Myocardial fibrosis as a therapeutic target

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Disclosures

• Research support from a) the American Heart Association and b) The Pittsburgh Foundation
• Current Scientific Advisory Board for Haya Therapeutics
• Consultant for PureTech LYT 100
Background: Several knowledge gaps in cardiology

- Heart Failure epidemic...
  - Increasing in prevalence
  - Costly
  - Incompletely understood
  - Not just sequelae of CAD with huge infarcts
  - “Conceptual homogenization of myocardium”—rather than considering components separately:
    - cardiomyocyte
    - Interstitium
    - microvasculature

- What is vulnerable remodeling...?
  - Among myriad changes in myocardium, what are the key components that really matter?
  - What are the causes and what are the effects?

Focus on Myocardial fibrosis

- Consider: the heart may be like other organs:
  - Lung $\rightarrow$ pulmonary fibrosis,
  - Liver $\rightarrow$ cirrhosis,
  - Kidney $\rightarrow$ glomerular fibrosis

- where disruption of its architecture through interstitial expansion leads to organ dysfunction and vulnerability to the patient

- "Amyloid-light"
Leveraging over a decade of CMR investigation
Association of myocardial fibrosis with outcomes → vulnerability

Apt Quote

When you can measure what you are speaking about and summarize it in numbers, you know something about it.

And when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind. It may be the beginning of knowledge, but you have scarcely in your thought advanced to the stage of science, whatever the matter may be.

—Lord Kelvin, Popular Lectures and Addresses Vol 1 (1889)
Electrical Units of measurement delivered 3 May 1883.
WE now have the tools to measure and follow myocardial fibrosis (and amyloidosis)

ECV

ExtraCellular Volume

Severe diffuse interstitial fibrosis

Normal

LGE misses the severe diffuse myocardial fibrosis

Schelbert EB, Fonarow GC, Bonow RO, Butler J, Gheorghiade M. JACC 2014
Conclusions

- Interstitial expansion from myocardial fibrosis likely causes:
  - Mechanical dysfunction (i.e., diastolic)
    - Systolic function and ECV are mostly independent
  - Microvascular dysfunction (↓ perfusion reserve, capillary rarefaction)
  - Electrical dysfunction (reentry)
  and the increases risks of death, hospitalization for HF, arrhythmia

- Similar strength of association with adverse outcomes between ECV and EF and/or GLS → Myocardial fibrosis likely causal

- CMR (and CCT) measure interstitial expansion with ECV reliably

- Anti-fibrotic Rx under development promise to reverse cardiac dysfunction & improve outcomes.

- ECV is critical for serial monitoring of disease progression / regression

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Key outcomes data → Conceptual model
A new taxonomy to conceptualize vulnerability related to myocardial disease
Potential for therapy to REVERSE myocardial fibrosis

- Renin angiotensin aldosterone inhibitors
- Other agents in development


A new age for understanding the role of the cardiac interstitium

- more than 30 phase 2 trials and observational ongoing studies leveraging change in ECV as an endpoint

The story starts with histopathology:

ECM expansion from diffuse fibrosis seems ubiquitous in diseased myocardium at autopsy

> distortion of micro-architecture

Nonischemic dilated cardiomyopathy

Control  DCM  DCM

Are energetics normal here??

Beltrami C, et al. J Mol Cell Cardiol 1995
Remote, non-infarcted myocardium in Ischemic CM


Are energetics normal here??

Remote, non-infarcted myocardium

15

16
Normal myocardium

Mild LVH from hypertensive heart disease

Rossi M et al. J Hypertens 1998
Worse LVH from hypertensive heart disease

Severe LVH from hypertensive heart disease

Rossi M et al. J Hypertens 1998
“Cardiac/myocardial cirrhosis” in hypertensive diabetic CM
van Hoeven and Factor, Circulation 1990

Mechanisms for myocardial fibrosis to cause vulnerability

- Capillary rarefaction and perivascular fibrosis that limit perfusion reserve


Mechanisms for myocardial fibrosis to cause vulnerability

- Increased space between the capillary and collagen-encircled cardiomyocyte, increasing the oxygen diffusion distance and rendering the cardiomyocyte prone to hypoxia, an important trigger of cardiomyocyte-programmed cell death or apoptosis, potentially promoting progressive MF


Mechanisms for myocardial fibrosis to cause vulnerability

- Myocardial stiffening from titin and collagen expansion with increased cross-linking in MF that leads to systolic and especially **diastolic dysfunction** and increased filling pressures


Mechanisms for myocardial fibrosis to cause vulnerability

• Impaired electric conduction from disarray in the collagen network architecture that predisposes to reentrant arrhythmia and sudden death


J.M. McLenachan, H.J. Dargie. Ventricular arrhythmias in hypertensive left ventricular hypertrophy. Relationship to coronary artery disease, left ventricular dysfunction, and myocardial fibrosis. Am J Hypertens, 3 (1990), pp. 735-740


• Final culmination of all of these insults:

• Likely impaired cardiomyocyte/mitochondrial energetics if interposing excess collagen isolates cardiomyocytes from capillaries in the setting of decreased perfusion reserve, arrhythmia, and myocardial stiffening, culminating in an engine out of fuel.
4 human histology studies showing fibrosis is reversible:
Lisinopril-Mediated Regression of Myocardial Fibrosis in Patients With Hypertensive Heart Disease

Christian G. Brilla, MD, PhD; Reinhard C. Funck, MD; Heinz Rupp, PhD

Background—In arterial hypertension, left ventricular hypertrophy (LVH) includes myocyte hypertrophy and fibrosis, which leads to LV diastolic dysfunction and, finally, heart failure. In spontaneously hypertensive rats, myocardial fibrosis was regress and LV diastolic function was improved by treatment with the angiotensin-converting enzyme inhibitor lisinopril. Whether this holds true for patients with hypertensive heart disease was addressed in this prospective, randomized, double-blind trial.

Methods and Results—A total of 35 patients with primary hypertension, LVH, and LV diastolic dysfunction were treated with either lisinopril (n=18) or hydrochlorothiazide (HCTZ; n=17). At baseline and after 6 months, LV catheterization with endomyocardial biopsy, Doppler echocardiography with measurements of LV peak flow velocities during early filling and atrial contraction and isovolumic relaxation time, and 24-hour blood pressure monitoring were performed. Myocardial fibrosis was measured by LV collagen volume fraction and myocardial hydroxyproline concentration. With lisinopril, collagen volume fraction decreased from 6.9±0.6% to 6.3±0.6% (P<0.05 versus HCTZ) and myocardial hydroxyproline concentration from 9.9±0.3 to 8.3±0.4 μg/mg of LV dry weight (P<0.0001 versus HCTZ); this was associated with an increase in the early filling and atrial contraction LV peak flow velocity ratio from 0.72±0.04 to 0.91±0.06 (P<0.05 versus HCTZ) and a decrease in isovolumic relaxation time from 123±7 to 81±5 ms (P<0.0002 versus HCTZ). Normalized blood pressure did not significantly change in either group. No LVH regression occurred in lisinopril-treated patients, whereas with HCTZ, myocyte diameter was reduced from 22.1±0.6 to 20.7±0.7 μm (P<0.01 versus lisinopril).

Conclusions—In patients with hypertensive heart disease, angiotensin-converting enzyme inhibition with lisinopril can regress myocardial fibrosis, irrespective of LVH regression, and it is accompanied by improved LV diastolic function. (Circulation. 2008;112:1388-1393.)

Losartan-Dependent Regression of Myocardial Fibrosis Is Associated With Reduction of Left Ventricular Chamber Stiffness in Hypertensive Patients

Javier Diez, MD, PhD; Ramón Querejeta, MD, PhD; Begoña López, BSc; Arantxa González, BSc; Mariano Larman, MD; Jose L. Martinez Ubago, MD

Background—This study was designed to investigate whether myocardial collagen content is related to myocardial stiffness in patients with essential hypertension.

Methods and Results—The study was performed in 34 patients with hypertensive heart disease. Nineteen of these patients were also evaluated after 12 months of treatment with losartan. Transthoracic endomyocardial biopsies of the interventricular septum were performed to quantify collagen volume fraction (CVF). Left ventricular (LV) chamber stiffness (KLV) was determined from the deceleration time of the early mitral filling wave as measured by Doppler echocardiography. Histological analysis at baseline revealed the presence of 2 subgroups of patients: 8 with severe fibrosis and 26 with nonsevere fibrosis. Values of CVF and KLV were significantly higher in the 2 subgroups of hypertensives than in normotensives. In addition, compared with patients with nonsevere fibrosis, patients with severe fibrosis exhibited significantly increased values of CVF and KLV. After treatment, CVF and KLV decreased significantly in patients with severe fibrosis (n=7). None of these parameters changed significantly after treatment in patients with nonsevere fibrosis (n=12). CVF was directly correlated with KLV (r=0.415, P<0.02) in all hypertensives.

Conclusions—These findings show a strong association between myocardial collagen content and LV chamber stiffness in patients with essential hypertension. Our results also suggest that the ability of losartan to induce regression of severe myocardial fibrosis is associated with diminution of myocardial stiffness in hypertensive patients. (Circulation. 2002; 105:2512-2517.)
Mineralocorticoid Receptor Antagonism Ameliorates Left Ventricular Diastolic Dysfunction and Myocardial Fibrosis in Mildly Symptomatic Patients With Idiopathic Dilated Cardiomyopathy
A Pilot Study
Hideo Iizuka, MD, PhD; Toshiyuki Murahara, MD, PhD; Kotetsu Nagata, MD, PhD; Satoshi Ito, MD, PhD; Hirofumi Asano, MD, Tetsuya Arano, MD, PhD; Saboko Ishihara, MD, PhD; Tomoko Kato, MD, PhD; Satoru Ohtsuka, MD; Yosuke Murase, MD, Shigeo Iino, MD, PhD; Koichi Obata, PhD; Akiko Noda, PhD; Kenji Okamura, MD, PhD; Mitsuhiro Yokota, MD, PhD

Background—Mineralocorticoid receptor antagonism reduces mortality associated with heart failure by mechanisms that remain unclear. The effects of the mineralocorticoid receptor antagonist spironolactone on left ventricular (LV) function and chamber stiffness associated with myocardial fibrosis were investigated in mildly symptomatic patients with idiopathic dilated cardiomyopathy (DCM).

Methods and Results—Twenty-five DCM patients with a New York Heart Association functional class of II or III were examined before and after treatment with spironolactone for 12 months. LV pressures and volumes were measured simultaneously, and LV endocardial biopsy specimens were obtained. Serum concentrations of the carboxy-terminal propeptide (P1P) and carboxy-terminal telopeptide (CTP) collagen type I were measured. The patients were divided into 2 groups on the basis of the serum PIP/CITP ratio (group A, n = 12; group B, n = 13). The index of myocardial collagen accumulation, LV diastolic stiffness, the collagen volume fraction, and abundance of collagen type I and III mRNA in biopsy tissue were greater and the LV early diastolic strain rate (tissue Doppler echocardiography) was smaller in group B than in group A at baseline. These differences and the difference in PIP/CITP were greatly reduced after treatment in patients in group B with spironolactone, with treatment having no effect on these parameters in group A. The collagen volume fraction was significantly correlated with PIP/CITP, LV early diastolic strain rate, and LV diastolic chamber stiffness for all patients before and after treatment with spironolactone.

Conclusions—Spironolactone ameliorated LV diastolic dysfunction and reduced chamber stiffness in association with myocardial fibrosis in mildly symptomatic patients with DCM. These effects appeared limited, however, to patients with increased myocardial collagen accumulation. (Circulation. 2005;112:2940-2945.)

Repair of Coronary Arterioles After Treatment With Perindopril in Hypertensive Heart Disease
Bodo Schwartzkopff, Michael Brehm, Markus Mundhenke, Bodo E. Strauer

Abstract—In hypertensive heart disease, no data are available on the repair of coronary resistance vessels in patients after long-term ACE inhibitor treatment. Fourteen patients with essential hypertension were studied with coronary flow reserve and with transvenous endomyocardial biopsy before and after 12 months of antihypertensive treatment with perindopril (4 to 8 mg/d, mean 5.9 ± 2.3 mg/d). Left ventricular muscle mass index decreased by 11% (from 145 ± 41 to 128 ± 36 g/m², P = 0.04). Maximal coronary blood flow was increased by 54% (from 170 ± 46 to 263 ± 142 mL · min⁻¹ · 100 g⁻¹, P = 0.001), and minimal coronary vascular resistance was diminished by 33% (from 0.67 ± 0.21 to 0.45 ± 0.19 mm Hg · min⁻¹ · 100 g · mL⁻¹, P = 0.001); consequently, coronary reserve increased by 67% from 2.1 ± 0.6 to 3.5 ± 1.9 (P = 0.001). Structural analysis revealed regression of perivascular collagen area by 54% (from 558 ± 270 to 290 ± 173 µm², P = 0.04) and of total interstitial collagen volume density by 22% (from 5.3 ± 1.3 to 4.3 ± 2.2%, P = 0.04), whereas arterial wall area was slightly but not significantly reduced. Long-term therapy with the ACE inhibitor perindopril induces structural repair of coronary arterioles that is mainly characterized by the regression of perivascular fibrosis and associated with a marked improvement in coronary reserve. These findings indicate the beneficial reparative effects of ACE inhibition on coronary microcirculation in hypertensive heart disease. (Hypertension. 2000;36:220-226.)

Key Words: arterioles ■ collagen ■ hypertension, arterial ■ angiotensin-converting enzyme inhibitors ■ coronary reserve
But only very modest changes with renin-angiotensin-aldosterone inhibition, ~1% absolute Δ

Table 2. Studies Examining the Extent of Myocardial Fibrosis Reversal by Histological Measures in Human With Angiotensin-Converting Enzyme Inhibitors, Angiotensin Receptor Blockers, or Mineralocorticoid Receptor Antagonism

<table>
<thead>
<tr>
<th>Drug</th>
<th>Investigators</th>
<th>Disease</th>
<th>Duration, mo</th>
<th>Collagen Volume Fraction, % Change</th>
<th>Absolute Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spironolactone</td>
<td>Izawa et al</td>
<td>Dilated cardiomyopathy</td>
<td>12</td>
<td>4.7 → 3.4</td>
<td>≈28% ≈1.3%</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Brilla et al</td>
<td>Hypertensive heart disease</td>
<td>6</td>
<td>6.9 → 6.3</td>
<td>9% 0.6%</td>
</tr>
<tr>
<td>Perindopril</td>
<td>Schwartzkopff et al</td>
<td>Hypertensive heart disease</td>
<td>12</td>
<td>5.5 → 4.3</td>
<td>22% 1.2%</td>
</tr>
<tr>
<td>Losartan</td>
<td>Diez et al</td>
<td>Hypertensive heart disease</td>
<td>12</td>
<td>4.32 → 3.72</td>
<td>14% 0.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average: 10.5</td>
<td>Average: 18%</td>
</tr>
</tbody>
</table>

Erik B. Schelbert, Hani N. Sabbah, Javed Butler, and Mihai Gheorghiade.  
https://doi.org/10.1161/CIRCIMAGING.116.005619 Circulation: Cardiovascular Imaging, 2017;10:e005619

2 more interesting fibrosis studies in HF
HFpEF vs. HFrEF
...collagen volume fraction is equally elevated...

Myocardial Structure and Function Differ in Systolic and Diastolic Heart Failure

Lock van Heerebeek, MD; Attila Borbély, MD; Hans W.M. Niessen, MD, PhD; Jean G.F. bronwen, MD, PhD; Jolanda van der Velden, PhD; Ger J.M. Steine, PhD; Wolfgang A. Linke, PhD; Gerrit J. Laarman, MD, PhD; Walter J. Paulus, MD, PhD

Background—To support the clinical distinction between systolic heart failure (SHF) and diastolic heart failure (DHF), left ventricular (LV) myocardial structure and function were compared in LV endomyocardial biopsy samples of patients with systolic and diastolic heart failure.

Methods and Results—Patients hospitalized for worsening heart failure were classified as having SHF (n=22; LV ejection fraction (EF) 34±29%) or DHF (n=22; LVEF 62±29%). No patient had coronary artery disease or biopsy evidence of inflammatory or infectious myocardial disease. More DHF patients had a history of arterial hypertension and were obese. Biopsy samples were analyzed with histomorphometry and electron microscopy. Single cardiomyocytes were isolated from the samples, stretched to a sarcomere length of 2.2 μm to measure passive force (Fpassive), and activated with calcium-containing solutions to measure total force. Cardiomyocyte diameter was higher in DHF (20.3±0.6 μm versus 19.1±0.4 μm, P<0.001), but collagen volume fraction was equally elevated. Myofibrillar density was lower in SHF (36±2%) versus 44±2%, P<0.001). Cardiomyocytes of DHF patients had higher Fpassive (7.1±0.6 versus 5.3±0.3 kN/m²; P<0.001), but their total force was comparable. After administration of protein kinase A to the cardiomyocytes, the drop in Fpassive was larger (P<0.01) in DHF than in SHF.

Conclusions—LV myocardial structure and function differ in SHF and DHF because of distinct cardiomyocyte abnormalities. These findings support the clinical separation of heart failure patients into SHF and DHF phenotypes.

(Circulation. 2006;113:1966-1973.)

Degree of Cardiac Fibrosis and Hypertrophy at Time of Implantation Predicts Myocardial Improvement During Left Ventricular Assist Device Support

Brian A. Bruckner, MD,* Peter Razeghi, MD,* Sonny Stenson, BS,* Larry Thompson, MD,* Javier Latinao, MD,* Mark Emman, MD,* Matthias Loeser, MD, PhD,* George Noon, MD,* Heinrich Taegtmeyer, MD, PhD,* O. H. Frazier, MD,* and Keith Youker, PhD*

Background: There has been increasing reports of cardiac improvement in heart failure patients supported by left ventricular assist devices (LVADs) i.e., including a number of patients who have tolerated removal of the device without the benefit of cardiac transplant. In the current study, we retrospectively investigated echocardiographic and histologic changes in patients supported by LVADs (n=18). The goal of our study was to determine if the degree of cardiac fibrosis and myocyte size in pre-implant biopsies could predict myocardial improvement as assessed by improvements in ejection fraction (EF) during LVAD support.

Methods: We determined total collagen content in myocardial biopsy specimens by a semi-quantitative analysis of positive Picro-Sirius Red-stained areas and myocyte size measurements by computerized edge detection software.

Results: During LVAD support, 9 of the 18 patients (Group A) were distinguished by significant improvement in ejection fraction (pre = 20% vs. unloaded 34±5%). In addition, Group A patients had significantly less fibrosis and smaller myocytes than their Group B counterparts, whose EF did not improve. There was an inverse correlation between pre-implant biopsy collagen levels and myocyte size with increases in EF during LVAD unloading.

Conclusions: We found that the patients who demonstrated the greatest improvements in EF during support had less fibrosis and smaller myocytes at the time of device implantation. We propose that tissue profiling a patient’s pre-implant biopsy for fibrosis and myocyte size may allow stratification in Stage IV heart failure and may predict myocardial improvement during LVAD support. J Heart Lung Transplant 2006;25; 36–42.
Histology suggests adverse associations between myocardial fibrosis and morbidity and mortality

Myocardial fibrosis is clearly ubiquitous in diseased myocardium, regardless of ‘stimulus’ or etiology

How do leverage this information clinically??

→ ECV!

Signal intensity (magnetization) = 1-2\cdot e^{-\frac{\text{time}}{T1}}

= 1-2\cdot e^{-\text{time} \cdot R1}

CMR ECV requires T1 or R1 measurement…exponentiated time constant

Phase reconstruction

Time (msec)

Magnitude
“MOLLI”
developed by Daniel Messroghli
permits pixelwise T1 maps since component images after an RF inversion pulse are acquired at same point in the cardiac cycle


**ExtraCellular Volume fraction (ECV)** measures myocardial interstitial expansion

= myocardial Gd uptake relative to plasma (not whole blood measured from images)

Schelbert EB, Fonarow GC, Bonow RO, Butler J, Gheorghiade M. JACC 2014
Imaging the interstitial space with Extracellular Volume Fraction (ECV)


A 38 year old volunteer with sleep apnea, no cardiac symptoms, ejection fraction=62%

B 77 year old patient with heart failure, nonischemic dilated cardiomyopathy, ejection fraction=37%

C Abnormally bright pixels highlighted in pink from the LGE image in row B are limited to the inferior right ventricular insertion point with a "full-width, half-maximum" threshold

Abnormally bright pixels highlighted in pink from the LGE image in row B are limited to the inferior right ventricular insertion point with a "6 standard deviation" threshold
ECV is validated against collagen volume fraction in human myocardium (many centers, many papers)


Generally high R2 values, despite the potential for 1) spatial heterogeneity of myocardial fibrosis, and 2) destructive histologic processing to introduce error

1. R2 = 0.893
2. R2 = 0.796
3. R2 = 0.767
4. R2 = 0.72
5. R2 = 0.69
6. R2 = 0.685
7. R2 = 0.608
8. R2 = 0.56
Spatial variation plagues needle and endomyocardial biopsies

Diffuse fibrosis is not homogeneous (Coefficient of variation=SD/mean)

- Left ventricular endomyocardial catheter biopsies from 73 patients with idiopathic dilated cardiomyopathy
- The coefficient of variation (several biopsies from the same patient) was:
  - 6% for determination of fiber diameter,
  - 43% for interstitial fibrosis,
  - 3% for volume fraction of myofibrils.
- Sampling error is high for evaluation of fibrosis,
- A reduction in the volume fraction of myofibrils and an increase in fibrosis are morphologic correlates of left ventricular dysfunction in patients with idiopathic dilated cardiomyopathy.

Diffuse ≠ homogenous

Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions which we know as disease.

—Sir William Osler

Analogous to HCM, amyloid, etc.

ECV in the clinical setting

HFpEF
On multivariate linear regression analyses including ECV, E/E', and left atrial volume index as the noninvasive imaging parameters potentially informing on LV stiffness, ECV emerged as the only independent predictor for intrinsic LV stiffness ($\beta_{\text{standardized}} = 0.75; \beta_{\text{nonstandardized}} = 0.21; p < 0.01$).

*Echo probably too load dependent...*
ECV better agrees with EDPVR than echo indices (too load dependent)

- ECV was significantly higher in patients with HFpEF

- HFpEF patients had higher LV EDPs at baseline and during exercise as well as a more pronounced increase in EDPVR in response to physical exertion ($\Delta$ EDPVR)

- On multivariate linear regression analyses including ECV, E/E', and left atrial volume index predicting LV stiffness, ECV was the only independent predictor for intrinsic LV stiffness ($p < 0.01$)
Among myriad changes occurring during the apparent evolution of HFP EF where elevated BNP is prevalent, MF was similarly prevalent in those with or at risk for HFP EF.

Conceivably, MF might precede clinical HFP EF diagnosis.

Regardless, MF was associated with disease severity (ie, BNP) and outcomes.

Whether cells and secretomes mediating MF represent therapeutic targets in HFP EF warrants further evaluation.
Outcomes in HFpEF (TOPCAT-like definition)

ECV in the clinical setting

Aortic Stenosis
In patients with severe aortic stenosis scheduled for aortic valve intervention, an increased ECV% is a measure of left ventricular decompensation and a powerful independent predictor of mortality.

Similar ECV distributions across 13 centers, 440 pts not so for native T1 (all over the “map”)

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Conclusions:

- **Machine learning** identified myocardial fibrosis (ECV) and biventricular remodeling markers as the top predictors of survival in AS and highlighted their nonlinear association with mortality.
ECV in the clinical setting

Degenerative Chronic Mitral regurgitation

Valvular Heart Disease

Quantification of Left Ventricular Interstitial Fibrosis in Asymptomatic Chronic Primary Degenerative Mitral Regurgitation

Nicola C. Edwards, PhD; William E. Moody, MBChB; Mengshi Yuan, MBBS; Peter Weale, BSc; Desley Neal, MD; Jonathan N. Townend, MD; Richard P. Steeds, MA, MD

Background—The optimum timing of surgery in asymptomatic patients with chronic severe primary degenerative mitral regurgitation (MR) remains controversial, and further markers are needed to improve decision making. There are limited data that wall stress is increased in MR and may result in ventricular fibrosis. We investigated the hypothesis that chronic volume overload in MR is a stimulus for myocardial fibrosis using T1-mapping cardiac MRI.

Methods and Results—A cross-sectional study of 35 patients (age 60±14 years) with asymptomatic moderate and severe primary degenerative MR (mean effective regurgitant orifice area, 0.45±0.25 cm²; with no class I indication for surgery were compared with age and sex controls. Subjects were studied with cardiopulmonary exercise testing, echocardiography, and cardiac MRI. Longitudinal and circumferential myocardial deformation was reduced with MR when left ventricular ejection fraction (67±10%) and N-terminal pro B Natriuretic peptide (126 [76–428] ng/L) were within the normal range. Myocardial extracellular volume was increased (0.32±0.07 versus 0.25±0.02, P<0.01) and was associated with increased left ventricular end-systolic volume index (r=0.62, P<0.01), left atrial volume index (r=0.41, P<0.05) but lower left ventricular ejection fraction (r=−0.60, P<0.01), longitudinal function (mitral anular plane systolic excursion, r=−0.46, P<0.01), and peak VO₂ peak (r=−0.55, P<0.05). In a multivariable regression model, left ventricular end-systolic volume index and left atrial volume index were independent predictors of extracellular volume (r²=0.42, P<0.01).

Conclusions—Patients with asymptomatic MR demonstrate a spectrum of myocardial fibrosis associated with reduced myocardial deformation and reduced exercise capacity. Future work is warranted to investigate whether left ventricle fibrosis affects clinical outcomes. (Circ Cardiovasc Imaging; 2014;7:456–463.)

Key Words: magnetic resonance imaging • mitral valve regurgitation • myocardial fibrosis
ECV associates with various disease severity metrics in chronic Mitral Regurgitation

- ECV was increased (0.32±0.07 versus 0.25±0.02, P<0.01)
- ECV associated with:
  - increased left ventricular end-systolic volume index (r=0.62, P<0.01),
  - left atrial volume index (r=0.41, P<0.05)
  - lower left ventricular ejection fraction (r=−0.60, P<0.01),
  - longitudinal function (mitral annular plane systolic excursion, r=−0.46, P<0.01), and
  - peak VO2 max (r=−0.51, P<0.05).

- In a multivariable regression model, LV-ESVindex and LA Vol index were independent predictors of ECV (r2=0.42, P<0.01).

ECV in the clinical setting

All comers
• GLS linearly related to EF

• GLS far more associated with outcomes compared to EF

• ECV and GLS are barely related (R2=0.04)

When separating the sample into 4 crude categories according to whether ECV and GLS were simply above or below the median, those with both elevated ECV and GLS experienced the highest incidence of death or heart failure, suggesting additive effects of combined diffuse fibrosis and contractile dysfunction. (The p values between strata do not adjust for multiple comparisons. Abbreviations as in Figures 1 and 2.)
## Multivariable modeling adjusting for every variable we collected

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Variable</th>
<th>Chi-Square Value</th>
<th>p value</th>
<th>HR (95% CI)</th>
<th>p value</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart failure with LVEF ≥ 55%</strong> &lt;br&gt; (n=130; 42 events)</td>
<td>GLS (per 5% increment)</td>
<td>3.4</td>
<td>0.004</td>
<td>1.51</td>
<td>0.064</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>11.1</td>
<td>0.001</td>
<td>1.52</td>
<td>1.50</td>
<td>1.55-1.90</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Heart failure with LVEF &lt;55%</strong> &lt;br&gt; (n=341; 153 events)</td>
<td>GLS (per 5% increment)</td>
<td>11.8</td>
<td>0.001</td>
<td>1.48</td>
<td>1.30</td>
<td>1.20-1.49</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>18.3</td>
<td>0.001</td>
<td>1.42</td>
<td>1.21</td>
<td>1.14-1.44</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Myocardial infarction present</strong> &lt;br&gt; (n=346; 130 events)</td>
<td>GLS (per 5% increment)</td>
<td>27.0</td>
<td>&lt;0.001</td>
<td>1.32</td>
<td>1.30</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>37.0</td>
<td>&lt;0.001</td>
<td>1.28</td>
<td>1.32</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Myocardial infarction absent</strong> &lt;br&gt; (n=1233; 209 events)</td>
<td>GLS (per 5% increment)</td>
<td>52.2</td>
<td>&lt;0.001</td>
<td>1.36</td>
<td>1.41</td>
<td>1.21-1.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>88.2</td>
<td>&lt;0.001</td>
<td>1.46</td>
<td>1.40</td>
<td>1.22-1.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes present</strong> &lt;br&gt; (n=315; 124 events)</td>
<td>GLS (per 5% increment)</td>
<td>38.4</td>
<td>&lt;0.001</td>
<td>1.32</td>
<td>1.32</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>23.9</td>
<td>&lt;0.001</td>
<td>1.32</td>
<td>1.30</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes absent</strong> &lt;br&gt; (n=1263; 215 events)</td>
<td>GLS (per 5% increment)</td>
<td>53.4</td>
<td>&lt;0.001</td>
<td>1.36</td>
<td>1.32</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>75.7</td>
<td>&lt;0.001</td>
<td>1.36</td>
<td>1.32</td>
<td>1.20-1.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Any evidence of obstructive coronary artery disease</strong> &lt;br&gt; (n=463; 162 events)</td>
<td>GLS (per 5% increment)</td>
<td>42.8</td>
<td>&lt;0.001</td>
<td>1.37</td>
<td>1.37</td>
<td>1.22-1.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>36.8</td>
<td>&lt;0.001</td>
<td>1.36</td>
<td>1.37</td>
<td>1.22-1.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>No evidence of obstructive coronary artery disease</strong> &lt;br&gt; (n=1115; 177 events)</td>
<td>GLS (per 5% increment)</td>
<td>104.7</td>
<td>&lt;0.001</td>
<td>1.38</td>
<td>1.38</td>
<td>1.17-1.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ECV (per 4% increment)</td>
<td>88.5</td>
<td>&lt;0.001</td>
<td>1.38</td>
<td>1.37</td>
<td>1.17-1.64</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Key outcomes data → Conceptual model
A new taxonomy to conceptualize vulnerability related to myocardial disease

Fröjdh F, Fukui M, Cavalcante JL, ... Ugander M, Schelbert EB. Extracellular Volume and Global Longitudinal Strain Both Associate With Outcomes But Correlate Minimally. JACC Imaging 2020

ECV in the clinical setting

Incident ventricular arrhythmia requiring ICD
Rx
ECV predicts serious ventricular arrhythmia (appropriate ICD shock)

ACC 2018 "Diffuse myocardial fibrosis measured by extracellular volume associates with incident ventricular arrhythmia in implantable cardioverter defibrillator recipients more than focal fibrosis"

Shock or anti-tachycardia pacing

ACC 2018 "Diffuse myocardial fibrosis measured by extracellular volume associates with incident ventricular arrhythmia in implantable cardioverter defibrillator recipients more than focal fibrosis"
ECV and ICD shock

- In multivariable Cox models, ECV remained associated with ICD shock HR 2.17 (95%CI 1.17-4.00) for every 5% increase in ECV, adjusted for:
  - age,
  - ejection fraction (EF),
  - myocardial infarction,
  - nonischemic scar on LGE,
  - ICD indication (primary prevention) and
  - ischemic cardiomyopathy

whereas myocardial infarction and focal fibrosis by LGE did not.
CONCEPTUAL MODEL: Inferring cardiomyocyte-ECM interactions by associations with cardiac dysfunction and adverse outcomes
Most robust measure for myocardial fibrosis?
ECV in the clinical setting

Athletic Heart
Assessing Myocardial Extracellular Volume by T1 Mapping to Distinguish Hypertrophic Cardiomyopathy From Athlete's Heart

Scatter plot shows maximal segmental thickness and ECV of the same segment for HCM subjects (orange) and athletes (blue). The gray area highlights the indeterminate zone of 12 to 15 mm. ECV = extracellular volume; HCM = hypertrophic cardiomyopathy.

Ventricular Structure and Function

Athletic Cardiac Adaptation in Males Is a Consequence of Elevated Myocyte Mass

Adam K. McDermid, MD; Peter P. Swoboda, MBBS; Bara Erhayem, BMBS; Rosalind E. Lancaster, PhD; Gemma K. Lyall, MSc; David A. Broadbent, BSc; Laura E. Dobson, MBChB; Tarique A. Musa, MBBS; David P. Ripley, MBChB; Paekj Garg, MD; John P. Greenwood, PhD; Carrie Ferguson, PhD; Sven Plein, PhD

Background—Cardiac remodeling occurs in response to regular athletic training, and the degree of remodeling is associated with fitness. Understanding the myocardial structural changes in athlete’s heart is important to develop tools that differentiate athletic from cardiomyopathic change. We hypothesized that athletic left ventricular hypertrophy is a consequence of increased myocardial cellular rather than extracellular mass as measured by cardiovascular magnetic resonance.

Methods and Results—Forty-five males (30 athletes and 15 sedentary age-matched healthy controls) underwent comprehensive cardiovascular magnetic resonance studies, including native and postcontrast T1 mapping for extracellular volume calculation. In addition, the 30 athletes performed a maximal exercise test to assess aerobic capacity and anerobic threshold. Participants were grouped by athletic status: untrained, low performance, and high performance (VO_{peak} <60 or>60 mL/kg/min, respectively). In athletes, indexed cellular mass was greater in high- than low-performance athletes 60.7±7.5 versus 48.6±6.3 g/m², P<0.001, whereas extracellular mass was constant (16.3±2.2 versus 15.3±2.2 g/m², P=0.20). Indexed left ventricular end-diastolic volume and mass correlated with VO_{peak} (r=0.45, P=0.01; r=0.55, P=0.002) and differed significantly by group (P=0.01; P=0.001, respectively). Extracellular volume had an inverse correlation with VO_{peak} (r=0.53, P=0.003 and left ventricular mass index (r=0.44, P=0.02).

Conclusions—Increasing left ventricular mass in athlete’s heart occurs because of an expansion of the cellular compartment while the extracellular volume becomes relatively smaller: a difference which becomes more marked as left ventricular mass increases. Athletic remodeling, both on a macroscopic and cellular level, is associated with the degree of an individual’s fitness. Cardiovascular magnetic resonance ECV quantification may have a future role in differentiating athlete’s heart from change secondary to cardiomyopathy. (Circ Cardiovasc Imaging. 2016;9:e003579. DOI: 10.1161/CIRCIMAGING.115.003579.)

Key Words: athlete’s heart • cardiovascular magnetic resonance imaging • ECV • exercise physiology • hypertrophy/remodeling • T1 mapping
Regression of Left Ventricular Mass in Athletes Undergoing Complete Detraining Is Mediated by Decrease in Intracellular but Not Extracellular Compartments

BACKGROUND: Athletic cardiac remodeling can occasionally be difficult to differentiate from pathological hypertrophy. Detraining is a commonly used diagnostic test to identify physiological hypertrophy, which can be diagnosed if hypertrophy regression. We aimed to establish whether athletic cardiac remodeling assessed by cardiovascular magnetic resonance is mediated by changes in intracellular or extracellular compartments and whether this occurs by 1 or 3 months of detraining.

METHODS: Twenty-eight athletes about to embark on a period of forced detraining due to incidental limb bone fracture underwent clinical assessment, ECG, and contrast-enhanced cardiovascular magnetic resonance within a week of their injury and then 1 month and 3 months later.

RESULTS: After 1 month of detraining, there was reduction in left ventricular (LV) mass (135a≤12 to 121±25 g; P<0.0001), increase in native T1 (121a≤10 to 129a±30 ms; P<0.02), and extracellular volume fraction (24.5±2.1% to 26.3±2.6%; P=0.002) with no further changes by 3 months. The decrease in LV mass was mediated by a decrease in intracellular compartment volume (91±12 to 85±19 mL; P=0.0003) with no significant change in the extracellular compartment volume. High LV mass index, low native T1, and low extracellular volume fraction at baseline were all predictive of regression in LV mass in the first month.

CONCLUSIONS: Regression of athletic LV hypertrophy can be detected after 1 month of complete detraining and is mediated by a decrease in the intracellular myocardial compartment with no change in the extracellular compartment. Further studies are needed in athletes with exert and pathological hypertrophy to establish whether native T1 and extracellular volume fraction may complement electrophysiology, echocardiography, cardiopulmonary exercise testing, and genetic testing in predicting the outcome of detraining.

Key Words: athletics ● magnetic resonance imaging ● hypertrophy ● sports

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Novel therapeutics for myocardial fibrosis on the horizon

- Mineralocorticoid antagonists
- RNA therapeutics (long non-coding)
- Pirfenidone - an oral antifibrotic agent without hemodynamic effect

J Cardiovasc Pharmacol 2014;64:69–78)
Aldosterone blockage without the hyperkalemia or renal dysfunction!

Histological assessment of fibrosis revealed a significant dose-dependent reduction of interstitial fibrosis suggesting improvements in the overall cardiac remodeling in the antiMiR132-treated groups.
Cardior Pharmaceuticals has a bigger goal—and has persuaded investors to commit €64 million ($75 million) to support its ambitions.

Cardior expects to have phase 2 data in the second half of 2024.
The long noncoding RNA Wisper controls cardiac fibrosis and remodeling

Rudi Micheletti,1 Isabelle Plaisance,1 Brian J. Abraham,2 Alexandre Sarre,3 Ching-Chia Ting,1 Michael Alexanian,1 Daniel Maric,1 Damien Maison,1 Mohamed Nemir,1 Richard A. Young,1,4 Blanche Schroen,6 Arantxa Gonzalez,6,7 Samir Unzain,6,7 Thierry Pedrazzini1

Long noncoding RNAs (lncRNAs) are emerging as powerful regulators of cardiac development and disease. However, our understanding of the importance of these molecules in cardiac fibrosis is limited. Using an integrated genomic screen, we identified Wisper (Wisp2 super-enhancer-associated RNA) as a cardiac fibroblast-enriched lncRNA that regulates cardiac fibrosis after injury. Wisper expression was correlated with cardiac fibrosis both in a murine model of myocardial infarction (MI) and in heart tissue from human patients suffering from aortic stenosis. Loss-of-function approaches in vitro using modified antisense oligonucleotides (ASOs) demonstrated that Wisper is a specific regulator of cardiac fibroblast proliferation, migration, and survival. Accordingly, ASO-mediated silencing of Wisper in vivo attenuated MI-induced fibrosis and cardiac dysfunction. Functionally, Wisper regulates cardiac fibroblast gene expression programs critical for cell identity, extracellular matrix deposition, proliferation, and survival. In addition, its association with TIA1-related protein allows it to control the expression of a profibrotic form of lysyl hydroxylase 2, implicated in collagen cross-linking and stabilization of the matrix. Together, our findings identify Wisper as a cardiac fibroblast-enriched super-enhancer-associated lncRNA that represents an attractive therapeutic target to reduce the pathological development of cardiac fibrosis in response to MI and prevent adverse remodeling in the damaged heart.


Heart failure as a consequence of myocardial fibrosis is the world's biggest killer and represents a significant unmet medical need. No therapies currently exist that either directly target the heart or the fibrotic process itself. We have discovered a heart specific regulator of fibrosis—the long noncoding RNA, Wisper. By using our first-in-class proprietary approach to target Wisper, we are able to block myocardial fibrosis and treat heart failure in pre-clinical animal models.

We are developing a first-in-class biopharmaceutical therapy to treat heart failure.

- Treats fibroblasts ONLY in the heart to reverse myocardial fibrosis with high potency
- No apparent off target effects
Undesirable off target effects

First Randomized Controlled double blinded Phase 2 trial to reverse myocardial fibrosis
Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial

Gavin A. Lewis, Susanna Dodd, Dannii Clayton, Emma Bedson, Helen Eccleson, Erik B. Schelbert, Josephine H. Naish, Beatriz Duran Jimenez, Simon G. Williams, Colin Cunnington, Fozia Zahir Ahmed, Anne Cooper, Rajavarma Viswesvaraiiah, Stuart Russell, Theresa McDonagh, Paula R. Williamson and Christopher A. Miller

In heart failure with preserved ejection fraction (HFrEF), the occurrence of myocardial fibrosis is associated with adverse outcome. Whether pirfenidone, an oral antifibrotic agent without hemodynamic effect, is efficacious and safe for the treatment of HFrEF is unknown. In this double-blind, phase 2 trial (NCT02932566), we enrolled patients with heart failure, an ejection fraction of 45% or higher and elevated levels of natriuretic peptides. Eligible patients underwent cardiovascular magnetic resonance and those with evidence of myocardial fibrosis, defined as a myocardial extracellular volume of 27% or greater, were randomly assigned to receive pirfenidone or placebo for 52 weeks. Forty-seven patients were randomized to each of the pirfenidone and placebo groups. The primary outcome was change in myocardial extracellular volume, from baseline to 52 weeks. In comparison to placebo, pirfenidone reduced myocardial extracellular volume between-group difference, −1.21%; 95% confidence interval, −2.12 to −0.31; P = 0.009, meeting the predefined primary outcome. Twelve patients (26%) in the pirfenidone group and 14 patients (30%) in the placebo group experienced one or more serious adverse events. The most common adverse events in the pirfenidone group were nausea, insomnia and rash. In conclusion, among patients with HFrEF and myocardial fibrosis, administration of pirfenidone for 52 weeks reduced myocardial fibrosis. The favorable effects of pirfenidone in patients with HFrEF will need to be confirmed in future trials.

Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial

• **Pirfenidone was associated with a reduction in log NT-proBNP** compared to placebo (P=0.02)

• the effect seen by week 13 (the reduction in median NT-proBNP from baseline to week 13 with pirfenidone was 415ng/L versus 326ng/L with placebo;


Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial

• among patients with HFP EF and myocardial fibrosis, administration of pirfenidone for 52 weeks reduced myocardial fibrosis $-1.21\%$;

• “The favorable effects of pirfenidone in patients with HFP EF will need to be confirmed in future trials” (e.g., phase 3).

Future Directions

• Assess how cardiomyocyte and interstitial/fibroblast domains of vulnerability change with interventions
  – Pharmacologic
  – Procedural (percutaneous, surgical)

• Understand the efficacy of these interventions on each domain as they both important

• Define high-ECV enriched populations without reliance on CMR/CCT for Phase 3 trials
  – (there definitely is a way to do this! — unpublished data)

Conclusions

• Interstitial expansion from myocardial fibrosis likely causes:
  – Mechanical dysfunction (i.e., diastolic)
    • Systolic function and ECV are mostly independent
  – Microvascular dysfunction (↓ perfusion reserve, capillary rarefaction)
  – Electrical dysfunction (reentry)

and the increases risks of death, hospitalization for HF, arrhythmia

• Similar strength of association with adverse outcomes between ECV and EF and/or GLS → Myocardial fibrosis likely causal

• CMR (and CCT) measure interstitial expansion with ECV reliably

• Anti-fibrotic Rx under development promise to reverse cardiac dysfunction & improve outcomes.

• ECV is critical for serial monitoring of disease progression / regression
Acknowledgments (alphabetical order)

- Javed Butler, MD, MPH, University of Mississippi
- Joao Cavalcante, MD, Minneapolis Heart Institute
- Fredrika Frojdh, MD, Karolinska Institutet
- Miho Fukui, MD, Minneapolis Heart Institute
- Mihai Gheorghiade, MD, Northwestern University (deceased)
- Peter Kellman, PhD, NHLBI
- Maren Maanja, MD, Karolinska Institutet
- Christopher Miller, MD, PhD, University of Manchester
- James Moon, MD, University College of London/Bart’s
- Eric Olausson, MD, Karolinska Institutet
- Kayla Piehler, MD
- Martin Ugander, MD, PhD, Karolinska Institutet
- Timothy Wong, MD, MS

Thank you
Omnipresent issue in research: Cause vs. Effect

Among cascading derangements in diseased myocardium:

– which "domains" confer vulnerability and are truly causal?

– which abnormalities simply represent downstream, noncausal effects of the above?

– How do we conceptualize these changes?

https://www.virginia.org/listings/OutdoorsAndSports/CascadesNationalRecreationTrail/
ECV in the clinical setting

Amyloidosis
Different story for Late Gadolinium Enhancement (LGE) and ExtraCellular Volume (ECV) cardiac MR


Prognostic Value of Late Gadolinium Enhancement (LGE) Cardiovascular Magnetic Resonance (CMR) in Both types of Cardiac Amyloidosis

Cardiac MR for ATTR diagnosis
LGE (qualitative) + ECV (quantitative measure of interstitial volume)


Cardiac MR with ECV for diagnosis as well as ATTR risk stratification

AUC 0.91 for ECV

ECV risk stratification for AL cardiac amyloidosis


ECV can track response to therapy!

Or lack thereof…

No change in ECV 1 yr after patisiran Rx for ATTR
Despite other testing suggesting regression
Pittsburgh experience with amyloidosis in aortic stenosis

Log-Rank X²=15.0, p < 0.0001

Aortic Stenosis + Cardiac Amyloidosis

Aortic Stenosis