Impact of Myocardial Fibrosis on Cardiovascular Structure, Function and Functional Status in Heart Failure With Preserved Ejection Fraction

Gavin Lewis

University of Manchester Faculty of Life Sciences: The University of Manchester Faculty of Biology Medicine and Health <https://orcid.org/0000-0001-6754-836X>

Anna Rosala-Hallas

University of Liverpool

Susanna Dodd

University of Liverpool

Erik B. Schelbert

University of Pittsburgh School of Medicine

Simon G. Williams

Manchester University NHS Foundation Trust

Colin Cunningham

Manchester University NHS Foundation Trust

Theresa McDonagh

King's College Hospital NHS Trust: King's College Hospital NHS Foundation Trust

Christopher A. Miller (✉ christopher.miller@manchester.ac.uk)

University of Manchester Faculty of Medical and Human Sciences: The University of Manchester Faculty of Biology Medicine and Health

---

Research Article

Keywords: Heart failure with preserved ejection fraction, myocardial fibrosis, magnetic resonance imaging (MRI), mediation analysis

Posted Date: February 9th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1332963/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Purpose: Myocardial fibrosis, measured using cardiovascular magnetic resonance extracellular volume (ECV), is associated with adverse outcome in heart failure with preserved ejection fraction (HFpEF), but the mechanisms by which myocardial fibrosis exerts this deleterious effect are unclear. We aimed to determine whether myocardial fibrosis causes changes in cardiovascular structure and function, circulating biomarkers and functional status.

Methods: Mediation analyses of data from the PIRouETTE (The Pirfenidone in Patients with Heart Failure and Preserved Left Ventricular Ejection Fraction) trial were conducted using the Baron and Kenny approach under a structural equation modelling framework to estimate average causal mediation effects, in order to determine whether changes in myocardial ECV, absolute myocardial extracellular matrix volume, and myocardial cellular volume (the mediator variables) following antifibrotic therapy, caused changes in cardiovascular structure and function, circulating biomarkers and functional status (the outcome variables).

Results: Regression of myocardial fibrosis correlated with improvements in functional status (6-minute walk test distance [$r=-0.28$, $p=0.021$] and KCCQ clinical summary score [$r=-0.23$, $P=0.045$]). Pirfenidone had significant effects on the mediator variables, but the only outcome variable that demonstrated a treatment effect was left ventricular ejection fraction (LVEF) ($p=0.011$). The estimated average causal mediation effects of myocardial ECV, absolute myocardial extracellular matrix volume and absolute myocardial cellular volume on LVEF were 6.1%, 21.5%, and 13.7% respectively, none of which were significant ($p=0.608$, $p=0.123$ and $p=0.186$ respectively).

Conclusion: Regression of myocardial fibrosis was associated with improvements in functional status. The small improvement in LVEF associated with pirfenidone was not mediated by myocardial fibrosis regression.

(PIROUETTE; NCT02932566).

Introduction

Myocardial fibrosis, measured using cardiovascular magnetic resonance (CMR) extracellular volume (ECV), is associated with adverse outcome in patients with heart failure with preserved ejection fraction (HFpEF), including hospitalisation for heart failure (HF) and death (1–5).

The mechanisms by which myocardial fibrosis exerts this deleterious effect in HFpEF are unclear, but observational studies have demonstrated associations between myocardial fibrosis and myocardial stiffness, diastolic dysfunction, microvascular dysfunction, and pulmonary hypertension (6–11).

The Pirfenidone in patients with heart failure and preserved left ventricular ejection fraction (PIROUETTE) study was a phase II, double-blind, placebo-controlled, randomised trial designed to evaluate the efficacy

and mechanism of the novel anti-fibrotic agent, pirfenidone, in patients with HFpEF and myocardial fibrosis (12). Pirfenidone is an orally bioavailable, small molecule anti-fibrotic agent that inhibits cardiac fibroblast synthesis and secretion of TGF- β 1, proliferation and activation of fibroblasts, and profibrotic pathways. Uniquely among cardiovascular interventions with antifibrotic effects that have been trialled in HFpEF, pirfenidone does not have a haemodynamic effect, thus in this regard, is a specific antifibrotic. As part of the trial protocol, participants underwent deep phenotyping, including detailed assessment of cardiovascular structure and function, circulating biomarkers and functional status.

Mediation analysis, conducted as part of a randomised controlled trial, allows estimation of the direct and indirect (via a mediator variable) effects of an intervention on outcome, and thus can be used to determine whether proposed pathophysiological mechanisms have causal effects (13).

The interventional nature of the PIROUETTE trial, and the specific antifibrotic nature of pirfenidone, in conjunction with mediation analysis, provide novel opportunity to investigate the causal impact of myocardial fibrosis on cardiovascular structure and function, circulating biomarkers and functional status, which has the potential to help understand why myocardial fibrosis is associated with adverse outcome.

This study aimed to determine whether myocardial fibrosis causes changes in cardiovascular structure and function, circulating biomarkers and functional status, by conducting a mediation analysis of data from the PIROUETTE trial.

Methods

Study design and patient selection

The trial design and results of the PIROUETTE trial (Clinicaltrials.gov NCT02932566) have been published previously (12, 14). In brief, between March 7, 2017, to December 19, 2018, 94 patients with HFpEF and myocardial fibrosis were randomised to pirfenidone or placebo for 52-weeks. Eligibility requirements included patients $\geq$ 40 years of age, symptoms and signs of heart failure, left ventricular ejection fraction of $\geq$ 45%, and elevated natriuretic peptides (brain natriuretic peptide (BNP) $\geq$ 100pg/ml or N-terminal pro-B-type natriuretic peptide (NT-proBNP) $\geq$ 300pg/ml; or BNP $\geq$ 300pg/ml or NT-proBNP $\geq$ 900pg/ml if atrial fibrillation (AF) present). Eligible patients underwent CMR and those with evidence of myocardial fibrosis, defined as an ECV of 27% or higher, were randomised in a 1:1 ratio to treatment with either pirfenidone or matching placebo for 52 weeks using block randomisation, stratified by sex. Key exclusion criteria included alternative causes of patients' symptoms such as significant pulmonary disease, anaemia, or obesity; pericardial constriction, hypertrophic cardiomyopathy or infiltrative cardiomyopathy; and contraindication to magnetic resonance imaging. The primary outcome was change in myocardial fibrosis, measured using CMR ECV, from baseline to 52-weeks.

The trial was sponsored by Manchester University NHS Foundation Trust. Trial management, independent data management and independent statistical analyses were performed by Liverpool Clinical Trials

Centre, a United Kingdom Clinical Research Collaboration fully-registered Clinical Trials Unit. The study protocol was approved by a research ethics committee and trial conduct was overseen by a trial steering committee. Patients were identified at six UK hospitals. Study visits took place at Manchester University NHS Foundation Trust. All patients provided written informed consent.

Study procedures and analysis

The protocol, trial procedures, analysis methods and outcome measurements have been described previously (12, 14). In brief, CMR, echocardiography, electrocardiography, 6-minute walk test, laboratory tests and the Kansas City Cardiomyopathy Questionnaire (KCCQ) were performed at baseline and repeated after 52-weeks of treatment. ³¹P-magnetic resonance spectroscopy (³¹P-MRS), was performed at baseline and 52-weeks in a subset of patients as part of a sub-study (n=60).

Myocardial ECV was calculated from basal and mid left ventricular (LV) short axis T1 maps (MODified Look-Locker Inversion recovery [MOLLI]), acquired before and 15-minutes following gadolinium contrast (0.15 mmol/kg of gadoterate meglumine), as: $ECV = (1 - \text{haematocrit}) \times [\Delta R1_{\text{myocardium}}] / [\Delta R1_{\text{bloodpool}}]$, where $\Delta R1$ is the difference in relaxation rates ($1 / T1$) between pre- and post-contrast (4). Haematocrit was measured on the same day as CMR scanning. Absolute myocardial extracellular matrix (ECM) volume was calculated as the product of LV myocardial volume (LV mass divided by the specific gravity of myocardium [1.05 g/ml]) and ECV. Absolute myocardial cellular volume was calculated as the product of LV myocardial volume (LV mass divided by the specific gravity of myocardium [1.05 g/ml]) and $(1 - ECV)$. Further details can be found in the trial protocol paper (14).

Statistical analysis

Analysis was conducted on an intention to treat basis, including all randomised patients retained in their randomised treatment groups. Continuous data are presented as mean $\pm$ standard deviation (SD) or as median (interquartile range (IQR)), as appropriate. Categorical data are presented as counts and percentages. Correlation analyses were used to assess associations between change in ECV (week 52 value minus baseline value), and change in selected secondary outcome variables that reflected cardiac mechanical and electrical function, circulating biomarkers, and functional status. Pearson's or Spearman's correlation coefficients were used as appropriate following Shapiro-Wilk testing for normality. Analyses were performed in Stata (Version 14.0, StataCorp, College Station, TX) and SAS (Version 9.4, SAS Institute, Inc; Cary,NC).

Mediation analysis

Mediation analyses were conducted in order to determine whether changes in myocardial fibrosis, measured using ECV and absolute ECM volume, and changes in myocardial cellular volume (the mediator variables) following antifibrotic therapy, caused changes in cardiovascular structure and function, circulating biomarkers and functional status (the outcome variables).

Mediation analyses were conducted using the Baron and Kenny approach (15), under a structural equation modelling (SEM) framework (Stata package medsem), in order to estimate the average causal mediation effect (ACME) of each mediator, adjusting for confounders (baseline covariates that predicted both the mediator and outcome at the 10% significance level). For each potential outcome, the analysis was only performed if both of the following conditions were satisfied:

1. The antifibrotic therapy had a significant effect on the outcome at the 5% level (i.e. $p < 0.05$ for the treatment effect in an analysis of covariance (ANCOVA) model of the outcome, adjusting for treatment, sex and baseline value of the outcome variable).
2. The antifibrotic therapy had a significant effect on the mediator variables (i.e. ECV, absolute ECM volume and myocardial cellular volume) at the 10% level (i.e. $p < 0.1$ for the treatment effect in the ANCOVA models of ECV, absolute ECM volume and myocardial cellular volume, adjusting for treatment, sex and baseline values of the mediator variables).

The outcome variables for this study were all secondary outcome measurements in the main PIROUETTE trial. The outcome variables reflect cardiovascular structure and function, circulating biomarkers and functional status. They were selected for use in this study because they are variables that, based on the published literature and clinical judgment, are associated with adverse outcome in HFpEF or were hypothesised to be impacted by myocardial fibrosis. The selection of the outcome variables was prospective i.e. outcome variables were selected before data lock for the trial had occurred, thus before the results of the trial were known, and were prespecified in an 'Additional Statistical Analysis Plan' that was written before data lock.

Results

Patients

Baseline characteristics of the 94 patients that were randomised are presented in Table 1. The mean age of patients was 78 years, and 46% were female. Nearly all patients had New York Heart Association functional class II or III symptoms (95%), mean LV ejection fraction was 64% and median NT-proBNP was 1104 pg/ml. Mean myocardial ECV was 30.1%. At the end of the trial, 12 patients had withdrawn from the study and two had died. No patient was lost to follow-up. Only patients with complete data were included in the mediation analysis (n=79).

Table 1
Baseline characteristics

Characteristic	Patients (N=94)
Age – years	78 ± 7.4
Female - no. (%)	43 (46)
White Race - no. (%)	88 (94)
Hypertension - no. (%)	79 (84)
Diabetes - no. (%)	28 (30)
Systolic blood pressure - mmHg	136 ± 22.5
Diastolic blood pressure - mmHg	76 ± 15.3
BMI – kg/m ²	31 ± 5.6
eGFR – ml/min	57 ± 16.7
Haemoglobin – g/dL	12.9 ± 1.5
Log-NT-proBNP – pg/ml	7.0 ± 0.9
HS-Troponin T – pg/ml	28.1 ± 31.0
QRS duration - ms	105 ± 16.8
Myocardial ECV - %	30.1 ± 2.7
Absolute myocardial ECM Volume - ml	36.7 ± 11.4
Absolute myocardial cellular volume - ml	85.1 ± 24.1
Left ventricular end-diastolic volume index – ml/m ²	63 ± 36.3
Left ventricular ejection fraction - %	64 ± 23.3
Left ventricular mass index – g/m ²	65 ± 15.4
Average e' – cm/s	8.8 ± 2.5
Average E/e' – cm/s	12.2 ± 3.4
Global longitudinal strain - %	-16.0 ± 3.5
Torsion – degrees/cm	1.5 ± 0.7
PCr:ATP ratio	1.3 ± 0.4
Right ventricular end-diastolic volume index – ml/m ²	69 ± 16.4
Right ventricular ejection fraction - %	52 ± 9.5

Characteristic	Patients (N=94)
Pulmonary artery systolic pressure - mmHg	33 ± 12.8
Left atrial volume index – ml/m ²	70.4 ± 18.6
Left atrial strain (reservoir) - %	16.9 ± 7.6
Left atrial strain (booster) - %	12.4 ± 4.3
Left atrial strain (conduit) - %	10.4 ± 3.8
Aortic distensibility – 10 ⁻³ /mmHg	1.6 ± 0.9
Pulse-wave velocity – m/s	12.6 ± 5.0
6-minute walk test - m	265 ± 114.6
KCCQ Clinical Summary Score	56.0 ± 19.9

Table 1 Legend. Values are presented as mean ±SD unless stated.

ATP – adenosine triphosphate; BMI – body mass index; ECM – extracellular matrix; ECV – extracellular matrix volume; eGFR – estimated glomerular filtration rate; HS-Troponin T – high-sensitivity troponin T; KCCQ – Kansas City Cardiomyopathy Questionnaire; NT-proBNP – n-terminal pro B-type natriuretic peptide; PCr – phosphocreatine.

Associations with change in ECV

Change in myocardial ECV from baseline to week 52 showed a positive correlation with change in left ventricular end diastolic volume indexed for body surface area ($r=0.23$, $P=0.039$), and inverse correlations with 6 minute walk test distance ($r=-0.28$, $P=0.021$), and KCCQ Clinical Summary Score ($r=-0.23$, $P=0.045$) (Figure 1 and Table S1 in Supplementary Appendix), although all the associations were weak.

Mediation analysis

Pirfenidone had a significant effect on the mediator variables measuring myocardial fibrosis (myocardial ECV and absolute myocardial ECM volume). The effect of pirfenidone on the mediator variable myocardial cellular volume was not significant at the conventional 5% level, but was significant at the 10% level, and thus, as prespecified, this mediator variable was also included in the mediation analysis (Table 2).

Table 2
Treatment effect on each mediator variable

Mediator variable	Estimated 95% CI	P-value associated with treatment effect
Myocardial ECV - %	-1.21 (-2.12 to -0.31)	0.009
Absolute myocardial ECM volume - ml	-3.06 (-4.96 to -1.16)	0.002
Absolute myocardial cellular volume - ml	-3.41 (-7.28 to 0.47)	0.084

Table 2 Legend. Analysis of covariance models, adjusted for baseline value of the mediator variable, sex and treatment group. CI – confidence interval; ECM – extracellular matrix; ECV – extracellular matrix volume.

The only outcome variable that demonstrated a treatment effect was LV ejection fraction (p=0.011) (Table 3).

Table 3
Treatment effect on each outcome variable

Outcome variable	P-value associated with treatment effect
Systolic blood pressure - mmHg	0.72
Diastolic blood pressure - mmHg	0.49
BMI - kg/m ²	0.31
eGFR - ml/min	0.13
Haemoglobin - g/dL	0.49
Log NT-proBNP - pg/ml	0.079
HS-Troponin T - pg/ml	0.64
QRS duration - ms	0.93
LV end diastolic volume index - ml/m ²	0.80
LV ejection fraction - %	0.011
LV mass index - g/m ²	0.10
Average e' - cm/s	0.73
Average E/e' - cm/s	0.76
Global Longitudinal Strain - %	0.10
Torsion - degrees/cm	0.55
PCr:ATP	0.62
RV end diastolic volume index - ml/m ²	0.58
RV ejection fraction - %	0.27
Pulmonary artery systolic pressure - mmHg	0.89
LA volume index - ml/m ²	0.83
LA strain (reservoir) - %	0.78
LA strain (booster) - %	0.75
LA strain (conduit) - %	0.50
Aortic distensibility - 10 ⁻³ /mmHg	0.08
Pulse Wave Velocity - m/s	0.80
6-minute walk test - m	0.22

Outcome variable	P-value associated with treatment effect
KCCQ Clinical Summary Score	0.09

Table 3 Legend. Analysis of covariance models, adjusted for baseline value of the outcome variable, sex and treatment group. ATP – adenosine triphosphate; BMI – body mass index; eGFR – estimated glomerular filtration rate; HS-Troponin T – high-sensitivity troponin T; KCCQ – Kansas City Cardiomyopathy Questionnaire; LA – left atrial; LV – left ventricular; NT-proBNP – N-terminal pro B-type natriuretic peptide; PCr – phosphocreatine; RV – right ventricular.

No baseline covariates were found to predict the mediator variables and LV ejection fraction, thus no baseline covariates were required to be included in the mediation analysis (Table S2 in Supplementary Appendix).

In the mediation analysis, the estimated average causal mediation effects of myocardial ECV, absolute myocardial ECM volume and absolute myocardial cellular volume on LV ejection fraction were 6.1%, 21.5%, and 13.7% respectively, none of which were significant ($p=0.608$, $p=0.123$ and $p=0.186$ respectively; Table 4 and Figure 2).

Table 4
Mediation analysis

Mediator	Direct effect of treatment on week 52 LVEF (%)	Direct effect of treatment on week 52 mediator	Indirect effect of treatment on week 52 LVEF (%)	Total effect of treatment on week 52 LVEF (%)	Proportion of effect mediated (%)
Myocardial ECV - %	1.89 (0.20 to 3.59); $p=0.028$	-1.14 (-2.03 to -0.26); $p=0.011$	0.12 (-0.35 to 0.60); $p=0.608$	2.02 (0.39 to 3.65); $p=0.015$	6.1; $p=0.608$
Absolute myocardial ECM volume - ml	1.88 (0.22 to 3.53); $p=0.026$	-3.13 (-4.99 to -1.28); $p=0.001$	0.51 (-0.14 to 1.17); $p=0.123$	2.39 (0.81 to 3.97); $p=0.003$	21.5; $p=0.123$
Absolute myocardial cellular volume - ml	1.93 (0.38 to 3.48); $p=0.015$	-3.83 (-7.56 to -0.10); $p=0.044$	0.30 (-0.15 to 0.76); $p=0.186$	2.23 (0.70 to 3.78); $p=0.004$	13.7; $p=0.186$

Table 4 Legend. Values are mean (95% confidence interval). ECM – extracellular matrix; ECV – extracellular matrix volume; LVEF – left ventricular ejection fraction.

Discussion

Over the past decade, observational data have demonstrated non-infarct myocardial fibrosis to be strongly associated with adverse prognosis across a range of cardiovascular conditions, including

HFpEF. As such, the myocardial interstitium has emerged as a potential therapeutic target, indeed this was the focus of the PIROUETTE trial, which targeted patients with HFpEF and evidence of myocardial fibrosis with a specific antifibrotic intervention. Nevertheless, while observational data are useful, the observed association between myocardial fibrosis and adverse cardiovascular outcomes does not establish causality. Randomised controlled trials provide an opportunity to do this.

The PIROUETTE trial established the efficacy of pirfenidone, an antifibrotic without blood pressure effect, to attenuate myocardial fibrosis in HFpEF. The associated reduction in natriuretic peptide levels over time, provides support for the extracellular matrix having a causal role in HFpEF and being an efficacious therapeutic target. The deep phenotyping conducted as part of the PIROUETTE trial, in conjunction with the described mediation analysis, potentially provided an opportunity to determine the causal impact myocardial fibrosis has on other aspects of cardiovascular structure and function, such as myocardial contractile, electrical and energetic function, as well as other factors such as functional status.

Unfortunately, the only secondary outcome of the trial prospectively selected as an outcome for this study that demonstrated a significant change from baseline to 52 weeks in response to pirfenidone, in comparison to placebo, was LV ejection fraction. There were notable trends towards improvement in other variables, such as KCCQ Clinical Summary Score ($p=0.09$) and global longitudinal strain ($p=0.10$), but none were significant, possibly reflecting lack of power. Indeed, the sample size for the PIROUETTE trial was calculated based on the primary outcome; the trial was not powered for secondary outcomes.

Despite being statistically significant, the magnitude of change in LV ejection fraction associated with treatment with pirfenidone was small (between-group difference, 2.16%; 95% confidence interval [CI], 0.51 to 3.81), and the clinical relevance is unclear. It is perhaps unsurprising, therefore, that regression of myocardial fibrosis was not found to mediate this effect.

Regression of myocardial fibrosis did correlate with improvements in functional status, such as 6-minute walk test distance and KCCQ clinical summary score. Whilst the associations were relatively weak, these findings are novel and provide more support for myocardial fibrosis having an important mechanistic role in HFpEF.

Limitations

As discussed, the PIROUETTE trial was not powered for secondary outcomes, thus the findings of this study are considered exploratory. The analyses conducted as part of the current study were not included in the Statistical Analysis Plan for PIROUETTE and thus are considered post-hoc. Nevertheless, the analyses conducted in this study were prespecified in an 'Additional Statistical Analysis Plan' that was written before trial data lock. As discussed, selection of outcome variables to include in this study was prospective and performed before data lock. Finally, no adjustment for multiple comparisons was performed therefore false positive results cannot be excluded.

Conclusion

In this analysis of the PIROUETTE trial, regression of myocardial fibrosis was associated with improvements in functional status. The small improvement in left ventricular ejection fraction associated with pirfenidone was not mediated by myocardial fibrosis regression.

Abbreviations List

³¹P-MRS - ³¹Phosphorous cardiac magnetic resonance spectroscopy

ANCOVA – analysis of covariance

BNP – brain natriuretic peptide

CMR – cardiac magnetic resonance

ECV – extracellular volume

HF – heart failure

HFpEF – heart failure with preserved ejection fraction

KCCQ – Kansas City Cardiomyopathy Questionnaire

LVEF – left ventricular ejection fraction

NT-proBNP – N-terminal pro B-type natriuretic peptide

Declarations

Funding

Dr Lewis is funded by a fellowship grant from the National Institute for Health Research. Dr Miller is funded by a Clinician Scientist Award (CS-2015-15-003) from the National Institute for Health Research. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.

Competing interests

The investigational medicinal product was gifted by Roche Products Limited. Immunoassay testing equipment and materials were gifted by Roche Diagnostics International Limited. Roche Products Limited and Roche Diagnostics International Limited had no role in study design, and were not involved in the preparation, drafting or editing of this manuscript. Roche Products Limited and Roche Diagnostics International Limited conducted a factual accuracy check of this manuscript, but any decisions to incorporate comments were made solely at the discretion of the authors. CAM has served on advisory

boards for Novartis, Boehringer Ingelheim and Lilly Alliance, and AstraZeneca, serves as an advisor for HAYA Therapeutics and PureTech Health and has received research support from Amicus Therapeutics, Guerbet Laboratories Limited, and Univar Solutions B.V.

Author contribution

Gavin Lewis: Conceptualisation, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - Original Draft, Visualisation, Project administration; **Anna Rosala-Hallas:** Software, Validation, Formal analysis, Writing - Review & Editing; **Susie Dodd:** Software, Validation, Formal analysis, Writing - Review & Editing; **Erik Schelbert:** Writing - Review & Editing; **Simon Williams:** Writing - Review & Editing; **Colin Cunningham:** Writing - Review & Editing; **Theresa McDonagh:** Writing - Review & Editing, **Christopher Miller:** Conceptualisation, Methodology, Validation, Writing - Review & Editing, Visualisation, Supervision, Project administration, Funding acquisition.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the NHS Health Research Authority regional ethics committee (North West - Liverpool Central, 16/NW/0717, 28th November 2016).

Consent to participate

Written informed consent was obtained from all individual participants include in this study.

Data availability statement

Anonymised data will be made available in full by reasonable request in writing to the corresponding author following appropriate completion of a data sharing agreement.

References

1. Fontana M, White SK, Banypersad SM et al. Comparison of T1 mapping techniques for ECV quantification. Histological validation and reproducibility of ShMOLLI versus multibreath-hold T1 quantification equilibrium contrast CMR. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2012;14:88.
2. Miller CA, Naish JH, Bishop P et al. Comprehensive validation of cardiovascular magnetic resonance techniques for the assessment of myocardial extracellular volume. Circ Cardiovasc Imaging 2013;6:373-83.
3. Schelbert EB, Fridman Y, Wong TC et al. Temporal Relation Between Myocardial Fibrosis and Heart Failure With Preserved Ejection Fraction: Association With Baseline Disease Severity and Subsequent Outcome. JAMA Cardiol 2017;2:995-1006.

4. Messroghli DR, Moon JC, Ferreira VM et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance* 2017;19:75.
5. Roy C, Slimani A, de Meester C et al. Associations and prognostic significance of diffuse myocardial fibrosis by cardiovascular magnetic resonance in heart failure with preserved ejection fraction. *J Cardiovasc Magn Reson* 2018;20:55.
6. Borbely A, van der Velden J, Papp Z et al. Cardiomyocyte stiffness in diastolic heart failure. *Circulation* 2005;111:774-81.
7. Zile MR, Baicu CF, Ikonomidis JS et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation* 2015;131:1247-59.
8. Rommel KP, von Roeder M, Latuscynski K et al. Extracellular Volume Fraction for Characterization of Patients With Heart Failure and Preserved Ejection Fraction. *Journal of the American College of Cardiology* 2016;67:1815-25.
9. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. *Circulation* 2015;131:550-9.
10. Su MY, Lin LY, Tseng YH et al. CMR-verified diffuse myocardial fibrosis is associated with diastolic dysfunction in HFpEF. *JACC Cardiovascular imaging* 2014;7:991-7.
11. Patel RB, Li E, Benefield BC et al. Diffuse right ventricular fibrosis in heart failure with preserved ejection fraction and pulmonary hypertension. *ESC heart failure* 2020;7:253-263.
12. Lewis GA, Dodd S, Clayton D et al. Pirfenidone in heart failure with preserved ejection fraction: a randomized phase 2 trial. *Nat Med* 2021;27:1477-1482.
13. Lewis GA, Dodd S, Naish JH, Selvanayagam JB, Dweck MR, Miller CA. Considerations for Clinical Trials Targeting the Myocardial Interstitium. *JACC Cardiovascular imaging* 2019;12:2319-2331.
14. Lewis GA, Schelbert EB, Naish JH et al. Pirfenidone in Heart Failure with Preserved Ejection Fraction- Rationale and Design of the PIROUETTE Trial. *Cardiovasc Drugs Ther* 2019;33:461-470.
15. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986;51:1173-82.

Figures

Figure 1

Associations with change in extracellular volume (ECV) from baseline to week 52.

Correlations between change in ECV and (A) change in left ventricular end-diastolic volume (LVEDV) index, (B) change in 6-minute walk test (6MWT), and (C) change in Kansas City cardiomyopathy questionnaire Clinical Summary Score (KCCQ-CSS).

Figure 2

Mediation model.

Illustration of the mediation model. Pirfenidone (treatment intervention) has a direct effect on left ventricular (LV) ejection fraction (outcome variable) and a direct effect on extracellular volume (ECV)

(mediator variable). Pirfenidone also has an indirect effect on LV ejection fraction via the effect on ECV. Mediation analysis determines whether the change in the mediator variable (ECV) causes the change in the outcome variable (LV ejection fraction), by calculating the proportion of the total effect of treatment on the outcome variable that is due to the indirect effect of treatment on the outcome variable acting via the mediator (ECV) i.e. the proportion of the total treatment effect that is mediated via the mediation variable (ECV). As per Table 4, the proportion of the total effect of pirfenidone on LV ejection fraction that was mediated by ECV was 6.1%, which was not significant ($p=0.608$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryAppendix.pdf](#)