

Evaluating the Utility of Circulating Biomarkers of Collagen Synthesis in Hypertrophic Cardiomyopathy

Andris H. Ellims, MBBS; Andrew J. Taylor, MBBS, PhD; Justin A. Mariani, MBBS, PhD; Liang-han Ling, MBBS, PhD; Leah M. Iles, MBBS; Micha T. Maeder, MD; David M. Kaye, MBBS, PhD

Background—In hypertrophic cardiomyopathy (HCM), accumulation of myocardial collagen may play a central role in the pathogenesis of diastolic dysfunction and arrhythmia. Previous studies have suggested that peripheral levels of byproducts of collagen synthesis are reflective of myocardial extracellular matrix metabolism, although this has not been validated in detail. Given the potential clinical utility of such biomarkers, we sought to validate the assumed relationship between peripheral markers and myocardial fibrosis in HCM.

Methods and Results—Fifty patients with HCM and 25 healthy controls underwent peripheral venous sampling to determine plasma concentrations of key collagen precursors (procollagen I and III N-terminal propeptides [PINP, PIIINP]). Contrast-enhanced cardiac magnetic resonance imaging was performed to quantify regional (by late-gadolinium enhancement) and diffuse (by T_1 mapping) myocardial fibrosis. Nineteen subjects also underwent simultaneous arterial and coronary sinus blood sampling (to derive transcardiac concentration gradients of PINP, PIIINP, and C-terminal telopeptide of type I collagen) and right heart catheterization. Despite cardiac magnetic resonance evidence of regional (late-gadolinium enhancement quantity, $6.4 \pm 8.0\%$) and diffuse (T_1 time, 478 ± 79 ms) myocardial fibrosis in patients with HCM, peripheral levels of collagen precursors were similar compared with control subjects (PINP, 45.9 ± 22.9 versus 53.4 ± 25.9 $\mu\text{g/L}$; $P=0.21$; PIIINP, 4.8 ± 1.7 versus 4.4 ± 1.1 $\mu\text{g/L}$; $P=0.26$). No significant net positive transcardiac concentration gradient was detected for either biomarker of collagen synthesis.

Conclusions—The cardiac contribution to peripheral levels of byproducts of collagen synthesis in patients with HCM is insignificant. Furthermore, peripheral levels of these biomarkers do not accurately reflect myocardial collagen content in these patients. (*Circ Heart Fail.* 2014;7:271-278.)

Key Words: cardiomyopathy, hypertrophic ■ collagen ■ endomyocardial fibrosis ■ magnetic resonance imaging

Left ventricular hypertrophy (LVH), most commonly the result of prolonged systemic hypertension, may also be due to primary myocardial disease, such as hypertrophic cardiomyopathy (HCM). Histopathologic studies have identified increased collagen content within thickened myocardium in both hypertension^{1,2} and HCM.^{1,3} Such expansion of the myocardial extracellular matrix (ECM) may result in increased passive myocardial stiffness resulting in diastolic dysfunction,⁴⁻⁷ while also providing a potential substrate for the development of ventricular arrhythmias.

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In patients with LVH, increased levels of peripheral byproducts of collagen metabolism, such as procollagen I and III N-terminal propeptide (PINP and PIIINP), have been assumed to reflect the expansion of myocardial ECM directly. For example, in hypertensive patients, procollagen propeptide concentrations have been found to be significantly higher

than in normotensives and correlated with LV mass, diastolic impairment, and ventricular arrhythmias.⁸ Additionally, treatment with an angiotensin-converting enzyme inhibitor reduced procollagen propeptide concentrations⁹ and reversed structural and physiological cardiac changes induced by hypertension.⁸ In patients with HCM too, elevated peripheral markers of collagen turnover have correlated with impaired diastolic function.^{10,11} Similarly, patients carrying HCM sarcomeric gene mutations, but without LVH or regional myocardial fibrosis, also had significantly higher serum concentrations of procollagen propeptides compared with healthy controls.¹²

In the majority of these studies, blood was only sampled from peripheral sites and their levels, therefore, reflect the integrated accumulation of collagen markers from throughout the body. As such, these levels may not be truly reflective of cardiac ECM turnover, and this issue could limit their utility as true biomarkers of myocardial matrix deposition or turnover. Furthermore, recent advances in tissue characterization capabilities of cardiac magnetic resonance (CMR) imaging

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From the Heart Centre, Alfred Hospital, Melbourne, Australia (A.H.E., A.J.T., J.A.M., L.L., L.M.I., D.M.K.); Baker IDI Heart and Diabetes Institute, Melbourne, Australia (A.H.E., A.J.T., J.A.M., L.L., L.M.I., M.T.M., D.M.K.); and Division of Cardiology, Kantonsspital, St Gallen, Switzerland (M.T.M.).

The Data Supplement is available at <http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.113.000665/-/DC1>. Correspondence to David M. Kaye, MBBS, PhD, Alfred Hospital and Baker IDI Heart and Diabetes Institute, Heart Centre, Alfred Hospital, Commercial Rd, Melbourne 3004, Australia. E-mail David.Kaye@bakeridi.edu.au

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provide an alternative approach to evaluating the extent of cardiac fibrosis in diseases such as HCM. Late-gadolinium enhancement (LGE) sequences can identify regions of dense replacement fibrosis in most patients with HCM, and its presence is associated with a worse prognosis.¹³ More recently, postcontrast T_1 mapping, a technique shown to correlate with histopathologic findings,¹⁴ has been used to quantify diffuse patterns of interstitial myocardial fibrosis in several cardiac disease states,^{15–17} including HCM,¹⁸ and that lower T_1 times, suggestive of more diffuse myocardial fibrosis, have correlated with higher LV filling pressures.¹⁸

In the present study, we sought to evaluate in HCM patients the relationship between peripheral markers of collagen turnover and CMR-derived measures of myocardial fibrosis, echocardiographic indices of diastolic performance, and invasively determined hemodynamic indices. Furthermore, we aimed to measure the precise degree of myocardial release of collagen biomarkers by determining their net transcardiac concentration gradients.

Methods

Patient Selection

All research was performed at the Alfred Hospital, Melbourne, Australia. Fifty consecutive patients referred to our CMR department for further evaluation of HCM and 25 asymptomatic healthy controls without a history of cardiovascular disease were invited to participate. Patients with HCM had asymmetric septal hypertrophy (interventricular septal thickness ≥ 15 mm with a ratio of septal-to-lateral ventricular wall thickness $\geq 1.3:1.0$ as measured by echocardiography), and the diagnosis of HCM required the absence of another condition that could cause the degree of hypertrophy observed.¹⁹

Exclusion criteria for all subjects included previously documented coronary artery disease or current symptoms suggestive of coronary artery disease, more than mild valvular heart disease, atrial fibrillation, previous septal reduction therapy, documented thyroid disease, recent trauma or surgery, documented bone or joint disease, forced expiratory volume within 1 second less than the lower limit of normal, contraindications to CMR including pacemaker and defibrillator implantation, and significant renal dysfunction (estimated glomerular filtration rate <30 mL/min per 1.73 m²).

Informed consent was obtained from all participants, and the study protocol conformed to the ethical guidelines of 1975 Declaration of Helsinki as reflected in a priori approval by the Alfred Hospital Ethics Committee's guidelines.

CMR Protocol

CMR was performed on the same day as blood sampling and echocardiography using a clinical 1.5-T scanner (Signa HD 1.5-T; GE Healthcare, Waukesha, WI). All sequences were acquired during a breathhold of 10 to 15 seconds. Initially, a contiguous short-axis steady-state free precession stack was acquired (8 mm slice thickness, no gap), extending from the mitral valve annulus to the LV apex, to enable volumetric analysis of the left ventricle using the summation of disc method.

LGE was evaluated 10 minutes after a bolus of gadolinium–diethylenetriamine pentaacetic acid (DTPA; 0.2 mmol/kg body weight; Magnevist, Schering, Germany) to identify regional myocardial fibrosis using a T_1 -weighted inversion recovery gradient echo technique (TR, 7.1 ms; TE, 3.1 ms; inversion time [TI] individually determined to null the myocardial signal; slice thickness, 8 mm; matrix 256×192; number of acquisitions, 2; Figure 1). A TI optimization sequence was performed 8 minutes post–gadolinium administration and was a fast gradient echo, inversion recovery, gated, multiphase acquisition, commencing at an inversion time of 150 ms and increasing in 25 ms increments to 250 ms, in a single midventricular

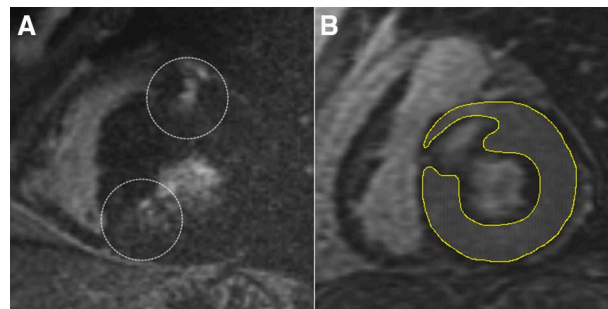


Figure 1. Evaluation of regional and diffuse myocardial fibrosis by contrast-enhanced cardiac magnetic resonance imaging in hypertrophic cardiomyopathy (HCM). **A**, Ventricular short-axis late-gadolinium enhancement image of a patient with HCM. Regional myocardial fibrosis (hyperintense areas; circled) is located within the left ventricular myocardium at the points of right ventricular free wall insertion. **B**, Ventricular short-axis T_1 mapping image of a patient with HCM. The region of interest (shaded) used to calculate postcontrast myocardial T_1 time excludes an area of LGE.

short-axis slice. A visual determination of optimum TI to null the myocardial signal was then made. LGE imaging was performed using standard long-axis views of the left ventricle and a contiguous stack of slices from the mitral valve annulus to the LV apex. Regional fibrosis was identified by LGE within the myocardium, defined quantitatively by a myocardial postcontrast signal intensity 6 SD above that within a reference region of remote myocardium (without LGE) within the same slice.²⁰

To evaluate diffuse interstitial myocardial fibrosis, a histologically validated postcontrast T_1 mapping sequence²¹ was used to cycle through the acquisition of images obtained at 3 standard LV short-axis levels (basal, mid, and apex) over a range of inversion times, as described previously.¹⁸ This ECG-triggered, inversion recovery–prepared, 2-dimensional, fast gradient echo sequence used variable temporal sampling of k-space (VAST)²² (GE Healthcare). Ten images at basal, mid, and apical LV short-axis levels were acquired sequentially at increasing inversion times, commencing 20 minutes after the bolus of gadolinium-DTPA (TI range, 75–750 ms), and each over a series of 3 to 5 breathholds. After image acquisition, the 3 sets of images of varying inversion times were transferred to an external computer for analysis using a dedicated research software package with a curve-fitting technique to generate T_1 maps (Cinetool; GE Healthcare). For each short-axis image, a region of interest was drawn around the entire LV myocardium (excluding regions of LGE) to calculate postcontrast myocardial T_1 time (Figure 1). A global postcontrast myocardial T_1 time was derived by calculating the mean T_1 time of all 3 short-axis levels. To account for potential effects of renal function and duration of time between contrast administration and image acquisition on gadolinium kinetics, correction values²³ were used to normalize postcontrast myocardial T_1 times to a matched state (estimated glomerular filtration rate, 90 mL/min per 1.73 m²; time from contrast administration to image acquisition, 20 minutes) for all 3 short-axis levels.

Echocardiography Protocol

Transthoracic echocardiography with a standard clinical protocol was performed on all patients. Diastolic function was assessed by a combination of mitral inflow pattern (E to A ratio) and early mitral annular velocities (e' , measured at septal and lateral aspects of mitral annulus in the apical 4-chamber view). Additionally, mitral E/ e' (septal and lateral) was chosen as an index of LV diastolic function. All measurements were made in accordance with American Society of Echocardiography guidelines.²⁴

Image Analysis

All echocardiogram and CMR images were interpreted by 2 experienced readers unaware of subjects' clinical information and results of other diagnostic tests. Endocardial and epicardial LV contours were

drawn manually for each diastolic and systolic frame, excluding papillary muscles.

Right Heart Catheterization and Coronary Sinus Sampling

A subgroup of 10 patients with HCM and 9 healthy controls also underwent right heart catheterization specifically for the purposes of the study protocol. For blood sampling and blood pressure measurement, a 3-F line was placed in a radial artery under local anesthesia. For the measurement of right atrial pressure, right ventricular pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure, an introducer sheath was placed in the right median cubital vein or the internal jugular vein under local anesthesia, and under fluoroscopy, a balloon-tipped thermodilution catheter (7 F Arrow; Edwards Inc) was introduced. Wedge position was confirmed fluoroscopically and by the profile of accompanying pressure waveform, and mean pulmonary capillary wedge pressure was recorded at end-expiration. Cardiac output was measured using the thermodilution technique. A 6-F coronary sinus catheter was advanced to the coronary sinus for blood sampling. The tip of the catheter was positioned ≥2 cm proximal to the orifice of the coronary sinus as confirmed by radiographic contrast injection.

Biochemical Assays

Blood samples were collected in tubes containing EDTA. Samples were subsequently centrifuged, and plasma was stored at -70°C until assay. For all subjects, plasma concentrations of PINP and PIIINP in peripheral venous, arterial, and coronary sinus plasma were measured by radioimmunoassay (Abbott, Abbott Park, IL). For the subgroup of 19 subjects who underwent coronary sinus sampling, plasma concentrations in peripheral arterial and coronary sinus plasma of C-terminal telopeptide of type I collagen (ICTP), a biomarker of collagen degradation, were measured by enzyme immunoassay (Orion Diagnostica, Espoo, Finland). Coefficients of variation were <10%. Transcardiac gradients for PINP, PIIINP, and ICTP were calculated as the difference between plasma concentration in the coronary sinus and that in the arterial sample. All analyses were conducted by researchers unaware of the clinical status of study subjects.

Statistical Methods

All data are expressed as mean±SD unless otherwise indicated. Comparison between groups used the 2 independent samples *t* test (normally distributed data), Wilcoxon–Mann–Whitney test (data with skewed distribution), or the Fisher exact test (categorical data) as appropriate. Comparisons within groups were made using the paired *t* test. Spearman correlation coefficients were calculated for all correlation analyses. For comparisons across multiple groups, 1-way ANOVA with Bonferroni correction was used for normally distributed variables. When performing statistical comparisons of means, we tested and, when necessary, accounted for unequal variances in the groups. For all comparisons, a *P* value <0.05 was considered significant, and all reported *P* values are 2-tailed. Statistical analyses were performed using Stata software version 11.1 (StataCorp, College Station, TX) and SPSS version 17 (SPSS Corp, Chicago, IL).

Results

Clinical and Demographic Data

Seventy-five patients were evaluated during the study period, comprising 50 patients with HCM and 25 healthy controls. Baseline characteristics are presented in Table 1. Mean duration from initial diagnosis of HCM to study involvement was 6.4±7.6 years. Patients with HCM had a similar age, heart rate, systolic blood pressure, and haematocrit compared with controls, but higher body mass index and lower estimated

Table 1. Baseline Characteristics

	HCM (n=50)	Control (n=25)	<i>P</i> Value
Age, y	48±13	47±17	0.8
Men, n (%)	38 (76%)	18 (72%)	0.7
Family history of HCM, n (%)	17 (34%)	0 (0%)	<0.01
Body mass index, kg/m ²	27.5±4.9	24.1±2.7	<0.01
History of systemic hypertension, n (%)	9 (18%)	0 (0%)	<0.05
History of diabetes mellitus, n (%)	1 (2%)	0 (0%)	0.7
Dyspnea, n (%)	28 (56%)	0 (0%)	<0.01
Medications, n (%)			
β-Blockers	29 (58%)	0 (0%)	<0.01
Calcium channel blocker	10 (20%)	0 (0%)	<0.05
ACE inhibitor	8 (16%)	0 (0%)	<0.05
ARB	4 (8%)	0 (0%)	0.3
Amiodarone	0 (0%)	0 (0%)	...
Statin	13 (26%)	0 (0%)	<0.01
Loop or thiazide diuretic	0 (0%)	0 (0%)	...
Aldosterone antagonist	0 (0%)	0 (0%)	...
Heart rate, beats/min	60±10	62±9	0.6
Systolic blood pressure, mm Hg	129±16	131±18	0.7
Hematocrit	0.42±0.03	0.42±0.03	0.6
eGFR, mL/min per 1.73 m ²	79±12	86±8	0.01

ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; and HCM, hypertrophic cardiomyopathy.

glomerular filtration rate. Nine patients with HCM had treated systemic hypertension, and the degree of their LVH was not explained by hypertension alone. More than half (56%) of the HCM group experienced dyspnea, but none worse than New York Heart Association (NYHA) class II severity, and most patients with HCM (76%) were receiving β-blocker or calcium channel blocker therapy. Twenty patients with HCM (including 5 patients who underwent right heart catheterization and coronary sinus sampling) were receiving a statin agent, angiotensin-converting enzyme (ACE) inhibitor, or angiotensin receptor blocker (ARB).

CMR, Echocardiography, and Peripheral Venous Collagen Precursor Data

Cardiac imaging and peripheral venous collagen precursor data are detailed in Table 2. The HCM group had a maximum LV wall thickness of 20±3 mm and significantly higher LV ejection fraction and indexed LV mass compared with the control group. All HCM patients had preserved LV systolic function (ie, LV ejection fraction >55%). LGE was identified in 84% of HCM patients (most commonly located within the interventricular septum or at the insertion points of the right ventricular free wall) and accounted for 6.4±8.0% of LV mass (based on complete volumetric data from 47 patients). T₁ mapping was successfully performed in all but 1 subject, a patient with HCM and frequent ventricular ectopy. Postcontrast myocardial T₁ time was significantly lower in the HCM group (478±79 versus 545±49 ms; *P*<0.001), indicative of more diffuse myocardial fibrosis. Postcontrast T₁ time of the LV

Table 2. Peripheral Venous Collagen Precursors, CMR, and Echocardiography Data

	HCM (n=50)	Control (n=25)	P Value
Peripheral venous collagen precursor levels			
PINP, $\mu\text{g/L}$	45.9 \pm 22.9	53.4 \pm 25.9	0.21
PIIINP, $\mu\text{g/L}$	4.8 \pm 1.7	4.4 \pm 1.1	0.26
CMR data			
LV end-diastolic volume indexed, mL/BSA	81 \pm 14	83 \pm 13	0.4
LV ejection fraction, %	69 \pm 7	60 \pm 6	<0.01
LV mass indexed, g/BSA	89 \pm 24	52 \pm 9	<0.01
Maximum LV wall thickness, mm	20 \pm 3	8 \pm 2	<0.01
LGE			
Presence, n (%)	42 (84%)	0 (0%)	<0.01
Quantity, % of LV mass	6.4 \pm 8.0	0 (0%)	<0.01
LGE distribution, n (%)			
Right ventricular insertion point(s)	28 (67%)	...	
Interventricular septum	26 (62%)	...	
LV apex	1 (2%)	...	
Other LV site	10 (24%)	...	
Postcontrast T ₁ time, ms			
LV myocardium	478 \pm 79	545 \pm 49	<0.001
LV blood pool	303 \pm 31	306 \pm 22	0.7
Echocardiography data			
Left atrial volume indexed, mL/m ²	52 \pm 18	32 \pm 9	<0.001
Peak LVOT gradient, mm Hg	41 \pm 47	4 \pm 1	<0.001
e', cm/s	0.07 \pm 0.02	0.10 \pm 0.03	<0.001
E/e' ratio	12.6 \pm 4.9	7.7 \pm 2.4	<0.001

BSA indicates body surface area; CMR, cardiac magnetic resonance; HCM, hypertrophic cardiomyopathy; LGE, late-gadolinium enhancement; LV, left ventricular; LVOT, LV outflow tract; PINP, procollagen I N-terminal propeptide; and PIIINP, procollagen III N-terminal propeptide.

blood pool did not differ significantly between the groups, implying similar contrast medium kinetics. The HCM group had significantly higher E/e', suggestive of higher LV filling pressure, but no patient exhibited echocardiographic features of severe (restrictive) LV diastolic filling. A peak LV outflow tract gradient >30 mm Hg at rest or with Valsalva, consistent with obstructive HCM, was observed in 22 (44%) patients with HCM. There were no significant differences between peripheral venous levels of PINP (45.9 \pm 22.9 versus 53.4 \pm 25.9 $\mu\text{g/L}$; $P=0.21$) or PIIINP (4.8 \pm 1.7 versus 4.4 \pm 1.1 $\mu\text{g/L}$; $P=0.26$). There were no significant differences in CMR, echocardiography, or peripheral venous collagen precursor data in HCM patients according to whether or not they were receiving statin, ACE inhibitor, or ARB therapy (Tables I and II in the Data Supplement).

Correlates of Peripheral Venous Levels of PINP and PIIINP

In patients with HCM, apart from a modest inverse correlation between peripheral venous level of PINP and patient age ($r=-0.35$; $P<0.05$), there were no significant correlations

Table 3. Correlates of Peripheral Levels of PINP and PIIINP in Patients With HCM

	PINP	PIIINP
Age	-0.35*	-0.13
Body mass index	-0.14	0.09
Heart rate	0.02	-0.13
Systolic blood pressure	-0.14	-0.14
eGFR	0.06	0.27
LV end-diastolic volume indexed	0.04	0.15
LV ejection fraction	0.18	0.06
LV mass indexed	0.01	0.09
Maximum LV wall thickness	0.07	0.02
Quantity of LGE	-0.16	-0.16
Postcontrast myocardial T ₁ time	0.03	-0.11
Left atrial volume indexed	-0.02	-0.14
Peak LVOT gradient	0.11	0.06
e'	0.12	-0.08
E/e'	0.05	0.20

eGFR indicates estimated glomerular filtration rate; HCM, hypertrophic cardiomyopathy; LGE, late-gadolinium enhancement; LV, left ventricular; LVOT, LV outflow tract; PINP, procollagen I N-terminal propeptide; and PIIINP, procollagen III N-terminal propeptide.

* $P<0.05$.

observed between collagen precursors and baseline patient characteristics, CMR data, or echocardiographic parameters (Table 3). In particular, no significant correlations were observed between PINP or PIIINP levels and CMR-determined quantities of regional or diffuse myocardial fibrosis, or echocardiographic indices of diastolic dysfunction.

Transcardiac Gradient of Collagen Precursors and Right Heart Catheterization Data

Arterial and coronary sinus levels and calculated transcardiac gradients for PINP, PIIINP, and ICTP are detailed in Table 4. Levels of PINP and PIIINP were similar in both groups, regardless of sampling site, and no significant net positive myocardial concentration gradient was detected for either collagen precursor (Figure 2). Coronary sinus levels of PINP were lower than arterial levels in both HCM and control groups, indicating negative myocardial concentration gradients for this biomarker. Although coronary sinus levels of PIIINP were higher than arterial levels in both groups, these differences were not statistically significant ($P=0.18$ for HCM group; $P=0.27$ for control group). Arterial levels of ICTP were also similar in both groups; however, in HCM patients, coronary sinus level of ICTP was significantly higher than the arterial level ($P<0.01$), resulting in a significant positive transcardiac gradient in this group. In patients with HCM, arterial and coronary sinus levels of PINP, PIIINP, and ICTP did not significantly differ according to whether or not they were receiving statin, ACE inhibitor, or ARB therapy.

There were no significant differences between HCM and control groups with respect to intracardiac pressures measured by right heart catheterization. Specifically, right atrial

Table 4. Arterial and Coronary Sinus Levels and Transcardiac Gradients of Collagen Biomarkers

	HCM (n=10)	Control (n=9)	P Value (HCM vs Control)
PINP level, µg/L			
Arterial	41.4±14.1	42.0±17.0	0.9
Coronary sinus	39.6±12.6	41.6±16.8	0.8
P value (arterial vs coronary sinus)	0.02	0.3	
Transcardiac gradient	-1.8±2.1	-0.4±0.9	0.09
PIIINP level, µg/L			
Arterial	4.6±0.7	4.3±0.7	0.3
Coronary sinus	4.8±0.8	4.4±0.9	0.4
P value (arterial vs coronary sinus)	0.18	0.3	
Transcardiac gradient	0.2±0.4	0.2±0.4	0.9
ICTP level, µg/L			
Arterial	3.3±1.2	3.2±1.6	0.8
Coronary sinus	4.3±0.9	3.1±1.5	<0.05
P value (arterial vs coronary sinus)	<0.01	0.7	
Transcardiac gradient	1.0±1.0	-0.04±0.3	<0.01

HCM indicates hypertrophic cardiomyopathy; ICTP, C-terminal telopeptide of type I collagen; PINP, procollagen I N-terminal propeptide; and PIIINP, procollagen III N-terminal propeptide.

pressure (4.9±2.8 versus 5.9±3.0 mmHg; *P*=0.5), mean pulmonary artery pressure (18.9±4.8 versus 16.8±3.6 mmHg; *P*=0.3), and pulmonary capillary wedge pressure (12.0±3.4 versus 10.3±3.8 mmHg; *P*=0.3) did not significantly differ. There was a trend toward lower cardiac output in HCM patients (5.7±0.9 versus 6.8±1.6 L/min; *P*=0.07). Peripheral levels of PINP, PIIINP, and ICTP showed no correlation either with any hemodynamic parameter or with transcardiac gradients for any collagen biomarker.

Discussion

In this study, we sought to measure peripheral plasma levels and transcardiac gradients of byproducts of collagen synthesis and investigate their relationships to CMR, echocardiographic, and hemodynamic correlates of myocardial fibrosis in patients with HCM. We found that plasma levels of PINP and PIIINP were similar in HCM patients compared with healthy controls and that neither biomarker exhibited a significant transcardiac gradient. No significant correlations were observed between these procollagen propeptides and CMR-determined quantities of myocardial fibrosis.

Myocardial fibrosis, along with myocyte disarray and small vessel disease, are characteristic histological findings in HCM. During an early study of ex vivo HCM hearts, Varnava et al³ concluded that although disarray was directly linked to sarcomeric protein mutations, fibrosis was more likely secondary to other factors, such as LV mass and local mediators. Despite subsequent studies of molecular biology and pathophysiology of HCM, the precipitant(s) for myocardial fibrosis in this condition remain uncertain. Putative mechanisms include impaired myocyte energy metabolism causing cell death,²⁵ coronary microvascular dysfunction

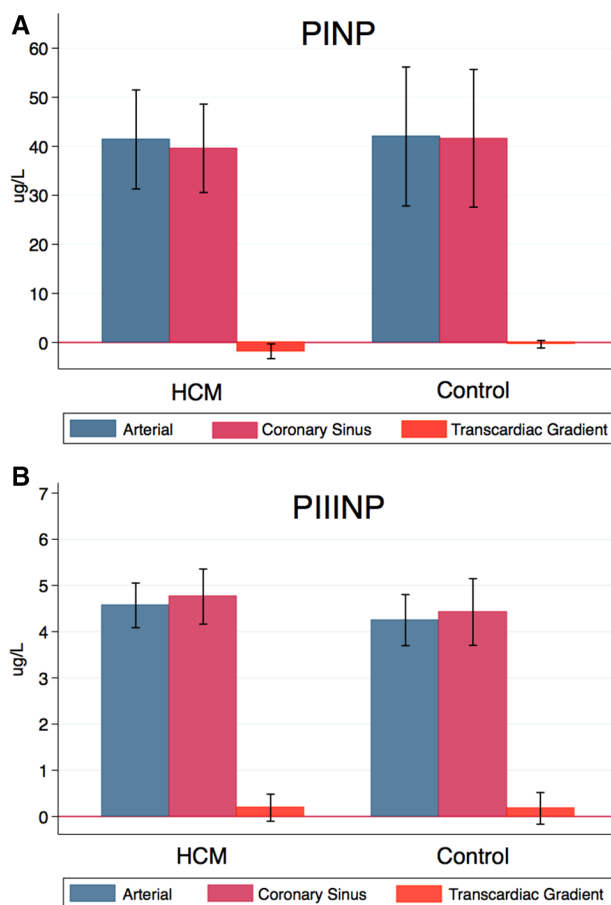


Figure 2. Procollagen I and III N-terminal propeptide (PINP and PIIINP) levels and transcardiac gradients. Bar graphs indicating mean arterial and coronary sinus levels and transcardiac gradients with 95% confidence intervals for PINP (A) and PIIINP (B) in patients with hypertrophic cardiomyopathy (HCM) and healthy controls. There were no significant differences in procollagen propeptide levels between the 2 groups and no significant net transcardiac gradients.

and resultant ischemia,²⁶ and activation of the transforming growth factor-β profibrotic signaling pathway by abnormal sarcomeric gene protein mutations.²⁷ With the advent of rapid next-generation genetic sequencing and the development of techniques to evaluate myocardial fibrosis noninvasively, a better understanding of the link between genotype and fibrotic phenotype may be obtained.

The calculation of transcardiac concentration gradients by simultaneous coronary sinus and arterial sampling can localize the source of collagen marker release to the myocardium. The absence of a net myocardial concentration gradient for either PINP or PIIINP is consistent with our observations in systolic heart failure.²⁸ If the myocardium is indeed a significant source of biomarkers of collagen synthesis, an increase in LV mass should theoretically result in higher peripheral levels and transcardiac gradients of PINP or PIIINP. We did not observe any such differences between our groups despite HCM patients having an indexed LV mass >70% greater than in healthy subjects. Interestingly, we observed transcardiac release of a biomarker of collagen degradation (ICTP) in HCM patients. This finding is consistent with increased collagen turnover in HCM patients; however, this observation

cannot be detected by examining the levels of ICTP in the systemic circulation.

Although transcatheter gradients did not reveal a significant release of procollagen propeptides, we provided evidence consistent with the presence of cardiac fibrosis in the HCM group. Using validated CMR techniques, we identified both regional and diffuse patterns of myocardial fibrosis in these patients; however, there were no correlations with peripheral PINP or PIIINP levels. Also, PINP and PIIINP levels failed to increase as CMR indices of regional and diffuse myocardial fibrosis increased. These findings suggest that peripheral plasma collagen biomarkers are not specific for the presence of myocardial fibrosis. There are several possible explanations for our findings. First, the magnitude of collagen metabolism occurring within the myocardium is likely small compared with the total amount within the body, and thus, the myocardial contribution to peripheral levels is also likely to be minor. Second, it is possible that myocardial collagen synthesis occurs in a relapsing-remitting pattern, increasing during periods of cardiac physiological stress, such as during exercise or higher blood pressure, and vice versa.

Our findings differ, to some degree, from those of Lombardi et al.¹⁰ In their study, a cohort of patients with HCM had significantly higher peripheral levels of PIIINP, active matrix metalloproteinases 2 and 9, and total tissue inhibitor of metalloproteinase 1 compared with healthy controls. Similar to the present study, there was no significant difference in PINP levels between the groups. Lombardi et al used echocardiography-derived LV diastolic dimensions, ratio of peak diastolic flow velocities (E/A), E-wave deceleration time, and pulmonary venous flow to demonstrate a link between PINP and PIIINP levels and LV diastolic dysfunction. Our study found no such associations between levels of these byproducts of collagen synthesis and CMR-derived LV dimensions, or with echocardiographic indices of LV diastolic dysfunction (including E/e').²⁹ We suggest that the associations observed between PINP and PIIINP levels and echocardiographic parameters of LV diastolic function are more likely to reflect a pulmonary site of increased collagen synthesis. In particular, previous studies indicate that in subjects with diastolic dysfunction, such as those with heart failure and a preserved ejection fraction, pulmonary pressures may rise dramatically on exertion,³⁰ which could trigger the release of collagen precursors and degradation products from the lungs.

In contrast to the present study, Ho et al¹² found higher peripheral levels of the C-terminal propeptide of procollagen I in patients with HCM compared with healthy controls and concluded that type I collagen synthesis was increased within myocardial tissue in HCM. However, because the samples were obtained from a peripheral vein, it is not necessarily possible to conclude that the origin was myocardial. Furthermore, in this study, the control group was not age-matched to their HCM cohort, and there were also other key differences such as the inclusion of patients with NYHA class III symptoms and LV systolic dysfunction, which could significantly influence the levels of biomarkers. As HCM reflects a complex interaction between genetic, biological, and physiological factors,

apparent discrepancies between studies may be accounted for by disparate subject cohorts.

Taken together, the present data provide further impetus for the identification of novel, specific biomarkers for the evaluation of myocardial fibrosis and its response to intervention. Although noninvasive modalities, including echocardiography and CMR, provide scope for assessment of the degree of cardiac fibrosis, each approach is accompanied by method-specific limitations. These include loading state sensitivity of echocardiographic parameters and general accessibility of CMR systems with the capacity for quantitative measurement of diffuse cardiac fibrosis. Moreover, it is likely that in response to antifibrotic therapies, cardiac imaging-based parameters would be modified gradually, whereas sensitive biochemical markers may respond rapidly.

Limitations

LGE is a well-validated technique for identifying regional myocardial fibrosis; however, different cut-off values for signal intensity have been investigated. In the present study, a 6 SD threshold was used.²⁰ Various approaches to T₁ mapping also exist, and each has its own strengths and weaknesses; however, no single method has been shown to be preferable in clinical practice. Blood sampling in our study occurred only once per patient, and the effect of fluctuations in physiological stress on myocardial collagen metabolism was not assessed. Serial blood sampling, both at rest and with exercise, may provide further information about variations in myocardial ECM metabolic activity. Further studies of patients with more myocardial fibrosis, worse symptoms, and higher intracardiac pressures compared with those in our study cohort would also be of interest. Peripheral concentrations of biomarkers related to collagen biosynthesis and degradation may be influenced by a variety of noncardiac conditions, including pulmonary, renal, and bone diseases, and certain medications. Subjects with these medical conditions were excluded from our study; however, those with subclinical disease could not be completely excluded (although these are unlikely to impact on biomarker levels). Furthermore, we observed no significant differences in CMR, echocardiography, or collagen precursor (peripheral venous, arterial, or coronary sinus) data according to whether or not HCM patients received statin, ACE inhibitor, or ARB therapy. Because of the invasive nature of coronary sinus sampling, transcatheter gradients were calculated in only a relatively small number of patients, and as such, further validation in a larger cohort would be useful. Also, comparisons in the present study were not made with histopathologic findings because endomyocardial biopsy was not performed due to the significant procedural risks. In a previous study of hypertensive patients,³¹ plasma levels of C-terminal propeptide of procollagen I in peripheral venous and coronary sinus blood correlated with myocardial collagen content in endomyocardial biopsies. However, in those studies, true arteriovenous transcatheter concentration gradient was not measured.

Conclusions

In patients with HCM, peripheral levels of key ECM precursors, PINP or PIIINP, do not correlate with either

CMR-derived measures of myocardial fibrosis or echocardiographic indices of diastolic dysfunction. Additionally, a net myocardial concentration release of either procollagen propeptide was not observed in these patients. The relatively small contribution of myocardial collagen metabolism to the body's overall activity, increased collagen metabolism at noncardiac sites, and temporal variations in the synthesis of myocardial collagen may account for these findings. Regardless, peripheral levels of collagen biomarkers do not reliably reflect myocardial collagen metabolism or content in patients with HCM.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Myocardial fibrosis is a feature of cardiac failure, irrespective of its cause. In hypertrophic cardiomyopathy (HCM), a disease characterized by an inherited tendency to develop otherwise unexplained left ventricular hypertrophy, myocardial collagen synthesis may contribute to the development of diastolic heart failure and arrhythmia. Previous studies have suggested that peripheral levels of byproducts of collagen synthesis reflect the burden of fibrotic myocardium. However, such peripheral levels represent collagen synthesis occurring throughout the body. In this study, we performed simultaneous arterial and coronary sinus sampling to discover that the cardiac contribution to peripheral levels of byproducts of collagen synthesis in patients with HCM is actually insignificant. Furthermore, we performed cardiac magnetic resonance (CMR) imaging to noninvasively quantify myocardial fibrosis and found that these peripheral levels do not accurately reflect actual myocardial collagen content in HCM patients. The small contribution of myocardial collagen metabolism to the body's total activity and temporal variations in the synthesis of myocardial collagen may account for these findings. Regardless, peripheral levels of collagen biomarkers do not reliably reflect myocardial collagen metabolism or content in patients with HCM. Accordingly, studies directed at exploring the potential effects of antifibrotic agents in HCM will require CMR or invasive transcardiac blood sampling for validation of effects.