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Short title: Biomarkers and interstitial fibrosis in hypertrophic cardiomyopathy

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WHAT'S NEW?

Cardiac fibrosis is present in many cardiovascular diseases and leads to numerous negative consequences such as cardiac remodeling, systolic and diastolic dysfunction, as well as life-threatening arrhythmias. Cardiac fibrosis is also present in hypertrophic cardiomyopathy (HCM), the most common genetic disease. This fibrosis can be assessed invasively and non-

invasively with cardiac magnetic resonance (CMR). Moreover, the use of blood parameters which could be indicative of fibrosis, is constantly being investigated. We distinguish two types of fibrosis — replacement and interstitial. While replacement fibrosis is largely explored, the interstitial type is much less studied. In this study, we observed an association between one of the plasma markers of fibrosis — galectin 3 and interstitial fibrosis as assessed in CMR studies in HCM patients. This paper could enable us to better understand the course of pathology leading to cardiac fibrosis, and further the development of more effective forms of fibrosis treatment in the future.

ABSTRACT

Background: Cardiac fibrosis is a hallmark of hypertrophic cardiomyopathy (HCM) and has proven unfavorable clinical significance. Replacement fibrosis is better known, and has already been studied on a larger scale, whereas interstitial fibrosis is less explored.

Aims: We aimed to analyze relationship between serum biomarkers and interstitial fibrosis, as assessed with cardiac magnetic resonance (CMR) in HCM.

Methods: We performed 3T CMR scans in 50 HCM patients to assess interstitial fibrosis as expressed by extracellular volume (ECV). In all patients, we determined levels of serum cardiac-specific (troponin T [TnT], N-terminal prohormone of brain natriuretic peptide [NT-proBNP]) and fibrosis-specific (procollagen I C-terminal propeptide, procollagen III N-terminal propeptide, transforming growth factor β1, galectin 3) biomarkers. Patients were divided based on their median value of ECV.

Results: The final study population consisted of 49 patients. The median value of ECV in our cohort was 28.1%. Patients stratified according to median ECV differed in terms of several variables: body mass index, late gadolinium extent, NT-proBNP and galectin 3 levels (all P <0.05). Cardiac biomarkers (TnT and NT-proBNP) and galectin 3 were significantly correlated with ECV (r_S = 0.34; P = 0.02; r_S = 0.39; P = 0.006; r_S = 0.43; P = 0.002, respectively). Galectin 3 and body mass index were found to be independent predictors of ECV (odds ratio [OR], 2.29 [1.07–4.91]; P = 0.03; OR, 0.81 [0.68–0.97]; P = 0.02, respectively).

Conclusions: Galectin 3 was an independent predictor of interstitial fibrosis in HCM patients expressed as elevated ECV values. The other fibrosis-specific biomarkers measured were not useful in detecting interstitial fibrosis in HCM. In addition, there was a positive correlation between classical cardiac biomarkers and interstitial fibrosis in HCM patients.

Key words: ECV, galectin 3, hypertrophic cardiomyopathy, myocardial fibrosis

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is one of the most common inherited myocardial diseases in the general population with a prevalence of approximately 1 in 500 [1]. Pathogenic mutations in sarcomeric genes are responsible for great majority of HCM cases [2]. The diagnosis of HCM is based on the detection of left ventricular hypertrophy (LVH) which cannot be explained by abnormal loading conditions [3, 4] via echocardiography or cardiac magnetic resonance (CMR). Besides LVH, myocardial fibrosis is also a hallmark of HCM, being a risk factor for ventricular arrhythmias, diastolic dysfunction and end-stage heart failure [3]. There are two types of fibrosis distinguished which differ from each other in terms of pathology and clinical significance: replacement and interstitial fibrosis [5]. Necrosis of myocytes leads to replacement (or local/reparative) fibrosis while general processes, such as hypertension, genetic mutations, and inflammation result in the interstitial (or diffuse/reactive) type. Both types of fibrosis are present in HCM. The negative effects of replacement fibrosis and its clinical significance have already been studied extensively, leading for instance to the recognition of qualitative evaluation of replacement fibrosis by means of CMR-based late gadolinium enhancement (LGE) in the American College of Cardiology guidelines on HCM, as an established and validated risk factor of sudden cardiac death (SCD) [4]. On the other hand, interstitial fibrosis is far less studied and more poorly understood.

Nowadays, thanks to tremendous strides in hardware and software, CMR is becoming the more preferable and most frequently utilized diagnostic modality in the detection and quantification of cardiac fibrosis. Both replacement fibrosis (via LGE quantification) and interstitial fibrosis (by means of T1 mapping) can be assessed. T1-based assessment of extracellular volume (ECV) allows for the precise assessment of global and regional (segmental) interstitial fibrosis. However, due to costs, limited availability, and the infeasibility of performing the CMR exams in certain groups of patients (e.g. with claustrophobia, hemodynamic compromise, certain ferromagnetic implants, etc.), there is ongoing research on the potential role of serum fibrosis biomarkers in the diagnosis and monitoring of the fibrosis process. The cardiac fibrosis process itself is a very complex, hence there are specific groups of biomarkers related to it, including markers of collagen metabolism (procollagen I C-terminal propeptide [PICP], procollagen III N-terminal propeptide [PIIINP]) or fibrosis-controlling and regulating factors (among these, transforming growth factor β1 [TGF-β1] or galectin 3 [gal-3]) [6, 7]. Although still not fully uncovered, associations between cardiac-specific (i.e. natriuretic peptides, troponin) and fibrosis-specific markers, and replacement fibrosis (as expressed as LGE) have already been explored to some extent in HCM. In contrast, any relationships between circulating fibrosis-

specific biomarkers and interstitial fibrosis in HCM have been much less studied and the results obtained thus far are rather unclear.

Given that there are already some proven relations between fibrosis-specific biomarkers and LGE, we hypothesized that cardiac- and fibrosis-specific biomarkers may also be related with interstitial fibrosis. Thus, the primary aim of the study was to compare the circulating levels of cardiac- and fibrosis-specific biomarkers between HCM patients with high and low burdens of interstitial fibrosis.

METHODS

Study population

In this prospective, single-center, observational study, we included 50 HCM patients. The study took place between December 2019 and April 2021. The diagnosis of HCM was made on the basis of the current guidelines of the European Society of Cardiology. Exclusion criteria were patients with previously implanted cardiac devices, severely reduced kidney function (GFR <30 ml/min/1.73 m^2), or HCM phenocopies, such as amyloidosis, hemochromatosis, Fabry disease, etc. All the patients underwent the following procedures: laboratory tests, echocardiography, a six-minute walk test, electrocardiographic Holter monitoring, and CMR. Echocardiographic examinations were performed on commonly available machines in accordance with the current European and American guidelines [8]. We received informed consent from all of the patients involved. The study was conducted in accordance with the Declaration of Helsinki, and prior to the study, the protocol was approved by the Jagiellonian University Ethical Committee (the protocol number 1072.6120.237.2019; date of approval: 24 October 2019.)

Cardiac magnetic resonance (CMR)

CMR imaging with cine CMR, native and post-contrast T1 mapping, and LGE imaging was performed on a 3T CMR scanner (Magnetom Skyra, Siemens, Erlangen, Germany) according to local imaging protocols, as previously described [9]. The analyses were conducted with the Syngo.VIA software, version VB 40 (Siemens, Erlangen, Germany). The CMR studies were analyzed based on the guidelines of the Society of Cardiovascular Magnetic Resonance [10]. The short-axis LGE scans were obtained approximately 15 min after the intravenous application of 0.1 mmol/kg of body weight of gadolinium-based contrast agent. The presence of LGE in both short and perpendicular long axis images indicated fibrosis. The quantitative extent of LGE was assessed with a 5-standard-deviation threshold in consecutive short-axis

images, and its value was computed as a percentage of the total left ventricle (LV) mass [10]. T1 mapping was acquired using a Modified Look Locker Inversion Recovery sequence before, and 15 mins after, gadolinium-based contrast agent administration. The following parameters of the sequence were used: breath-hold TR/TE of 281/1.1 ms, slice thickness of 8 mm, matrix of 144 × 256 pixels, FOV from 320 × 260 mm^2, and a flip angle of 35°. The native and post-contrast T1 values were obtained by drawing regions of interest in the mid-wall regions of every segment according to the AHA 16-segment. Drawings from the center of the LV cavity determined T1 blood pools. Between the pre- and post-contrast, T1 maps of the regions of interest were copied. We excluded artifact segments. The global values of native and post-contrast T1 times were computed as the means of all segments. The ECV was calculated with the formula [15]: ECV = ([1/(post-contrast T1] − 1/[native T1])/(1/[blood post-contrast T1]) − 1/(blood native T1)*(1 − Hct).

Laboratory measurements

The blood samples were centrifuged (1600 × g) for 10 min at 4°C. The material was stored at –20°C until the analysis. The quantification of collagen type I and III synthesis markers in blood samples was performed using an Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with the manufacturer's directions (Bioassay Technology Laboratory, Shanghai, China). The level of sensitivity of the assays was 2.26 ng/ml for PICP, and 2.52 ng/l for PIIINP. The detection range for the PICP ELISA kit was 5–1500 ng/l and for the PIIINP ELISA kit 5–2000 ng/l. The intra-assay and inter-assay coefficients of variation were <8% and <9% for PICP, and <7% and <10% for PIIINP, respectively. Plasma concentrations of gal-3 and TGF-β1 were assessed using the Nori Human ELISA Kit in line with the manufacturer's protocol (Genorise Scientific, Inc.; Glen Mills, PA, US). The assay sensitivity of the Nori Human TGF-β1 ELISA Kit was 6 pg/ml, and the detection range was 31–2000 pg/ml. The sensitivity of the Nori Human gal-3 ELISA Kit was 30 pg/ml, and the detection range was 156-10000 pg/ml. In accordance with the standards of our laboratory, normal values for N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were defined as <125 pg/ml, and for troponin T (TnT) <14 pg/ml.

Statistical analysis

Values are presented as percentages (counts) or mean (standard deviations) or median (interquartile range). The Shapiro–Wilk test was used for the assessment of the normal distribution of quantitative variables. The continuous variables were compared with a t-test or

U-Mann–Whitney test when appropriate, and the qualitative ones with the χ^2 test or Fisher's exact test. The correlation analyses were performed based on the Spearman rank correlation. All parameters (presented in Tables 1 and 2) differentiating groups by their ECV median with *P*-values <0.1 were included in the regression analyses. Uni- and multivariable logistic regression methods were used to analyze the associations between the analyzed parameters and greater ECV burdens. Redundant parameters (correlated with other predictors with R >0.4) were not included in multivariable logistic regression models. The results were statistically significant if the p-value was <0.05. The analysis was performed with the Statistica package, version 13.3 (StatSoft, TIBCO Software Inc., Palo Alto, CA, US).

RESULTS

Baseline characteristics

Due to incomplete data, the final study population from whom ECV and biomarkers values were obtained consisted of 49 patients. One patient was not included in the analysis due to not obtaining complete CMR data. Patients were divided based on their median value of ECV, which was 28.1%. The comparison of the baseline parameters between the groups with lower and higher values of ECV is presented in Table 1. Patients with higher ECV had a lower body mass index (BMI) (mean [SD], 28.2 [5.4] kg/m^{2}vs. 31.9 [5.7] kg/m^2; *P* = 0.03) and larger LGE extent (median [SD], 5.21% [1.6%–9.38%] vs. 2.82% [0%–4.83%]; *P* = 0.04).

Relationships between cardiac-specific and fibrosis-specific markers, and fibrosis

Among established cardiac-specific markers, NT-proBNP was more elevated in patients with higher ECV (median [SD], 823 [440–1465] pg/ml vs. 199.5 [116–817.5] pg/ml; *P* = 0.007), and TnT showed a trend towards significance (*P* = 0.08) (Table 2) in these patients in comparison to those with lower ECV. As for serum fibrosis biomarkers, only gal-3 clearly distinguished the groups (median [SD], 2.93 [1.9–4.25] ng/ml vs. 1.93 [1.68–2.97] ng/ml; *P* = 0.03), whereas all other fibrosis-specific markers ~~were~~ comparable between the groups. In the correlation analysis between biomarkers and ECV, a moderate correlation between gal-3 and ECV (r_S = 0.43; *P* = 0.002), and also weaker correlations both between NT-proBNP and ECV (r_S = 0.39; *P* = 0.006) and, TnT and ECV (r_S = 0.34; *P* = 0.02) were observed (Table 3). Only TnT weakly correlated with the LGE extent (r_S = 0.35; *P* = 0.01). Neither cardiac-specific nor fibrosis-specific markers correlated with LV mass (Table 3). LGE and ECV were correlated with each other (r_S = 0.47; *P* <0.001).

Predictor factors for elevated ECV

Among all the parameters differentiating patients with lower and higher ECV values, univariable regression analysis revealed significant associations between ECV and BMI, LGE extent, left ventricular outflow tract gradient and gal-3 (Table 4). However, in the multivariable regression model, only BMI and gal-3 were independently associated with ECV (odds ratio [OR], 0.81 [0.68–0.97]; *P* = 0.02; OR, 2.29 [1.07–4.91]; *P* = 0.03, respectively) (Table 4). Nonetheless, it is worth highlighting that the LGE extent was also very close to being significant.

Parameters associated with higher LGE extent

In the group of patients with confirmed LGE (n = 37), we conducted an analysis aimed at identifying parameters associated with higher LGE extent, defined as equal or greater than the median in our subgroup, which was 5.21%. In the univariable analysis, the following variables were associated with the LGE extent: ejection fraction (EF) (OR, 0.91 [0.84–0.99]; *P* = 0.03), ventricular tachycardia (VT) (OR, 5.56 [1.14–27.16]; *P* = 0.03), with TnT being the only biomarker differing between the groups and showing a trend toward significance (OR, 1.06 [0.999–1.12]; *P* = 0.05) (Supplementary material, *Tables S1, S2*). Due to the small size of the subgroup, we did not perform a multivariable analysis identifying independent predictors of higher LGE extent.

DISCUSSION

The study findings can be summarized as follows. Firstly, HCM patients stratified according to median ECV differ in terms of several key clinically-relevant variables, such as BMI, LGE extent and mass, as well as NT-proBNP and gal-3 levels. Secondly, cardiac-specific (NT-proBNP and TnT) as well as fibrosis-related (gal-3) markers are correlated with ECV, whereas only TnT was correlated with LGE extent. Thirdly, EF and VT were found to be associated with replacement fibrosis (LGE extent), whereas gal-3 and BMI were found to be independently associated with interstitial fibrosis (ECV).

Biomarkers and replacement fibrosis

So far, the issue of the relationship between cardiac-specific biomarkers and replacement fibrosis expressed as LGE has been quite widely studied, mainly due to the fundamental role of replacement fibrosis in HCM pathology. Despite this extensive investigation, the topic is still far from resolved, with conflicting results being reported. In our study, we observed a

positive correlation between LGE extent and TnT levels. In the sub-analysis involving only patients with LGE presence, troponin levels presented a tendency to predict higher LGE extent in the univariable analysis and only EF and VT were associated with LGE extent. It is worth remarking that this observed association could well be interpreted in the following way: LGE extent is a VT predictor (which we and others have shown previously), not the other way round [9, 11]. Several authors have also observed higher levels of TnT in HCM patients with LGE presence [12, 13], and in yet other studies, there have been associations reported between TnT levels and LGE mass or extent [14, 15]. However, Kawasaki et al., despite the presence of higher troponin levels in LGE positive HCM patients, observed no correlation between TnT and LGE extent [12]. Interestingly, Gommans et al. observed the association between LGE extent and elevated TnT in their whole group of HCM patients; however, this association was no longer seen when only patients with LGE were considered [16].

Moving on to the question of natriuretic peptides, numerous authors have found that there is a higher level of BNP or NT-proBNP in LGE positive patients (e.g. via qualitative classification into LGE positive and negative patients) as well as finding relationships between quantitative LGE evaluation (LGE extent or LGE mass) and NT-proBNP [12, 14, 17]. Despite that, we did not observe any correlation between NT-proBNP levels and LGE, which is similar to the report of Miyaji et al., who observed no differences in the percentage of LGE extent among the tertiles of BNP levels of HCM patients [18]. In the study of Roldan et al., NT-proBNP was increased in those patients with fibrosis as assessed by CMR; still, NT-proBNP values did not correlate with LGE extent [19]. Similarly, Gommas et al. did not report differences in the NT-proBNP levels in the patients with LGE extent <15% and ≥15% [15].

Significantly, among the plasma fibrosis-specific biomarkers analyzed in our study, no association with LGE was observed, nor have other researchers shown such a relationship with respect to gal-3, PICP, PIIINP or TGF-β1 [14, 15, 20]. Thus, the question concerning the relationship between fibrosis-specific markers and replacement fibrosis seems to have been answered somewhat by this and previous studies since no such associations have been found in HCM.

Biomarkers and interstitial fibrosis

As previously mentioned, the topic of interstitial fibrosis and its relationship with serum biomarkers is much less studied, with few papers centred on cardiac-specific markers in the setting of interstitial fibrosis in HCM. Neubauer et al. presented data from a large registry on HCM, and reported a significant trend of higher NT-proBNP values with increasing ECV

quartiles as well as a significant trend of higher TnT with higher ECV quartiles, but only in males rather than in females [21]. Ho et al. [22] reported correlations between ECV and NT-proBNP, but no relations were observed between ECV or LGE and serum PICP or troponin levels in a cohort of 77 people including sarcomere mutation carriers with and without LVH. Importantly, we found that TnT and NT-proBNP were elevated in HCM patients. Similarly, we found higher values of NT-proBNP in those with higher ECV values. In terms of troponins, we noted only a trend towards significance in patients with higher ECV, and a weak but significant correlation with ECV. In the recent paper on this topic by Shi et al. [23], no differences within LGE, native T1, and the ECV were observed in HCM patients stratified into normal and elevated troponin levels.

Our observations on the lack of associations between collagen turnover-related biomarkers (PICP, PIIINP) and TGF-β1 with interstitial fibrosis are consistent with other studies conducted so far. Apart from the work cited above by Ho et al., Fang et al., who studied a population of a similar size to ours, observed no differences in levels of PINP and PIIINP in patients with lower and higher ECV values [22, 24]. In another paper, Ellims et al. reported no correlations between PINP or PIIINP levels, and CMR-determined replacement (LGE extent) and diffuse (post-contrast T1 mapping) fibrosis [25].

Our observation of the association of lower BMI with higher ECV values is consistent with the study of Neubauer et al. [21]. Since, apart from gal-3, only BMI emerged as a predictor of ECV, this observation requires additional attention and further study.

Galectin 3 in HCM

Galectin 3 has recently become a “hot topic”, being a key molecule integrating cardiac stress injury, inflammation, and fibrosis. As such, its significance and role has been studied in various cardiovascular diseases, including heart failure and cardiomyopathies. In our previous study in dilated cardiomyopathy (DCM) patients, we observed **no correlation of** gal-3 with biopsy-determined fibrosis while circulating gal-3 was independently associated with cardiovascular outcomes in DCM; its serial measurements also correlated with markers of fibrosis, including markers of collagen synthesis [26]. Several studies reported gal-3 levels to be significantly higher in patients with HCM than in controls [27, 28]. Data regarding gal-3 and fibrosis assessed by CMR in HCM are scarce. Gawor et al. [14] reported no correlation between gal-3, sST2, GDF-15 levels and LGE extent in 60 HCM patient. Also in the study of Hu et al. [29], HCM patients with and without LGE did not differ in terms of gal-3 levels. Thus, our observations on the lack of gal-3 association with LGE are consistent with the above-mentioned

authors. To the best of our knowledge, our study is the first to reveal a rather strong relationship between gal-3 and interstitial fibrosis in HCM. The observed association between gal-3 and ECV (interstitial fibrosis) and the lack of any relationship between gal-3 and LGE (replacement fibrosis) clearly points to distinct metabolic pathways and the significance of these two types of fibrosis in HCM. In our opinion, this relationship deserves further attention and in-depth research, especially bearing in mind that the data on the relationship of gal-3 with HCM SCD-risk are to date contradictory - Gawor et al. observed no significant relationships between sST2 and gal-3 levels, and HCM SCD-risk while Emet et al. found a significant correlation between the estimated 5-year risk of SCD and serum levels of gal-3 [27, 30].

Limitations

We admit that there are several limitations in our study. Firstly, our group is relatively small, and this is a single-center study. Secondly, due to the impaired quality of T1 mapping images in patients with implantable devices, we excluded such patients, who are high-risk; still, a sub-analysis of such patients would probably be valuable. Thirdly, blood markers concentrations may vary daily or weekly; thus, sequential measurements could provide additional data. Fourthly, due to low number of cases with LGE we did not perform a multivariable analysis and results of the univariable logistic regression analysis could be less sound and conclusion should be interpreted with caution.

CONCLUSIONS

Cardiac-specific biomarkers (troponin, NT-proBNP) are weakly related with both replacement and interstitial fibrosis, and markers of collagen turnover as well as TGF-β1 seem to be inadequate as fibrosis-related biomarkers in HCM. On the other hand, Galectin-3 appears to be strongly related with interstitial fibrosis in HCM, making it a strong candidate for being a potential biomarker in this setting.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/kardiologia_polska.

Article information

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Table 1. Baseline characteristics. Comparison of HCM patients stratified according to ECV

Parameter	**All (n = 49)**	**ECV <28.1% (n = 24)**	**ECV ≥28.1% (n = 25)**	***P*-value**
Age, years, mean (SD)	51.9 (14.4)	53 (15.2)	50.8 (13.8)	0.59
Male sex, n (%)	34 (69.4)	18 (75)	16 (64)	0.4
BMI, kg/m^2, mean (SD)	30.0 (5.8)	31.9 (5.7)	28.2 (5.4)	**0.03**
NYHA class, median (IQR)	1 (1–2)	1.5 (1–2)	1 (1–2)	0.61
Left ventricular outflow track obstruction, n (%)	18 (36.7)	11 (45.8)	7 (28)	0.2
Family history of sudden cardiac death, n (%)	4 (8.2)	2 (8.3)	2 (8)	1
Ventricular tachycardia, n (%)	14 (28.6)	7 (29.2)	7 (28)	0.93
Syncope, n (%)	6 (12.2)	4 (16.7)	2 (8)	0.42
Estimated 5-year risk of sudden cardiac death, %, median (IQR)	2.8 (1.9–4.5)	3.0 (2–3.9)	2.4 (1.4–5)	0.33
Diabetes mellutis, n (%)	6 (12.2)	2 (8.3)	4 (16)	0.67
Coronary artery disease, n (%)	8 (16.3)	5 (20.8)	3 (12)	0.46
Hypertension, n (%)	29 (59.2)	17 (70.8)	12 (48)	0.1
Atrial fibrillation, n (%)	6 (12.2)	4 (16.7)	2 (8)	0.42
Dyslipidaemia, n (%)	23 (46.9)	13 (54.2)	10 (40)	0.32
SBP, mm Hg, mean (SD)	131.2 (21.6)	136.6 (20.7)	126 (21.7)	0.09
6MWT distance, m, mean (SD)	431.5 (121.2)	447.5 (101.4)	416.2 (138.1)	0.39
Hb, g/dL, median (IQR)	14.4 (13.6–15.4)	14.6 (13.8–15.6)	14.3 (13.4–15.2)	0.25
Creatinine, µmol/l, median (IQR)	84 (78–94)	83.5 (78–90)	85 (78–94)	0.62
Echocardiographic data				
Indexed LVEDd, mm/m^2, mean (SD)	22.5 (3.4)	21.8 (3)	23.2 (3.6)	0.14
Max. wall thickness, mm, mean (SD)	19.9 (4.0)	19.5 (3.8)	20.3 (4.2)	0.48
Left atrium diameter, mm, mean (SD)	43.5 (6.6)	44.3 (6.6)	42.8 (6.7)	0.43
Left atrial volume index, ml/m², median (IQR)	41.6 (33.7–64.3)	51.7 (33.7–67.6)	40.5 (33.5–56.5)	0.36
Max. LVOT gradient, mm Hg, median (IQR)	20 (8–55)	37 (11.5–78.5)	15 (8–36)	0.07
E/A, median (IQR)	1.05 (0.76–1.83)	0.97 (0.7–1.76)	1.1 (0.77–1.9)	0.66
E’ intraventricular septum, m/s, median (IQR)	0.06 (0.04–0.08)	0.07 (0.06–0.08)	0.05 (0.04–0.07)	0.05
E/E’, median (IQR)	10.1 (8–14)	8.8 (6.8–13.9)	11.3 (8.8–14)	0.21

Right ventricular systolic pressure, mmHg, median (IQR)	23 (16–28)	20 (15–26)	24 (19–29)	0.14
Cardiac magnetic resonance data				
Indexed LVED volume, ml/m^2, mean (SD)	79.7 (12.4)	79.8 (13.6)	79.6 (11.4)	0.96
Indexed LVES volume, ml/m^2 , median (IQR)	23.9 (20.5–30.6)	25.3 (21.6–30.6)	23.3 (19.9–30.3)	0.71
Indexed stroke volume, ml/m^2, mean (SD)	53.1 (10.3)	53.6 (10.4)	52.6 (10.4)	0.73
EF, %, median (IQR)	69 (61–73)	68.5 (61–72.5)	69 (63– 74)	0.8
Left ventricular mass, grams, mean (SD)	196.5 (53.3)	205.8 (54.4)	187.8 (51.8)	0.25
LGE presence, n (%)	37 (75.5)	16 (66.7)	21 (84)	0.16
LGE mass, grams, median (IQR)	6.65 (0.5–13)	5.6 (0–8.65)	8.65 (2.55–20.5)	0.05
LGE extent, %, median (IQR)	3.80 (0.45–8.29)	2.82 (0–4.83)	5.21 (1.6–9.38)	**0.04**
Native T1 time, ms, median (IQR)	1262.4 (1238.8–1315)	1241 (1217.1–1275.7)	1289.2 (1258–1326.6)	**0.002**
Post-contrast T1 time, ms, mean (SD)	470.4 (55.5)	479.4 (53.1)	461.6 (57.4)	0.27
Native blood T1 time, ms, median (IQR)	1851.7 (1820.3–1919.3)	1836.2 (1787.3–1903.5)	1854.7 (1825.3–1960.7)	0.12
Post-contrast blood T1 time, ms, mean (SD)	306.8 (43.2)	289.9 (42.4)	323 (38)	**0.006**
ECV, %, median (IQR)	28.1 (25.6–30.2)	25.5 (23.4–26.6)	30.2 (29–32.1)	**<0.001**
Pharmacotherapy				
BB, n (%)	42 (85.7)	22 (91.7)	20 (80)	0.42
Diltiazem/verapamil, n (%)	7 (14.3)	4 (16.7)	3 (12)	0.7
ASA, n (%)	8 (16.3)	4 (16.7)	4 (16)	1
ACEi/ARB, n (%)	22 (44.9)	12 (50)	10 (40)	0.48
MRA, n (%)	14 (28.6)	7 (29.2)	7 (28)	0.93
Loop diuretics, n (%)	14 (28.6)	6 (25)	8 (32)	0.59
Amiodarone, n (%)	2 (4.1)	2 (8.3)	0	0.23
OAC, n (%)	6 (12.2)	4 (16.7)	2 (8)	0.42
Statins, n (%)	20 (40.8)	9 (37.5)	11 (44)	0.64

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; ASA, acetylsalicylic acid; BB, beta-blocker; BMI, body mass index; E/A, ratio of early mitral inflow E-wave and late mitral inflow A-wave velocity; ECV, extracellular volume; E/E', ratio of early mitral inflow velocity to early mitral myocardial velocity; EF, left ventricle

ejection fraction; Hb, hemoglobin; LGE, late gadolinium enhancement; LVED, left ventricle end-diastolic; LVEDd, left ventricle end-diastolic diameter; LVES, left ventricle end-systolic; LVOT, left ventricular outflow tract; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association class; SBP, systolic blood pressure; OAC (VKA and non-VKA) oral anticoagulants; 6MWT, 6-minute walk test

Table 2. Comparison of biomarkers between HCM patients, stratified according to ECV

Parameter	**All (n = 49)**	**ECV <28.1% (n = 24)**	**ECV ≥28.1% (n = 25)**	***P*-value**
Troponin T, pg/ml	15 (8–25)	13 (7.5–19)	20 (8–28)	0.08
NT-proBNP, pg/ml	481 (197–1117)	199.5 (116–817.5)	823 (440–1465)	**0.007**
PICP, ng/ml	268.9 (203.7–350.2)	267.7 (201.6–350)	268.9 (228.3–354.1)	0.73
PIIINP, ng/l	399.6 (328.2–476.8)	408.4 (335.8–469.4)	376.2 (328.2–546)	0.98
Gal-3, ng/ml	2.40 (1.83–3.38)	1.93 (1.68–2.97)	2.93 (1.9–4.25)	**0.03**
TGF-β1, pg/ml	50.5 (21.6v110.1)	51.2 (21.4–126.9)	50.5 (28.7–110.1)	0.9

Values are presented as median (IQR)

Abbreviations: Gal-3, galectin-3; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PICP, procollagen I C-terminal propeptide; PIIINP, procollagen III N-terminal propeptide; TGF-β1, transforming growth factor β1

Table 3. Correlation between biomarkers and selected CMR data

	LV mass		**LGE extent**		**ECV**	
	r_S	*P*-value	r_S	*P*-value	r_S	*P*-value
Troponin T	0.18	0.21	**0.35**	**0.01**	**0.34**	**0.02**
NT-proBNP	–0.005	0.97	0.16	0.26	**0.39**	**0.006**
PICP	–0.06	0.7	0.15	0.3	0.06	0.67
PIIINP	–0.17	0.25	–0.04	0.81	-0.04	0.79
Gal-3	0.02	0.87	0.11	0.45	**0.43**	**0.002**
TGF-β1	–0.1	0.5	0.01	0.94	0.07	0.62

Abbreviations: LV, left ventricular; r_S – R Spearman coefficient; other — see Table 1 and 2

Table 4. Uni- and multivariable regression models for presence of higher value of ECV.

	Univariable		**Multivariable**	
Parameter	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**
BMI	0.89 (0.79–0.99)	0.03	0.81 (0.68–0.97)	0.02
SBP	0.97 (0.95–1.005)	0.09	–	–
LGE extent	1.16 (1.006–1.34)	**0.04**	1.24 (0.996–1.55)	0.05
Max. LVOT gradient	0.98 (0.96–0.999)	**0.03**	0.98 (0.96–1.006)	0.13
E' intraventricular septum	2.66 (0.003–2610.67)	0.78	–	–
Troponin T	1.03 (0.99–1.07)	0.1	–	–
NT-proBNP	1.0002 (0.9997–1.0007)	0.41	–	–
Gal-3	1.73 (1.03–2.89)	0.03	2.29 (1.07–4.91)	0.03

Abbreviations: see Table 1 and 2