(0.46–0.55)	82(82–83)	83(81–84)	0.0001
ED maximal WT index	18(10–28)	94.5(91–96)	0.56(0.52–0.61)	40(27–56)	84(83–86)	0.0001
Maximal apex/base ratio	17(10–27)	100(99–100)	0.59(0.54–0.63)	100(77–100)	85(83–86)	<0.0001

*Based on Shiwani *et al.* (ref.[9]) ED, end-diastolic, WT wall thickness, SD standard deviation. With the exception of demographic-based personalized threshold, the cut-off of other parameters are shown in *Table 4*.
ED maximal WT not included because it was used for diagnosis of all patients with HCM (≥15 mm or ≥13 mm in those with family history of HCM or with sarcomere pathogen mutation) and it was <13 mm in all the mutation carriers without a diagnosis of HCM.

≥13 mm (if carriers of pathogenic sarcomeric mutations) or ≥15 mm. The WTSD showed a high sensitivity (97%) and specificity (99%), with an AUC of 0.98 that was significantly greater than that of all other parameters. WTSD allowed identification of 287 out of 297 HCM patients with 4 false positive, while the demographic-based personalized threshold identified 248 out of 297 HCM with 2 false positive.

WTSD proved to be the most effective parameter also to identify sarcomeric mutation carriers from controls, showing a sensitivity of 64%, a specificity of 99%, and an AUC of 0.82, which was significantly superior to all other parameters. WTSD allowed identification of 53 out of 82 mutation carriers, with only 4 false-positive cases, whereas the demographic-based personalized threshold allowed identification of only 9 out of 82 mutation carriers, with 2 false-positive cases. As evident in *Figure 2*, WTSD was significantly higher in mutation carriers than in healthy controls for each class of age. An apex/base ratio >1 was identified in 14 mutation carriers (17%), but in none of the patients did the ED wall thickness of the apical segments indexed to BSA exceed the reference cut-off values proposed by Hughes and colleagues.[10]

WTSD was also the parameters with the highest AUC to identify patients with sarcomeric mutations with and without overt HCM (see Supplementary data online, *Table S2*).

Wall-thickness derived parameters in females and males

In the female population of patients diagnosed with HCM (*Table 6*), WTSD proved to be the best parameter, identifying 91 out of 92 patients without any false positives, achieving a sensitivity of 98.9%, a specificity of 100%, and an AUC of 0.995, which was significantly superior to all other parameters. The demographic-based personalized threshold with a > 95th percentile cut-off and the ED maximal WT m indexed by BSA showed identical performance, identifying 85 out of 92 patients with only one false positive. *Figure 3* shows WTSD between

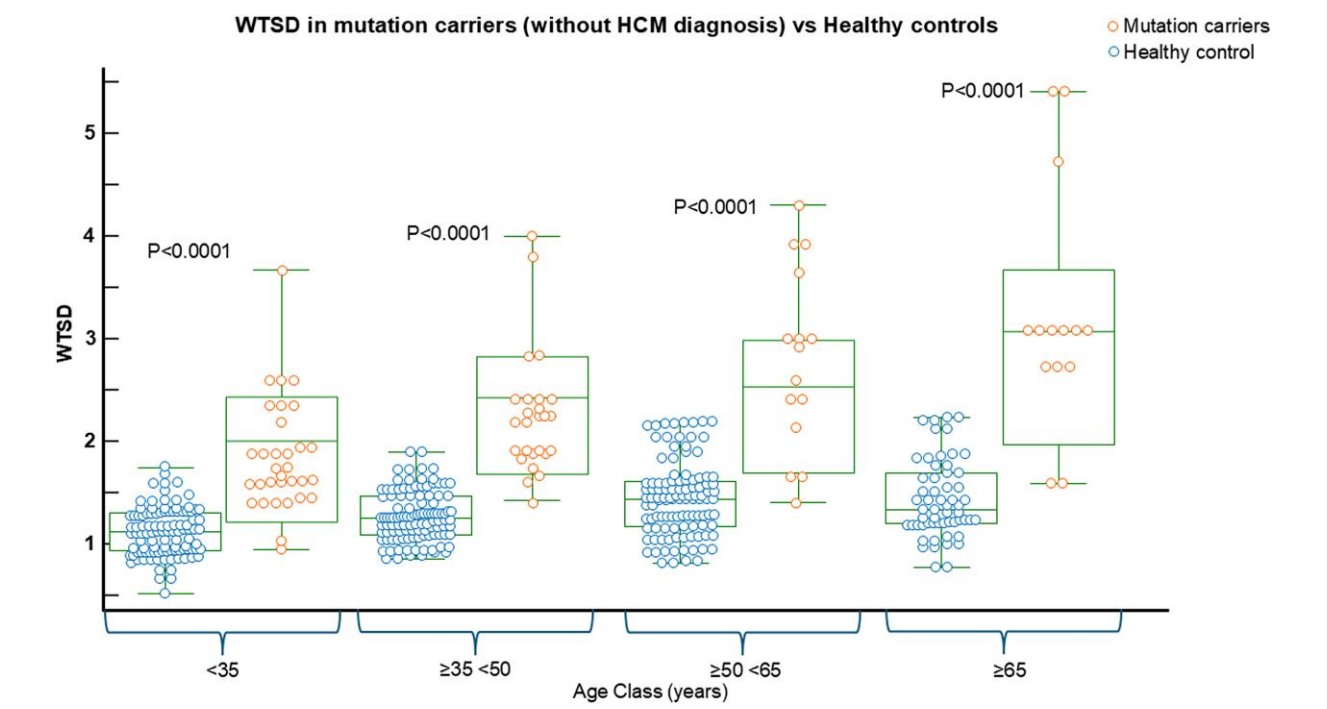


Figure 2 Box-and-whisker plots showing WTSD in sarcomere mutation carriers and healthy controls, stratified by age group. WTSD was significantly higher in mutation carriers compared with controls in all age categories.

Table 6 Diagnostic accuracy of wall thickness derived parameters in females

Females with a guideline-based diagnosis of HCM vs. controls:	Sensitivity	Specificity	AUC	PPV	NPV	AUC comparison vs. WTSD (*P* value)
Demographic-based Personalized Thresholds of maximal WT (95% PI)*	92(84–97)	99(97–99.9)	0.96(0.93–0.98)	98.8(92–99.8)	96(92–99)	0.012
Demographic-Based Personalized Thresholds of maximal WT (99.7%PI)*	81(72–89)	99(95–99.9)	0.90(0.86–0.94)	98.6(91–99.8)	90.7(86–94)	<0.0001
WTSD	98.9(94–99.9)	100(98–100)	0.995(0.98–1.0)	100(96–100)	99(96–99.9)	—
ED minimal WT	7(1–14)	97(93–99)	0.52(0.46–0.58)	54(27–79)	65(63–66)	<0.0001
Maximal-minimal WT	98.9(94–99.9)	90(85–94)	0.95(0.91–0.97)	85(78–90)	99(95–99.9)	0.005
ED mean WT	46(35–56)	96(92–98.6)	0.71(0.65–0.77)	88(76–94)	76(72–79)	<0.0001
ED maximal WT indexed by BSA	92(84–97)	99(97–99.9)	0.96(0.93–0.98)	98.8(92–99.8)	96(92–98)	<0.0001

Female carriers of sarcomere pathogen mutation without a HCM diagnosis vs. controls:	Sensitivity	Specificity	AUC	PPV	NPV	AUC comparison vs. WTSD (*P* value)
Demographic-based Personalized Thresholds of maximal WT (95%PI)*	7(2–18)	98(96–99)	0.53(0.46–0.60)	67(27–91)	77(75–78)	<0.0001
	2(1–10)	99(95–99.9)	0.50(0.43–0.57)	33(4–84)	76(75–76)	<0.0001

Continued

Table 6 Continued

Female carriers of sarcomere pathogen mutation without a HCM diagnosis vs. controls:	Sensitivity	Specificity	AUC	PPV	NPV	AUC comparison vs. WTSD (*P* value)
Demographic-Based Personalized Thresholds of maximal WT (99.7%PI)*						
WTSD	74(60–85)	99(97–99.9)	0.87(0.82–0.91)	98(85–99.6)	92(85–99.6)	–
ED minimal WT	0	95(91–98)	0.47(0.41–0.54)	0	75(73–75)	<0.0001
Maximal-minimal WT	48(34–62)	93(88–96)	0.71(0.64–0.76)	68(54–80)	84(79–88)	<0.0001
ED mean WT	6(1–15)	99(98–99.8)	0.53(0.49–0.55)	33(11–66)	96(95–96)	<0.0001
ED maximal WT indexed by BSA	11(4–22)	98(94–99.6)	0.55(0.48–0.61)	67(34–88)	77(75–79)	<0.0001

*Based on Shiwani *et al.* (ref [9]). ED, end-diastolic, WT wall thickness. With the exception of demographic-based personalized threshold, the cut-off of other parameters are shown in *Table 4*. ED maximal WT not included because it was used for diagnosis of all patients with HCM (≥15 mm or ≥13 mm in those with family history of HCM or with sarcomere pathogen mutation) and it was <13 mm in all the mutation carriers without a diagnosis of HCM.

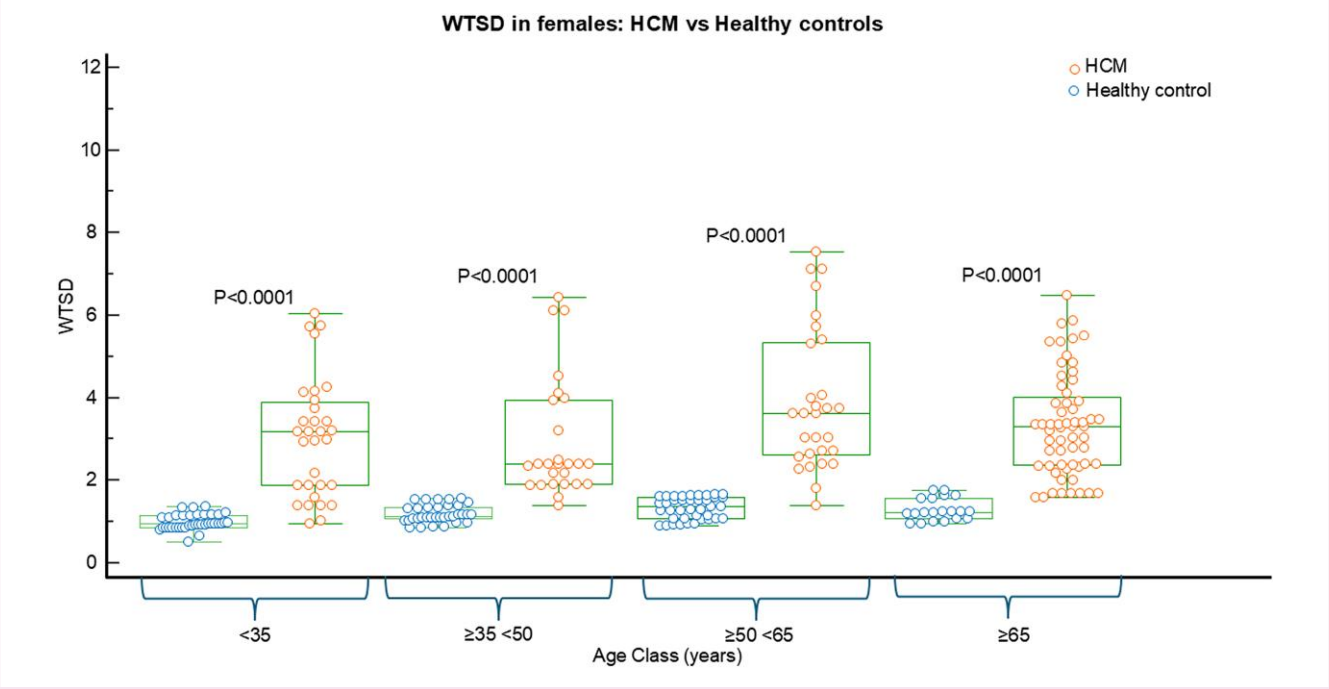


Figure 3 WTSD in females with HCM compared with healthy controls, across age groups. WTSD was consistently higher in HCM patients compared to controls.

females with HCM and healthy controls across different age groups.

The diagnostic effectiveness of WTSD was particularly evident in female carriers of pathogenic sarcomeric mutations without an HCM diagnosis (*Table 6*), in whom this parameter identified 40 out of 54 patients with one false positive, achieving a sensitivity of 74%, a specificity of 99%, and an AUC of 0.87, which was significantly higher than in all other parameters (*Table 5*).

In male subjects (*Table 7*) diagnosed with HCM, WTSD proved to be the best-performing parameter, identifying 196 out of 205 patients with only 4 false positives, and showing an AUC of 0.97, which was significantly higher than that of all other parameters (*Table 7*). *Figure 4* shows the differences in WTSD between males with HCM and healthy controls across different age groups. similar results observed in females were reproduced.

WTSD and the maximal–minimal ED WT difference were the best parameters to identify male carriers of pathogenic sarcomeric mutations without an HCM diagnosis with respectively AUC of 0.72 and 0.77 ($P = 0.23$). WTSD allowed identification of 13 out of 28 male mutation carriers with 4 false positive, while the maximal–minimal ED WT difference identified 19 out of 28 carriers with 28 false positive.

Table 7 Diagnostic accuracy of wall thickness derived in males

Males with a guideline-based diagnosis of HCM vs. controls:	Sensitivity	Specificity	AUC	PPV	NPV	AUC comparison vs. WTSD (*P* value)
Demographic-Based Personalized Thresholds of maximal WT (95%PI)*	81(75–87)	100(98–100)	0.91(0.88–0.93)	100(98–100)	85(91–89)	<0.0001
Demographic-Based Personalized Thresholds of maximal WT (99.7%PI)*	70(63–76)	100(99–100)	0.85(0.82–0.88)	100(97–100)	80(77–83)	<0.0001
WTSD	96(92–98)	98(96–99.6)	0.97(0.95–0.98)	98(95–99)	96(94–99)	—
ED minimal WT	94(89–97)	97(94–99)	0.95(0.93–0.97)	96(92–98)	95(92–97)	<0.0001
ED Maximal-minimal WT difference	96(92–98)	89(84–92)	0.92(0.90–0.95)	88(83–91)	97(93–98)	<0.0001
ED mean WT	40(33–46)	98(95–99)	0.69(0.64–0,73)	93(86–97)	66(64–69)	<0.0001
ED maximal WT indexed by BSA	88(83–92)	95(92–98)	0.92(0.89–0.94)	95(91–97)	91(87–93)	<0.0001
Male carriers of sarcomere pathogen mutation without a HCM phenotype vs. controls:	**Sensitivity**	**Specificity**	**AUC**	**PPV**	**NPV**	**AUC comparison vs. WTSD (*P* value)**
Demographic-Based Personalized Thresholds of maximal WT (95%PI)*	18(6–37)	100(98–100)	0.59(0.53–0.65)	100(47–100)	92(90–93)	<0.0001
Demographic-Based Personalized Thresholds of maximal WT (99.7%PI)*	4(1–18)	100(98–100)	0.52(0.46–0.58)	100(2.5–100)	90(89–91)	<0.0001
WTSD	46(27–66)	98(95–99)	0.72(0.66–0.78)	76(53–90)	93(90–95)	—
ED minimal WT	0	97(94–99)	0.48(0–43–0.54)	0	89(89–90)	<0.0001
Maximal-minimal WT	67(47–84)	87(81–91)	0.77(0.72–0.83)	40(30–51)	95(92–97)	0.24
ED mean WT	0	97(94–99)	0.49(0–43–0.55)	0	89(89–90)	<0.0001
ED maximal WT indexed by BSA	21(8–41)	96(93–98)	0.59(0.53–0.65)	38(90–93)	92(90–93)	<0.0001

*Based on Shiwani *et al.* (ref [9]). ED, end-diastolic, WT wall thickness. With the exception of demographic-based personalized threshold, the cut-off of other parameters are shown in *Table 4*. ED maximal WT not included because it was used for diagnosis of HCM (≥15 mm or ≥13 mm in those with family history of HCM or with sarcomere pathogen mutation).

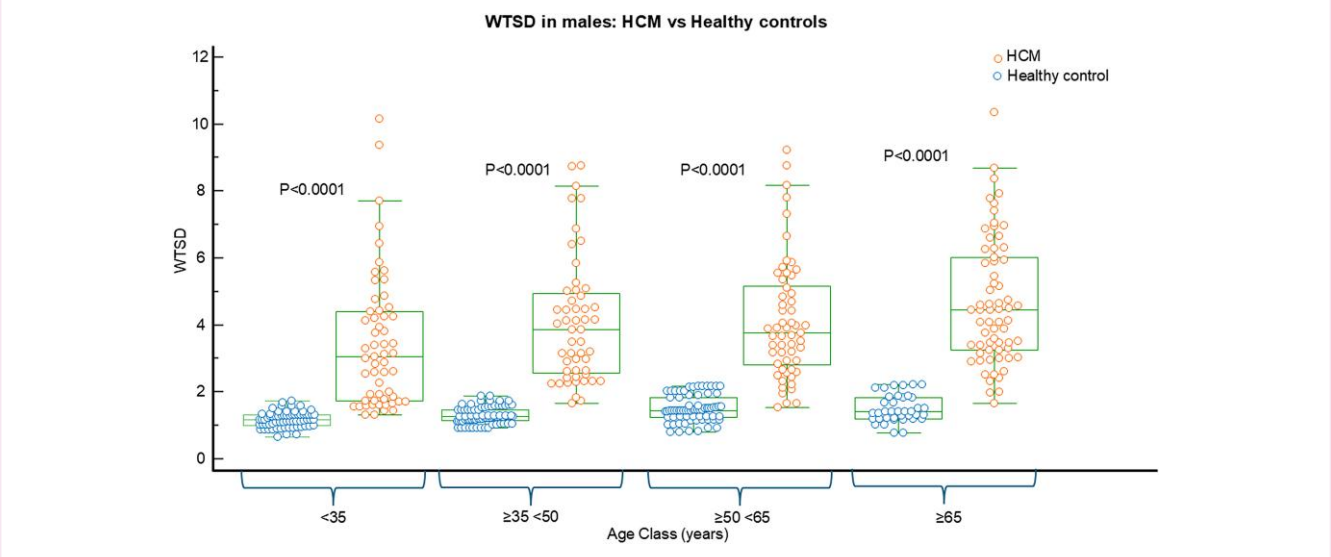


Figure 4 WTSD in males with HCM compared with healthy controls, across age groups. WTSD was consistently higher in HCM patients compared to controls.

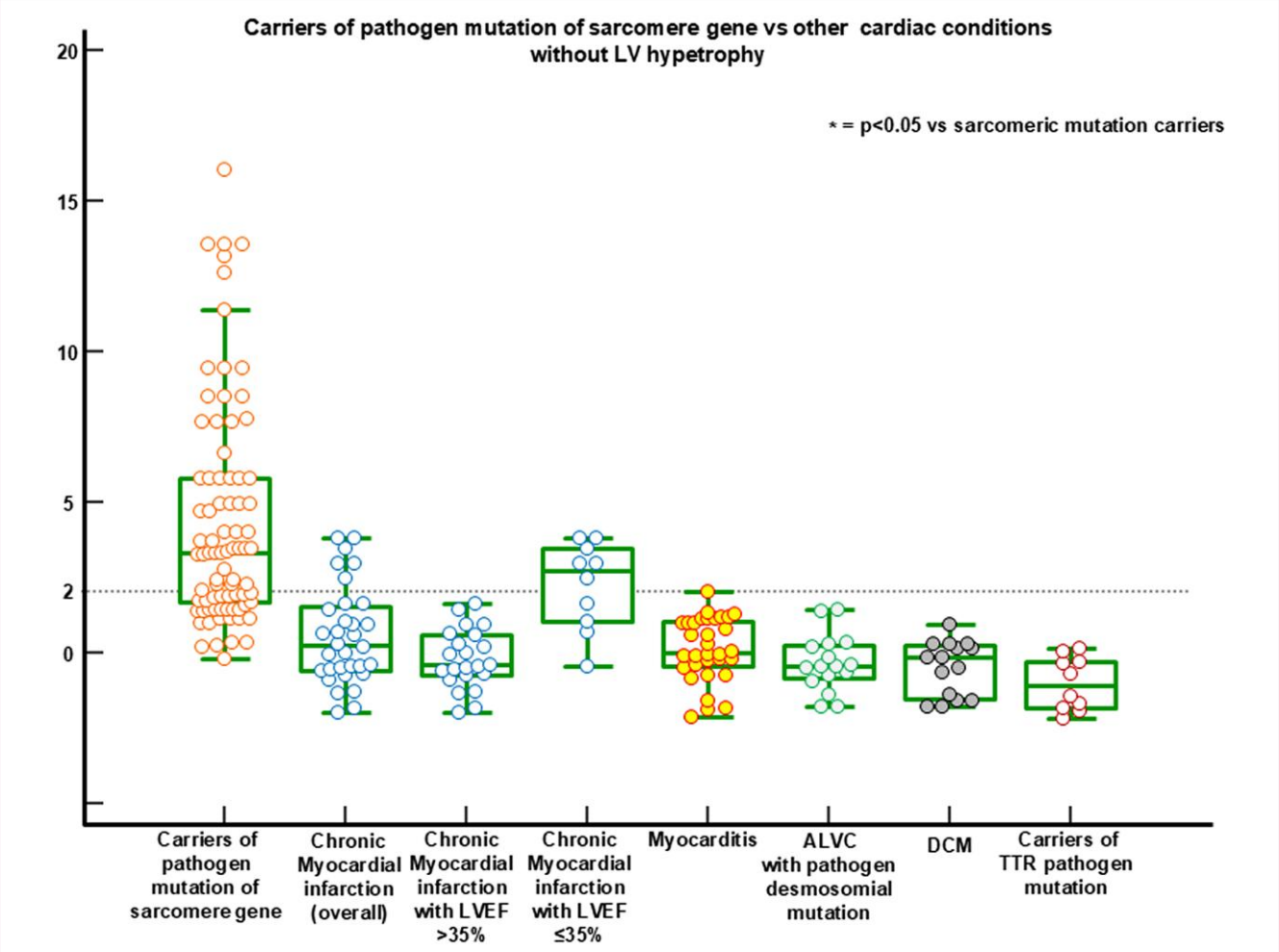


Figure 5 Box-and-whisker plots showing the Z-score of WTSD in sarcomere mutation carriers and in other cardiac conditions without left ventricular hypertrophy. As in the upper graph, carriers of pathogen mutation of sarcomeric gene had greater Z-score of WTSD than other cardiac conditions without hypertrophy, with the exception of severe post-ischaemic dysfunction (with LV EF ≤35%) (DCM, dilated cardiomyopathy, ALVD, arrhythmogenic left ventricular cardiomyopathy, TTR, transthyretin).

WTSD in HCM, sarcomeric mutation carriers, and other cardiac conditions

The *Figures 5* and *6* presents box-and-whisker plots comparing the WTSD z-scores across HCM patients, sarcomeric mutation carriers, and other cardiac conditions. As evident in the *Table 4*, WTSD was the only parameters that was higher in sarcomeric mutation carries than all other conditions without LV hypertrophy as well as the only parameter higher in HCM compared all other cardiac conditions. As shown in the plot of *Figure 5*, carriers of pathogenic sarcomeric mutations had higher WTSD z-scores (median 3.3, 25th–75th 1.7–5.8) than patients with all other non-hypertrophic cardiac conditions (median −0.18, 25th–75th −0.75–0.75) including those with prior myocardial infarction, except for patients with myocardial infarction and severe LV dysfunction (LVEF ≤35%), in whom no significant difference was observed (median 2.7, 25th–75th 1–3.4, $P = 0.16$). Among the patients with cardiac conditions without LV hypertrophy, only 6 out of 105 had WTSD above the normality limit for age class and sex (all of these 6 in the group with previous myocardial infarction and severe LV dysfunction), while it was found in 53 out of 82 sarcomeric mutation carriers.

As evident in the plot of *Figure 6*, patients with HCM had significantly higher WTSD z-score (median 8.1, 25th–75th 5–13) than all other conditions with LV hypertrophy. In HCM the median z-score of WTSD was 8.1 (4.9–13), while it was 1.8 (1–5.3) in patients with other cause of LV hypertrophy. Among patients with cardiac conditions with LV hypertrophy only 31 out of 75 had WTSD higher than the normality limit for age class and sex (1 with sigmoidal septal hypertrophy, 6 with aortic valve stenosis, 4 Fabry disease, 11 cardiac amyloidosis, and 9 with cardiac sarcoidosis) while it was found in 287 out of 297 HCM patients.

In *Table 8*, it is shown the sensitivity, specificity, AUC, PPV and NPV of WTSD to identify HCM and mutation carriers from all other cardiac conditions. WTSD demonstrated a higher diagnostic accuracy than all other wall thickness parameters (as evident also in *Figure 7*). To distinguish sarcomeric mutation carriers from other non-hypertrophic conditions, WTSD achieved an AUC of 0.81, outperforming the demographic-based personalized threshold with a > 95th percentile cut-off (AUC 0.56,

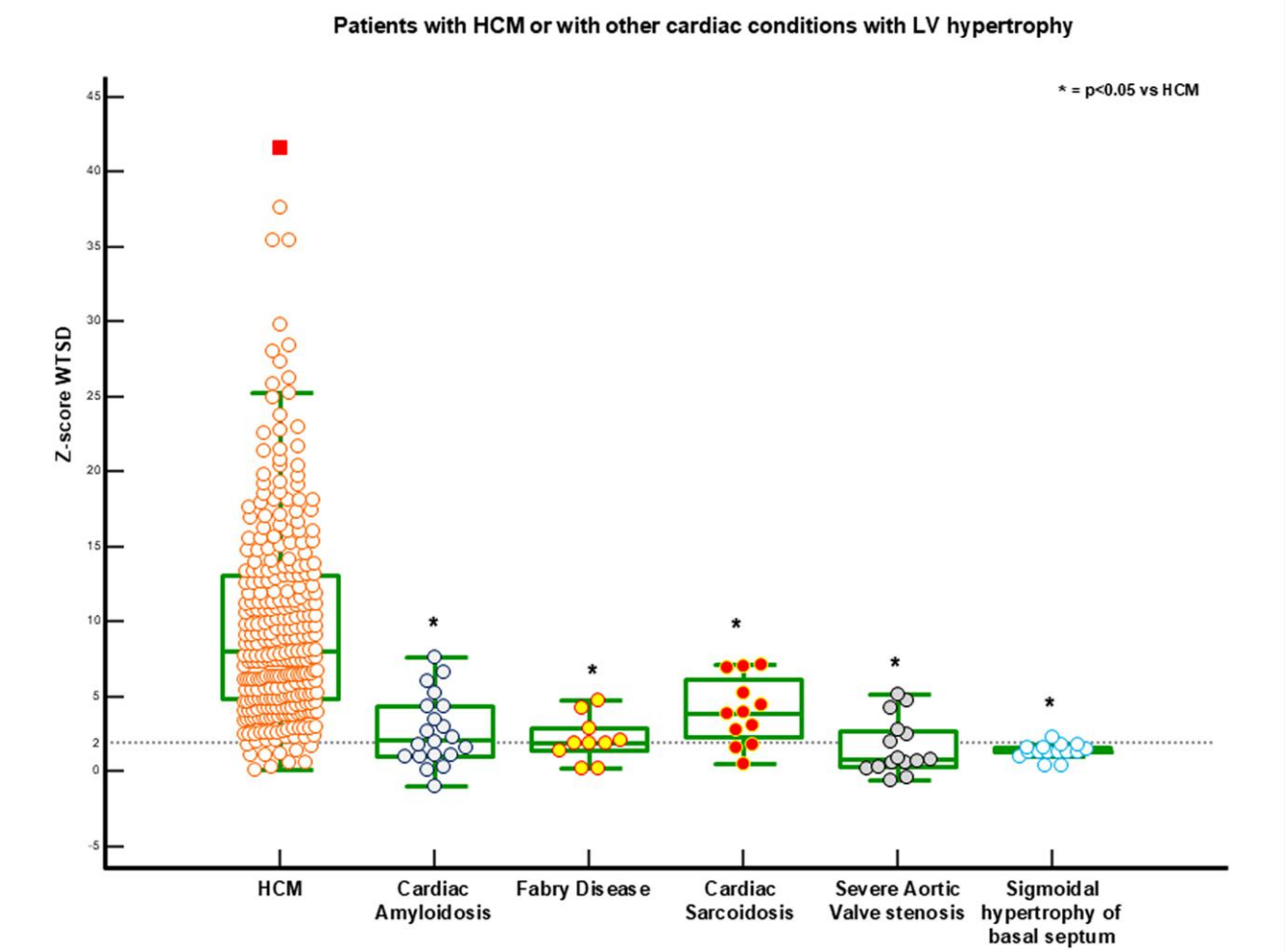


Figure 6 Box-and-whisker plots showing the Z-score of WTSD in patients with HCM and in other cardiac conditions with left ventricular hypertrophy. The graph shows that HCM had greater Z-scores of WTSD compared to all cardiac conditions with LV hypertrophy.

$P < 0.0001$), maximal WT (AUC 0.63, $P < 0.0001$), maximal WT indexed to BSA (AUC 0.74, $P < 0.0001$), minimal WT (AUC 0.71, $P < 0.0001$), and maximal–minimal WT difference (AUC 0.74, $P = 0.0002$). Similarly, WTSD had the highest discriminative power to differentiate HCM from other hypertrophic conditions (AUC 0.79), confirming higher AUC than the demographic-based personalized threshold with a > 95th percentile cut-off (AUC 0.68, $P < 0.0001$), maximal WT (AUC 0.71, $P = 0.02$), maximal WT indexed to BSA (AUC 0.68, $P = 0.008$), minimal WT (AUC 0.72, $P = 0.03$), and maximal–minimal WT difference (AUC 0.71, $P = 0.02$).

Finally, sex-specific diagnostic thresholds in the overall cohort to identify sarcomeric disease (overt HCM or pathogenic sarcomere mutation carriers) vs. all other participants were derived. In men, a WTSD Z-score >2 provided excellent discrimination, with an AUC of 0.96 (95% CI 0.94–0.97), sensitivity 85% (80–90), specificity 95% (92–97), PPV 92% (87–94) and NPV 91% (88–93). The corresponding optimal threshold for the raw WTSD was 2.23 mm, yielding very similar performance [AUC 0.95 (0.93–0.97), sensitivity 85% (80–90), specificity 93% (90–96), PPV 88% (84–92), NPV 91% (88–93)]. In women, a WTSD Z-score >2 again showed excellent discriminative ability [AUC 0.96 (0.94–0.98)], with sensitivity 88% (81–93), specificity 94% (90–97), PPV 92% (87–95) and NPV 91% (87–94). The optimal raw WTSD cut-off was 1.8 mm, with an AUC of 0.96 (0.93–0.98), sensitivity 88% (81–93), specificity 95% (91–97), PPV 93% (87–96) and NPV 91% (87–94).

Reproducibility

WTSD showed excellent reproducibility: intra-observer ICC was 0.9578 (95% CI 0.9181–0.9785) for single measures and 0.9784 (0.9573–0.9891) for average measures; inter-observer ICC was 0.9178 (0.8435–0.9577) for single measures and 0.9571 (0.9151–0.9784) for average measures (see Supplementary data online, *Table S2*).

Discussion

The results of this study suggest that increased left ventricular wall thickness heterogeneity, as reflected by high WTSD values, represents a phenotypic hallmark of sarcomeric cardiomyopathy.

WTSD was increased in patients with definite HCM compared to age- and sex-matched healthy controls, and it was also elevated in the majority (64%) of individuals carrying a pathogenic

Table 8 Diagnostic accuracy of WTSD to identify mutation carriers and patients with HCM from healthy controls and other cardiac conditions# with and without LV hypertrophy

	Sensitivity	Specificity	AUC	PPV	NPV
Whole population of HCM or mutation carriers* vs. healthy controls and other cardiac conditions	90(86–93)	93(90–95)	0.91(0.89–0.93)	89(86–92)	93(91–95)
Whole population of female patients with HCM or mutation carriers* vs. healthy controls and other cardiac conditions	90(84–94)	94(90–97)	0.92(0.89–0.95)	92(87–95)	92(87–95)
Whole population of male patients with HCM or mutation carriers* vs. healthy controls and other cardiac conditions	90(85–93)	92(89–94)	0.91(0.88–0.93)	87(83–91)	93(90–95)
HCM vs. other cardiac conditions with LV hypertrophy	97(94–98)	62(51–74)	0.79(75–0.83)	92(89–94)	80(68–89)
Mutation carriers* vs. healthy controls and other cardiac conditions without LV hypertrophy	64(53–75)	98(96–99)	0.81(0.78–0.84)	84(74–91)	94(92–96)
Female mutation carriers* vs. healthy controls and other cardiac conditions without LV hypertrophy	74(60–85)	99(97–99.9)	0.87(0.82–0.91)	98(85–99.6)	92(88–95)
Male mutation carriers* vs. healthy controls and other cardiac conditions without LV hypertrophy	46(28–66)	97(95–99)	0.72(0.67–0.77)	59(40–75)	95(94–97)

*Patients with pathogen mutation of sarcomeric gene; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

sarcomeric mutation but without left ventricular hypertrophy. WTSD was also significantly higher in patients with HCM compared with other cardiac conditions characterized by LV hypertrophy, and in subjects carrying pathogenic sarcomeric mutations without LV hypertrophy against patients with other cardiac conditions also lacking hypertrophy. The only exception was the subgroup of patients with prior myocardial infarction and severe LV dysfunction (EF ≤35%), in whom no significant difference was observed from mutation carriers.

In patients with definite HCM, the WT heterogeneity is driven by marked differences in wall thickness among hypertrophic segments, as well as the coexistence of hypertrophic segments with segments of normal or even reduced thickness. In mutation carriers, on the other hand, no hypertrophic segments are present; rather, heterogeneity results from the coexistence of normal and thinned segments.

The observation that HCM often exhibits asymmetric or regional hypertrophy with adjacent areas of relative thinning is not novel and reflects the typical pattern of hypertrophic development, which frequently follows a spiral trajectory from base to apex in a counterclockwise direction.[14] In HCM, the LV mass index is often increased, but it may also be within the normal range due to the coexistence of wall hypertrophy and areas of thinning[15]

In post-mortem CMR, hearts of patients with HCM and pathogen sarcomeric mutation have significantly greater wall thickness dispersion than both normal hearts and non-HCM hypertrophy controls.[12] Similarly, a living CMR study of genotype-positive relatives reported subtle structural areas of abnormalities in all carriers, including abrupt regional changes in wall thickness and focal 'inappropriate' thinning that are never observed in healthy controls.[16]

Current guidelines rely on an end-diastolic wall thickness ≥15 mm (or ≥13 mm with a familial mutation) to define HCM,[2] a threshold that by definition misses those in the preclinical phase. Many sarcomere mutation carriers show no hypertrophy for years, with one longitudinal study estimating only 46% penetrance at 15-year follow-up.[17] Notably, a subset of 32% of carriers in that study fulfilled HCM criteria by CMR (often due to apical or subtle hypertrophy) that were not apparent by echocardiography, emphasizing the superior sensitivity of CMR in early disease detection. In the previously mentioned study, Shiwani *et al.* created a personalized threshold accounting for sex, age, and BSA, showing to maintain high specificity.[9] However, even such refined metrics remain predicated on surpassing a hypertrophy threshold.

Genotype-positive individuals traditionally classified as 'phenotype-negative' may, in fact, represent an early phenotypic stage of HCM that eludes current diagnostic thresholds. In our cohort, the demographic-based 95th-percentile cut-off identified only 11% of mutation carriers, whereas WTSD was increased in 64% of them, achieving and maintaining an excellent diagnostic specificity. Our findings, together with emerging imaging data, suggest a continuum of disease expression from genotype-positive/phenotype-negative to overt HCM more than a mere binary classification. The WTSD provides a sensitive and reproducible means to detect these subtle yet clinically relevant abnormalities. The ability to identify mutation carriers who have not yet developed the hypertrophic phenotype may have important prognostic implications. It is reasonable to assume that some of these individuals, particularly the younger ones, may go on to develop hypertrophy over time. However, we cannot determine if or how many mutation carriers would eventually develop the phenotype, as the design of the present study did not include longitudinal follow-up of these patients. Future studies are needed to assess whether the novel therapies for HCM, as cardiac myosin inhibitors, can prevent the development of the hypertrophic phenotype and reduce the associated risks in mutation carriers with marked wall thickness heterogeneity. The implications are also significant for individuals who may never develop the phenotype, as the imaging-based identification of a possible sarcomeric mutation would prompt genetic testing in first-degree relatives, allowing for the early identification of those who may eventually develop the disease.

Sex-specific results of the present study provided additional diagnostic insight. Female HCM patients are known to present later and with more advanced disease than males,[7,8] largely

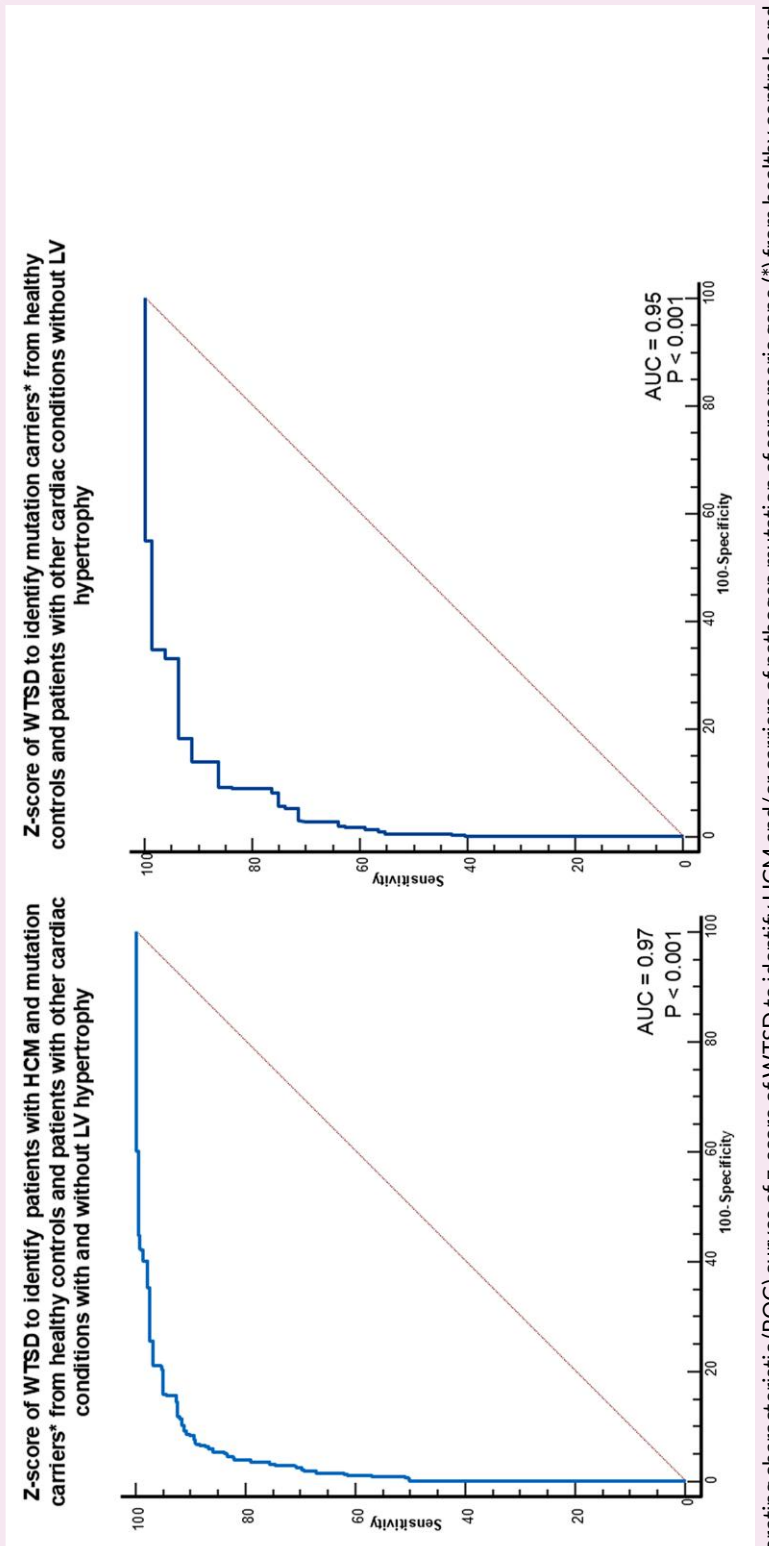


Figure 7 Receiver operating characteristic (ROC) curves of z-score of WTSD to identify HCM and/or carriers of pathogen mutation of sarcomeric gene (*) from healthy controls and patients with other cardiac conditions.

due to smaller LV size and under-detection by wall thickness–based criteria. By applying sex-specific WTSD thresholds, we achieved near-perfect accuracy in women with overt HCM and identified about three-quarters of female mutation carriers who would otherwise remain undiagnosed. In contrast, conventional or demographic-adjusted cut-offs tend to miss women until marked hypertrophy develops, consistent with their historical under-recognition.[8] Beyond highlighting sex-specific diagnostic performance, our ROC analyses showed that a WTSD Z-score >2 is essentially the optimal threshold for detecting sarcomeric cardiomyopathy in both sexes. This corresponds to simple absolute cut-offs of approximately 2.2 mm in men and 1.8 mm in women. Thus, WTSD can be readily implemented either as a normalized Z-score (using sex- and age-specific reference ranges) or, in centres without automated Z-score calculation, by applying these straightforward sex-specific millimetre thresholds.

In men, WTSD also outperformed other metrics but detected a smaller proportion of carriers, likely reflecting higher normal wall thickness and earlier phenotypic conversion. Male sex has been associated with a nearly threefold higher risk of transitioning to overt HCM[17]; accordingly, only one-third of mutation carriers in our cohort were male. Thus, while WTSD enhances detection in both sexes, its impact is especially relevant for women, who show slower and less diffuse hypertrophy progression.

An additional observation was the coexistence of abnormally thin and mildly thickened myocardial segments in mutation carriers, contributing to elevated WTSD. Such localized thinning and myocardial crypts, confined mainly to the basal septum or posterior wall, have been described as early markers of preclinical HCM.[18] These microstructural abnormalities, enriched in mutation carriers and absent in controls, highlight subtle remodelling long before overt hypertrophy.

In our analysis, WT was measured on the compact myocardium, excluding trabeculations. The combination of thinning, crypts, and mild thickening likely reflects uneven regional development, yielding the distinctive structural fingerprint of HCM. Clinically, this underscores that marked heterogeneity in wall thickness, even within normal absolute values, should raise suspicion in at-risk individuals. Advanced echocardiographic techniques have also shown promise in this arena: for instance, abnormal diastolic flow propagation and heightened apical rotation can distinguish mutation carriers with 83% sensitivity before hypertrophy develops.[19] Future studies are needed to assess the combined diagnostic role to identify mutation carriers of these novel functional echocardiographic techniques with the evaluation of wall thickness heterogeneity through WTSD measured by CMR.

Although WTSD was derived from CMR, an echocardiographic analogue appears feasible, either by segmental end-diastolic thickness sampling (facilitated by contrast to optimize endocardial delineation) or by 3D echo regional mapping; however, modality-specific reference ranges and cross-modality validation will be required before clinical adoption.

Finally, WTSD complements, not replaces, genetic testing by detecting a structural intermediate phenotype in genotype-positive individuals before absolute hypertrophy thresholds are reached. This is particularly impactful in women, who are disproportionately diagnosed later with worse functional status, as WTSD achieved near-perfect diagnostic accuracy in overt HCM and identified the majority of genotype-positive/phenotype-negative women in our cohort. Clinically, elevated WTSD can (i) prompt genetic testing in patients imaged for other indications, (ii) justify shorter surveillance intervals and closer rhythm monitoring in carriers, and (iii) could serve as imaging endpoint for future prevention trials, even in the current absence of approved therapies for pre-hypertrophic stage.

Within current ESC and AHA/ACC guideline frameworks, these sex-specific WTSD thresholds would therefore be applied as an adjunctive imaging criterion in individuals with suspected or genotype-positive HCM whose maximal wall thickness remains below conventional diagnostic cut-offs, helping to prioritize genetic testing, CMR, and closer follow-up in those with markedly increased wall thickness heterogeneity (*Figure 8*).

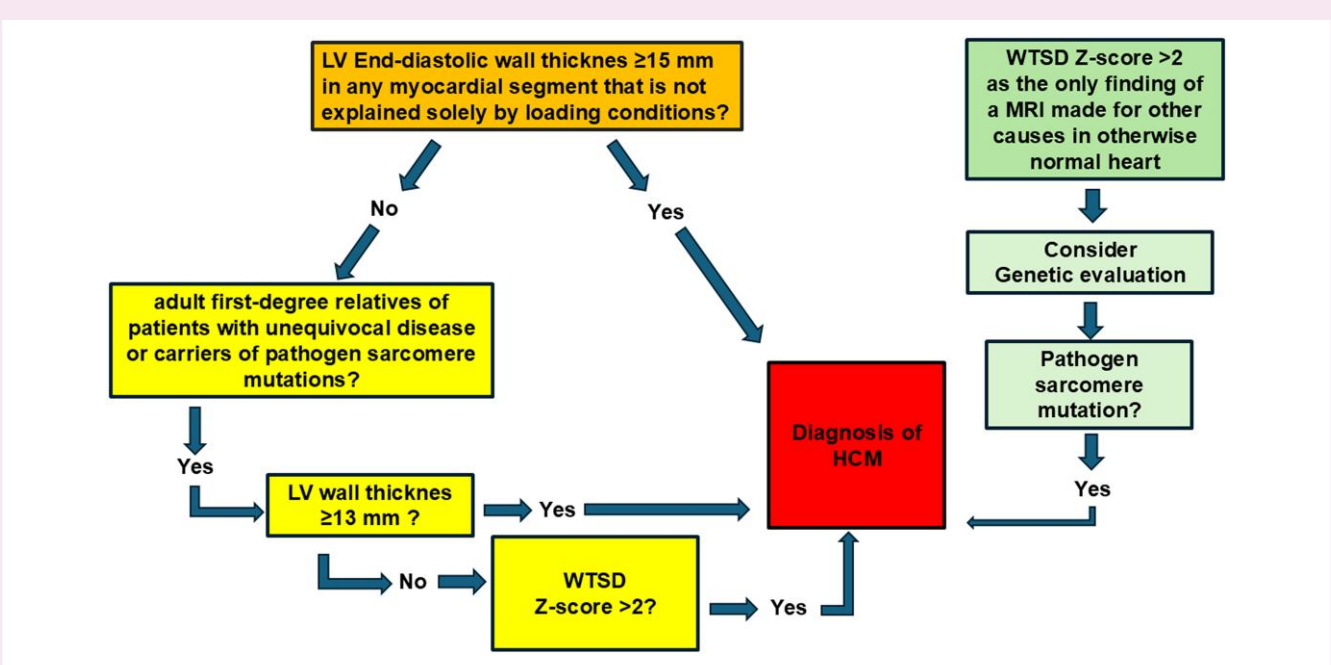


Figure 8 Potential modifications of the diagnostic algorithm in HCM based on the results of the present study.

Some study limitations need to be mentioned. First, the results of the present study were obtained in relatively small patient cohorts, particularly considering the limited number of mutation carriers. Larger population-based studies are needed to confirm our findings in both mutation carriers and patients with overt HCM. Second, although we did not exclude healthy controls who engaged in regular recreational physical activity, we cannot confirm or rule out whether WTSD can distinguish between HCM and elite athletes with physiological (eccentric) hypertrophy. Systematic drug-history data were not prospectively collected in mutation carriers. On review of available records, the majority were not receiving cardiac medications, and none were treated with disease-specific myosin inhibitors. Third, our cohort was almost exclusively Caucasian. Therefore, the thresholds we propose for WTSD may not be directly generalizable to populations with different ethnic backgrounds, as prior studies have reported ethnic differences in cardiac morphology and hypertrophic cardiomyopathy expression. Fourth, because no carriers of pathogenic variants were identified for the rarer HCM-associated sarcomeric genes, the study was underpowered to assess potential gene-specific differences in WTSD expression, despite comprehensive panel testing. Finally, we did not specifically design the study to enrol patients with borderline wall thickness, in whom diagnostic uncertainty is often greatest. Prospective studies in unselected populations, enriched for such diagnostically challenging cases, are needed to formally quantify the incremental diagnostic value of WTSD over current guideline-based criteria

Conclusions

Our study demonstrates that wall thickness heterogeneity, quantified as WTSD, is a powerful and versatile imaging biomarker across the full spectrum of sarcomeric HCM. WTSD outperforms conventional wall thickness–based criteria not only in detecting early phenotypic expression among genotype-positive individuals, but also in accurately identifying overt disease. These findings, if confirmed by future large population study, may support the integration of WTSD into diagnostic algorithms, promoting a shift from rigid threshold-based definitions towards a more dynamic, sex-based, and sensitive approach to HCM diagnosis and risk stratification.

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All authors meet the authorship criteria.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

Author contributions

Giovanni Donato Aquaro [Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing—original draft, Writing—review & editing (lead)], Licordari Roberto [Methodology, Writing—original draft, Writing—review & editing (equal)], Giancarlo Todiere [Investigation, Methodology (equal)], Umberto Ianni (Conceptualization, Writing—original draft [supporting], Data curation, Methodology [equal]), Carmelo De Gori (Investigation [equal], Methodology [supporting]), Marco Merlo [Investigation, Supervision (lead)], Andrea Barison (Formal analysis, Investigation [equal], Methodology [supporting]), Antonio De Luca [Investigation, Methodology (equal)], Chrysanthos Grigoratos (Investigation [equal], Methodology [supporting]), Francesca Raimondi (Investigation, Methodology [equal], Writing—review & editing [supporting]), Lamia Ait Ali [Investigation, Methodology (equal)], Pierluigi Festa [Methodology, Writing—review & editing (equal)], Francesco Negri [Investigation (supporting)], Francesco Bianco (Investigation [equal], Methodology [supporting]), Fabrizio Ricci [Investigation, Methodology (equal)], Nicoletta Botto [Investigation, Methodology (equal)], Lapo Taddei (Methodology [equal], Writing—original draft [supporting]), Mattia Alberti [Investigation, Methodology (supporting)], Andrea Italiano [Investigation (equal)], Luna Gargani [Writing—review & editing (supporting)], Lorenzo Faggioni [Supervision (supporting)], Dania Cioni (Validation [supporting], Visualization [equal]), Riccardo Lencioni [Supervision (equal)], Michele Emdin [Supervision (supporting)], Emanuele Neri (Data curation [equal], Writing—review & editing [supporting]), Gianfranco Sinagra [Supervision (equal)], Raffaele De Caterina [Methodology, Supervision (equal)], and Gianluca Di Bella [Investigation, Supervision, Writing—review & editing (equal)]

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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