Cardiovascular Pathology 43 (2019) 107150

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Procollagen type I carboxy-terminal propeptide (PICP) and MMP-2 are potential biomarkers of myocardial fibrosis in patients with hypertrophic cardiomyopathy☆

Chengzhi Yang [a], Shubin Qiao [b,*], Yunhu Song [c,*], Yun Liu [c], Yajie Tang [c], Long Deng [c], Jiansong Yuan [b], Fenghuan Hu [b], Weixian Yang [b]

[a] Department of Cardiology and Macrovascular Disease, Beijing Tiantan Hospital, Capital Medical University, Beijing, China
[b] Department of Cardiology, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College
[c] Department of Cardiovascular Surgery, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College

ARTICLE INFO

Article history:
Received 21 May 2019
Received in revised form
15 July 2019
Accepted 19 August 2019

Keywords:
Hypertrophic cardiomyopathy
Myocardial fibrosis
Procollagen type I carboxy-terminal propeptide (PICP)
MMP-2

ABSTRACT

Background: Whether current proposed biomarkers of myocardial fibrosis (BMFs) actually reflect the changes in fibrous characteristics of myocardial tissue remains unclear. The relation between peripheral BMFs and histological myocardial fibrosis in patients with hypertrophic cardiomyopathy (HCM) has been unknown.

Methods and results: We studied 52 HCM patients who underwent a transaortic extended septal myectomy. Complete medical history was collected, and related examinations were performed. Echocardiography and cardiovascular magnetic resonance were employed to characterize cardiac morphology and function. Procollagen type I carboxy-terminal propeptide (PICP), C-terminal telopeptide of type I collagen (CITP), matrix metalloproteinases (total MMP-2 and total MMP-9), and tissue inhibitor of metalloproteinase 1 (TIMP-1) levels in both plasma and myocardial tissues were determined and compared. Myocardial fibrosis was detected with Masson's trichrome staining, and collagen volume fraction (CVF) was calculated. There was a significant correlation between plasma PICP levels and myocardial PICP contents (r=0.382, P=.007). Besides, plasma PICP (r=0.332, P=.020) levels correlated positively with CVF. In addition, plasma TIMP-1 levels were significantly correlated with myocardial TIMP-1 contents (r=0.282, P=.043). Plasma MMP-2 levels correlated positively with CVF (r=0.379, P=.006). Patients who took calcium channel blockers (CCBs; diltiazem or verapamil) had significantly lower plasma PICP levels, myocardial PICP content, and CVF in comparison with those who did not take CCBs.

Conclusions: In patients with HCM, plasma PICP and MMP-2 levels quantitatively reflect myocardial fibrosis, suggesting that PICP and MMP-2 may be used as reliable BMFs. CCBs may attenuate cardiac fibrosis in patients with HCM.

1. Introduction

Hypertrophic cardiomyopathy (HCM), with a prevalence of about 1/500 in the general population, is characterized by asymmetrical left ventricular (LV) hypertrophy, myocyte disarray, and fibrosis [1]. In the past years, it has been demonstrated that myocardial fibrosis contributes to heart failure, ventricular arrhythmias, and cardiac death in HCM [2–4]. Therefore, many studies have been performed to detect myocardial fibrosis and improve risk stratification in HCM [5].

Recent literature has suggested that focal myocardial fibrosis in HCM can be identified using cardiac magnetic resonance imaging (CMRI) with late gadolinium enhancement (LGE) [6,7]. However, LGE is unable to visualize diffuse fibrosis due to its limitation of tissue resolution [8]. Besides, CMRI is very expensive, time consuming, and not often accessible [9].

☆ This work was supported by the National Natural Science Foundation of China (81370327).

* Corresponding authors at: Fuwai Hospital, Beilishi Road No.167, Xicheng District, Beijing 100037, China.
E-mail addresses: qsbfw@sina.com (S. Qiao), heartcenter_song@hotmail.com (Y. Song).

https://doi.org/10.1016/j.carpath.2019.107150
1054-8807/

Circulating biomarkers are molecules that are objectively and easily measured by laboratory department in hospital. Therefore, the search for circulating biomarkers of myocardial fibrosis (BMFs) has attracted intense interest. To date, a large number of studies have investigated BMFs that reflect collagen synthesis, such as procollagen type I carboxy-terminal propeptide (PICP); and collagen degradation, including C-terminal telopeptide of type I collagen (CITP) [10,11]. In addition, matrix metalloproteinases (MMPs) play an important role in collagen degradation [10,12]. MMPs are inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs) [10,13]. Thus, MMPs and TIMPs are also proposed as potential BMFs. However, findings concerning the role of these BMFs are conflicting among previous studies. A study performed by Lombardi et al. showed that serum CITP levels were elevated in patients with HCM, whereas PICP levels were not different from those of controls [14]. In contrast, Carolyn Y. Ho et al. found higher serum PICP levels in HCM patients, while CITP levels were not elevated [15]. However, few studies have investigated BMFs content of myocardial tissue [11]. Consequently, there are scanty data regarding the relationship between BMFs content in peripheral blood and those in myocardial tissue. Therefore, the basic question remains as to whether current proposed BMFs actually reflect the changes in fibrous characteristics of myocardial tissue [11,16].

In the present study, we aimed to explore peripheral BMFs that can reflect the BMFs content in myocardial tissue in HCM patients who underwent extended Morrow procedures. The levels of PICP, CITP, total MMP-2, total MMP-9, and TIMP-1 in both plasma and myocardial tissue were determined. The relation between BMFs in plasma and myocardial tissue was analyzed. These five molecules were investigated because they might be promising as BMFs according to previous studies [10,17]. Furthermore, given that there are no reports of relation between peripheral BMFs and histological myocardial collagen volume fraction (CVF) in patients with HCM, the extent of myocardial fibrosis was also assessed with Masson's trichrome staining.

2. Methods

2.1. Study population

The protocol of this study was approved by Fuwai Hospital (Beijing, China) ethics committee and complied with the Declaration of Helsinki. Informed consents were obtained from all participants.

We studied patients with HCM who underwent a transaortic extended septal myectomy (Morrow operation) in Fuwai Hospital from November 2015 to August 2016. The diagnosis of HCM was based on a maximum LV wall thickness $\geq$15 mm (or $\geq$13 mm with an unequivocal family history of HCM), as measured by echocardiography or CMR), in the absence of other cardiac or systemic diseases capable of producing comparable magnitude of hypertrophy [18]. Evaluation of patients included complete medical history, physical examination, 12-lead electrocardiography, 24-h ambulatory electrocardiographic monitoring, transthoracic echocardiography, blood examination, CMRI, and coronary angiography.

Patients with coronary artery disease, valvular heart disease, documented bone or joint disease, connective tissue diseases, inflammatory states, post-surgery or trauma (<4 weeks), or permanent mechanical device implantation were excluded. Finally, a total of 52 patients with HCM were recruited in the present study.

2.2. Plasma and myocardial tissue sampling

It has been suggested that some BMFs might be released by platelets or leukocytes after activation [19,20]. Thus, we used plasma samples instead of serum to measure BMFs in this study. Venous blood samples of HCM patients were collected 1 h before surgery in tubes containing EDTA. Samples were subsequently centrifuged for 15 min at 3000*g*, and plasma was stored at −80°C until assay.

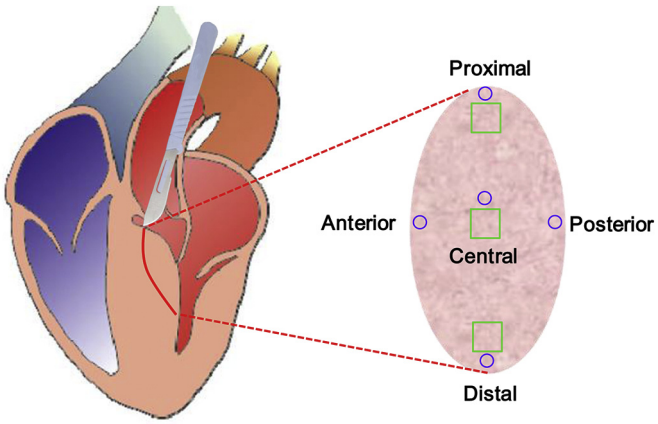

Fig. 1. Working model of myocardial tissue sampling following extended septal myectomy (Morrow operation). As indicated with blue rings, about 25 mg of tissue was obtained from each of four sides (proximal, distal, anterior, and posterior) and center of the excised septal tissue for ELISA assays. Besides, myocardial tissue with size of about 1 cm×1 cm×0.5 cm was taken from proximal, central, and distal part of the excised tissue (green blocks) for histological analysis.

Following septal myectomy [21], excised myocardial tissue was flushed with cold normal saline to remove blood. Then, about 25 mg tissue was obtained from each of four sides (proximal, distal, anterior, and posterior) and the center of the excised septal tissue (Fig. 1). The five specimens were mixed together, frozen in liquid nitrogen, and stored at −80°C for later enzyme-linked immunosorbent assay (ELISA) analysis. Besides, myocardial tissue with a size of about 1 cm×1 cm×0.5 cm was taken from proximal, central, and distal parts of the excised myocardial tissue (Fig. 1) and fixed in 10% buffered formalin. Twenty-four hours later, the three specimens were dehydrated with graded ethanol and embedded in paraffin. In all tissue samples, endocardium was excluded.

2.3. Determination of biomarkers in plasma and myocardial tissue

All biomarkers were assayed by ELISA. The following assays were obtained from R&D Systems (Minneapolis, MN, USA): total MMP-2 (catalog no. MMP200), human MMP-9 (catalog no. DMP900), and human TIMP-1 (catalog no. DTL100). PICP and CITP levels were measured with assays from Elabscience (Wuhan, China; catalog no. E-EL-H0196c) and CUSABIO (Wuhan, China; catalog no. CSB-E10363h), respectively. Biomarkers in plasma were measured according to the manufacturers' recommendations. For biomarkers in myocardial tissue, each sample was transferred to a tube, homogenized in 1-ml lysis buffer, and then centrifuged at 12,000 rpm for 15 min at 4°C temperature, after which the upper layer was collected for further analysis. The protein concentration was determined by BCA protein assay kit (Pierce, Rockford, IL, USA). The supernatant can be determined directly for MMP-2, MMP-9, TIMP-1, and ICTP, while a 1:20 dilution was used for PICP assay. The content of BMF in myocardial tissue was calculated as the ratio of BMF concentration to protein concentration in the supernatant. In addition, preliminary experiments showed that lysis buffer used in the study did not affect ELISA assays.

2.4. Quantitative histological analysis

Myocardial samples in paraffin were sectioned in 5-mm thickness. Then, the sections were stained with Masson's trichrome for

detection of collagen. Photographs of myocardial sections were obtained under ×40 magnification (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). All images were analyzed using a color image analysis system (Image Pro Plus 6.0; Media Cybernetics Inc., Silver Spring, MD, USA). Myocardial CVF was calculated as the ratio of the stained fibrotic area to total myocardial area. A CVF higher than mean value was defined as severe myocardial fibrosis, and a CVF not higher than mean value was defined as mild myocardial fibrosis.

2.5. Echocardiography

Standard transthoracic M-mode, two-dimensional, and pulse-wave and continuous-wave Doppler images were obtained with an iE33 Color Doppler Ultrasound System (Philips Healthcare, Andover, MA, USA). All measurements were analyzed following the guidelines of the American Society of Echocardiography [22]. The peak velocity across the left ventricular outflow tract (LVOT) was measured, and the peak pressure gradient was estimated using the simplified Bernouilli equation. The presence of LVOT obstruction was defined as an instantaneous peak Doppler LVOT gradient ≥30 mmHg at rest or during physiological provocation, such as Valsalva maneuver, standing, and exercise.

2.6. Statistical analysis

Continuous variables are expressed as mean±S.D. or median (interquartile range) according to their normality. Categorical variables are shown as frequencies (percentages). Comparisons of continuous variables between two groups were assessed using independent Student's *t* test or Mann−Whitney *U* test depending on the distribution of variables. Pearson's correlation test or Spearman's correlation test was used to examine correlations between two continuous variables (as appropriate). A two-tailed *P* value <.05 was considered as statistically significant. Statistical analysis was performed with SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA).

Table 1
Baseline characteristics of patients with HCM

Variable	HCM patients (n=52)
Age, y	45.5±15.0
Male, *n* (%)	36 (69.2%)
Body mass index, kg/m^2	25.2±3.8
Systolic blood pressure, mmHg	117.1±12.6
Diastolic blood pressure, mmHg	71.2±9.0
Heart rate, beats/min	73.0±9.5
Dyspnea, *n* (%)	48 (92.3%)
Angina, *n* (%)	17 (32.7%)
Syncope, *n* (%)	10 (19.2%)
Atrial fibrillation, *n* (%)	6 (11.5%)
Hypertension, *n* (%)	8 (15.4%)
Diabetes mellitus, *n* (%)	3 (5.8%)
Medications, *n* (%)	
β-Blockers	36 (69.2%)
CCBs	23 (44.2%)
ACEI/ARB	2 (3.8%)
Amiodarone	0 (0%)
Statins	4 (7.7%)
Diuretics	4 (7.7%)
Echocardiography data	
Septal wall thickness (mm)	23.3±5.4
LV end-diastolic diameter (mm)	42.1±5.6
Left atrium diameter (mm)	41.5±7.9
Pulmonary artery diameter (mm)	23.5±3.3
LV ejection fraction (%)	69.3±5.3
LVOT obstruction	52 (100%)
Systolic anterior motion	50 (96.2%)
LVOT gradient at rest (mmHg)	87.1±37.8
Mitral regurgitation	50 (96.2%)

Data are expressed as mean±S.D. or number (percentage). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

3. Results

3.1. Baseline characteristics

Demographic and clinical baseline characteristics of the study population are presented in Table 1. There were 52 HCM patients, and 36 (69.2%) of them were male. The mean age of the patients was 45.5±15.0 years (range 8−68). Most of patients (92.3%) had dyspnea. Angina was present in 17 (32.7%) patients, and 10 (19.2%) of them experienced syncope. Eight (15.4%) patients with HCM had systemic hypertension. However, the hypertension could not explain their magnitude of hypertrophy. β-Blockers were taken in 36 (69.2%) patients and calcium channel blockers (CCBs) in 23 (44.2%).

Echocardiography data showed that the septal wall thickness of HCM patients was 23.3±5.4 mm and the left atrium diameter was 41.5±7.9. All HCM patients had LVOT obstruction, and LVOT gradient at rest was 87.1±37.8 mmHg. Fifty (96.2%) of patients had mitral regurgitation. These data indicate that HCM patients in our study suffered from severe LV hypertrophy and LVOT obstruction.

3.2. Relation between concentration of plasma BMFs and content of myocardial BMFs

Concentration of plasma BMFs and content of myocardial BMFs of patients with HCM are summarized in Table 2. There was a significant correlation between plasma PICP levels and myocardial PICP content (r=0.382, P=.007; Fig. 2A). Plasma TIMP-1 levels were also correlated with myocardial TIMP-1 content (r=0.282, P=.043). However, the levels of plasma CITP, MMP-2, and MMP-9 were not significantly correlated with those of myocardial tissues.

It has been reported that peripheral BMF levels might correlated with each other [14]. Therefore, correlations between plasma BMF levels in patients with HCM were studied. Plasma MMP-9 levels were found to be significantly related to TIMP-1levels (r=0.381, P=.006; Table 1 in the Supplemental Data).

3.3. Correlation between plasma BMF levels and CVF

Histological analysis of CVF was detected with Masson's trichrome staining to study relation between plasma BMF levels and myocardial fibrosis (Table 3). Several studies have revealed that interstitial fibrosis is the main type of myocardial fibrosis in HCM. And interstitial fibrosis was also predominant in the hematoxylin and eosin images in the present study (Fig. 3). Plasma PICP (r=0.332, P=.020; Fig. 2 B) and MMP-2 (r=0.379, P=.006) levels

Table 2
Correlations between concentration of plasma BMFs and content of myocardial BMFs

Variable	Concentration of BMF in plasma	Content of BMF in myocardial tissue	r	P
PICP	525.3±138.7 ng/ml	12.5±4.7 ng/mg tissue	0.382	.007
CITP	1035.5 (611.7 −1773.7) ng/ml	90.1 (60.0−117.5) ng/mg tissue	0.170	.228
MMP-2	214.5±63.4 pg/ml	74.8±20.7 pg/mg tissue	−0.086	.550
MMP-9	88.9 (53.2−129.4) ng/ml	1414.0 (1071.5−2234.0) pg/mg tissue	0.181	.199
TIMP-1	60.6±15.9 ng/ml	487.8±273.6 pg/mg tissue	0.282	.043

Data are expressed as mean±S.D. or median (interquartile range).

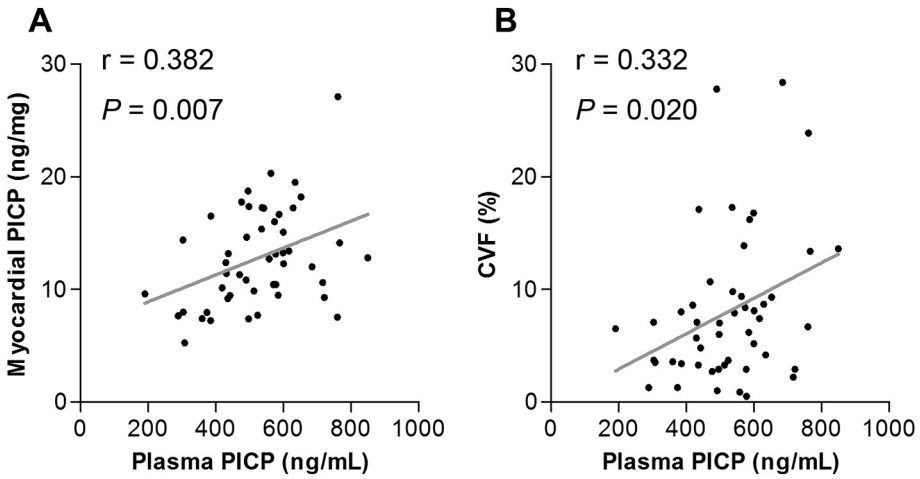

Fig. 2. Correlation between plasma PICP levels and myocardial PICP contents (A) and CVF (B) in HCM patients.

correlated positively with CVF, whereas plasma CITP, MMP-9, and TIMP-1 were nonsignificantly correlated with CVF.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate plasma PICP levels for predicting severe myocardial fibrosis (Fig. 4). Based on ROC curve analysis, the optimal cutoff value of plasma to predict severe myocardial fibrosis was 529.5 ng/ml, with a sensitivity of 72% and specificity of 65% (area under curve=0.71, 95% confidence interval: 0.56–0.85, *P*=.012). Then, we analyzed the clinical characteristics between patients with severe fibrosis and patients with mild fibrosis. We found that there were no significant differences of clinical characteristics between the two groups, such as age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, septal wall thickness, LV ejection fraction, left ventricular end-diastolic diameter, LVOT gradient at rest, the presence of male patients, dyspnea, angina, using β-blockers, and using CCBs. Of note, patients with mild fibrosis had a tendency of taking CCBs more frequently (54.8% vs. 28.6%, *P*=.089), which is in line with the latter finding that patients who took CCBs had significantly lower myocardial CVF.

3.4. Relation between plasma BMFs levels and LGE

Several studies have shown that LGE on CMRI in patients with HCM is associated with myocardial fibrosis [6]. Forty-eight of the 52 patients in the present study received CMRI assessment, and 41 (85.4%) of them had LGE. The mass of myocardium with LGE (LGEm) was measured, and the LGE fraction (LGEf) was calculated. We found that LGEm (*r*=0.512, *P*=.001) and LGEf (*r*=0.525, *P*<.001) were positively correlated with CVF. In addition, plasma levels of MMP-2 correlated positively with LGEm (*r*=0.463, *P*=.003) and LGEf (*r*=0.413, *P*=.008).

3.5. Relation between plasma BMF levels and clinical characteristics

Previous studies have indicated that some peripheral BMF levels are related to clinical characteristics [23]. Thus, we analyzed the relation between plasma BMF levels and clinical characteristics, including CMRI parameters. As shown in Table 4, plasma MMP-9 levels correlated inversely with age (*r*=−0.332, *P*=.017), and CITP correlated positively with BMI (*r*=0.409, *P*=.004). Interestingly, we observed that patients who took CCBs (diltiazem or verapamil) had significantly lower plasma PICP levels (476.9±145.9 vs, 564.8±121.3, *P*=.026), myocardial PICP content (11.1±4.7 vs. 13.8±4.4, *P*=.043), and CVF (5.7%±3.8% vs. 9.9%±7.6%, *P*=.022) in comparison with those who did not take CCBs (Fig. 5), indicating that CCBs may relieve cardiac fibrosis in patients with HCM. In contrast, β-blockers did not have significant effect on BMFs of the studied population.

Table 3
Correlations between plasma BMF levels and histopathological CVF of myocardial tissue from patients with HCM

Variable	*r*	*P*
PICP	0.332	.020
CITP	0.025	.860
MMP-2	0.379	.006
MMP-9	0.178	.208
TIMP-1	0.008	.958

4. Discussion

To the best of our knowledge, this is the first study to compare peripheral BMF levels with myocardial BMF content and also the first study to compare peripheral BMF levels with CVF in patients with HCM. We found that plasma PICP levels were significantly correlated with myocardial PICP content and histological CVF. Plasma MMP-2 was positively correlated with CVF, LGEm, and LGEr. CCBs may relieve cardiac fibrosis in patients with HCM.

It is well established that myocardial fibrosis is one of main pathological features of HCM [24]. Previous studies based on CMRI have revealed that more than 60% of HCM patients had LGE, which indicates overt focal fibrosis [2,6,25]. Considering that diffuse myocardial fibrosis is not well detected by LGE [26], there are actually more HCM patients who had myocardial fibrosis. Previous literature has demonstrated that myocardial fibrosis is an important predictor of adverse cardiovascular events, including ventricular tachycardia or ventricular fibrillation, sudden cardiac death, and heart failure [27]. As such, it is pivotal to assess myocardial fibrosis for risk stratification in HCM patients. Although CMRI is being developed to detect myocardial fibrosis, it is obvious that biomarkers in peripheral blood are far easier to be quantitatively measured.

Most of studied BMFs are molecules involved in collagen synthesis and degradation [13]. As direct products of collagen metabolism, PICP and CITP have attracted lots of attention. PICP and CITP are cleavages during type I collagen synthesis and degradation, respectively [10]. Thus, it seems likely that PICP levels would be increased and CITP levels might be decreased in HCM patients. Carolyn Y. Ho et al. reported that serum PICP levels were significantly higher in HCM patients and their “healthy” relatives with positive mutation in comparison with control subjects [15]. However, there was a drawback that the control population was much

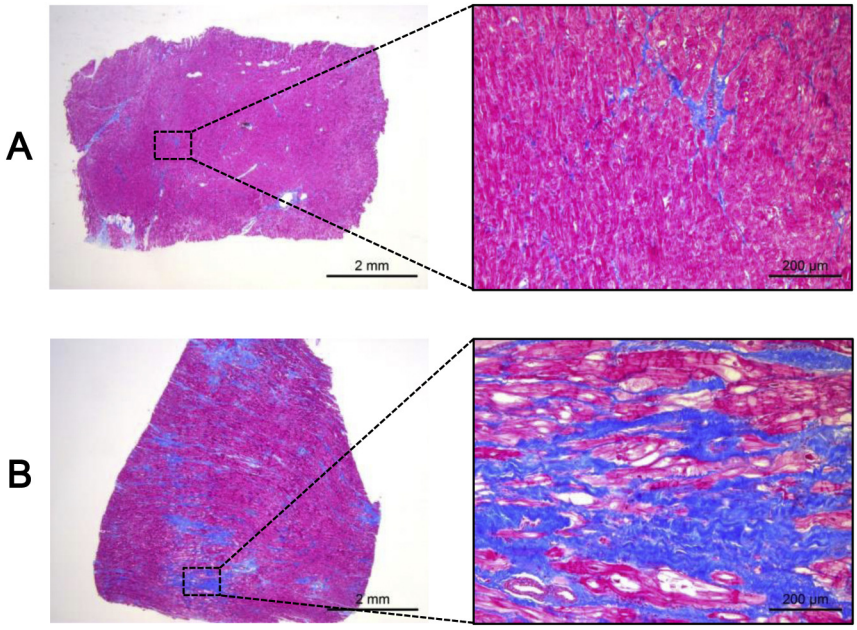

Fig. 3. Representative micrographs of Masson's trichrome-stained sections of myocardial tissues from two HCM patients with nonsevere myocardial fibrosis (A) and severe myocardial fibrosis (B). Blue parts represent collagen.

younger than the HCM patients in their study. And they found no differences in serum CITP levels between the HCM patients and the control subjects. In contrast, Lombardi et al. reported that serum PICP levels in HCM patients were not significantly different from those in control subjects [14]. Besides, they found that CITP levels were elevated in HCM patients. Therefore, the role of PICP and CITP in cardiac fibrosis of HCM is far from being defined. Furthermore, little is known about the correlation between peripheral BMF levels and myocardial CVF in patients with HCM. In a study of patients with systematic hypertension, authors observed that serum PICP levels correlated significantly with myocardial CVF [28]. In contrast, Rubis et al. studied 70 patients with dilated cardiomyopathy and found no single serum BMF correlated with CVF [29]. Here, we found that plasma PICP levels were significantly correlated with myocardial CVF in HCM patients. Ramón Querejeta et al. reported that the heart is the main source of circulating PICP in patents with hypertensive heart disease [30]. Consistently, we observed that plasma PICP levels correlated significantly with PICP contents of myocardial tissue. Taken together with previous studies, our data suggest that peripheral PICP could be used as a reliable marker for myocardial fibrosis in patients with HCM.

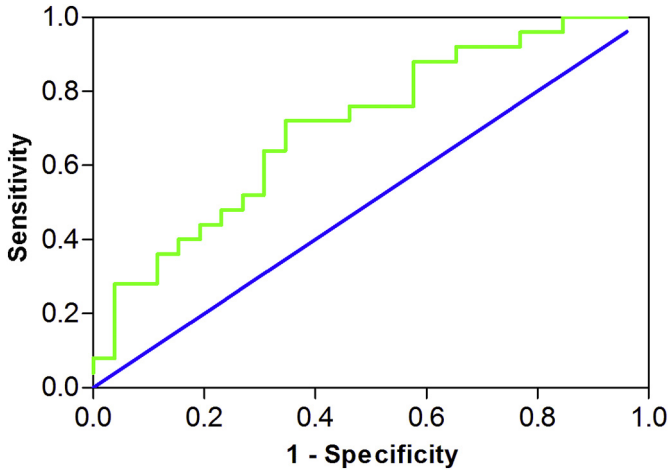

Fig. 4. ROC curves of plasma PICP levels for predicting severe myocardial fibrosis. AUC indicates area under ROC curve.

MMPs are zinc-dependent endopeptidases that play an important role in degrading various collagens [10,11]. MMP activity can be inhibited by a family of molecules known as endogenous TIMPs [10,31]. Several studies have explored MMPs and TIMPs as potential BMFs. The study by Lombardi et al. showed that serum active MMP-2, MMP-9, and TIMP-1 levels were significantly higher in HCM patients [14]. However, Carolyn Y. Ho et al. found no difference in serum TIMP-1 levels between HCM patients and control subjects [15]. In a study of hypertensive patients, authors found that patients with diastolic heart failure had elevated active MMP-2 and MMP-9, whereas TIMP-1 was not significantly different between groups [32]. Collectively, peripheral MMP-2 and MMP-9 levels seemed higher in HCM patients but not consistent in other cardiovascular diseases. The role of TIMP-1 levels has not been well defined in detecting myocardial fibrosis. Besides, data concerning association of these potential BMF with their content in myocardial tissue and CVF are lacking [17]. In this study, we observed that plasma MMP-9 levels were not correlated with myocardial MMP-9 content or CVF, suggesting that peripheral levels of MMP-9 might not reflect myocardial MMP-9 content or cardiac fibrosis. In fact, this finding may be reinforced by a study by Kaye et al., who revealed that arterial MMP-9 and TIMP-1 levels were both not significantly different between patients with advanced heart failure and controls [33]. Furthermore, the present study revealed that

Table 4
Correlations of plasma BMF with clinical and cardiac morphological characteristics in HCM patients

	PICP	CITP	MMP-2	MMP-9	TIMP-1
Age	0.115	−0.002	−0.115	−0.332*	0.063
BMI	0.053	0.409**	0.035	−0.009	−0.239
Maximum wall thickness	0.057	−0.061	0.121	0.332*	−0.186
LV mass index	0.046	−0.181	0.187	0.219	−0.108
Left atrium diameter	0.198	−0.089	−0.188	0.089	−0.023
LV ejection fraction	0.013	−0.086	0.138	−0.147	−0.116
Cardiac index	0.198	0.063	0.040	−0.103	−0.216

* $P<.05$.
** $P<.01$.

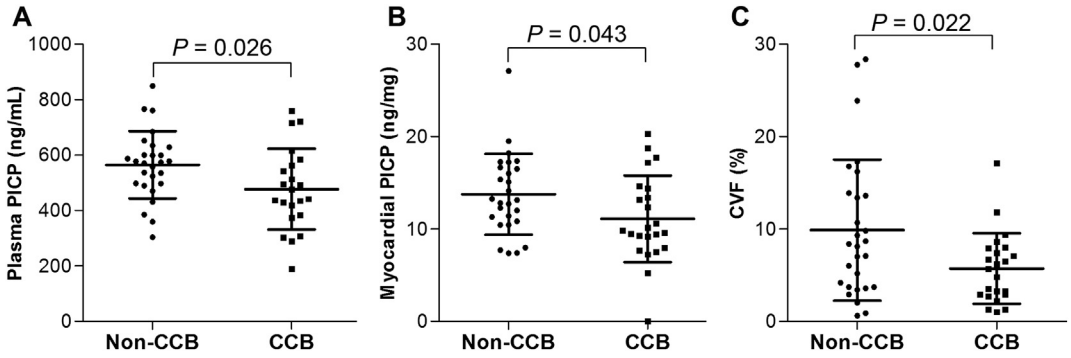

Fig. 5. HCM patients who took CCBs (diltiazem or verapamil) had significantly lower plasma PICP levels (A), myocardial PICP content (B), and CVF (C) in comparison with those who did not take CCBs (non-CCB).

plasma TIMP-1 levels correlated significantly with myocardial TIMP-1content but not with CVF. These findings indicate that peripheral TIMP-1 levels may reflect TIMP-1 content in myocardial tissue, but they are not suitable as a BMF.

In the present investigation, although plasma MMP-2 levels in HCM patients were not significantly correlated with myocardial MMP-2 content, they did correlate significantly with myocardial CVF. In addition, plasma MMP-2 levels correlated positively with LGEm and LGEf, which may reflect focal fibrosis in the myocardium. Currently, scant information is available in the literature about the direct relationship between peripheral MMP-2 and myocardial CVF [10]. Our study raises the possibility that MMP-2 may be a potential biomarker of focal fibrous in the heart of patients with HCM.

Several studies have explored the effect of CCBs on prevention and treatment of HCM. The study by Christopher Semsarian demonstrated that early use of diltiazem could prevent cardiac fibrosis in a mouse model of HCM [34]. Another investigation in mice model of HCM showed that pretreatment with diltiazem could prevent diastolic heart failure [35]. Besides, a pilot randomized trial found that preclinical administration of diltiazem may improve diastolic function of LV in HCM [36]. It is well known that cardiac fibrosis is one of the physiopathological mechanisms that lead to diastolic heart failure [32]. In line with previous study, our data suggest that CCBs may attenuate cardiac fibrosis in patients with HCM. Therefore, the efficacy of CCBs treatment in attenuating cardiac fibrosis of HCM merits further exploration in larger trials.

It has been reported that LGE on CMR could detect focal fibrosis in the myocardium [6]. The current study also observed that LGEm and LGEf correlated positively with CVF. Furthermore, their correlation coefficients were greater than that of PICP with CVF. These results are not surprising given that all the studied myocardial tissues were taken from the left septum, where LGE is usually observed in HCM. In contrast, plasma PICP may reflect global cardiac fibrosis better.

5. Limitations

This study has some limitations that warrant discussion. First, all the myocardial tissues analyzed were taken from the left septum. Thus, myocardial BMF content and CVF of the free wall were not included. Second, our measurements of MMP levels, as performed in the majority of studies, reflect the total MMP content rather than activity; that is, it is possible that our data did not reveal myocardial MMP activity. Third, the BMFs were determined only at one time point. It will be intriguing to investigate BMFs at different time points to elucidate dynamic process of myocardial fibrosis in experimental studies. Finally, some other potential BMFs, particularly procollagen type I amino-terminal propeptide (PINP) and procollagen type III amino-terminal propeptide (PIIINP), were not investigated in this study. However, previous studies have suggested that peripheral PINP and PIIINP levels might give an unreliable value as they are not always cleaved during collagen synthesis [10,37].

6. Conclusions

In patients with HCM, plasma PICP quantitatively reflects myocardial PICP content and fibrosis, suggesting that PICP could be used as a BMF. Correlating positively with CVF and the mass of LGE and LGE fraction, plasma MMP-2 may also be a potential BMF in patients with HCM. CCBs may attenuate cardiac fibrosis in patients with HCM.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carpath.2019.107150.

- Plasma MMP-2 may be a potential biomarker of focal fibrosis in the heart of patients with HCM.
- Calcium channel blockers may attenuate cardiac fibrosis in patients with HCM.

Acknowledgments

The authors thank Tiewei Li for providing some reagents.

Disclosures

None.

References

[1] Maron BJ, Maron MS, Maron BA, Loscalzo J. Moving beyond the sarcomere to explain heterogeneity in hypertrophic cardiomyopathy. JACC Review Topic of the Week J Am Coll Cardiol 2019;73:1978–86.
[2] O'Hanlon R, Grasso A, Roughton M, Moon JC, Clark SWage R, et al. Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. J Am Coll Cardiol 2010;56:867–74.
[3] Azevedo CF, Nigri M, Higuchi ML, Pomerantzeff PM, Spina GS, Sampaio RO, et al. Prognostic significance of myocardial fibrosis quantification by histopathology and magnetic resonance imaging in patients with severe aortic valve disease. J Am Coll Cardiol 2010;56:278–87.
[4] Munch J, Avanesov M, Bannas P, Saring D, Kramer E, Mearini G, et al. Serum matrix metalloproteinases as quantitative biomarkers for myocardial fibrosis and sudden cardiac death risk stratification in patients with hypertrophic cardiomyopathy. J Card Fail 2016;22:845–50.
[5] Zhou Y, Yuan J, Wang Y, Qiao S. Predictive values of apelin for myocardial fibrosis in hypertrophic cardiomyopathy. Int Heart J 2019;60:648–55.
[6] Bruder O, Wagner A, Jensen CJ, Schneider S, Ong P, Kispert EM, et al. Myocardial scar visualized by cardiovascular magnetic resonance imaging predicts major adverse events in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2010;56:875–87.
[7] Chan RH, Maron BJ, Olivotto I, Pencina MJ, Assenza GE, Haas T, et al. Prognostic value of quantitative contrast-enhanced cardiovascular magnetic resonance

for the evaluation of sudden death risk in patients with hypertrophic cardiomyopathy. Circulation 2014;130:484–95.
[8] Moon JC, Treibel TA, Schelbert EB. T1 mapping for diffuse myocardial fibrosis: a key biomarker in cardiac disease? J Am Coll Cardiol 2013;62:1288–9.
[9] Maron BJ, Maron MS. The 20 advances that have defined contemporary hypertrophic cardiomyopathy. Trends Cardiovasc Med 2015;25:54–64.
[10] de Jong S, van Veen TA, de Bakker JM. Vos MA, van Rijen HV. Biomarkers of myocardial fibrosis. J Cardiovasc Pharmacol 2011;57:522–35.
[11] Lopez B, Gonzalez A, Ravassa S, Beaumont J, Moreno MU, San JG, et al. Circulating biomarkers of myocardial fibrosis. The Need for a Reappraisal J Am Coll Cardiol 2015;65:2449–56.
[12] Cambronero F, Marin F, Roldan V, Hernandez-Romero D, Valdes M, Lip GY. Biomarkers of pathophysiology in hypertrophic cardiomyopathy: implications for clinical management and prognosis. Eur Heart J 2009;30:139–51.
[13] Dhingra R, Vasan RS. Biomarkers in cardiovascular disease: statistical assessment and section on key novel heart failure biomarkers. Trends Cardiovasc Med 2017;27:123–33.
[14] Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Miranda M, et al. Myocardial collagen turnover in hypertrophic cardiomyopathy. Circulation 2003;108:1455–60.
[15] Ho CY, Lopez B, Coelho-Filho OR, Lakdawala NK, Cirino AL, Jarolim P, et al. Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. N Engl J Med 2010;363:552–63.
[16] Spinale FG. Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. Physiol Rev 2007;87:1285–342.
[17] Chalikias GK, Tziakas DN. Biomarkers of the extracellular matrix and of collagen fragments. Clin Chim Acta 2015;443:39–47.
[18] Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J 2014;35:2733–79.
[19] Gerlach RF, Tanus-Santos JE. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in congestive heart failure. Am Heart J 2006;152:e9.
[20] Chubb SA. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. Clin Biochem 2012;45:928–35.
[21] Liu Y, Song Y, Gao G, Ran J, Su W, Li H, et al. Outcomes of an extended morrow procedure without a concomitant mitral valve procedure for hypertrophic obstructive cardiomyopathy. Sci Rep 2016;6:29031.
[22] Nagueh SF, Bierig SM, Budoff MJ, Desai M, Dilsizian V, Eidem B, et al. American Society of Echocardiography clinical recommendations for multimodality cardiovascular imaging of patients with hypertrophic cardiomyopathy. Endorsed by the American Society of Nuclear Cardiology, Society for Cardiovascular Magnetic Resonance, and Society of Cardiovascular Computed Tomography J Am Soc Echocardiogr 2011;24:473–98.
[23] Biolo A, Rohde LE, Goldraich LA, Mascarenhas M, Palombini DV, Clausell N. Serum procollagen type III is associated with elevated right-sided filling pressures in stable outpatients with congestive heart failure. Biomarkers 2009;14:438–42.
[24] Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. N Engl J Med 2018;379:655–68.
[25] Rudolph A, Abdel-Aty H, Bohl S, Boye P, Zagrosek A, Dietz R, et al. Noninvasive detection of fibrosis applying contrast-enhanced cardiac magnetic resonance in different forms of left ventricular hypertrophy. relation to remodeling J Am Coll Cardiol 2009;53:284–91.
[26] Zhou H, Lin X, Fang L, Zhao X, Ding H, Chen W, et al. Characterization of compacted myocardial abnormalities by cardiac magnetic resonance with native T1 mapping in left ventricular non-compaction patients—a comparison with late gadolinium enhancement. Circ J 2016;80:1210–6.
[27] Moore B, Semsarian C, Chan KH, Sy RW. Sudden cardiac death and ventricular arrhythmias in hypertrophic cardiomyopathy. Heart Lung Circ 2019;28:146–54.
[28] Querejeta R, Varo N, Lopez B, Larman M, Artinano E, Etayo JC, et al. Serum carboxy-terminal propeptide of procollagen type I is a marker of myocardial fibrosis in hypertensive heart disease. Circulation 2000;101:1729–35.
[29] Rubis P, Wisniowska-Smialek S, Wypasek E, Biernacka-Fijalkowska B, Rudnicka-Sosin L, Dziewiecka E, et al. Fibrosis of extracellular matrix is related to the duration of the disease but is unrelated to the dynamics of collagen metabolism in dilated cardiomyopathy. Inflamm Res 2016;65:941–9.
[30] Querejeta R, Lopez B, Gonzalez A, Sanchez E, Larman M, Martinez UJ, et al. Increased collagen type I synthesis in patients with heart failure of hypertensive origin: relation to myocardial fibrosis. Circulation 2004;110:1263–8.
[31] Lopez B, Gonzalez A, Diez J. Circulating biomarkers of collagen metabolism in cardiac diseases. Circulation 2010;121:1645–54.
[32] Martos R, Baugh J, Ledwidge M, O'Loughlin C, Conlon C, Patle A, et al. Diastolic heart failure: evidence of increased myocardial collagen turnover linked to diastolic dysfunction. Circulation 2007;115:888–95.
[33] Kaye DM, Khammy O, Mariani J, Maeder MT. Relationship of circulating matrix biomarkers to myocardial matrix metabolism in advanced heart failure. Eur J Heart Fail 2013;15:292–8.
[34] Semsarian C, Ahmad I, Giewat M, Georgakopoulos D, Schmitt JP, McConnell BK, et al. The L-type calcium channel inhibitor diltiazem prevents cardiomyopathy in a mouse model. J Clin Invest 2002;109:1013–20.
[35] Westermann D, Knollmann BC, Steendijk P, Rutschow S, Riad A, Pauschinger M, et al. Diltiazem treatment prevents diastolic heart failure in mice with familial hypertrophic cardiomyopathy. Eur J Heart Fail 2006;8:115–21.
[36] Ho CY, Lakdawala NK, Cirino AL, Lipshultz SE, Sparks E, Abbasi SA, et al. Diltiazem treatment for pre-clinical hypertrophic cardiomyopathy sarcomere mutation carriers. a pilot randomized trial to modify disease expression JACC Heart Fail 2015;3:180–8.
[37] Risteli J, Risteli L. Analysing connective tissue metabolites in human serum. Biochemical, physiological and methodological aspects. J Hepatol 1995;22:77–81.