Title Page

Full Title

Evaluating the utility of circulating biomarkers of collagen synthesis in hypertrophic cardiomyopathy.

Short Title

Ellims. Biomarkers of collagen synthesis in HCM.

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Total Word Count

5185

Journal Subject Codes

[16] myocardial cardiomyopathy disease; [30] CT and MRI; [31] echocardiography; [33] other diagnostic testing.

Abstract

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 twenty-five healthy control subjects underwent peripheral venous sampling to determine the plasma concentrations of key collagen precursors (procollagen I and III N-terminal propeptides [PINP, PIIINP]). Contrast-enhanced cardiac magnetic resonance (CMR) imaging was performed to quantify regional (by late gadolinium enhancement [LGE]) 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 [ICTP]) and right heart catheterization. Despite CMR evidence of regional (LGE quantity: 6.4±8.0%) and diffuse (T_1 time: 478±79 ms) myocardial fibrosis in HCM patients, peripheral levels of collagen precursors were similar compared to control subjects (PINP 45.9±22.9 μg/L vs. 53.4±25.9 μg/L, p=0.21; PIIINP 4.8±1.7 μg/L vs. 4.4±1.1 μg/L, p=0.26). While PINP levels correlated with intra-cardiac pressures, no significant net positive transcardiac concentration gradient was detected for any 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.

Key Words

Hypertrophic cardiomyopathy; collagen; myocardial fibrosis; cardiac magnetic resonance imaging.

Introduction

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], whilst also providing a potential substrate for the development of ventricular arrhythmias.

In patients with LVH, increased levels of peripheral byproducts of collagen metabolism, such as procollagen I N-terminal propeptide (PINP) and procollagen III N-terminal propeptide (PIIINP), have been assumed to directly reflect expansion of the myocardial ECM. For example, in hypertensive patients, procollagen propeptide concentrations have been found to be significantly higher than in normotensives, and correlated with left ventricular (LV) mass, diastolic impairment and ventricular arrhythmias[8]. Additionally, treatment with an angiotensin-converting enzyme inhibitor reduced procollagen propeptide concentrations[9] and reversed the structural and physiologic cardiac changes induced by hypertension[8]. In HCM patients 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 than 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 the advanced tissue characterization capabilities of cardiac magnetic resonance (CMR) imaging 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, post-contrast T_1 mapping, a technique shown to correlate with histopathologic findings[14], has been used to quantify diffuse patterns of interstitial myocardial fibrosis in a number of cardiac disease states[15-17], including HCM[18], and 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 the further evaluation of HCM, and twenty-five asymptomatic healthy control subjects without a history of cardiovascular disease were invited to participate. HCM patients had asymmetric septal hypertrophy (interventricular septal thickness ≥ 15 mm with a ratio of septal-to-lateral ventricular wall thickness of ≥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 s less than the lower limit of normal; contraindications to CMR, including pacemaker and defibrillator implantation; and significant renal dysfunction (estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2).

Informed consent was obtained from all participants and the study protocol conformed to the ethical guidelines of the 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, Wisconsin, USA). All sequences were acquired during a breathhold of 10–15 s. 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 LV using the summation of disc method.

Late gadolinium enhancement (LGE) was evaluated 10 min after a bolus of gadolinium-diethylene triamine penta-acetic acid (DTPA) (0.2 mmol/kg BW 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) (see Figure 1). The TI optimization sequence was performed 8 min post-gadolinium administration and was a fast gradient echo, inversion recovery, gated, multi-phase acquisition, commencing at an inversion time of 150 ms and increasing in 25 ms increments to 250 ms, in a single mid-ventricular short-axis slice. A visual determination of the optimum TI to null the myocardial signal was then made. LGE imaging was performed using standard long-axis views of the LV 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 post-contrast 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 post-contrast T_1 mapping sequence[21] was used to cycle through acquisition of images obtained at three standard LV short-axis levels (basal, mid and apex) over a range of inversion times, as described previously[18]. This electrocardiogram-triggered, inversion-recovery prepared, 2-dimensional fast gradient echo sequence employed variable temporal sampling of k-space (VAST)[22] (GE Healthcare). Ten images at the basal, mid and apical LV short-axis levels were acquired sequentially at increasing inversion times, commencing 20 min after the bolus of

gadolinium-DTPA (TI range 75 to 750 ms), and each over a series of 3 to 5 breath-holds. Following image acquisition, the three 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 (ROI) was drawn around the entire LV myocardium (excluding regions of LGE) to calculate post-contrast myocardial T_1 time (see Figure 1). A global post-contrast myocardial T_1 time was derived by calculating the mean T_1 time of all three short-axis levels. To account for the potential effects of renal function and duration of time between contrast administration and image acquisition on gadolinium kinetics, correction values[23] were used to normalize post-contrast myocardial T_1 times to a matched state (eGFR = 90 mL/min/1.73m^2, time from contrast administration to image acquisition = 20 min) for all three 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 the septal and lateral aspects of the 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 two experienced readers unaware of the subjects' clinical information and the 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 ten patients with HCM and nine healthy control subjects 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 (PCWP), 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. The wedge position was confirmed fluoroscopically and by the profile of the accompanying pressure waveform and the mean PCWP was recorded at end-expiration. Cardiac output was measured using the thermodilution technique. A 6 F coronary sinus catheter was next advanced to the coronary sinus for blood sampling. The tip of the catheter was positioned at least 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 ethylene diamine tetra-acetate (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, USA). For the subgroup of nineteen 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

the plasma concentration in the coronary sinus and that in the arterial sample. All analyses were conducted by researchers unaware of the clinical status of the study subjects.

Statistical methods

All data are expressed as mean ± standard deviation (SD) unless otherwise indicated. Comparison between groups utilized the two independent samples t-test (normally distributed data), the Wilcoxon-Mann-Whitney test (data with skewed distribution) or the Fisher's exact test (categorical data) as appropriate. Comparisons within groups were made using the unpaired t-test. Pearson or Spearman correlation coefficients between parameters of interest were calculated as appropriate. For comparisons across multiple groups, one-way analysis of variance with Bonferroni correction was used for parametric variables. Analysis of covariance was performed to examine between group differences in the presence of potential confounding covariates. For all comparisons, a p value of < 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, Texas) and SPSS version 17 (SPSS Corp, Chicago, Illinois)

Results

Clinical and demographic data

Seventy-five patients were evaluated during the study period, comprising fifty patients with HCM and twenty-five healthy controls subjects. Baseline characteristics are presented in Table 1. Mean duration from the initial diagnosis of HCM to study involvement was 6.4 ± 7.6 years. HCM patients had a similar age, heart rate, systolic blood pressure and haematocrit compared to controls, but higher body mass index (BMI) and lower eGFR. Nine patients with HCM had treated systemic hypertension and the degree of their LVH was not explained by hypertension alone. Over half (56%) of the HCM group experienced dyspnea, but none worse than New York Heart Association class II severity, and most patients with HCM (76%) were receiving beta-blocker and/or calcium channel blocker therapy.

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 (LVEF) and indexed LV mass than the control group. All HCM patients had preserved LV systolic function (ie. LVEF > 55%). LGE was identified in 84% of HCM patients (most commonly located within the interventricular septum and/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 forty-seven patients). T_1 mapping was successfully performed in all but one subject, a patient with HCM and frequent ventricular ectopy. Post-contrast myocardial T_1 time was significantly lower in the HCM group (478 ± 79 ms vs. 545 ± 49 ms, $p < 0.001$), indicative of more diffuse myocardial fibrosis. Post-contrast T_1 time of the LV 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 mmHg at rest or with Valsalva, consistent with obstructive HCM, was observed in twenty-two (44%) patients with HCM. There were no significant differences between peripheral venous levels of PINP (45.9 ± 22.9 μg/L vs. 53.4 ± 25.9 μg/L, p = 0.21) or PIIINP (4.8 ± 1.7 μg/L vs. 4.4 ± 1.1 μg/L, p = 0.26).

Peripheral venous collagen precursor levels according to extent of myocardial fibrosis in HCM group

Peripheral venous levels of PINP and PIIINP in HCM patients according to LGE quantity and post-contrast myocardial T_1 time are detailed in Table 3. We observed no significant increases in levels of either PINP or PIIINP despite CMR evidence of increasing LGE. Patients with the highest post-contrast myocardial T_1 times (> 550 ms), suggestive of the least amount of diffuse myocardial fibrosis, actually had the highest PINP levels. Again, levels of PINP and PIIINP did not increase as post-contrast myocardial T_1 time fell.

Correlates of peripheral venous levels of PINP and PIIINP

In patients with HCM, apart from a modest inverse correlation between the peripheral venous level of PINP and patient age ($r = -0.37$, $p < 0.01$), there were no significant correlations observed between collagen precursors and baseline patient characteristics, CMR data or echocardiographic parameters (see Table 4). 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. A modest inverse correlation between PINP and BMI was observed in healthy control subjects ($r = -0.42$, $p = 0.04$), but not in patients with HCM ($r = -0.21$, $p = 0.14$). To adjust for the potential confounding effects of differing BMI, we conducted an analysis of covariance which again confirmed a lack of difference in PINP levels by group ($F = 0.01$, $p = 0.93$).

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 5. 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 any collagen precursor (see Figure 2). As shown in Table 5, arterial levels of ICTP were also similar in both groups, however coronary sinus levels in HCM patients were significantly higher, resulting in a significant positive transcardiac gradient. There were no significant differences between the HCM and control groups with respect to intra-cardiac pressures measured by right heart catheterization. Specifically, right atrial pressure (4.9 ± 2.8 mmHg vs. 5.9 ± 3.0 mmHg, $p = 0.5$), mean pulmonary artery pressure (18.9 ± 4.8 mmHg vs. 16.8 ± 3.6 mmHg, $p = 0.3$), and PCWP (12.0 ± 3.4 mmHg vs. 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 L/min vs. 6.8 ± 1.6 L/min, $p = 0.07$). Arterial and coronary sinus levels of PINP correlated with right atrial pressure (arterial, $r = 0.48$, $p = 0.04$; coronary sinus, $r = 0.51$, $p = 0.03$) and PCWP (arterial, $r = 0.50$, $p = 0.03$; coronary sinus, $r = 0.49$, $p = 0.04$). Peripheral levels of PIIINP and ICTP showed no correlation with any hemodynamic parameter, nor did transcardiac gradients for any collagen biomarker.

Discussion

In this study, we sought to measure the 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 to 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. Plasma levels, but not the transcardiac gradient, of PINP positively correlated with invasively determined intra-cardiac pressures.

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 al concluded that while disarray was directly linked to sarcomeric protein mutations, fibrosis was more likely secondary to other factors, such as LV mass and local mediators[3]. Despite subsequent studies of the 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 and resultant ischemia[26]; and activation of the transforming growth factor beta (TGF-β) profibrotic signalling pathway by abnormal sarcomeric gene protein mutations[27]. With the advent of rapid next-generation genetic sequencing and the development of techniques to non-invasively evaluate myocardial fibrosis, 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 localise 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 and/or PIIINP. We did not observe any such differences between our groups despite HCM patients having an indexed LV mass more than 70% greater than 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 the transcardiac 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. Firstly, the magnitude of collagen metabolism occurring within the myocardium is likely small compared to the total amount within the body and, thus, the myocardial contribution to peripheral levels is also likely to be minor. Secondly, 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 pressures, and vice-versa.

Despite the absence of a net myocardial concentration gradient for any collagen precursor, there were significant correlations between the plasma levels of PINP and invasively determined hemodynamic parameters. Arterial and coronary sinus levels of PINP positively

correlated with both right atrial pressure and PCWP. These findings suggest that, while transcardiac gradients of PINP and PIIINP were not apparent, a link between collagen synthesis and myocardial stiffness in these patients exists, probably reflecting underlying pulmonary release in the setting of elevated pulmonary artery or venous pressures.

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 metalloproteinase 2 (MMP-2), active MMP-9 and total tissue inhibitor of metalloproteinase 1 (TIMP-1) compared to healthy controls. Similarly to the present study, there was no significant difference in PINP levels between the groups. Lombardi et al employed echocardiography-derived LV diastolic dimensions, the 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, nor with echocardiographic indices of LV diastolic dysfunction (including E/e'[29]). Taken together we suggest that the associations observed between PINP and PIIINP levels and invasively determined or 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 al found higher peripheral levels of the C-terminal propeptide of procollagen I (PICP) in patients with HCM compared to healthy controls and concluded that type I collagen synthesis was increased within myocardial tissue in HCM[12]. However, given the samples were obtained from a peripheral vein, it is not possible

necessarily 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 levels of the 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. Whilst non-invasive 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 the loading state sensitivity of echocardiographic parameters and the general accessibility of CMR systems with capacity for quantitative measurement of diffuse cardiac fibrosis. Moreover, it is likely that in response to anti-fibrotic therapies, cardiac imaging-based parameters would be modified gradually whilst 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 utilized[20]. Varying approaches to T_1 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 physiologic 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 intra-cardiac pressures compared to 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 non-cardiac conditions, including pulmonary, renal and bone diseases. Subjects with these conditions were excluded from our study, however those with subclinical disease could not be completely excluded although these are unlikely to impact upon biomarker levels. Due to the invasive nature of coronary sinus sampling, transcardiac 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 as endomyocardial biopsy was not performed due to the significant procedural risks. In a previous study of hypertensive patients[31], plasma levels of PICP in peripheral venous and coronary sinus blood correlated with myocardial collagen content in endomyocardial biopsies. However in those studies the true arteriovenous transcardiac 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. Levels of PINP, however, did correlate with invasively determined intra-cardiac and, thereby, pulmonary vascular pressures. The relatively small contribution of myocardial collagen metabolism to the body's overall activity, increased collagen metabolism at non-cardiac 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.

Acknowledgements

We thank Donna Vizi, Jenny Starr, Sofie Karapanagiotidis, and Liz Dewar for their technical assistance in coronary sinus sampling, echocardiographic imaging, and blood sample processing.

Sources of Funding

The study was supported by grants from the National Health and Medical Research Council, Australia (AHE, LMI, AJT, DMK), National Heart Foundation, Australia (AHE, AJT, LMI), and Swiss National Science Foundation, Switzerland (MTM). Baker IDI Heart and Diabetes Institute is supported by a Victorian Government Operational Infrastructure Support grant.

Disclosures

None

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Figure Legends

Figure 1. Evaluation of regional and diffuse myocardial fibrosis by contrast-enhanced cardiac magnetic resonance (CMR) imaging in hypertrophic cardiomyopathy (HCM).

(A) Ventricular short-axis late gadolinium enhancement (LGE) image of a patient with HCM. Regional myocardial fibrosis (hyperintense areas, *circled*) is located within the left ventricular (LV) 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 post-contrast myocardial T_1 time excludes an area of LGE.

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 control subjects. There were no significant differences in procollagen propeptide levels between the two groups, and no significant net transcardiac gradients.

Tables

Table 1. Baseline characteristics

	HCM (n=50)	Control (n=25)	p value
Age, y	48 ± 13	47 ± 17	0.8
Males, 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 (%)			
Beta-blocker	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/1.73 m2	79 ± 12	86 ± 8	0.01

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

Table 2. Peripheral venous collagen precursors, CMR and echocardiography data

	HCM (n=50)	Control (n=25)	p value
Peripheral Venous Collagen Precursor Levels			
PINP, μg/L	45.9 ± 22.9	53.4 ± 25.9	0.21
PIIINP, μg/L	4.8 ± 1.7	4.4 ± 1.1	0.26
CMR Data			
LV end-diastolic volume indexed, ml/BSA	81 ± 14	83 ± 13	0.4
LV ejection fraction, %	69 ± 7	60 ± 6	< 0.01
LV mass indexed, g/BSA	89 ± 24	52 ± 9	< 0.01
Maximum LV wall thickness, mm	20 ± 3	8 ± 2	< 0.01
LGE			
Presence, n (%)	42 (84%)	0 (0%)	< 0.01
Quantity, % of LV mass	6.4 ± 8.0	0 (0%)	< 0.01
Distribution, n (%)			
RV insertion point(s)	28 (67%)	-	
Interventricular septum	26 (62%)	-	
LV apex	1 (2%)	-	
Other LV site	10 (24%)	-	
Post-contrast T_1 time, ms			
LV myocardium	478 ± 79	545 ± 49	< 0.001
LV blood pool	303 ± 31	306 ± 22	0.7
Echocardiography Data			
Left atrial volume indexed, ml/m^2	52 ± 18	32 ± 9	< 0.001
Peak LVOT gradient, mm Hg	41 ± 47	4 ± 1	< 0.001

e', cm/s	0.07 ± 0.02	0.10 ± 0.03	< 0.001
E/e' ratio	12.6 ± 4.9	7.7 ± 2.4	< 0.001

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

Table 3. Peripheral venous collagen precursors and extent of myocardial fibrosis in HCM group

	n	PINP	p	PIIINP	p
LGE quantity, % of LV mass					
0%	9	40.9 ± 13.2	} ns	4.6 ± 1.1	} ns
> 0% and ≤ 5%	20	56.4 ± 29.5	} ns	5.0 ± 2.2	} ns
> 5% and ≤ 10%	8	37.9 ± 17.3	} ns	4.7 ± 1.0	} ns
> 10% and ≤ 20%	6	38.7 ± 10.5	} ns	4.8 ± 2.4	} ns
> 20%	4	36.3 ± 10.2		4.5 ± 0.8	
Post-contrast myocardial T_1 time, ms					
> 550	7	66.4 ± 38.5	} < 0.05	5.1 ± 1.7	} ns
> 475 and ≤ 550	14	38.2 ± 13.2	} ns	4.3 ± 1.0	} ns
> 400 and ≤ 475	22	46.5 ± 20.5	} ns	5.0 ± 2.2	} ns
≤ 400	6	41.5 ± 16.3		5.0 ± 0.5	

HCM indicates hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LV, left ventricular; PINP, procollagen I N-terminal propeptide; PIIINP, procollagen III N-terminal propeptide; and ns, non-significant.

Table 4. Correlates of peripheral levels of PINP and PIIINP in HCM patients

	PINP	PIIINP
Age	-0.37**	-0.10
Body mass index	-0.21	0.08
Heart rate	0.06	0.08
Systolic blood pressure	-0.16	-0.18
eGFR	0.13	0.25
LV end-diastolic volume indexed	0.03	0.10
LV ejection fraction	0.20	0.06
LV mass indexed	0.01	0.05
Maximum LV wall thickness	0.18	0.18
Quantity of LGE	-0.22	-0.07
Post-contrast myocardial T_1 time	0.17	-0.03
Left atrial volume indexed	-0.10	-0.20
Peak LVOT gradient	-0.10	-0.06
e’	0.09	-0.11
E/e’	-0.06	0.04

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

*$p < 0.05$; **$p < 0.01$.

Table 5. Arterial and coronary sinus levels, and transcardiac gradients of collagen biomarkers

	HCM (n = 10)	Control (n = 9)	p value
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
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
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
Transcardiac gradient	1.0 ± 1.0	-0.04 ± 0.3	< 0.01

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

Figure 1

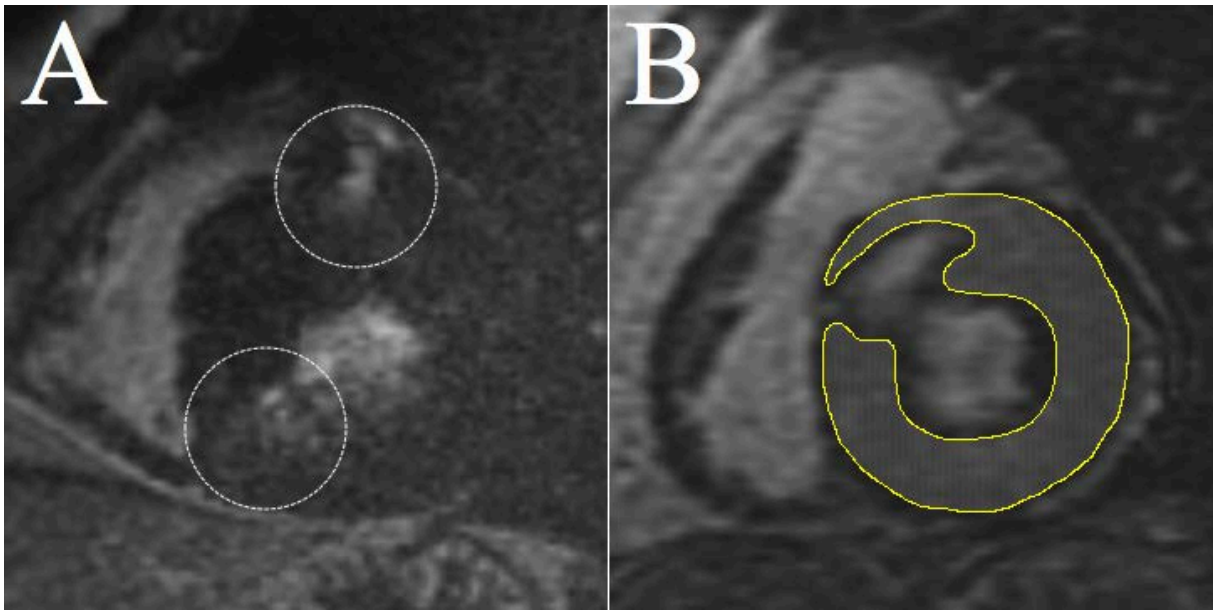
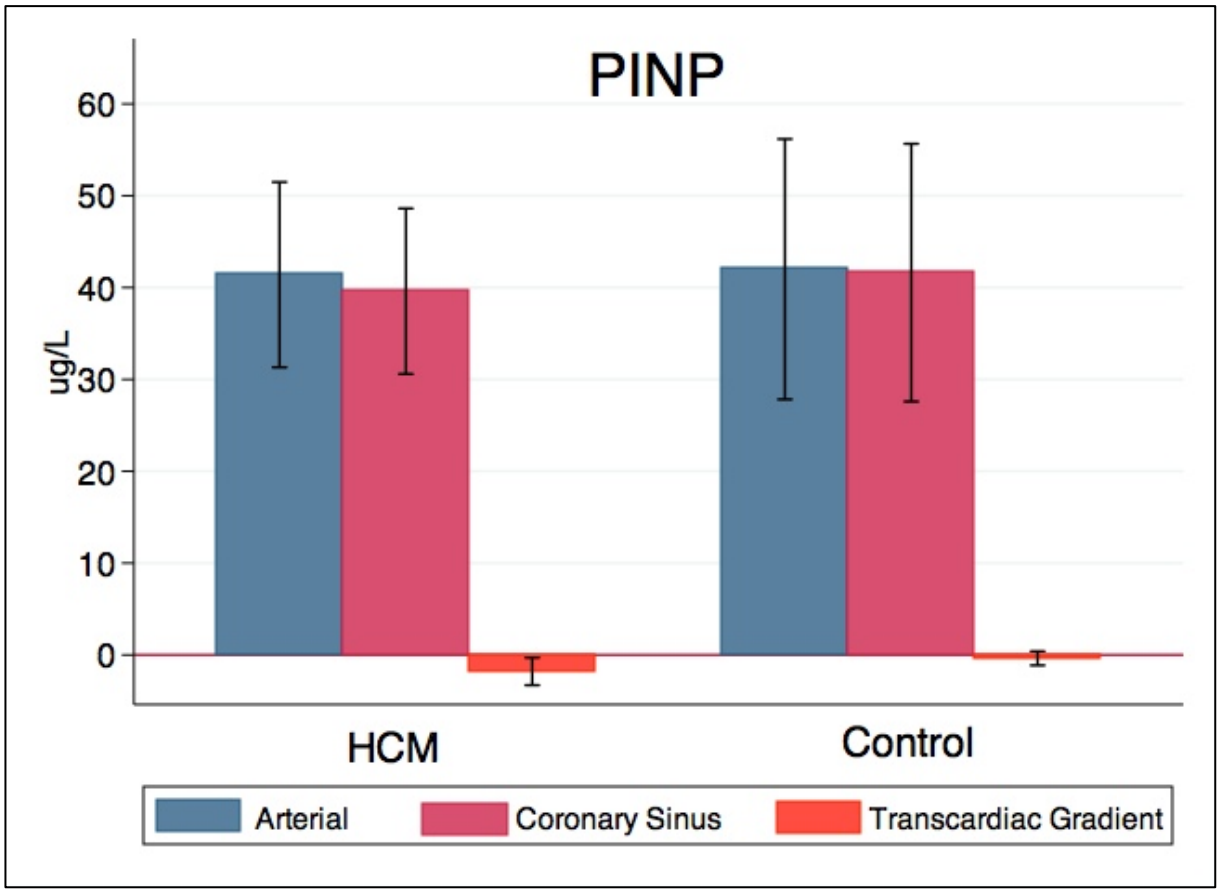


Figure 2A


PINP
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40
30
20
10
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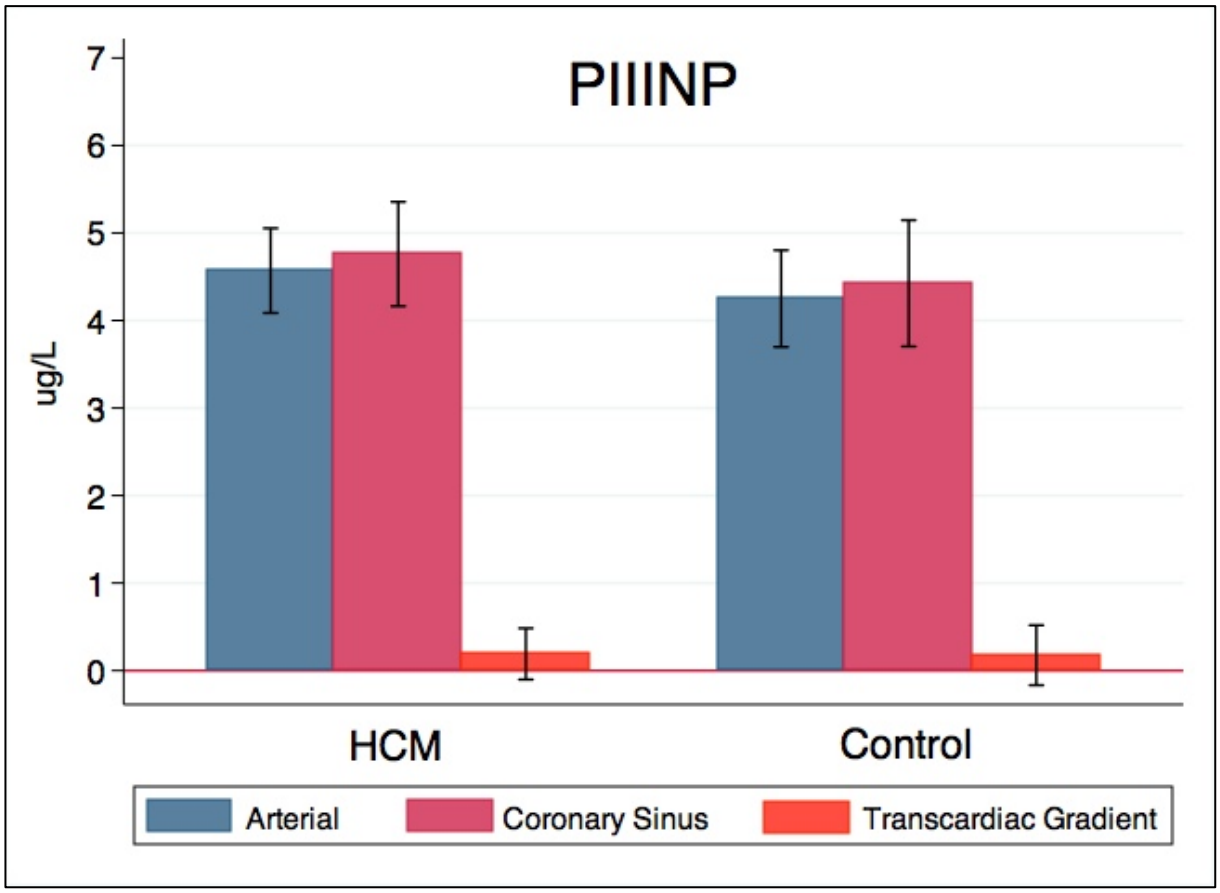
ug/L
HCM
Control
Arterial
Coronary Sinus
Transcardiac Gradient

Figure 2B


PIIINP
ug/L
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HCM
Control
Arterial
Coronary Sinus
Transcardiac Gradient