Predictive Values of Apelin for Myocardial Fibrosis in Hypertrophic Cardiomyopathy

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Summary

Apelin was proved to attenuate cardiac interstitial fibrosis. However, the association between apelin level and myocardial fibrosis in patients with hypertrophic cardiomyopathy (HCM) is still unclear.

This study aims to determine whether apelin is associated with myocardial fibrosis in HCM and investigate the predictive values of apelin for myocardial fibrosis in HCM.

One hundred sixteen patients with HCM were enrolled in this study. Plasma apelin-13 and high-sensitivity cardiac troponin I (cTNI) were measured. The cardiac systolic and diastolic functions were evaluated by echocardiography, and the presence and extent of cardiac fibrosis were assessed by cardiac magnetic resonance. All statistical data were analyzed by SPSS version 21.0.

The percentage of late gadolinium enhancement (LGE) was negatively correlated with apelin and positively correlated with cTNI, maximum wall thickness (MWT), and left ventricular mass index in the overall patients with HCM and LGE. Apelin, cTNI, MWT, and left ventricular ejection fraction were independent predictors of the presence of LGE. The combined measurements of MWT ≥ 19 mm and/or apelin ≥ 1.24 pg/mL, as well as the combined measurements of MWT ≥ 19 mm and/or cTNI ≥ 0.031 ng/mL, obtained higher specificity and higher sensitivity, thus, indicating the presence of LGE.

Plasma apelin and cTNI are independent predictors of myocardial fibrosis. The combined measurements of serum apelin and MWT, as well as cTNI and MWT, showed higher predictive values for predicting myocardial fibrosis in patients with HCM.

Key words: cTNI, MWT, Prediction of fibrosis in heart

Hypertrophic cardiomyopathy (HCM), which is a genetic disease caused by mutations in sarcomeric proteins, is the most common cause of sudden cardiac death (SCD) in the young.1-3 Hypertrophy and fibrosis are the major determinants of mortality, morbidity, and SCD in HCM.4

Myocardial fibrosis has been implicated in the pathogenesis of SCD and is thought to play a key role in arrhythmia and the development of systolic dysfunction. This information provides a strong pathophysiological rationale for evaluating myocardial fibrosis as a biomarker of HCM risk.4,5 Late gadolinium enhancement (LGE) on cardiac magnetic resonance (CMR) plays an important role in the assessment of the myocardial fibrosis of HCM.4 However, the need for the administration of contrast agents for LGE assessment increases the cost of the study and may lead to nephrogenic systemic fibrosis in patients with end-stage chronic kidney disease or to adverse allergic reactions.5 Furthermore, the inherent features of the LGE method lower its utility in cases of diffused localized fibrosis rather than localized fibrosis.6

The pressing need to improve risk stratification in HCM has driven the search for better biomarkers that identify the underlying substrate responsible for heart failure and SCD.6 These features are thought to be mediated by elevated cardiac trophic factors, including angiotensin II.1 Its progression over the disease course is associated with worsening diastolic function, increasing left ventricular outflow tract gradient (LVOTG), higher New York Heart Association (NYHA) class, and higher incidence of SCD.6

Apelin, which is the endogenous ligand of the orphan G protein-coupled receptor APJ, was discovered in 1998.8 As the natural antagonist of angiotensin II, apelin has both direct and indirect effects on cardiovascular physiology.9 Previous studies showed that apelin could prevent cardiac fibroblast activation and collagen production.10 The experiments performed in pressure-overloaded mice showed that pretreatment with low doses of apelin significantly decreased myocardial fibrosis and downregulated
The study of our research group found that treatment with apelin could attenuate cardiac interstitial fibrosis via the TGF-β1-dependent signaling transduction pathway in the hearts of cTnT-Q92 transgenic mice. These findings in the transgenic mouse model of human HCM illustrate the potential role of apelin in the treatment of cardiac interstitial fibrosis.

However, the association between apelin level and myocardial fibrosis in patients with HCM is unclear. In this study, we determined whether apelin is associated with myocardial fibrosis in HCM and investigated the predictive values of apelin for myocardial fibrosis in HCM.

Methods

Patients: We included patients with HCM in Fuwai Hospital (Beijing, China) between January 2012 and January 2015. The diagnosis of HCM was defined as a maximum left ventricular wall thickness of 15 mm (or 13 mm with an unequivocal family history of HCM), as measured by echocardiography or CMR. Patients with hypertension (patients with systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg or patients receiving antihypertensive drugs); established coronary artery disease (previous myocardial infarction, previous percutaneous coronary intervention and/or coronary artery bypass grafting, or > 70% stenosis in any major coronary artery on angiography); systolic heart failure (left ventricular fraction [LVEF] < 50% as measured by echocardiography); renal dysfunction (estimated glomerular filtration rate < 60 mL/minute/1.73 m²); valvular heart disease (aortic stenosis, mitral valve regurgitation, mitral valve stenosis); connective tissue disease; and previous myocardial infarction were also excluded. The exclusion criteria were applied to all groups.

The patients’ demographic characteristics; cardiovascular risk factors (such as diabetes, primary hypertension, hyperlipidemia, previous MI, previous stroke, chronic renal failure, smoking, and family history of CAD); physical examination; laboratory data; and cardiovascular medication history were collected. Twelve-lead electrocardiography (ECG), 24-hour ambulatory ECG monitoring, transthoracic echocardiography, CMR, and coronary angiography were performed. Eventually, 116 patients were enrolled in the study.

The Ethics Committee of Fuwai Hospital approved the study protocol, and all enrolled patients signed the informed consent. This study was also performed in accordance with the Declaration of Helsinki.

Blood chemistry and apelin measurements: Venous peripheral blood samples were drawn from all patients under resting conditions within 2 hours upon admission to this study. Samples were taken from the peripheral vein to measure blood chemistry, apelin-13, and high-sensitivity cardiac troponin I (cTNI).

Blood was collected in chilled tubes containing potassium ethylenediaminetetraacetic acid (1 mg/mL blood) and aprotinin (50 kIU/mL blood) for apelin analysis. These tubes were centrifuged immediately at 3000 rpm for 15 minutes at ambient temperature. Plasma was extracted and frozen in aliquots at −80 °C until analysis. Plasma apelin-13 levels were determined using a commercially available enzyme immunoassay without extraction (Phoenix Pharmaceuticals, Burlingame, CA, USA; catalog number: FEK-057-15) according to the manufacturer’s instructions. The apelin ELA kit has cross-reactivity with apelin-12, apelin-13, and apelin-36. The intra-assay coefficient of variance (CV) was 3.8%, the interassay CV was 12.5%, and the limit of detection was 20 pg/mL. cTNI was measured using a fourth-generation assay on an Elecsys 2010/cobas e 411 instrument (Roche Diagnostics). Plasma cholesterol and creatinine concentrations were measured using an automated chemistry analyzer (Abbott Aeroset, Minnesota) and commercial kits (Abbott).

Hypercholesterolemia was defined as fasting serum total cholesterol level > 5.5 mmol/L or the use of lipid-lowering therapy at the time of the procedure. Diabetes mellitus was defined as hyperglycemia requiring antidiabetic drugs or fasting blood sugar > 7.0 mmol/L.

Echocardiographic evaluation: Transthoracic Doppler echocardiography was performed on all patients by using an ultrasound imaging system (iE33, Philips Healthcare, Andover, MA, USA). We analyzed LVEF, left ventricular fractional shortening, peak velocity across the left ventricular outflow tract (LVOT), mitral systolic peak velocity E/A, and E/e′. The peak early diastolic annular velocity (e′) was obtained using tissue Doppler at the lateral mitral annulus. The peak velocity across the LVOT was also measured, and the peak pressure gradient was estimated using the simplified Bernoulli equation. LVEF was measured using Simpson’s method according to the suggestions of the American Society of Echocardiography.

CMR evaluation: CMR imaging was performed using a 1.5-T speed clinical scanner (Siemens Medical Solutions, Erlangen, Germany) under breath control and electrocardiographic gating. Cine imaging was performed in four-chamber, three-chamber, and two-chamber long- and short-axis views, and the typical imaging parameters were as follows: 360 × 315 mm² field of view, 6-mm-thick sections with a 2-mm gap between sections, 2.7 ms repetition time, 1.2 ms echo time, 40 ms temporal resolution, 192 × 162 pixels image matrix, 70° flip angle, and 1.9 × 1.3 mm² pixel size.

The LGE images were obtained 10-15 minutes after a bolus injection of 0.2 mmol/kg gadolinium diethylenetriamine pentaacetic acid (Magnevist, Schering AG, Berlin, Germany) by using a segmented, phase-sensitive, inversion-recovery, spoiled-gradient echo sequence at the same position as the long- and short-axis cines in the end-diastole. The inversion time was adjusted per patient to an optimally null signal from a normal myocardium typically between 250 and 350 ms. The typical imaging parameters were as follows: 380 × 320 mm² field of view, 6-mm-thick sections with a 2-mm gap between sections, 8.6 ms repetition time, 3.36 ms echo time, 256 × 162 pixels matrix, 25° flip angle, 2 × 1.5 mm² pixel size, and parallel acquisition technique factor of 2. The average total acquisition time was 45 minutes.

All MR image analysis was performed using commercial software (Medis Medical Imaging systems, Netherlands).
Table I. Baseline Characteristics of Patients with or without LGE

| Variables                      | Overall patients (n = 116) | Patients with LGE (n = 85) | Patients without LGE (n = 31) | P value |
|--------------------------------|----------------------------|-----------------------------|-------------------------------|---------|
| Age, years                     | 47.6 ± 10.1                | 42.7 ± 10.4                 | 51.3 ± 9.5                    | < 0.001 |
| Men, n (%)                     | 71 (61.2%)                 | 54 (63.5%)                  | 17 (54.8%)                   | 0.220   |
| Body mass index, kg/m²         | 20.4 ± 4.9                 | 23.4 ± 4.5                  | 17.9 ± 5.7                   | < 0.001 |
| Body surface area, m²          | 1.76 ± 0.18                | 1.77 ± 0.10                 | 1.72 ± 0.23                  | 0.340   |
| Hypertension, n (%)            | 34 (29.3%)                 | 25 (29.4%)                  | 9 (29.0%)                    | 0.120   |
| Diabetes mellitus, n (%)       | 4 (3.4%)                   | 3 (3.5%)                    | 1 (3.2%)                     | 0.230   |
| Hypercholesterolemia, n (%)    | 37 (31.9%)                 | 27 (31.7%)                  | 10 (32.2%)                   | 0.541   |
| Current smokers, n (%)         | 45 (38.8%)                 | 34 (40.0%)                  | 11 (35.3%)                   | 0.090   |
| Time of diagnosis, mean (IQR), months | 12 (1–46)          | 12 (3–46)                   | 4 (1–46)                     | 0.037   |
| Family history of HCM, n (%)   | 23 (19.8%)                 | 20 (23.5%)                  | 3 (9.7%)                     | 0.025   |
| Family history of sudden death, n (%) | 10 (10.3%)          | 9 (10.5%)                   | 3 (9.7%)                     | 0.506   |
| NYHA function class III-IV, n (%) | 40 (34.5%)             | 34 (40.0%)                  | 6 (19.3%)                    | 0.030   |
| AF, n (%)                      | 9 (7.6%)                   | 7 (8.2%)                    | 2 (6.5%)                     | 0.070   |
| Non-sustained VT, n (%)        | 8 (6.7%)                   | 6 (7.1%)                    | 2 (6.5%)                     | 0.803   |
| SBP, mmHg                      | 119.3 ± 13.8               | 121.3 ± 15.5                | 118.5 ± 17.9                 | 0.540   |
| DBP, mmHg                      | 75.1 ± 11.3                | 76.6 ± 9.8                  | 73.5 ± 10.4                  | 0.621   |
| HR, beats/minute               | 65.9 ± 10.9                | 64.9 ± 11.4                 | 67.8 ± 8.6                   | 0.345   |
| Medication, n (%)              |                           |                             |                              |         |
| Beta blocker                   | 92 (79.3%)                 | 68 (80.0%)                  | 24 (77.1%)                   | 0.145   |
| Calcium antagonist             | 35 (30.2%)                 | 26 (30.6%)                  | 9 (29.0%)                    | 0.879   |
| ARB /ACEI                      | 23 (21.5%)                 | 17 (20.0%)                  | 8 (25.8%)                    | 0.544   |
| Statins                        | 13 (11.2%)                 | 9 (10.6%)                   | 4 (12.9%)                    | 0.503   |
| Aspirin                        | 38 (32.7%)                 | 28 (32.9%)                  | 10 (32.2%)                   | 0.337   |
| Diuretics                      | 6 (5.2%)                   | 4 (4.7%)                    | 2 (6.4%)                     | 0.261   |

LGE indicates late gadolinium enhancement; IQR, interquartile range; HCM, hypertrophic cardiomyopathy; NYHA, New York Heart Association; AF, atrial fibrillation; VT, ventricular tachycardia; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; ACEI, angiotensin converting enzyme inhibitor; and ARB, angiotensin receptor blocker.

Image analysis: For the assessment of left ventricular filling patterns, endocardial and epicardial contours were semiautomatically drawn and manually corrected. End-diastolic and end-systolic frames were identified according to the ventricular blood pool area, excluding papillary and trabecular structures, across all end-diastolic temporal phases by using short-axis images from the base to apex. Left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), stroke volume, cardiac output, LVEF, and left ventricular mass (LVM) were quantified and indexed to the body surface area with the software package.\(^{35}\)

The presence of left ventricular LGE was first assessed visually by two observers who were blinded to all patient details. The presence of higher and lower signal intensity areas compared with the nulleld normal myocardium were defined as LGE positive and LGE negative, respectively. The extent of hyperenhancement in each segment was scored as 0 (no enhancement), 1 (0%-25% enhancement), 2 (26%-50% enhancement), 3 (51%-75% enhancement), or 4 (76%-100% enhancement).\(^{36}\) The global volume of LGE (LGE score) was expressed as a percentage of the total maximum score (4 × 17 = 68): 100 × (LGE score)/68. From the short-axis planes, the locations of LGE in the left ventricle were recorded as follows: (1) right ventricular insertion points (the anterior and inferior attachment points of the right ventricle to the interventricular septum), (2) septum (all other septal hyperenhancements), (3) apical (confined to the left ventricular apex), and (4) all other left ventricular locations.\(^{35}\)

Statistical analysis: Statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Continuous variables including age, heart rate, body mass index (BMI), blood pressure, ejection fraction, and laboratory data were expressed as the mean ± standard deviation or median (with interquartile range). Student’s t-test was used if continuous variables were normally distributed, whereas the Wilcoxon two-sample test was used if continuous variables were not normally distributed. Categorical data were summarized as frequency. The chi-square test was used to compare categorical variables including sex, risk factors, and medication.

Multivariate, stepwise, backward, conditional, logistic regression analysis was used to determine the independent predictors of the presence of LGE. All significant parameters in the univariate analysis were selected in the multivariate model. A receiver operator characteristic (ROC) curve analysis was performed to identify the optimal cutoff point of apelin in the prediction of LGE. The area under the curve (AUC) was calculated as a measure of the accuracy of the test. A two-tailed P value < 0.05 was considered significant.

Results

Clinical characteristics: One hundred sixteen patients with HCM were included in this study. Table I presents the baseline characteristics.

LGE was identified in 85 of 116 patients (71.5%). Patients with LGE were younger (42.7 ± 10.4 versus 51.3 ± 9.5 years, \(P < 0.001\)), had larger BMI (23.4 ± 4.5 versus 17.9 ± 5.7, \(P < 0.001\)), and were more likely to have...
a family history of HCM (23.5% versus 9.7%, \( P = 0.025 \)) than those without LGE. The median time from the initial diagnosis of HCM to this study entry was longer in patients with LGE compared with patients without LGE [12 (IQR 3-46) versus 4 (IQR 1-46) months, \( P = 0.037 \)]. Patients with LGE were more likely to be in NYHA functional class IV than patients without LGE (\( P = 0.037 \)). No other significant differences were observed between these two groups concerning family history of sudden death, history of other cardiovascular diseases, nonsustained ventricular tachycardia or atrial fibrillation, and medications taken.

Levels of circulating biomarkers in patients with and without LGE: The median levels of apelin was significantly lower in patients with LGE than in those without LGE [0.44 (IQR 0.12-0.8) versus 1.54 (IQR 0.76-2.33) pg/mL, \( P = 0.001 \)]. By contrast, the median levels of cTNI were significantly higher in patients with LGE than in those without LGE [0.027 (IQR 0.009-0.052) versus 0.011 (IQR 0.004-0.023) ng/mL, \( P = 0.001 \)] (Table II).

Echocardiographic results in patients with and without LGE: Table III shows the echocardiographic results in LGE-positive and negative patients. There were no significant differences with respect to the prevalence of LVOT obstruction, presence of systolic anterior motion, and level of LVOTG in the two groups. Compared with patients without LGE, patients with LGE had lower LVEF (60.6 ± 7.8 versus 65.3 ± 4.9, \( P = 0.025 \)). The prevalence of mitral systolic peak velocity E/A < 1 in LGE-positive patients was higher in LGE-negative patients (70.6% versus 51.6%, \( P = 0.020 \)). The cardiac diastolic function (presented as E/e') was worse in patients with LGE than in patients without LGE [16.3 (IQR 9.5-22.9) versus 10.4 (IQR 4.2-16.4), \( P = 0.030 \)].

CMR results in patients with and without LGE: Patients with LGE had greater MWT (26.6 ± 5.1 versus 20.3 ± 3.3 mm, \( P = 0.001 \)) than patients without LGE. The LVEDV index (LVEDVI) and LVESV index (LVESVI) were larger in patients with LGE than in patients without LGE (67.5 ± 14.2 versus 60.3 ± 11.6 mL/m², \( P = 0.035 \); 18.5 ± 5.6 versus 13.8 ± 3.5 mL/m², \( P = 0.002 \); respectively). The LVEF measured by CMR in LGE-positive patients was lower than LGE-negative patients (61.4 ± 6.3 versus 66.3 ± 5.1, \( P = 0.015 \)). By contrast, the left ventricular remodeling index in patients with LGE was higher than those without LGE (1.46 ± 0.28 versus 1.16 ± 0.35, \( P = 0.029 \)). The most frequent locations of LGE observed by CMR were the RV insertion points identified in 76 of 85 patients (89.4%), followed by other septal locations in 39 patients (45.9%), other left ventricular locations in 17 patients (20%), and left ventricular apex in 16 patients (18.8%). The extent of LGE (presented as LGE%) involved 30.9 ± 15.8% of left ventricular myocardium within patients with LGE (Table IV).

Relationship between LGE% and variables: The extent of LGE was inversely correlated with age (\( r = -0.042, P = 0.353 \); time of diagnosis (\( r = -0.012, P = 0.625 \); \( r = -0.068, P = 0.447 \); LAD (\( r = 0.008, P = 0.876 \); \( r = -0.012, P = 0.849 \); LVESVI (\( r = 0.065, P = 0.251 \); \( r = 0.045, P = 0.533 \); or LVESVI (\( r = 0.108, P = 0.329 \); \( r = 0.094, P = 0.570 \) in the overall patients and LGE-positive patients (Table V).

### Table II. Levels of Biomarkers

| Variables       | Overall patients (n = 116) | Patients with LGE (n = 85) | Patients without LGE (n = 31) | \( P \) value |
|-----------------|---------------------------|---------------------------|-----------------------------|---------------|
| Apelin, pg/mL   | 1.25 (0.67-1.98)          | 0.44 (0.12-0.8)           | 1.54 (0.76-2.33)            | 0.001         |
| cTNI, ng/mL     | 0.019 (0.007-0.048)       | 0.027 (0.009-0.052)       | 0.011 (0.004-0.023)         | 0.001         |

### Table III. Echocardiography Results

| Variables               | Overall patients (n = 116) | Patients with LGE (n = 85) | Patients without LGE (n = 31) | \( P \) value |
|-------------------------|---------------------------|---------------------------|-----------------------------|---------------|
| SAM                     | 38 (32.7%)                | 28 (32.9%)                | 10 (32.2%)                  | 0.979         |
| LVEF (%)                | 63.5 ± 6.2                | 60.6 ± 7.8                | 65.3 ± 4.9                  | 0.025         |
| mitral systolic peak velocity E/A< 1, n (%) | 76 (65.5%)                | 60 (70.6%)                | 16 (51.6%)                  | 0.020         |
| Ea'                     | 13.5 (6.7-19.5)           | 16.3 (9.5-22.9)           | 10.4 (4.2-16.4)             | 0.030         |
| LVOT obstruction, n (%) | 41 (35.3%)                | 30 (35.2%)                | 11 (35.4%)                  | 0.977         |
| LVOTG at rest, mmHg     | 60.4 ± 27.7               | 59.4 ± 34.6               | 62.3 ± 30.5                 | 0.836         |

SAM indicates systolic anterior motion; MR, mitral regurgitation; LVEF, left ventricular ejection fraction; LVFS, left ventricular; and LVOTG, left ventricular outflow tract gradient.
analysis was performed to identify the potential predictors of the presence of LGE. Multivariate logistic regression analysis was performed to identify the variables OR, 95%CI, and P value for each predictor.

Table IV. CMR Results

| Variables         | Overall patients (n = 116) | Patients with LGE (n = 85) | Patients without LGE (n = 31) | P value |
|-------------------|---------------------------|---------------------------|------------------------------|---------|
| LAD, mm           | 40.7 ± 5.8                | 41.6 ± 6.9                | 40.9 ± 7.1                   | 0.690   |
| LVEDD, mm         | 46.0 ± 4.3                | 46.7 ± 4.2                | 45.9 ± 4.8                   | 0.582   |
| MWT, mm           | 23.9 ± 4.2                | 26.6 ± 5.1                | 20.3 ± 3.3                   | 0.001   |
| LVMI, g/m²        | 142.6 (136.5-146.4)       | 140.6 (136.4-144.9)       | 137.3 (134.2-139.3)          | 0.337   |
| LVEDVI, mL/m²     | 63.4 ± 12.5               | 67.5 ± 14.2               | 60.3 ± 11.6                  | 0.035   |
| LVESVI, mL/m²     | 16.4 ± 4.7                | 18.5 ± 5.6                | 13.8 ± 3.5                   | 0.002   |
| LVEF (%)          | 62.8 ± 5.9                | 61.4 ± 6.3                | 66.3 ± 5.1                   | 0.015   |
| Apelin, ng/mL     | 1.36 ± 0.43               | 1.46 ± 0.28               | 1.16 ± 0.35                  | 0.029   |
| SVI, mL/minute    | 48.0 ± 9.4                | 48.5 ± 9.1                | 47.9 ± 10.2                  | 0.507   |
| LVMI, mL/m²       | 3.20 ± 0.79               | 3.19 ± 0.74               | 3.25 ± 0.81                  | 0.675   |
| LGE score         | 16.2 ± 5.9                | 23.0 ± 7.6                | 0 ± 0                        | NA      |
| LGE (%)           | 24.7 ± 13.5               | 30.9 ± 15.8               | 0 ± 0                        | NA      |
| Location of LGE, n (%) | 16 (13.8%)           | 16 (18.8%)                | 0 (0%)                       | NA      |
| LV apex           | 76 (65.5%)                | 76 (89.4%)                | 0 (0%)                       | NA      |
| RV insertion points | 39 (33.6%)           | 39 (45.9%)                | 0 (0%)                       | NA      |
| Septum            | 17 (14.7%)                | 17 (20%)                  | 0 (0%)                       | NA      |

LGE indicates late gadolinium enhancement; LAD, left atrium diameter; LVEDD, left ventricular end-diastolic diameter; MWT, maximum wall thickness; LVMI, left ventricle mass index; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LVRI, left ventricular remodeling index; SVI, stroke volume index; CI, cardiac index; RV, right ventricle; and NA, not applicable.

Table V. Correlation of LGE% with Variables

| Variables         | Overall patients (n = 116) | Patients with LGE (n = 85) | P value |
|-------------------|---------------------------|---------------------------|---------|
| Age, years        | −0.325                    | < 0.001                   | 0.217 | 0.051 |
| BMI, kg/m²        | −0.074                    | 0.529                     | 0.036 | 0.748 |
| BSA, m²           | 0.183                     | 0.034                     | 0.268 | 0.005 |
| Time of diagnosis, months | −0.042                  | 0.353                     | 0.052 | 0.276 |
| Apelin, pg/mL     | −0.417                    | < 0.001                   | −0.392 | < 0.001 |
| cTNI, ng/mL       | 0.357                     | < 0.001                   | 0.268 | 0.021 |
| LAD, mm           | 0.008                     | 0.876                     | 0.012 | 0.849 |
| MWT, mm           | 0.399                     | < 0.001                   | 0.312 | < 0.001 |
| LVEDV index, mL/m²| 0.096                     | 0.251                     | 0.045 | 0.533 |
| LVESV index, mL/m²| 0.108                     | 0.329                     | 0.094 | 0.570 |
| LVEF, %           | −0.370                    | < 0.001                   | −0.276 | 0.001 |
| LVMI, g/m²        | 0.349                     | < 0.001                   | 0.298 | < 0.001 |

LGE indicates late gadolinium enhancement; BMI, body mass index; BSA, body surface area; cTNI, cardiac troponin; LVOTG, left ventricular outflow tract gradient; LAD, left atrium diameter; MWT, maximum wall thickness; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; and LVMI, left ventricular myocardium mass index.

Table VI. Multivariable Logistic Regression Analysis for Prediction of LGE

| Variables | OR      | 95%CI     | P value |
|-----------|---------|-----------|---------|
| Apelin    | 1.247   | 0.957-1.428 | < 0.001 |
| cTNI      | 1.994   | 1.284-4.539 | 0.023   |
| MWT       | 1.508   | 1.094-1.845 | 0.001   |
| LVEF      | 0.923   | 0.673-0.995 | 0.001   |

CI indicates confidence interval; OR, odds ratio; LVOTG, left ventricular outflow tract gradient; cTNI, cardiac troponin; MWT, maximum wall thickness; and LVEF, left ventricular ejection fraction.

Multivariate logistic regression analysis for identifying the predictors of the presence of LGE: Multivariable analysis was performed to identify the potential predictors of the presence of LGE. All significant parameters in the univariate analysis (BSA, apelin, cTNI, MWT, LVEF, and LVMI) were selected in the multivariable model. Both apelin and cTNI were independent predictors of the presence of LGE in patients with HCM (OR = 1.247, P < 0.001; OR = 1.994, P = 0.023, respectively). MWT (OR = 1.508, P = 0.001) and LVEF (OR = 0.923, P = 0.001) were also independently associated with the presence of LGE (Table VI).

ROC curve analysis: ROC curve analysis was performed to evaluate the utility of apelin, cTNI, and MWT as predictors of the presence of LGE. The cutoff value of apelin obtained by the ROC curve analysis was 1.24 pg/mL for the prediction of LGE (specificity of 81% and sensitivity of 68%; AUC = 0.78, 95% CI = 0.70-0.82, P < 0.001). The optimal cutoff value of cTNI was 0.031 ng/mL, with a specificity of 85% and sensitivity of 53% (AUC = 0.73,
Table VII. Value of Apelin, cTNI, and MWT in the Prediction of LGE in HCM patients

| Variables                  | Specificity | Sensitivity | PPV   | NPV   |
|----------------------------|-------------|-------------|-------|-------|
| cTNI ≥ 0.031 ng/mL         | 85%         | 53%         | 90%   | 36%   |
| Apelin ≤ 1.24 pg/mL        | 81%         | 68%         | 85%   | 45%   |
| MWT ≥ 19 mm                | 71%         | 76%         | 86%   | 49%   |
| MWT ≥ 19 mm and apelin ≤ 1.24 pg/mL | 91%     | 40%         | 85%   | 59%   |
| MWT ≥ 19 mm or apelin ≤ 1.24 pg/mL | 54%     | 83%         | 81%   | 41%   |
| MWT ≥ 19 mm and cTNI ≥ 0.031 ng/mL | 93%     | 35%         | 94%   | 36%   |
| MWT ≥ 19 mm or cTNI ≥ 0.031 ng/mL | 56%     | 87%         | 88%   | 62%   |

cTNI indicates cardiac troponin I; MWT, maximum wall thickness; HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; NPV, negative predictive value; and PPV, positive predictive value.

95% CI = 0.65-0.83, P < 0.001). The optimal cutoff value of MWT was 19 mm, with a specificity of 71% and sensitivity of 76% (AUC = 0.77, 95% CI = 0.68-0.85, P < 0.001). Compared with apelin, cTNI, or MWT alone, the combined measurements of both MWT ≥ 19 mm and apelin ≤ 1.24 pg/mL obtained a higher specificity of 91% to identify the presence of LGE, whereas the combination of either MWT ≥ 19 mm or apelin ≤ 1.24 pg/mL obtained a higher sensitivity of 83%. Similarly, the combination of both MWT ≥ 19 mm and cTNI ≥ 0.031 ng/mL obtained a higher specificity of 93% to identify the presence of LGE, whereas the combination of either MWT ≥ 19 mm or cTNI ≥ 0.031 ng/mL obtained a higher sensitivity of 87% (Table VII).

Discussion

Myocardial fibrosis was shown to influence prognosis in HCM. It is typically assessed by LGE on CMR. However, for patients with advanced heart failure and chronic renal insufficiency, the use of gadolinium as a contrast agent for LGE assessment is inappropriate and harmful. Therefore, continuous efforts were made to create simpler, safer, and faster methods of myocardial fibrosis detection.

Multiple studies have been performed to determine the serological markers of collagen metabolism in patients with HCM; however, their results are inconsistent. Considering that myocardial fibrosis in HCM is partially mediated by elevated cardiac trophic factors, including angiotensin II, apelin, which is the internal antagonist of angiotensin II, was proved to play essential roles in the inhibition of myocardial fibrosis and may be associated with myocardial fibrosis in HCM.

To the best of our knowledge, this is the first study to investigate the association between apelin and myocardial fibrosis by using LGE-CMR in patients with HCM. The levels of plasma apelin and cTNI were significantly associated with the presence and severity of LGE in HCM patients. In multivariate logistic regression analysis, serum apelin and cTNI were independent predictors of the presence of LGE. Moreover, MWT and LVEF were also independently associated with the presence of LGE. ROC curve analysis revealed that the combined measurements of MWT ≥ 19 mm and/or apelin ≤ 1.24 pg/mL showed higher predictive values for the presence of LGE (specificity of 91% and sensitivity of 83%) than MWT ≥ 19 mm or apelin ≤ 1.24 pg/mL alone. Furthermore, the combined measurements of MWT ≥ 19 mm and/or cTNI ≥ 0.031 ng/mL showed higher predictive values for the presence of LGE (specificity of 93% and sensitivity of 87%) than MWT ≥ 19 mm or cTNI ≥ 0.031 ng/mL alone.

The potential mechanisms of the relationship between decreased apelin and cardiac fibrosis in HCM remain unclear. The immunocytometry analysis of human hearts revealed that apelin is expressed in cardiomyocytes and in the vascular and endocardial endothelia. The heart, particularly the atria, and adipose tissue are the predominant sources of plasma apelin in humans. Mass spectrometry analysis revealed that (pyr)-apelin-13 is the major isof orm present in healthy human plasma, with concentrations ranging from 7.7-23.3 pg/mL.

It has been reported that reduced apelin expression was associated with cardiac, renal, and pulmonary artery fibrosis. CKD patients with renal fibrosis have a significant reduction in the plasma level of apelin, which may be attributed to the vascular endothelial dysfunction caused by uremic toxins. Heart failure is usually accompanied by cardiac fibrosis. In clinical research on heart failure patients, apelin level is increased in early stage disease but gradually decreases with the development of the disease. For HCM patients with LGE, the atria, which is the main source of plasma apelin, is also affected by fibrosis.

This is the possible reason for the decreased level of apelin in LGE-positive patients with HCM. This result is consistent with reduced apelin level in end-stage heart failure patients with cardiac fibrosis.

Cardiac troponins, including cardiac troponin T and troponin I, are specific and sensitive markers of myocardial damage. Previous studies found that cardiac troponin level also increased in patients with HCM. Elevated cardiac troponins are proved to be prognostic biomarkers of major adverse cardiovascular events in HCM patients. The conclusions of previous studies on the relationship between cardiac troponin and HCM LGE were inconsistent. Hs-cTnT levels were reported to be significantly higher in HCM patients with LGE than in those without LGE. HCM patients with elevated hs-cTnT level were found to have a higher extent of fibrosis. However, a study on 62 patients with HCM showed that LGE prevalence and hs-cTnT level have no significant relationship. In the present study, cTNI levels significantly increased in patients with LGE compared with patients without LGE and were positively correlated with the extent of LGE. cTNI level was an independent predictor of the presence...
of LGE in patients with HCM.

The underlying mechanisms of correlations between myocardial fibrosis and cardiac troponins in HCM remain unclear. Myocardial perfusion was reduced in the relatively hypertrophic myocardium in patients with HCM owing to abnormal coronary arteries and microvascular dysfunction. These abnormal coronary arteries could not meet the increased oxygen demand of the hypertrophic myocardium, thus, possibly resulting in myocardial ischemia, cardiomyocyte necrosis and apoptosis, release of cardiac troponins, and myocardial fibrosis.

For the inconsistent results of previous studies and the present study concerning the relationship between cTNI and cardiac fibrosis in HCM patients, there is also an important factor that should be considered seriously. cTNI is one of the pathological genes of HCM. Gene mutations in cardiac troponin I (cTNI) account for up to 5% of genotyped families with familial hypertrophic cardiomyopathy. As a result, the cTNI protein produced by a mutated gene will be different from the normal cTNI protein in patients without HCM and will not be detected by regular clinical tests via antigen-antibody reactions. A previous study showed that in cTNI transgenic mice, the ratio of mutant to wild-type cTNI protein was approximately 1:3 (mutant:WT). The overall expression of the mutant cTNI was 25% of the total expression of the protein. Considering that phosphorylated mutant cTNI protein was undetectable, the level of WT cTNI in a transgenic animal was significantly decreased compared with nontransgenic mice. Therefore, the plasma cTNI tested by a regular method in clinical use may not reflect the real cTNI level in HCM patients who had cTNI mutation. The cTNI level may vary according to whether the HCM patient has gene mutations on cTNI.

HCM patients enrolled in the present study and whose gene mutations were not analyzed were selected by clinical characteristics. Some patients who have cTNI mutation may have a relatively lower cTNI level because of the above reasons. The bias caused by gene mutation variation and ratio might be improved by a larger study population.

Apelin has not been found to be associated with the pathological gene mutation of HCM, thus, indicating that apelin level in HCM patients is stable and not influenced by the ratio of gene mutations compared with cTNI level. In this sense, apelin has some advantages in predicting cardiac fibrosis compared with cTNI.

In this study, LGE-positive patients had lower LVEF and larger LVEDVI than LGE-negative patients. In accordance with a previous study, the present study showed a positive correlation between MWT and the extent of LGE. In a previous study, fibrosis was highly associated with significant hypertrophy, which is defined in terms of maximal left ventricular wall thickness and indexed LVM; this finding is consistent with that of the present study. We also found that LVEF was negatively correlated with the extent of LGE. The possible mechanism is that fibrous tissue contributes to increased ventricular stiffness. Microscopic fibrosis is greater in the hearts of patients with the dilated phase of HCM than in nondilated hearts.

The severity and extent of cardiac fibrosis are directly related to diastolic and systolic dysfunctions in HCM. The superior predictive value of LVEF over and above fibrosis may reflect the fact that it provides a better synthesis of the various ventricular effects of HCM, including fibrosis and genetically mediated contractile dysfunction and energetic abnormalities. In this study, we found that BSA was positively related to LGE in patients with cardiac hypertrophy. The underlying mechanism is unknown. It was reported that BSA is positively related to the size of a human heart, including intracardiac areas, as well as to cardiac volume and cardiac hypertrophy. Given that LGE is positively related to MWT, which is one of the most important representatives of myocardial hypertrophy. It is possible that BSA is positively associated with LGE. More studies with larger groups are needed to confirm this result and find the underlying reason.

We also found that patients with LGE were significantly younger than those without LGE. The extent of LGE was negatively correlated with age in the overall patients but not in LGE-positive patients. A previous study found that intramyocardial fibrosis is one of the histopathologic characteristics of HCM and is commonly found in the necropsies of young patients who experienced unexpected cardiac death. This finding is consistent with our study.

Conclusion

The levels of plasma apelin and cTNI were significantly associated with the presence and severity of LGE in HCM patients. Apelin, which is an antagonist of angiotensin II, and cTNI, which is a biomarker of myocardial injury, are independent predictors of myocardial fibrosis, and the combined measurements of serum apelin and MWT, as well as cTNI and MWT, showed higher predictive values for predicting myocardial fibrosis in patients with HCM. Considering the gene mutation variety and ratio in HCM patients, apelin may be a more stable factor in predicting cardiac fibrosis in HCM than cTNI. This finding may provide a new approach for estimating myocardial fibrosis in HCM in clinical practice, in addition to CMR.

Disclosures

Conflicts of interest: None.

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