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ORIGINAL ARTICLE

CARDIAC MULTIMARKER TESTING IN NON-OBSTRUCTIVE HYPERTROPHIC CARDIOMYOPATHY

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Summary

The natural history of hypertrophic cardiomyopathy (HCM) varies from an asymptomatic and benign clinical course to sudden premature death. Therefore, the new markers are searched with the aim to detect risk patients and improve their prognosis. The aim of this study was to test a cardiac multimarker testing strategy in detection of initial structural changes in patients with nonobstructive hypertrophic cardiomyopathy (HCM). In the group of 47 patients with nonobstructive HCM (58.4 ± 12.4 years, 12 emales) the mean left ventricle mass was 344.8 ± 129.9 g, the mean left ventricle mass index was 171.4 ± 60.2 $g.m^{-2}$. We observed increased concentration of cardiac markers in peripheral blood: high sensitivity troponin T (hsTnT): median: 9 ng/L (IQR: 5 - 16 ng/L), vs. controls: 7 (5 - 9) ng/L, p 0.03; creatine kinase MB isoenzyme (CK MB): 2 (1.4 - 2.7) µg/L vs. 1.6 (1.1 - 2.2) µg/L, p 0.04; myoglobin 46.4 (33.3 - 65.2) µg/L vs. 35.6 (22.8 - 43.7) µg/L, p 0.001; heart type of fatty acid binding protein (hFABP): 1.8 (1.4 - 3.3) µg/L vs. 1.6 (1.3 - 2.1) µg/L, p 0.05; glycogen phosphorylase BB (GPBB): 3.9 (2.5 - 6.3) µg/L vs. 2.3 (1.9 - 4.2) µg/L, p 0.001. The analysis of the associations of left ventricle mass index and cardiac markers revealed its significant association with hFABP (r = 0.41, 95% CI: 0.07-0.66, p 0.01), CKMB (r = 0.33, 95% CI: 0.11-0.59, p 0.05), and with hsTnT (r = 0.39, 95%CI: 0.12 - 0.62, p 0.008). This study indicates potential clinical use of the multimarker testing in diagnosis and screening of the hypertrophic cardiomyopathy.

Key words: cardiac markers; hypertrophic cardiomyopathy; morphology

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a heterogeneous myocardial disorder characterized by myocardial hypertrophy with structural and functional abnormalities. The incidence of HCM is approximately 1 in 500 (0.2 %) of the general population (1).

The clinical outcome of HCM is diverse, ranging from asymptomatic patients to cardiac arrhythmias, congestive heart failure, and sudden cardiac death. Patients with HCM require lifelong follow-up to detect changes in symptoms, risk of adverse events, left ventricle outflow tract obstruction (LVOTO), LV function and cardiac rhythm (1,2). There is a broad spectrum of biomarkers in peripheral blood, which are potentially useful for diagnosis and risk stratification in patients with HCM. But, only cardiac troponins and natriuretic peptides have the most robust data (3-5). The association of myocardial hypertrophy, cardiac troponin and natriuretic peptide levels is not specific for the degree of left ventricle remodeling in HCM (6-8). The increase of these parameters is probably driven more by myocardial damage or pressure overload in patients with obstructive type of LV hypertrophy and not by hypertrophy *per se* (9,10). Therefore, new markers or multimarker testing is performed with the aim to detect even initial structural changes of the myocardium (5,11). Because of these facts, we performed the study with the aim to use multimarker testing strategy in detection of the initial structural changes in patients with nonobstructive hypertrophic cardiomyopathy.

SUBJECTS AND METHODS

Study population

Study population consisted of patients with nonobstructive hypertrophic cardiomyopathy. The diagnosis of hypertrophic cardiomyopathy was based on history of illness, physical examination, echocardiography, and cardiac catheterization in accordance with European Society of Cardiology recommendations (2). Based on the results of previous cardiac catheterization, patients with resting or provoked LVOTO were not included in the study. Also, patients with significant concomitant disease, such as pulmonary disease, arterial hypertension, malignancy, autoimmune disorders, neurodegenerative disorders, thyroid disease, or concurrent viral disease were excluded. Plasma cardiac marker levels were compared to control group of healthy 21 blood donors (40.4 ± 8.5 years) with no evidence of cardiovascular disease according to ECG stress test and echocardiography. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the ethical committee of our institution. Informed consent was obtained from each patient. Baseline demography and clinical characteristics of the study population are shown in table 1.

Table 1. Patient demographic and clinical characteristics

	n = 47
Age (years)	58.4 ± 12.4
Female gender, n (%)	12 (26)
NYHA functional class, n (%)	
- I	21 (45)
- II	18 (38)
- III-IV	8 (17)
Atrial fibrillation, n (%)	14 (30)
Plasma creatine (μmol.L-1)	94.0 ± 29.8
Diabetes mellitus, n (%)	13 (28)
Hyperlipidemia, n (%)	23 (49)
Smoking, n (%)	20 (43)
Medication, n (%)	
Ca blockers	22 (47)
β-blockers	30 (64)
Diuretics	20 (43)
ACE inhibitors/sartans	23 (49)
Implantable devices, n (%)	
DDD pacing	7 (15)
ICD/BiV	5 (11)

Abbreviations: NYHA, New York Heart Association; ACE, angiotensin converting enzyme; DDD, dual chamber pacing; ICD, implantable cardioverter/defibrillator; BiV, biventricular pacing.

Assessment of cardiac markers

Blood samples were obtained from venous catheters, introduced into tube collectors containing no preservatives. Within 1 h, the blood samples were centrifuged for 10 min at 2500g and the supernatant was removed and kept at –70°C until the assay was performed.

Cardiac troponin T was measured by high-sensitivity electrochemiluminiscence imunoassay using the Troponin T hs STAT assay for Elecsys 2010 analyser (Roche Diagnostics, Mannheim, Germany). The hs-cTnT assay had an analytical range from 3

to 10 000 ng/L. The cut-off value according to the manufacturer was 14 ng/L (the 99th percentile of healthy reference population values, n = 616, CV = 9.0%).

Plasma concentrations of cardiac markers were determined by the Evidence Investigator protein biochip system (Randox Laboratories, Crumlin, UK). The cardiac array included the following markers: creatine kinase MB isoenzyme (CKMB), myoglobin (MYO), glycogenphosphorylase BB isoenzyme (GPBB), and heart type of fatty acid binding protein (hFABP). Concentrations of all cardiac markers are given in µg/L. The assay analytical parameters - analytical range, cut-off value were as follows: CKMB: 0.4-100 µg/L, 3.9 µg/L, MYO: 1.8-700 µg/L, 59.0 µg/L, GPBB: 2-290 µg/L, 7.3 µg/L, and hFABP: 0.15 – 150 µg/L, 4.5 µg/L. The interassay coefficients of variability (n = 10) were in accordance with data provided by the manufacturers in the range of CV=2.0-9.8 % with the exception of myoglobin assay where the measured CV was 11.6-15.6 % (CKMB: 3.9 µg/L – 7.5 %, 30.9 µg/L – 8.6 %, MYO: 86.3 µg/L – 13.5 %, 120.7 µg/L – 15,6 %, GPBB: 8.4 µg/L – 6.6 %, 68.1 µg/L – 7.9 %, hFABP 3.2 µg/L – 5.0 %, 43.8 µg/L – 8.7 %). Internal quality control measurements were carried out by using samples provided by the kit manufacturers.

Echocardiography

Echocardiography was performed in agreement with the American Society of Echocardiography and European Association of Cardiovascular Imaging standards evaluating the following parameters: left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) diameters, left atrium diameter (LA), right ventricle diameter (RV), end-diastolic interventricular septum (IVST) and posterior wall thickness (PWT), LV ejection fraction (LV EF), left ventricle fractional shortening (FS), left ventricle mass (LVM) and left ventricle mass index (LVMI) (12). Left ventricle outflow tract gradient was evaluated in accordance with European Society of Cardiology guidelines at rest and after provocation by Valsalva maneuver [2]. For estimation of the left ventricle mass, we used formula: 0.8 x {1.04 [(LVID + PWT + IVST)3- (LVID)^3]} + 0.6 g, where LVID is left ventricular internal diastolic diameter, PWT is posterior wall thickness, and IVST means interventricular septal thickness diameter in diastole.

Statistics

Statistical analysis was performed by MedCalc Software, version 14 (MedCalc Software bvba,

Table 2. Plasma cardiac markers in in patients with non-obstructive hypertrophic cardiomyopathy

Parameter	HCM (n = 47) median IQR min-max	Controls (n = 21) median IQR min - max	p
CK MB (µg/L)	2.0 1.4-2.7 0.7-5.58	1.6 1.1-2.2 0.4-3.8	0.045
Myoglobin (µg/L)	46.4 33.3-65.2 20.4-162.4	35.6 22.8-43.7 13.5-92.7	0.0013
hFABP (µg/L)	1.8 1.4-3.3 0.6-8.1	1.6 1.3-2.1 0.7-3.2	0.05
GPBB (µg/L)	3.9 2.5-6.3 2-13.5	2.2 1.9-4.2 1.2-10.6	0.001
hsTnT (ng/L)	9 5 - 16 3 - 67	7 5 - 9 3 - 12	0.03

Abbreviations: CK MB, creatine kinase MB isoenzyme;
GPBB, glycogenphosphorylase BB isoenzyme;
hFABP, heart type of fatty acid binding protein;
hsTnT, high sensitivity troponin T.

Ostend, Belgium). Normally distributed variables are expressed as means ± standard deviation, while non-normally distributed variables are expressed as median (interquartile range). Categorical variables are presented as percentages. Continuous variables were compared using Student's t-test, Mann-Whitney or Wilcoxon's tests, where appropriate. Linear regression was applied to evaluate the relationship between continuous variables. The degree of association between continuous variables was calculated using Pearson's correlation coefficient. A p-value < 0.05 was considered statistically significant.

RESULTS

In patients with non-obstructive hypertrophic cardiomyopathy, all plasma cardiac markers were increased when compared with the control group. In patients with HCM, plasma high sensitivity troponin T level was significantly increased [median: 9 ng/L, IQR: 5 - 16 ng/L, vs. median: 7 ng/L, IQR: 5 - 9 ng/L, p 0.03]. Also other parameters were significantly increased (Table 2).

Table 3. Echocardiographic parameters of the study population

	n = 47
LA (mm)	48.2 ± 7.2
RV (mm)	26.1 ± 3.8
IVST (mm)	19.4 ± 4.4
LV ESD (mm)	31.9 ± 7.1
LV EDD (mm)	47.1 ± 7.3
PWT (mm)	13.2 ± 2.7
LV EF (%)	67.1 ± 9.9
LV FS	33.7 ± 8.7
TAPSE (mm)	22.7 ± 3.2
LVM (g)	344.8 ± 129.9
LVMI($g.m^{-2}$)	171.4 ± 60.2

Abbreviations: LA, left atrium;
RV, right ventricle diameter;
IVST, interventricular septal thickness;
LV ESD, left ventricular end-systolic diameter;
LV EDD, left ventricular end-diastolic diameter;
PWT, posterior wall thickness;
LV EF, left ventricular ejection fraction;
TAPSE, tricuspid annular plane systolic motion;
LV FS, left ventricular fractional shortening;
LVM, left ventricle mass;
LVMI, left ventricle mass index.

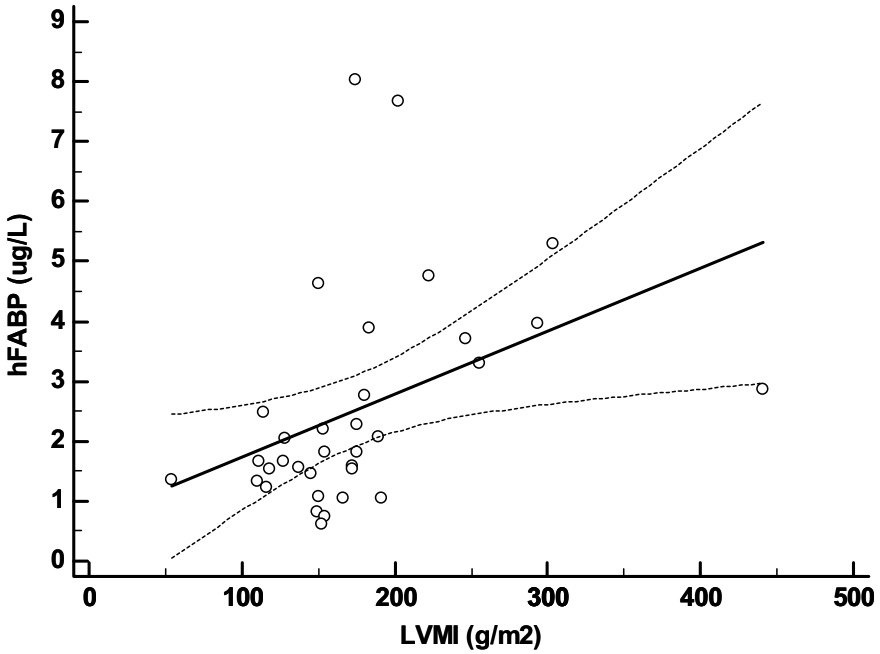
All patients underwent two-dimensional and Doppler echocardiography. Table 3 shows the summary information of echocardiographic parameters of patients with hypertrophic cardiomyopathy. The mean of the anteroposterior left atrium dimension was 48.2 ± 7.2 mm, and it exceeded reference values (female: 38 mm, male: 40 mm) in 37 (78 %) patients. The mean internal diameter of right ventricle was 26.1 ± 3.8 mm, and it exceeded reference value (31 mm) in 2 (4 %) patients. The mean of the internal end systolic left ventricle dimension was 31.9 ± 7.1 mm, and it exceeded reference values (female: 34.8 mm, male: 39.8 mm) in 4 (8 %) patients. The mean of the internal end diastolic left ventricle dimension was 47.1 ± 7.3 mm, and it exceeded reference values (female: 52.2 mm, male: 58.4 mm) in 3 (6 %) patients. The mean of the interventricular septum thickness was 19.4 ± 4.4 mm, and it exceeded recommended thickness for diagnosis of hypertrophic cardiomyopathy (≥ 15 mm) in all patients. The mean left ventricle mass was 344.8 ± 129.9 g, and it exceeded reference values for two-dimensional method (female: 150 g, male: 200 g) in all patients. The mean left ventricle mass index was 171.4 ± 60.2 $g.m^{-2}$, and it exceeded reference values for two-dimensional method (female: 88 $g.m^{-2}$, male: 102 $g.m^{-2}$) in all patients. The mean of the left ventricle ejection fraction was 67.1 ± 9.9 %, and, only in 2 (4%) patients, the LV ejection fraction was below the reference values (female: 54 %, male: 52 %). The mean of the left ventricle fractional shortening was 33.7 ± 8.7, and it exceeded the reference values (female: 27 - 45, male: 25 - 43) in 3 (6 %) patients. None of the patients had left ventricle outflow tract obstruction.

The analysis of the associations of left ventricle mass index and cardiac markers revealed its significant association with hFABP (r = 0.41, 95% CI: 0.07 - 0.66, p 0.01), CKMB (r = 0.33, 95% CI: 0.11 - 0.59, p 0.05), and with hsTnT (r = 0.39, 95% CI: 0.12 - 0.62, p 0.008) (Table 4, Fig. 1-3).

Table 4. Association of the left ventricle mass index and cardiac markers

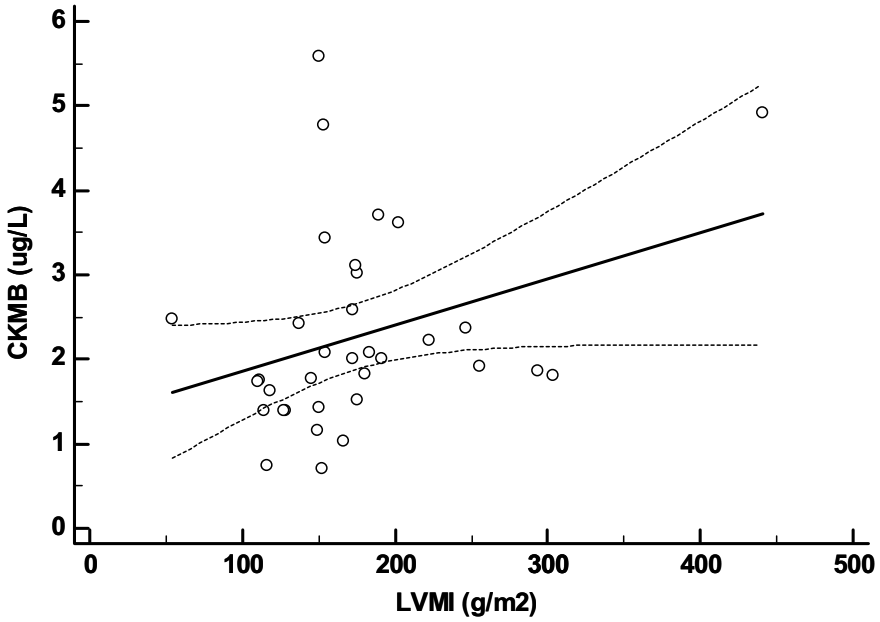
Parametr	r	95% CI	p
CKMB	0.33	0.011-0.59	0.05
Myoglobin	0.2	-0.14 - 0.51	0.25
hFABP	0.41	0.07 - 0.66	0.01
GPBB	0.21	-0.12 - 0.51	0.21
hs TnT	0.39	0.12 - 0.62	0.008

Figure 1. Association of the hFABP and left ventricle mass index



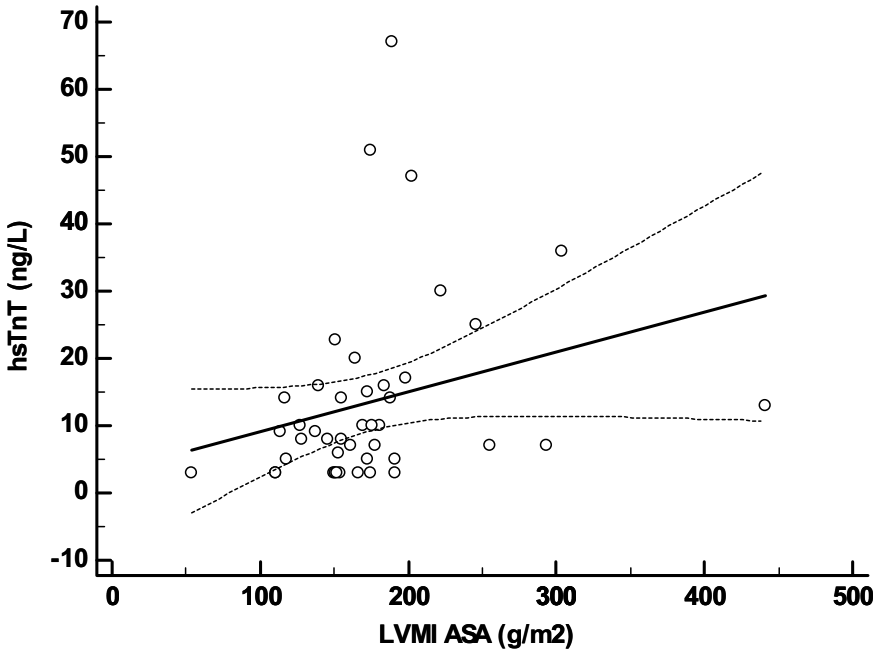
Abbreviations: hFABP, heart type of fatty acid binding protein;
LVMI, left ventricle mass index.

Figure 2. Association of the CKMB and left ventricle mass index



Abbreviations: CKMB, creatine kinase MB isoenyzme;
LVMI, left ventricle mass index.

Figure 3. Association of the hsTnT and left ventricle mass index



Abbreviations: hsTnT, high-sensitivity troponin T;
LVMI, left ventricle mass index

DISCUSSION

Hypertrophic cardiomyopathy is an autosomal dominant inherited myocardial disease defined by the presence of increased left ventricular wall thickness that is not solely explained by abnormal loading conditions (2). Therefore, genetic screening is an important part of the diagnostic procedure and patient relatives follow up. According to the guidelines, HCM is inherited as an autosomal dominant genetic trait with a 50% risk of transmission to off springs. Some cases are explained by de novo mutations, but apparently sporadic cases can arise because of incomplete penetrance in a parent and, less commonly, autosomal recessive inheritance. In patients fulfilling HCM diagnostic criteria, sequencing of sarcomere protein genes identifies a disease-causing mutation in up to 60% of cases (1,2). The likelihood of finding a causal mutation is highest in patients with familial disease and lowest in older patients and individuals with non-classical features. But, the lack of robust data on genotype–phenotype associations means that the impact of genetic testing on clinical management is limited mostly to some of the rare genetic causes of HCM. Because of this, clinical follow up of all patient relatives is recommended. It includes regular clinical, electrocardiography and echocardiography examination. Therefore, the new strategies for detection of early stages of myocardial hypertrophy are investigated. One of the methods to detect the early myocardial structural changes is the use of multimarker strategy (3,5,6).

In our study, we analyzed simultaneous assessment of cardiac markers which can reflect structural changes of myocardium. Troponins (T and I) and CK isoenzyme MB (CK-MB) are the gold standards for diagnosis of myocardial injury (3,13). Recently, a heart type of fatty acid binding protein (hFABP) and glycogen phosphorylase isoenzyme BB (GPBB) have been evaluated as new markers of myocardial injury (3). hFABP is a novel small cytosolic protein that is abundant in the heart, and is released into the circulation following myocardial ischemia and necrosis (13). GPBB is a key enzyme of glycogenolysis. Its degree of association with the sarcoplasmatic reticulum glycogenolysis complex depends essentially on the metabolic state of the myocardium. With the onset of tissue hypoxia, GPBB is converted from a structurally bound into a cytoplasmatic form and released into circulation (14). All these markers seem to be promising in detection of the structural changes of myocardium.

In our study, all markers were increased in patients with nonobstructive hypertrophic cardiomyopathy. The fact that these markers were tested in patients without left ventricle obstruction is very important because it reflects that these markers are increased as a result of myocardial hypertrophy and not as a result of pressure overload caused by left ventricle tract obstruction.

CONCLUSION

This study showed that plasma concentrations of cardiac markers (hsTnT, hFABP, GPBB, CKMB and myoglobin) are increased in patients with hypertrophic cardiomyopathy. Furthermore, this increase is present even in the absence of left ventricle tract obstruction. This study indicates potential clinical use of the multimarker testing in diagnosis and screening of the hypertrophic cardiomyopathy.

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