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Original article

Coronary perivascular fibrosis is associated with impairment of coronary blood flow in patients with non-ischemic heart failure

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ABSTRACT

Background: Although myocardial interstitial fibrosis has been considered to play a pathogenic role in chronic heart failure (HF), the role of perivascular fibrosis, another form of fibrosis, remains to be elucidated.

Methods: We examined 64 consecutive patients with non-ischemic HF caused by hypertrophic cardiomyopathy (HCM, $n = 16$), hypertensive heart disease (HHD, $n = 11$), or dilated cardiomyopathy (DCM, $n = 37$), diagnosed by both cardiac catheterization and endomyocardial biopsy (right ventricular side of the interventricular septum) in the Tohoku University Hospital between January 2001 and April 2009. We calculated the collagen volume fraction (CVF) and perivascular fibrosis ratio (PFR) in biopsy samples and also examined Thrombolysis in Myocardial Infarction (TIMI) frame count to evaluate coronary blood flow.

Results: There was no significant correlation between CVF and PFR ($r^2 = 0.0007$). Although CVF was comparable among HCM, HHD, and DCM (1.11 ± 1.04, 1.89 ± 1.61, and 1.41 ± 1.48, respectively), PFR was significantly higher in HCM than in DCM (1.78 ± 1.09 vs. 1.23 ± 0.44, $p < 0.05$). PFR was not correlated with cardiac function parameters, such as left ventricular (LV) ejection fraction, cardiac output, LV end-diastolic pressure, LV end-diastolic volume, aortic pressure, or pulmonary artery pressure. However, PFR was significantly correlated with coronary flow in the left anterior descending coronary artery (as evaluated by TIMI frame count) ($r^2 = 0.3351$, $p < 0.0001$, in all-cases combined population), but not with that in the left circumflex or right coronary artery. This correlation remained significant in a logistic regression model tested in 7 variables (body mass index, PVR, CVF, presence of hypertension, dyslipidemia, diabetes mellitus, and atrial fibrillation).

Conclusions: These results indicate that coronary perivascular fibrosis is associated with the impairment of coronary blood flow although not associated with interstitial fibrosis or cardiac function, suggesting that it can be a new therapeutic target to improve coronary microcirculation.

Introduction

Chronic heart failure (HF) is a complex clinical syndrome that can result from any structural or functional cardiac disorders, including coronary artery disease, hypertensive heart disease, myocardial disease, and valvular heart disease [1], where not only HF with reduced ejection fraction (HFrEF) but also HF with preserved ejection fraction (HFpEF) are substantially involved [2–4]. Indeed, HFrEF and HFpEF respectively account for approximately half of chronic HF patients [5–8].

In HF patients, coronary flow reserve is impaired during the acute phase of HF and is strongly correlated with mortality [9,10]. Coronary microvascular dysfunction has been recognized as an important contributor to impaired coronary blood flow [11], which occurs not only in coronary artery disease, but also in various myocardial diseases, such as hypertension, hypertrophic cardiomyopathy (HCM) and infiltrative heart disease (e.g. Anderson–Fabry cardiomyopathy) [11]. Also, it has been reported that myocardial diseases are associated with abnormal coronary microvascular structures such as thickened wall and proliferation of vascular smooth muscle cells [11]. However, the correlation between organic and functional coronary microvascular abnormalities has not been well documented.

Thrombolysis in Myocardial Infarction (TIMI) frame count is a simple, objective, and quantitative index of coronary blood flow, and has been reported to increase not only in myocardial infarction

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[12], but also in coronary microvascular dysfunction [11,13–15]. It is important to exclude the contribution of organic coronary artery disease when evaluating coronary blood flow and microvascular dysfunction in HF patients. In the present study, we thus aimed to examine the impact of perivascular fibrosis on coronary microvascular dysfunction in patients with chronic and non-ischemic HF by using TIMI frame count.

Methods

The ethical committees of Tohoku University Hospital approved the study protocol and all patients provided written informed consent.

Study subjects

We examined 132 consecutive patients with stages B/C/D chronic HF defined by the American College of Cardiology/American Heart Association 2005 Guidelines [1,4], caused by non-ischemic HF, including hypertrophic cardiomyopathy (HCM, $n=45$), hypertensive heart disease (HHD, $n=25$), or dilated cardiomyopathy (DCM, $n=62$) enrolled in our database, who were hospitalized at our hospital and underwent both cardiac catheterization and endomyocardial biopsy (right ventricular side of the interventricular septum) to diagnose the etiology of HF from January 2001 to April 2009. In the present study, 68 out of the 132 patients were excluded; 66 for the absence of coronary arteries in the biopsy samples and 2 for significant coronary artery disease. Finally, we enrolled the remaining 64 patients, including 16 HCM, 11 HHD, and 37 DCM patients.

Data collection

Baseline demographic data, hemodynamic data obtained by catheterization, medications, and comorbidities (hypertension, diabetes mellitus, dyslipidemia, and atrial fibrillation) were obtained from their medical records. Hypertension was diagnosed by the use of antihypertensive drugs and/or systolic blood pressure $\geq$130 mmHg and/or diastolic blood pressure $\geq$80 mmHg. Diabetes mellitus was diagnosed by the use of anti-diabetic drugs, fasting glucose $\geq$110 mg/dl, and/or glucose $\geq$200 mg/dl 2 h after a 75 g oral glucose tolerance test. Dyslipidemia was diagnosed by the use of lipid-lowering drugs and/or elevated lipid levels, defined as plasma low-density lipoprotein (LDL) cholesterol $\geq$140 mg/dl, triglycerides $\geq$150 mg/dl, or high-density lipoprotein (HDL) <40 mg/dl. The hemodynamic parameters obtained by cardiac catheterization included left ventricular ejection fraction (LVEF), LV end-diastolic volume index (LVEDVI), aortic pressure (AoP), LV end-diastolic pressure (LVEDP), pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and cardiac index (CI). Before cardiac catheterization, we measured serum levels of hemoglobin, brain natriuretic peptide (BNP), creatinine, and high-sensitivity C-reactive protein (hsCRP), and estimated creatinine clearance (eGFR) by Cockroft–Gault formula.

Histological analysis of biopsy samples

The acquisition, fixation, and staining of myocardial biopsy samples was previously described [16]. Collagen volume fraction (CVF), as an index of myocardial interstitial fibrosis, was calculated and averaged in representative fields containing no endocardium or blood vessel, as previously described [16].

Furthermore, we have analyzed images of the stained sections using ImageJ 1.45 s (W. Rasband, NIH, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997–2012, 400×) to determine perivascular fibrosis around arteries, expressed as perivascular fibrosis ratio (PFR) (Fig. 1). PFR was defined as the area of perivascular fibrosis divided by the area of the vascular wall, averaged over all quantifiable images of arteries taken from a section [17] (mean, 1.72 ± 0.84 quantifiable images of arteries). All histological evaluation was performed by a single well-trained observer without knowing whose samples were analyzed.

Determination of delayed enhancement on cardiac magnetic resonance imaging

Out of the 64 patients enrolled, 27 also underwent cardiac magnetic resonance (CMR) imaging using a 1.5-T CMR system (Siemens Magnetom, Erlangen, Germany). Delayed enhancement images captured 15 min after intravenous injection of gadolinium were used to determine the presence of delayed enhancement [18–20].

Evaluation of coronary blood flow

Coronary blood flow was quantified by a single well-trained observer in a blind manner, using TIMI frame count (TFC) method [12]. TFC was determined separately for the left anterior descending (LAD) and circumflex coronary artery (LCx), and the right coronary artery (RCA), according to the method described by Gibson et al. [12]. We calculated corrected TFC (CTFC) for LAD by dividing TFC by 1.7, in order to adjust that for LCx and RCA [12]. All coronary angiograms were obtained before endomyocardial biopsy at a speed of 15 frames/s. To be universally comparable, TFCs and CTFCs were converted to a frame acquisition rate of 30 frames/sec by multiplying by 2.0 [15].

Statistical analysis

Continuous variables were presented as mean $\pm$ SD. Comparison of 2 groups was made by unpaired t-test for continuous variables and Pearson's chi-square test for categorical variables. Comparison among 3 groups was made by ANOVA test. A multivariate analysis was conducted to examine the effectors of CTFC for LAD, using a logistic regression model, in which the following were included as variables: body mass index (BMI), PFR, CVF, and the presence of comorbidities (hypertension, dyslipidemia, diabetes mellitus, and atrial fibrillation). A p-value less than 0.05 was considered to be statistically significant.

Results

Patient characteristics

There were no statistically significant differences among the 3 groups in age, gender, or BMI (Table 1). Expectedly, the prevalence of hypertension was significantly higher in the HHD group compared with the other 2 groups (Table 1). The DCM group had a higher prevalence of atrial fibrillation than the HCM group (Table 1). LVEF was the highest in the HCM group (Table 1).

Coronary perivascular fibrosis and myocardial interstitial fibrosis

Although there was no significant difference in myocardial interstitial CVF among the 3 groups (Fig. 2A), PFR was significantly more severe in the HCM group than in the DCM group (Fig. 2B). No correlation was observed between CVF and PFR in all-cases combined population (Fig. 2C) or in each group (Fig. 2D–F). Of the 27 patients who underwent CMR, 16 presented delayed enhancement and PFR was comparable between the patients with and those without delayed enhancement (1.18 ± 0.67 vs. 1.55 ± 0.78, $p=0.21$).

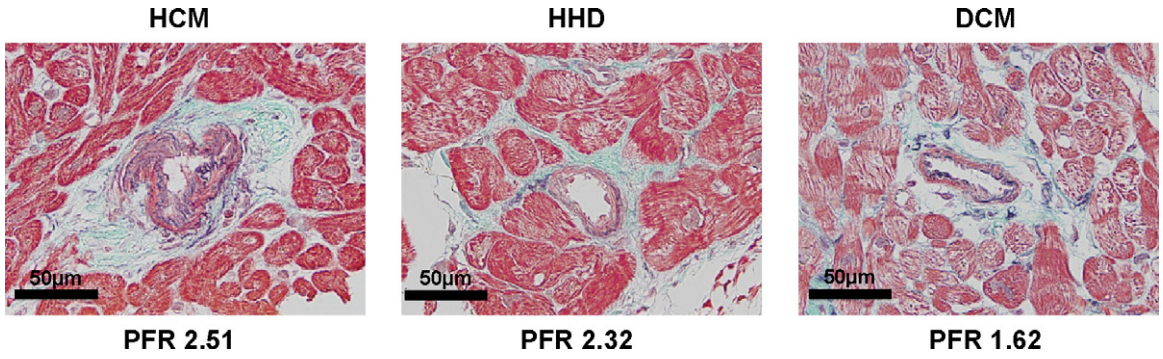

Fig. 1. Representative histology of perivascular fibrosis. Perivascular fibrosis in hypertrophic cardiomyopathy (HCM) (left), hypertensive heart disease (HHD) (middle), and dilated cardiomyopathy (DCM) (right) patients, stained in blue by Elastica–Masson staining. Scale bar, 50 μm.

Coronary perivascular fibrosis and cardiac function

PFR was not significantly correlated with LVEF ($r^2 = 0.0003$, $p = 0.90$), CO ($r^2 = 0.0095$, $p = 0.45$), LVEDP ($r^2 = 0.0042$, $p = 0.62$), LVEDV ($r^2 = 0.0019$, $p = 0.75$), systolic and diastolic AoP ($r^2 = 0.0038$, $p = 0.63$; $r^2 = 0.0102$, $p = 0.43$, respectively), systolic or diastolic PAP ($r^2 = 0.0102$, $p = 0.43$; $r^2 = 0.0072$, $p = 0.51$, respectively), in all-cases combined population as well as in each group. The prognosis was comparable between mild and severe perivascular fibrosis groups (divided by the median of 1.255), for all-cause death (log-rank test, $p = 0.49$) or cardiac events, including cardiac or sudden death and admission for HF (log-rank test, $p = 0.29$), as was the case with patient characteristics such as gender, age, BMI, comorbidities, medication, and laboratory data (data not shown).

Perivascular fibrosis and TIMI frame count

TFCs measured in each group are shown in Table 2. There were no significant differences among the 3 groups in each coronary artery. Importantly, PFR was significantly correlated with CTFCs for LAD (Fig. 3A), but not with those for LCx or RCA (Fig. 3B and C). The correlation between PFR and CTFC for LAD was also observed in each group (Fig. 3D–F). Multivariate analysis was performed using a logistic regression model to examine the difference between high and low CTFC for LAD (divided by median of 21.18, with median attributed into the lower group) with 7 comorbidities, demonstrating that only PFR and atrial fibrillation were significantly correlated with CTFC for LAD (Table 3). PFR in patients with and without atrial fibrillation were comparable (1.42 ± 0.53 vs. 1.45 ± 0.91, $p = 0.92$).

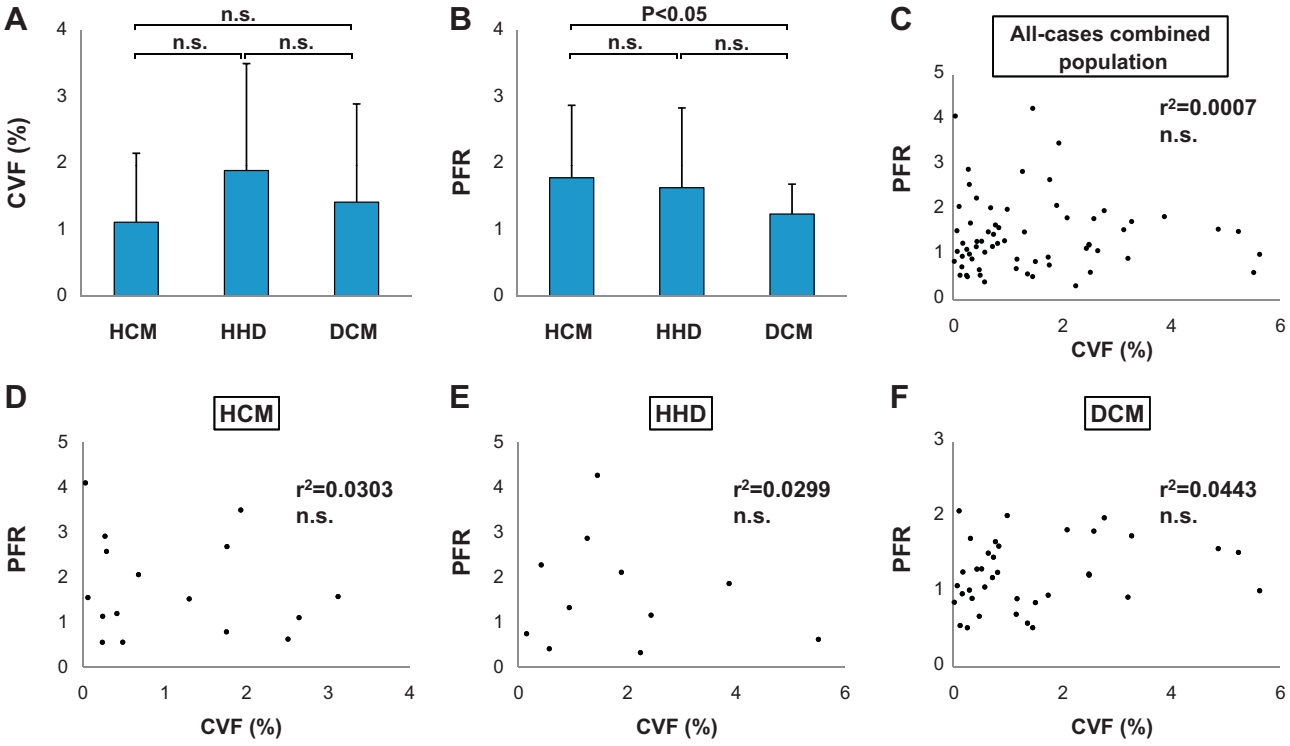

Fig. 2. Interstitial collagen volume fraction and perivascular fibrosis ratio. (A) Interstitial collagen volume fraction (CVF) was not significantly different among the 3 groups. (B) Perivascular fibrosis ratio (PFR) was higher in the HCM group than in the DCM group. (C) No correlation was observed between CVF and PFR in all-cases combined population ($r^2 = 0.0007$, $p = 0.84$), HCM (D), HHD (E), or DCM (F). HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease; DCM, dilated cardiomyopathy.

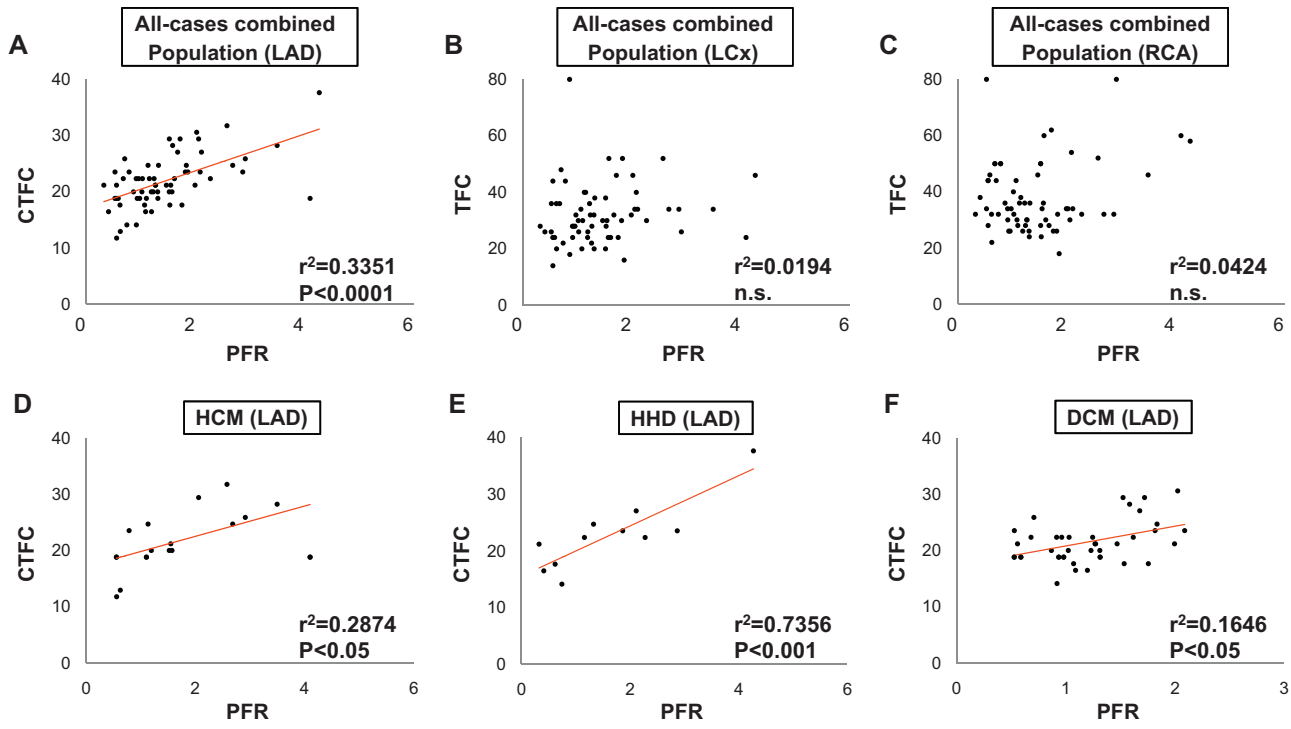

Fig. 3. Correlation between perivascular fibrosis ratio and thrombolysis in myocardial infarction (TIMI) frame counts (TFC). (A) Significant correlation was noted between perivascular fibrosis ratio (PFR) and corrected TIMI frame count (CTFC) for the left anterior descending coronary artery (LAD) in all study population ($r^2 = 0.3351$, $p < 0.0001$), but not for the left circumflex coronary artery (LCx) (B) ($r^2 = 0.0194$, $p = 0.27$) or the right coronary artery (RCA) (C) ($r^2 = 0.0424$, $p = 0.11$). A correlation between PFR and CTFCs for LAD was also noted in each HCM (D), HHD (E), and DCM group (F), respectively. HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease; DCM, dilated cardiomyopathy.

Discussion

The novel findings of the present study are as follows: (1) although interstitial CVF was comparable among the HCM, HHD, and DCM groups, perivascular fibrosis was more severe in the HCM group than in the DCM group; (2) PFR was independent of CVF; and (3) PFR was significantly correlated with CTFC for LAD. To the best of our knowledge, this is the first report that demonstrates the histopathological impact of perivascular fibrosis of small coronary arteries on impaired coronary blood flow in non-ischemic HF patients. Thus, the present results suggest that impaired coronary microcirculation could be caused not only by vascular remodeling, endothelial dysfunction, and microvascular spasm [11,21], but also by perivascular fibrosis.

Coronary perivascular fibrosis and interstitial myocardial fibrosis

Several studies have shown that myocardial collagen content is correlated with LV stiffness [22,23] and is involved in the progression of HF [24–26]. We also have recently demonstrated that CVF is correlated with LVEDP in HFrEF and is an important predictor of poor prognosis [16]. Previous studies using autopsy samples have demonstrated that interstitial CVF was higher in HCM than in HHD [27,28] and that ventricular fibrosis in HCM is known to concentrate in the ventricular septum, whereas it is equally distributed in the septum and the LV free wall in HHD [27]. The present study demonstrates that interstitial CVF was comparable between HCM and HHD, probably because our biopsy samples were obtained from the ventricular septum. Compared with interstitial myocardial fibrosis, not much attention has been paid to the possible importance of perivascular fibrosis in the pathogenesis of non-ischemic HF.

As demonstrated in the present study, neither CVF nor delayed enhancement on CMR was correlated with perivascular fibrosis. Furthermore, no correlation was observed between PFR and cardiac function such as LVEF, CO, or LVEDP. These results suggest that coronary perivascular fibrosis occurs independently of interstitial myocardial fibrosis or cardiac function.

Coronary perivascular fibrosis and coronary blood flow

TFC is a simple, effective and quantitative index of coronary blood flow that is able to detect coronary microvascular dysfunction [11–15]. The present study demonstrates for the first time the significant correlation between PFR and CTFC for LAD, indicating that perivascular fibrosis is substantially associated with the impairment of coronary blood flow in patients with non-ischemic HF.

In the present study, PFR in biopsy samples from the septum was associated with CTFC for LAD, but not TFC for LCx or RCA. This finding appears to be reasonable as blood flow in the septum is supplied mainly by LAD [29]. As the present study indicated, PFR in the area supplied by LAD was significantly associated with CTFC. Thus, if we could examine PFR in the free walls supplied by LCx or RCA, PFR in such areas might be associated with TFC in the corresponding areas. Conversely, this finding also suggests that the evaluation of perivascular fibrosis of the septum does not reflect that of the whole heart.

In addition to PFR, the presence of atrial fibrillation, which was irrelevant to PFR, was also significantly correlated with CTFC for LAD, a consistent finding with the previous study that atrial fibrillation was associated with increased coronary resistance and impaired myocardial blood flow [30]. The present study did not show that hypertension, diabetes mellitus, and dyslipidemia were related to CTFC for LAD, probably due to the limited number of patients in the present study.

Table 1
Patient characteristics.

	HCM ($n=16$)	HHD ($n=11$)	DCM ($n=37$)	Notes
Male gender	13 (81)	8 (73)	27 (75)	
Age (years)	55.9 ± 12.4	53.4 ± 14.9	55.5 ± 13.5	
BMI (kg/m^2)	24.2 ± 4.0	25.6 ± 4.8	24.7 ± 4.2	
Hypertension	5 (31)	11 (100)	17 (46)	*,‡
Diabetes mellitus	2 (13)	5 (45)	6 (16)	‡
Dyslipidemia	2 (13)	3 (27)	10 (27)	
Atrial fibrillation	1 (6)	1 (9)	14 (38)	†
Medication				
ACEI	6 (38)	6 (55)	22 (59)	
ARB	2 (13)	8 (73)	13 (35)	*,‡
β-blocker	10 (63)	9 (82)	26 (70)	
Diuretics	0 (0)	3 (27)	21 (57)	†
Spironolactone	2 (13)	1 (9)	11 (30)	
Warfarin	2 (13)	2 (18)	18 (49)	†
CCB	7 (44)	6 (55)	4 (11)	†,‡
Antiplatelet	4 (25)	4 (36)	10 (27)	
Statin	0 (0)	1 (9)	9 (24)	†
Amiodarone	0 (0)	0 (0)	4 (11)	
Laboratory data				
Hemoglobin (g/dl)	14.4 ± 1.4	14.3 ± 1.9	14.3 ± 1.6	
hsCRP (mg/dl)	0.10 ± 0.14	0.15 ± 0.14	0.15 ± 0.17	
BNP (pg/ml)	218.7 ± 247.3	50.7 ± 33.9	240.6 ± 373.3	*
LDL (mg/dl)	77.8 ± 62.8	89.7 ± 75.0	85.2 ± 74.8	
HDL (mg/dl)	56.7 ± 23.5	39.3 ± 9.4	42.0 ± 12.5	*,†
TG (mg/dl)	130.9 ± 91.9	143.9 ± 65.2	138.3 ± 74.4	
Glucose (mg/dl)	94.5 ± 25.8	110.9 ± 19.5	106.4 ± 30.6	
CCr (ml/min)	89.4 ± 34.4	95.5 ± 24.2	93.8 ± 37.7	
Hemodynamic data				
LVEDVI (ml/m^2)	72.5 ± 18.3	72.7 ± 19.0	111.8 ± 28.0	†,‡
EF (%)	71.2 ± 10.1	54.4 ± 4.9	36.8 ± 11.8	*,†,‡
mAoP (mmHg)	103.8 ± 17.5	120.2 ± 19.7	98.8 ± 17.8	*,‡
LVEDP (mmHg)	16.6 ± 5.7	10.3 ± 5.1	10.6 ± 4.1	*,†
mPAP (mmHg)	19.9 ± 4.5	20.9 ± 7.0	18.9 ± 5.3	
PCWP (mmHg)	11.3 ± 6.2	9.3 ± 5.0	9.0 ± 5.3	
Cardiac output (L/min)	4.7 ± 1.0	5.9 ± 1.7	4.6 ± 1.4	*,‡
Cardiac index (L min^{-1} m^{-2})	2.7 ± 0.4	3.3 ± 0.6	2.7 ± 0.7	*,‡
Morphometric data				
CVF (%)	1.11 ± 1.04	1.89 ± 1.61	1.41 ± 1.48	
PFR	1.78 ± 1.09	1.63 ± 1.20	1.24 ± 0.45	†
All-cause death	1 (6)	0 (0)	2 (5)	
Cardiac events				
Cardiac or sudden death	0 (0)	0 (0)	2 (5)	
Admission for HF	2 (13)	0 (0)	3 (8)	

Results are presented as mean ± SD or n (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; CCB, calcium channel blocker; CCr, creatinine clearance; CVF, collagen volume fraction; DCM, dilated cardiomyopathy; EF, ejection fraction; HCM, hypertrophic cardiomyopathy; HDL, high-density lipoprotein; HF, heart failure; HHD, hypertensive heart disease; hsCRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; LVEDP, left ventricular end-diastolic pressure; LVEDVI, left ventricular end-diastolic volume index; mAoP, mean aortic pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PFR, perivascular fibrosis ratio; TG, triglyceride.

* $p<0.05$, HCM vs. HHD.
† $p<0.05$, HCM vs. DCM.
‡ $p<0.05$, HHD vs. DCM.

Table 2
Thrombolysis in myocardial infarction frame counts.

Coronary artery	HCM ($n=16$)	HHD ($n=11$)	DCM ($n=37$[a])	p-Value
LAD (corrected)	21.91 ± 5.48	22.78 ± 6.23	21.67 ± 3.87	n.s.
LCx	29.63 ± 3.80	31.82 ± 8.55	33.30 ± 11.82	n.s.
RCA	39.50 ± 15.22	34.72 ± 9.39	38.72 ± 14.64	n.s.

Results are presented as mean ± SD. HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease; DCM, dilated cardiomyopathy; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; RCA, right coronary artery; n.s., not statistically significant.

[a] Data of LAD in one DCM case and that of RCA in another DCM case failed to be obtained.

Table 3
Logistic regression model for factors of corrected thrombolysis in myocardial infarction frame count for the left anterior descending coronary artery.

Variables	p-Value	Odds ratio (95% CI)
Hypertension	0.23	
Dyslipidemia	0.21	
Diabetes mellitus	0.63	
Atrial fibrillation	0.02	5.78 (1.33–30.53)
Body mass index (kg/m^2)	0.20	
PFR	<0.002	3.28 (1.52–9.00) per 1.0 increase
CVF (%)	0.81	

95% CI, 95% confidence interval; PFR, perivascular fibrosis ratio; CVF, collagen volume fraction.

Heart failure and coronary blood flow

It has been reported that coronary blood flow is impaired in aortic valve diseases because coronary blood flow depends on hemodynamic changes [31] and that coronary blood flow is determined by heart rate, peak stress, and ventricular performance in cardiomyopathy with normal coronary angiogram [32]. Coronary microvascular dysfunction has attracted much attention as a contributor to impaired coronary blood flow in various etiologies of HF [11]. The presence of microvascular dysfunction after acute myocardial infarction is now considered to be an important prognostic factor [33]. Coronary flow reserve, as an indicator of coronary blood flow, has been demonstrated to be impaired during acute phase of HF, and to be strongly correlated with the mortality of HF patients [9,10]. Thus, it is important to improve coronary blood flow in non-ischemic HF patients as well and to pay much attention to perivascular fibrosis as it is an important determinant of coronary blood flow.

Study limitations

Several limitations should be mentioned for the present study. First, coronary arteries in biopsy samples were partially crushed during the procedure, which might have affected the quantitative analyses of perivascular fibrosis, although we did not use the lumen area for the present analysis. Furthermore, myocardial biopsy samples from healthy controls were not available for apparent ethical reasons. Second, we only examined the role of perivascular fibrosis quantity but not its quality. Indeed, it has been reported that not only the quantity but also the quality of interstitial myocardial fibrosis (e.g. cross-linking and type I/III collagen ratio) are important determinants of myocardial stiffness [34–36]. Future studies are required to address this issue. Third, we used TFC method to evaluate coronary blood flow because we did not directly evaluate coronary flow velocity with a flow wire. This was based on the previous studies that TFC well represents coronary flow velocity [12,15,37,38]. Fourth, we were unable to elucidate the mechanisms and factors related to perivascular fibrosis. This issue should also be examined in future studies. Finally, the present study was an observational study with a relatively small number of patients. The relatively small numbers of all-cause deaths and admissions (Table 1) prevented us from investigating whether perivascular fibrosis has a prognostic impact. This issue also should be addressed in future studies with a large number of patients, including the effects of drugs to modulate perivascular fibrosis.

In conclusion, we were able to demonstrate that coronary perivascular fibrosis is significantly associated with impairment of coronary blood flow in non-ischemic HF patients. Thus, coronary perivascular fibrosis can be a new therapeutic target to improve coronary microcirculation in HF.

Disclosures

None.

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