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ORIGINAL RESEARCH

Relationship Between Focal and Diffuse Fibrosis Assessed by CMR and Clinical Outcomes in Heart Failure With Preserved Ejection Fraction

Prathap Kanagala, MBBS, PhD,[a,b] Adrian S.H. Cheng, MBBS, MD,[a,c] Anvesha Singh, MBChB,[a] Jamal N. Khan, MBChB, PhD,[a] Gaurav S. Gulsin,[a] Prashanth Patel, MBBS,[a] Pankaj Gupta, DPB,[a] Jayanth R. Arnold, BMBChB, DPhil,[a] Iain B. Squire, MBChB, MD,[a] Leong L. Ng, MBBChir, MD,[a] Gerry P. McCann, MBChB, MD[a]

ABSTRACT

OBJECTIVES This study sought to assess the presence and extent of focal and diffuse fibrosis in heart failure in patients with preserved ejection fraction (HFpEF) compared to asymptomatic control subjects, and the relationship of fibrosis to clinical outcome.

BACKGROUND Myocardial fibrosis has been implicated in the pathophysiology of HFpEF.

METHODS In this prospective, observational study, 140 subjects of similar age and sex (HFpEF: n = 96; control subjects: n = 44; 73 ± 8 years of age; 49% males) underwent cardiac magnetic resonance imaging. Late gadolinium-enhanced (LGE) imaging and T1 mapping to calculate myocardial extracellular volume indexed to body surface area (iECV) were used to assess fibrosis.

RESULTS Patients with HFpEF had more concentric remodeling and worse diastolic function. Focal fibrosis was more frequent in HFpEF subjects (overall: n = 49; infarction: n = 17; nonischemic cases: n = 36; mixed patterns: n = 4) than in control subjects (overall: n = 3). Diffuse fibrosis was also greater in HFpEF subjects than control subjects (iECV: 13.7 ± 4.4 ml/m^2 versus 10.9 ± 2.8 ml/m^2; p < 0.0001). During median follow-up (1,429 days), there were 42 composite events (14 deaths; 28 heart failure hospitalizations) in cases of HFpEF. Myocardial infarction revealed on LGE imaging was a predictor of outcomes on univariate analysis only. With multivariate analysis, iECV (hazard ratio [HR]: 1.689; 95% confidence interval [CI]: 1.141 to 2.501; p = 0.009) was an independent predictor of outcome along with mitral peak velocity of early filling (E)-to-early diastolic mitral annular velocity (E′) (E/E′) ratio (HR: 1.716; 95% CI: 1.191 to 2.472; p = 0.004) and prior HF hospitalization (HR: 2.537; 95% CI: 1.090 to 5.902; p = 0.031). iECV was also significantly associated with ventricular/left atrial remodeling and renal dysfunction: right ventricular end-diastolic volume indexed (r = 0.456; p < 0.0001), left ventricular mass/volume (r = 0.348; p = 0.001), maximal left atrial volume indexed (r = 0. 269; p = 0.009), and creatinine (r = 0.271; p = 0.009).

CONCLUSIONS Both focal and diffuse myocardial fibrosis are more prevalent in HFpEF subjects than in control subjects of similar age and sex. iECV significantly correlates with indices of ventricular/left atrial remodeling and renal dysfunction and is an independent predictor of adverse outcome in HFpEF. (Developing Imaging and plasMa biOmarkers iN Describing Heart Failure With Preserved Ejection Fraction [DIAMONDHFpEF]; NCT03050593)
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From the [a]Department of Cardiovascular Sciences, University of Leicester, National Institute for Health Research, Leicester Biomedical Research Centre, Leicester, United Kingdom; [b]Department of Cardiology, Aintree University Hospital, Liverpool, United Kingdom; and the [c]Department of Cardiology, Kettering General Hospital National Health Service Foundation Trust, Kettering, United Kingdom. Supported by the National Institute for Health Research (NIHR) Leicester Cardiovascular Biomedical Research Centre and NIHR Comprehensive Local Research Network. Dr. McCann is supported by British Heart Foundation, Medical Research Council, and NIHR Career Development fellowship 2014-07-045. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

BNP = B-type natriuretic peptide

CMR = cardiac magnetic resonance imaging

ECV = extracellular volume

iECV = indexed extracellular volume

HFpEF = heart failure with preserved ejection fraction

LAVImax = maximal left atrial volume indexed

LGE = late gadolinium enhancement

LV = left ventricle

RV = right ventricle

MI = myocardial infarction

Heart failure with preserved ejection fraction (HFpEF) accounts for up to half of all heart failure patients in the community, and outcomes remain poor (1). Current prognostic markers in HFpEF relate largely to clinical and echocardiographic parameters (2,3). However, cardiac magnetic resonance (CMR) imaging is the recognized gold standard for most of the imaging parameters that comprise the latest guidance for assessing HFpEF (4). Both focal fibrosis (myocardial infarction [MI] and "nonischemic" fibrosis) and interstitial myocardial fibrosis have been implicated in the pathophysiology of HFpEF by promoting adverse ventricular remodeling, increasing myocardial stiffness, and in turn, causing diastolic dysfunction (5). Focal fibrosis (6), including MI (7), can be detected by late gadolinium enhanced (LGE) imaging, and pre- and post-contrast T1 mapping allow calculation of myocardial extracellular volume (ECV), a surrogate marker of interstitial fibrosis (8,9). LGE is associated with reduced survival across a range of clinical conditions (6), including heart failure with reduced ejection fraction, hypertrophic cardiomyopathy, and HFpEF, in a single study with a small sample size (10).

In HFpEF, however, the pattern of interstitial fibrosis tends to be more diffuse, which cannot be detected using the LGE technique (6,9). CMR T1 parametric mapping techniques enable quantification of the extracellular matrix (9), a surrogate marker of diffuse fibrosis, and have been validated histologically (8). To date, only 4 small prospective HFpEF outcome studies (11–14), including only 1 study (13) with phenotyped, healthy control reference groups have evaluated diffuse fibrosis using either post-contrast T1 times (12) or ECV (11,13,14). Recently, in a further refinement, iECV (ECV indexed to body surface area) was related to outcomes in patients with aortic stenosis (15), but this has not been studied in HFpEF and related to clinical outcomes.

The present study sought to evaluate whether there were differences between the presence and extent of both focal and diffuse fibrosis in HFpEF subjects and those in matched control subjects without heart failure and to determine whether fibrosis provided additional prognostic value beyond conventional clinical and echocardiographic indices.

METHODS

PATIENT POPULATION. HFpEF patients were recruited as part of a prospective, observational cohort study conducted at a tertiary cardiac center and were compared with asymptomatic control subjects. HFpEF inclusion criteria were clinical or radiographic evidence of HF, left ventricular ejection fraction (LVEF) >50% on transthoracic echocardiography, and ≥18 years of age. Exclusion criteria were documented MI in the preceding 6 months, suspected or confirmed cardiomyopathy (e.g., hypertrophic cardiomyopathy, amyloid) or constrictive pericarditis, severe native valve disease, noncardiovascular life expectancy <6 months, severe pulmonary disease (a predicted forced expiratory volume in 1 s of <30% or a predicted forced vital capacity of <50%), estimated glomerular filtration rate of <30 ml/min/m^2, and contraindications to CMR. Asymptomatic control subjects of similar age and sex without known cardiac disease were recruited. Hypertensive control subjects (n = 19) were not excluded because hypertension is highly prevalent in the general population (16), and the authors wanted to identify factors that were different between those with and those without HFpEF.

All subjects were examined for medical history, a review of medical notes, blood sample analysis, transthoracic echocardiography, and CMR during the same visit. The study was approved by the National Research Ethics Service. Written informed consent was provided by all subjects prior to participation.

BLOOD SAMPLES. Blood was sampled for B-type natriuretic peptide (BNP) (immunoassay, Siemens, Erlangen, Germany), hematocrit, hemoglobin, and renal function.

TRANSTHORACIC ECHOCARDIOGRAPHY. Echocardiography was performed as previously reported (17), using an iE 33 system (Philips Medical Systems, Best, The Netherlands). LVEF for study inclusion was calculated using the biplane method or estimated visually where endocardial border definition was poor.

CMR PROTOCOL. The CMR protocol was similar to that previously published by the present authors (17). All scans were performed using a 3-T scanner (Siemens Skyra, Erlangen, Germany) with an 18-channel cardiac coil. Briefly, the protocol consisted of standard breath-held steady-state free precession cine imaging; basal, mid-ventricular, and apical short-axis modified Look-Locker inversion recovery (MOLLI) images pre- and post-contrast; and LGE imaging. The MOLLI sequence (18) was performed with the following parameters: breath-held or free breathing, single-shot sequence, 3,5(3)3(3)5 sampling pattern, 8-mm slice thickness, 300 × 400-mm field of view, 50°-flip angle, 120-ms minimum TI, and 80-ms

increments of inversion time. Similar techniques were used to minimize artifacts, as previously reported by the present authors, and the T1 times were calculated from motion-corrected parametric maps with excellent reproducibility (19). LGE was performed at least 10 min after an injection of 0.15 mmol/kg contrast (Gadovist, Bayer Healthcare, Berlin, Germany) was administered in the same slice positions as the cine images. A 2D phase-sensitive inversion recovery (PSIR) gradient echo sequence was used, and the optimal TI was determined following a standard Look-Locker sequence. Single-shot multislice acquisitions were obtained in patients with poor breath-hold technique or arrhythmia.

CMR IMAGE ANALYSIS. Images were analyzed by a single observer (P.K.), who was blinded to all clinical data, using CVI42 software (Circle Cardiovascular Imaging, Calgary, Canada). Ventricular volumes, EF, and LV mass (excluding papillary muscles) were calculated from the short-axis cine stack (17). Left atrial volumes were calculated using the biplane method, excluding the appendage and pulmonary veins (20). All volumetric and mass data were indexed to body surface area. Indexed LV end-diastolic mass was divided by 1.05, the specific density of myocardial tissue, to derive myocardial volume.

LGE ANALYSIS OF FOCAL MYOCARDIAL FIBROSIS. As described previously (19), qualitative assessment of LGE images was first undertaken by 2 experienced observers (P.K., A.S.H.C.) to achieve consensus for identifying the presence and pattern of LGE (i.e., ischemia vs. nonischemia). If there was disagreement, a third observer (G.P.M.) adjudicated. Fibrosis was considered present if LGE was visualized on both short- and orthogonal long-axis LGE images. Insertion point fibrosis was included in the analysis. The full-width half-maximum technique was then used to quantify fibrosis (21). Examples of focal fibrosis and measurements are shown in **Figure 1**. In some cases, both of the patterns of fibrosis were evident in the same subject and were therefore reanalyzed separately, that is, MI was quantified first, and nonischemic fibrosis was analyzed subsequently after drawing exclusion zone contours around MI areas.

ANALYSIS OF DIFFUSE MYOCARDIAL FIBROSIS. Only the mid-ventricular slice MOLLI images were analyzed to minimize errors (19,22). As described previously by the present authors (19) (**Figure 2**), regions of interest were drawn in the myocardium and blood pool to generate native and post-contrast T1 values from parametric maps. The software provided T1 results for 6 mid-ventricular segments (7-12) corresponding to the American Heart Association nomenclature after use of the anterior RV insertion point as a reference marker. After blood hematocrit values were entered, the software generated segmental ECV values (19). Segments with MI and artifacts were excluded from final T1 and ECV calculation, and segmental values were then averaged. Regions of focal nonischemic fibrosis were included in the ECV calculations, consistent with other studies (9,22). iECV was derived using the formula: [ECV (%) $\times$ left ventricular end-diastolic myocardial volume indexed to body surface area] (15).

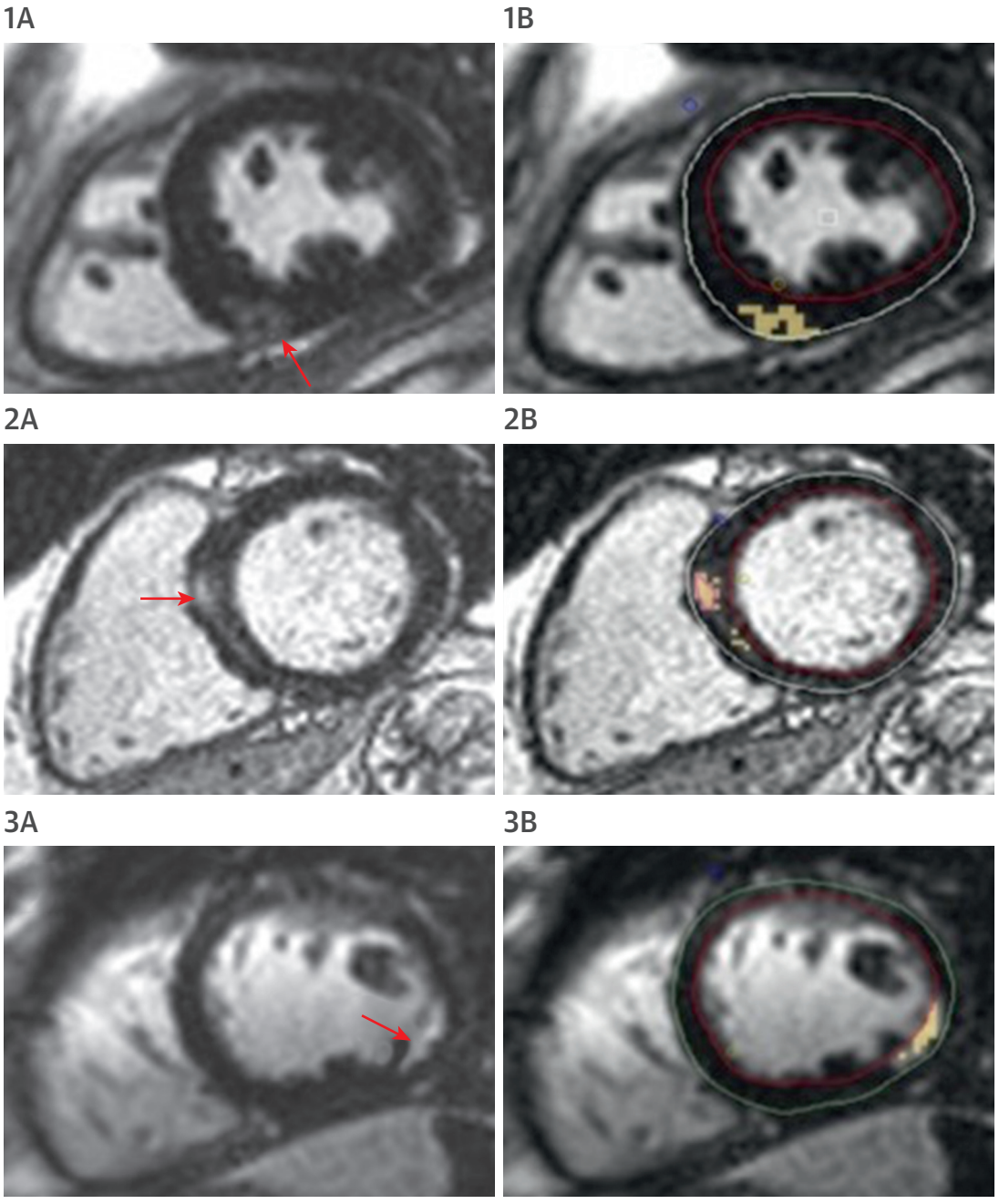
OUTCOME DATA. The clinical endpoint was a composite of mortality or repeat hospitalization for HF. Hospital databases and patient records were selected to obtain outcome data. Patients were followed for a minimum of 6 months post-study entry.

STATISTICAL ANALYSIS. Statistical tests were performed using SPSS version 22 software (IBM Corp., Armonk, New York). Continuous data were assessed for normality by using histograms, Q-Q plots, and the Shapiro-Wilk test. Summary data are mean $\pm$ SD or median (25% to 75% interquartile range [IQR]). Between-group differences were compared using the *t*-test, the Mann-Whitney *U* test, and the chi-square test as appropriate. BNP and creatinine concentrations were $\log_{10}$ transformed before analysis.

Kaplan-Meier analysis was undertaken to calculate event rates. The log-rank test was used to test differences in survival curves. Univariate Cox regression modeling was initially performed to identify variables associated with outcome. Variables tested were those shown to have prognostic importance from published reports with the intention of preventing model overfitting (2,3). Covariates associated with the endpoint at a p value of <0.10 were then entered into subsequent multivariate analyses to identify independent predictors, using both backward and forward stepwise elimination methods. Furthermore, multivariate models were limited to no more than 4 parameters, allowing for approximately 1 parameter per 10 composite events. Four separate clinically relevant multivariate models were generated, including a final model incorporating the strongest predictors. Continuous variables were z-standardized to enable comparison of hazard ratios (HR) on the basis of 1 SD increase in the predictor variable. The combined accuracy of the independent variables to predict events was then tested by receiver operator characteristics (ROC) analysis.

Pearson's correlation tests were performed to check for potential associations between iECV and

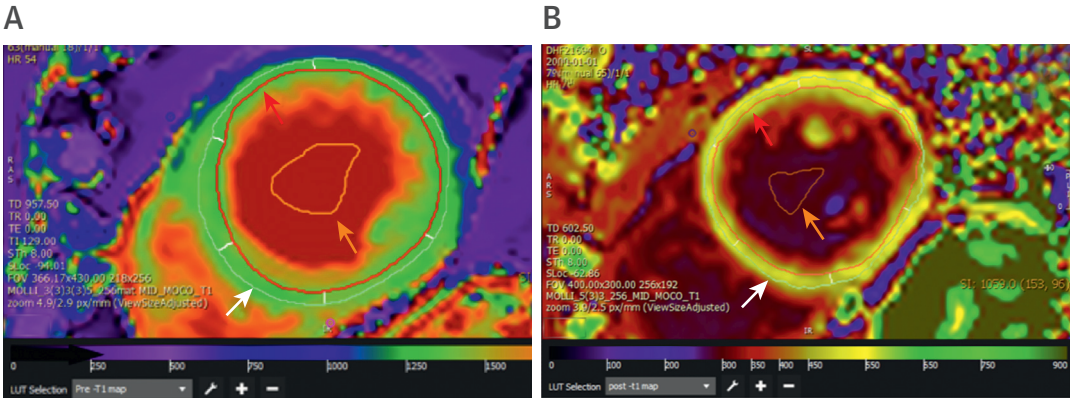
FIGURE 1 Different Patterns of Focal Fibrosis And Quantification



Late gadolinium enhancement images demonstrating **(A)** focal fibrosis **(red arrows)** and **(B)** corresponding, quantified burden **(highlighted in yellow)** using the full width half-maximum technique: 1 = insertion point fibrosis; 2 = mid-wall fibrosis; 3 = subendocardial myocardial infarction.

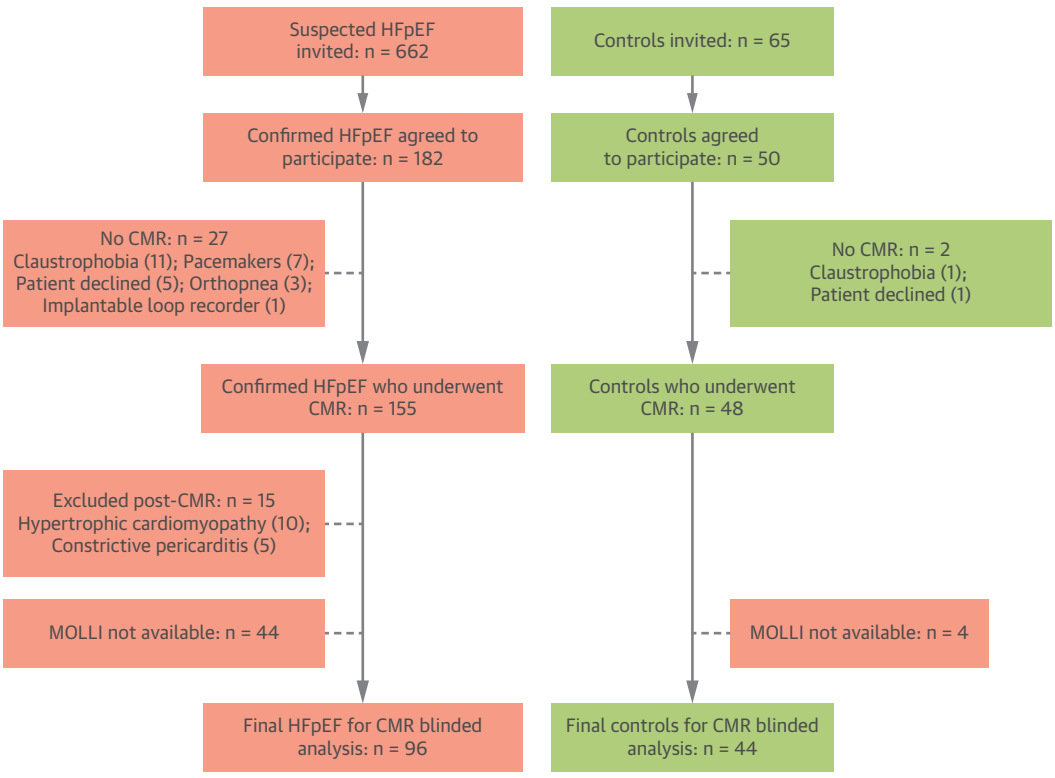
ECV and other variables. Linear regression modeling with stepwise selection methods was undertaken to identify the strongest independent associations. In cases of collinearity, the variable with the highest coefficient was entered into multivariate analysis. A p value of <0.05 was considered significant. Assessments of intraobserver and interobserver variability for focal fibrosis and ECV calculation were undertaken from 10 randomly selected patients, a minimum of 4 weeks apart (by P.K. and J.R.A.).

FIGURE 2 **Calculation of Extracellular Volume**



(A) Pre- and **(B)** post-contrast contours in the mid-myocardial area are indicated by **white** and **red arrows**, respectively. The left ventricular cavity is shown **(orange)** to enable derivation of T1 values and extracellular volume.

FIGURE 3 **Study Recruitment Overview**



Flow chart shows recruitment and CMR assessments. CMR = cardiac magnetic resonance imaging; HFpEF = heart failure with preserved ejection fraction; MOLLI = modified Look-Locker inversion recovery sequence.

TABLE 1 Baseline Clinical Characteristics

	HFpEF Subjects (n = 96)	Control Subjects (n = 44)	p Value
Demographics			
Age, yrs	73 ± 9	73 ± 5	0.784
Males	46 (48)	21 (48)	0.983
Body mass index, kg/m^2	34 ± 7	25 ± 3	<0.0001
Clinical findings			
Heart rate, beats/min	69 ± 14	68 ± 11	0.614
Systolic blood pressure, mm Hg	146 ± 25	151 ± 24	0.282
Diastolic blood pressure, mm Hg	75 ± 12	80 ± 11	0.025
Sinus rhythm	64 (67)	44 (100)	<0.0001
Atrial fibrillation	32 (33)	0 (0)	<0.0001
Medical history			
Diabetes	48 (50)	0 (0)	<0.0001
Hypertension	86 (90)	19 (43)	<0.0001
Angina	19 (20)	0 (0)	0.002
Known myocardial infarction	13 (14)	0 (0)	0.010
Asthma or COPD	18 (19)	2 (5)	0.026
Smoking	52 (54)	16 (36)	0.050
Hypercholesterolemia	45 (47)	16 (36)	0.244
Peripheral vascular disease	2 (2)	0 (0)	0.335
TIA or CVA	9 (9)	1 (2)	0.006
Medication			
Beta-blocker	68 (71)	1 (2)	<0.0001
ACE inhibitor or ARB	82 (85)	9 (20)	<0.0001
Aldosterone antagonist	31 (32)	0 (0)	<0.0001
Loop diuretic	76 (79)	0 (0)	<0.0001
Functional status			
NYHA functional class I/II	68 (71)	NA	NA
NYHA functional class III/IV	28 (29)	NA	NA
Sera values			
Sodium, mmol/l	139.5 ± 3.4	140.2 ± 1.8	0.084
Urea, mmol/l	8.5 ± 3.6	6.1 ± 1.5	<0.0001
Median creatinine, mmol/l (IQR)	87 (71-113)	69 (56-85)	<0.0001
Hemoglobin, g/l	129 ± 19	140 ± 14	<0.0001
Hematocrit	38 ± 5	41 ± 4	0.013
Median BNP, ng/l	144 (66-250)	33 (24-44)	<0.0001

Values are mean ± SD, n (%), or median (interquartile range). The p values are results for the *t*-test or the chi-square test.

ACE = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; CMR = cardiac magnetic resonance imaging; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident; HFpEF = heart failure with preserved ejection fraction; NA = not applicable; NYHA = New York Heart Association; TIA = transient ischemic attack.

RESULTS

A total of 232 subjects were enrolled (HFpEF n = 182; control subjects n = 50), of whom 96 patients with HFpEF and 44 control subjects had complete datasets including T1 maps. Reasons for exclusion are shown in Figure 3.

Baseline clinical and imaging characteristics are summarized in Tables 1 and 2. In the HFpEF group, there was a high burden of obesity, hypertension, diabetes, and atrial fibrillation. Nearly one-fifth of the group had a history of angina or lung disease. More than two-thirds (71%) had evidence of prior pulmonary congestion on chest radiography, and a substantial minority (29%) assessed were New York Heart Association (NYHA) functional class III/IV. Compared to control subjects, HFpEF patients had worse renal function, increased LV end-diastolic mass indexed but not LV volumes and more concentric remodeling (mass/volume). HFpEF patients also had higher filling pressures (E/E′), BNP concentrations, and maximal left atrial volume indexed (LAVImax), consistent with worse diastolic function.

FOCAL FIBROSIS. Results are shown in Table 2. Approximately half (n = 49 [51%]) of the HFpEF cohort had evidence of focal fibrosis (vs. n = 3 [7%] in the control subjects; p < 0.0001). The predominant pattern of fibrosis in the HFpEF group was non-ischemic in 36 patients (38%). MI was present in 17 patients (18%), including 7 patients with previously unknown MI, but in none of the control subjects. Both MI and nonischemic fibrosis were present in 4 patients. In those with HFpEF exhibiting LGE hyper-enhancement, the quantified fibrotic burden was relatively small: 3% of LV mass (3% ischemic patients and 2.9% nonischemic patients).

DIFFUSE FIBROSIS. Native T1, post-contrast T1, ECV, and iECV values in HFpEF subjects were all significantly different from those in control subjects, overall. Both ECV (27.8%) and iECV (13.7 ml/m^2) values were also elevated in HFpEF subjects compared to those in hypertensive (24.8%; p = 0.007; 11.3 ml/m^2; p = 0.002) and nonhypertensive (25.7%; p = 0.032; 10.7 ml/m^2; p < 0.0001) control subjects. There were no differences between ECV (p = 0.346) and iECV (p = 0.505) values in hypertensive control subjects and those in nonhypertensive control subjects.

INTEROBSERVER AND INTRAOBSERVER ASSESSMENTS. Data quantification for T1 mapping and LGE are shown in Supplemental Table 1. Interobserver and intraobserver variability were excellent for all measurements (intraclass correlation coefficients: >0.95).

OUTCOMES. During median follow-up of 1,429 days (IQR: 1,157 to 1,657 days), there were 42 events (14 deaths, 28 HF hospitalizations) in patients with HFpEF. There were no events in the control group.

PREDICTORS OF CLINICAL OUTCOME. On univariate analysis (Supplemental Table 2), 10 variables were associated with adverse outcomes: prior HF hospitalization, lung disease, hemoglobin, log BNP, E/E′, LV mass/volume, LAVImax, MI, ECV, and iECV. The presence of (or quantified) nonischemic focal fibrosis was not associated with outcome (p = 0.164 and p = 0.210, respectively). Kaplan-Meier survival curves stratified according to quartiles of iECV and ECV are shown in Figure 4. The highest quartiles of

both iECV (>16.8 ml/m^2) and ECV (>30.7%) were associated with the greatest risk of adverse outcome (log-rank p = 0.017 and 0.009, respectively). On multivariate analysis (Table 3), iECV remained significantly associated with outcome in 3 separate models incorporating clinical factors, markers of diastolic dysfunction, and LV structural/remodeling parameters. In a final model consisting of the strongest predictors overall, iECV (HR: 1.689; 95% confidence interval [CI]: 1.141 to 2.501; p = 0.009) remained an independent predictor along with E/E′ (HR: 1.716; 95% CI: 1.191 to 2.472; p = 0.004) and prior HF hospitalization (HR: 2.537; 95% CI: 1.090 to 5.902; p = 0.031). The final multivariate model predicted outcomes with an area under the ROC curve of 0.724; p = 0.001. Although ECV was also a strong predictor, it was not independent in the diastolic dysfunction or final combined model inclusive of the strongest predictors (Supplemental Table 3).

ASSOCIATIONS OF iECV (AND ECV). Univariate associations of iECV in HFpEF are shown in Table 4 (see also Supplemental Table 4 for ECV). Because the calculation of iECV is dependent on LV mass, which in turn is closely linked with LV volumetric measurements, both of these metrics were excluded from further analysis. Indeed, the correlations of iECV with LV mass and left ventricular end-diastolic volume indexed (LVEDVI) yielded Pearson's $r = 0.888$ and $r = 0.578$, respectively ($p < 0.0001$ for both). For LV mass correlation with LVEDVI, r was 0.582; $p < 0.0001$.

Heart rate, systolic blood pressure, diabetes, history of hypertension, creatinine level, LV mass/volume, right ventricular end-diastolic volume indexed (RVEDVI) and LAVImax remained significant on multivariate analysis. Of those independently associated with iECV (Supplemental Figure 1), the strongest correlations were with RVEDVI ($r = 0.456$; $p < 0.0001$), LV mass/volume ($r = 0.348$; p = 0.001), creatinine level ($r = 0.271$; p = 0.009), and LAVImax ($r = 0.269$; p = 0.009).

TABLE 2 Baseline Imaging Characteristics

	HFpEF Subjects (n = 96)	Control Subjects (n = 44)	p Value
Previous chest radiography			
Pulmonary edema	68 (71)	NA	NA
Raised cardiothoracic ratio	65 (68)	NA	NA
Pleural effusion	33 (34)	NA	NA
Echocardiography			
E/E′ ratio	12.8 ± 4.8	9.0 ± 2.9	<0.0001
CMR volumes, function, and LV mass			
LVEF	56 ± 6	58 ± 5	0.406
LVEDVI, ml/m^2	78 ± 18	81 ± 14	0.409
LVESVI, ml/m^2	34 ± 11	34 ± 8	0.708
LVMI, g/m^2	51 ± 13	46 ± 9	0.004
LV mass/volume	0.68 ± 0.15	0.57 ± 0.09	<0.0001
RVEF	54 ± 10	56 ± 6	0.090
RVEDVI, ml/m^2	79 ± 20	83 ± 15	0.307
RVESVI, ml/m^2	37 ± 14	37 ± 9	0.922
LAVImax, ml/m^2	54 ± 27	35 ± 12	<0.0001
LGE focal fibrosis			
Total focal fibrosis	49 (51)	3 (7)	<0.0001
Total focal fibrosis, g	3.6 (2.0-6.4)	2.5 (0.5-2.6)	<0.0001
Total focal fibrosis, % of LV mass	3.0 (2.0-6.3)	2.0 (0.8-3.0)	<0.0001
Ischemic pattern	17 (18)	0 (0)	<0.0001
Ischemic pattern, % of LV mass	3.0 (2.2-4.6)	NA	NA
Nonischemic pattern	36 (38)	3 (7)	<0.0001
Nonischemic pattern, % of LV mass	2.9 (1.4-6.5)	2.0 (0.8-3.0)	<0.0001
T1 mapping of diffuse fibrosis			
Native myocardial T1, ms	1,234 ± 73	1,197 ± 91	0.021
Post-contrast myocardial T1, ms	461 ± 63	495 ± 85	0.011
ECV	27.8 ± 4.6	25.3 ± 3.2	<0.0001
iECV, ml/m^2	13.7 ± 4.4	10.9 ± 2.8	<0.0001

Values are mean ± SD, n (%), or median (IQR).

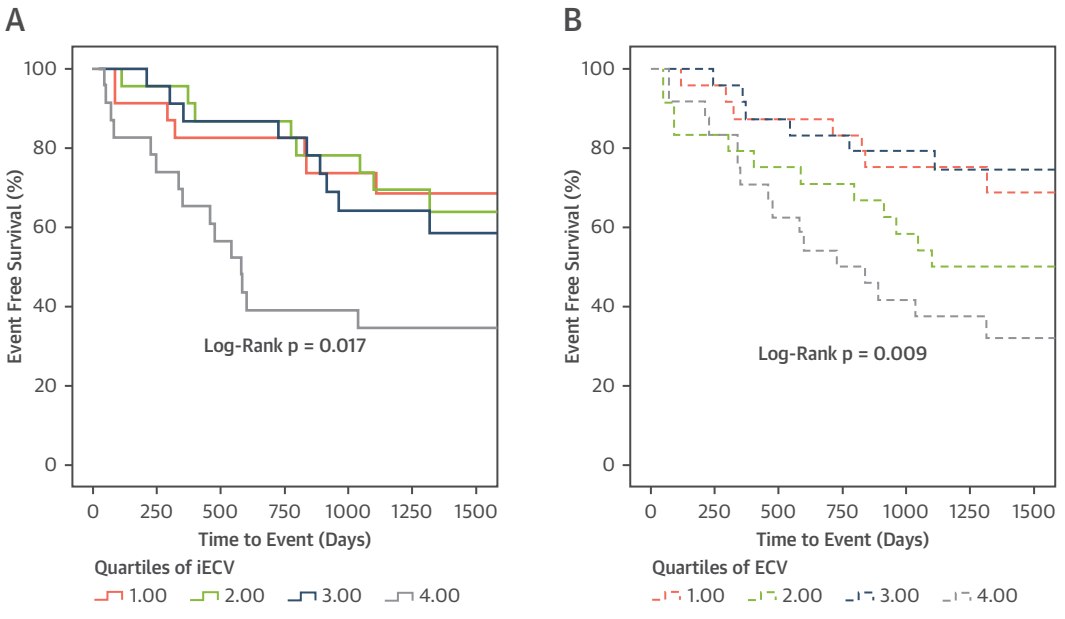
ECV = extracellular volume; E/E′ = mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E'); iECV = indexed extracellular volume; LAVImax = maximal left atrial volume indexed to body surface area; LGE = late gadolinium enhancement imaging; LVEF = left ventricular ejection fraction; LVMI = left ventricular end-diastolic mass indexed to body surface area; LVEDVI = left ventricular end-diastolic volume indexed to body surface area; LVESVI = left ventricular end-systolic volume indexed to body surface area NA = not applicable; RVEF = right ventricular ejection fraction; RVEDVI = right ventricular end-diastolic volume indexed to body surface area; RVESVI = right ventricular end-systolic volume indexed to body surface area; other abbreviations as in Table 1.

DISCUSSION

This is the first outcome study to systematically evaluate fibrotic burden in well-phenotyped cohorts of HFpEF subjects and control subjects of similar age and sex, using CMR. Furthermore, iECV was evaluated as a newer marker of diffuse fibrosis for the first time in HFpEF subjects and this was related to clinical outcome. The following are the principal findings: 1) both focal and diffuse fibrosis are elevated in HFpEF subjects compared to asymptomatic control subjects; 2) diffuse fibrosis as assessed by iECV independently predicted prognosis in HFpEF; and 3) iECV was associated with markers of ventricular and left atrial remodeling, as well as renal dysfunction.

FOCAL FIBROSIS. Overall, there was more focal fibrosis (ischemic and nonischemic) in HFpEF subjects than in control subjects. New cases of previously unknown MI were also detected, which were generally small, in keeping with overall preservation of LVEF. Unlike the only previous study to demonstrate independent prediction of outcomes with LGE-quantified focal fibrosis in HFpEF subjects (10), the present patients had less burden of nonischemic fibrosis, and LGE was not related to outcomes. The reason for this difference may be due to the quantification method used for focal fibrosis. Although various semiautomated quantification methods exist,

FIGURE 4 Survival Analysis for the Composite of Mortality or Repeat Hospitalization for Heart Failure



Kaplan-Meier survival curves stratified according to quartiles of iECV and ECV. ECV = extracellular volume; iECV = indexed extracellular volume.

the full-width half-maximum technique was used, which is the most reproducible across the spectrum of both ischemic and nonischemic causes (21). The previous study used a threshold of > 2 SD of signal intensity above remote myocardium to define fibrosis, which can result in overestimation and measurement errors from partial volume effects (21), and in addition, as the ECV in these patients is diffusely increased, defining normal myocardium is problematic.

TABLE 3 Multivariate Predictor Models Inclusive of iECV for the Composite Endpoint of Death and/or Hospitalization With Heart Failure

	Hazard Ratio (95% CI)	p Value
Model 1: Clinical		
Prior HF hospitalization	2.573 (1.135-5.837)	0.024
Asthma or COPD	2.085 (0.972-4.473)	0.059
Hemoglobin, g/l	0.747 (0.525-1.063)	0.105
+ iECV, ml/m^2	1.530 (1.107-2.113)	0.010
Model 2: Diastolic Dysfunction		
BNP, ng/l	1.020 (0.702-1.484)	0.916
E/E′ ratio	1.717 (1.178-2.503)	0.005
LAVImax	1.222 (0.894-1.670)	0.209
+ iECV, ml/m^2	1.613 (1.111-2.342)	0.012
Model 3: LV Structural/Remodeling		
LV mass/volume	1.251 (0.905-1.731)	0.176
Presence of MI	1.590 (0.765-3.307)	0.214
+ iECV (ml/m^2)	1.463 (1.060-2.020)	0.021
Model 4: Strongest Parameters Combined		
Prior HF hospitalization	2.537 (1.090-5.902)	0.031
Asthma or COPD	2.083 (0.883-4.915)	0.094
E/E′ ratio	1.716 (1.191-2.472)	0.004
+ iECV (ml/m^2)	1.689 (1.141-2.501)	0.009

CI = confidence interval; other abbreviations as in Tables 1 and 2.

DIFFUSE FIBROSIS. Recently, iECV has been proposed as a novel marker of diffuse fibrosis (15). In a cohort of patients with aortic stenosis, iECV correlated well with histological fibrosis, discriminated between patients with disease and healthy control subjects, and was the only T1 mapping parameter to differentiate between differing grades of valve stenosis. Furthermore, it demonstrated association with clinical outcomes (15).

ECV (and iECV) were quantifiable in 97% in the present subjects and with a high degree of reproducibility, which is clinically relevant. Recently, ECV was shown to predict outcome in a large retrospective study (n = 1,172), encompassing all-comers referred for CMR (22). In that study, ECV analysis was similar to the present method and predicted outcomes independently across the whole cohort, regardless of EF. Similar to the present results, diffuse fibrosis was more strongly associated with outcomes than nonischemic focal fibrosis. However, as well as selection bias in only recruiting patients referred for clinical

CMR, the proportion of subjects with clinical HF was small, and it is unclear how many patients had HFpEF.

In the present study, although native T1 was also significantly increased, ECV and iECV were the only markers of diffuse fibrosis to provide prognostic value in multivariate analysis. Native T1 values reflect both intra- and extracellular changes, whereas post-contrast T1 is subject to a variety of confounders (8). On the other hand, ECV is effectively a ratio, taking into account both pre- and post-contrast values and canceling out systematic biases in T1 measurements. ECV and iECV are therefore more likely to provide a better reflection of diffuse fibrosis (9). This is further supported by evidence showing better correlation of ECV with histologically measured fibrosis than for native or post-contrast T1 values (8,9).

To date, only 4 prior prospective studies have demonstrated the association between diffuse fibrosis and clinical outcomes in HFpEF. The first study (12) used post-contrast T1 times as a measurement of diffuse fibrosis in a much smaller cohort of HFpEF subjects (n = 61) and had the intrinsic limitations outlined above. In the second study (n = 117) (11), ECV was also associated with adverse events. In contrast to the present study, focal fibrosis was defined as myocardial signal intensity of >5 SD above the mean intensity of healthy myocardium, and such regions were excluded from ECV calculations. Furthermore, ECV was associated with outcomes when confined to CMR parameters only but not in a combined multivariate model including clinical variables, unlike the present study in which iECV was used. In the third study (14), diffuse fibrosis, that is, elevated ECV, was suggested to precede overt clinical HFpEF and was also related to outcomes in those (n = 160) with or who were "at risk" (n = 250) of HFpEF but in all comers with preserved ejection fraction referred for clinical CMR, again highlighting concerns regarding referral bias. In the recently published final study, ECV independently predicted adverse outcomes in HFpEF (n = 118). However, the control group was much smaller (n = 26), and median follow-up (11 months) was substantially shorter than that in the present study.

IMPORTANCE OF FIBROTIC ASSESSMENT IN HFpEF. Our results shed further insight into the pathophysiology of HFpEF, with diffuse fibrosis playing a central role. Diabetes (23), pressure overload (increasing systolic blood pressure and/or history of hypertension) (24) and renal impairment (25) as likely causative factors for diffuse myocardial fibrosis development have previously been reported. Furthermore, this hypothesis is supported by epidemiological and clinical trial data. whereby HFpEF is heavily laden with such co-morbidities (2). Myocardial fibrosis is further intrinsically linked to LV stiffness and chamber modification, which likely explains the greater adverse remodeling and diastolic dysfunction seen in the present predominantly hypertensive HFpEF cohort (5,26). Additionally, right ventricular and left atrial remodeling are likely downstream consequences (27).

Previous studies have also highlighted the association between ECV and strain measurements of both systolic and diastolic dysfunctions in hypertensive LVH subjects at risk of developing HFpEF (28). In a recent small study, ECV was the imaging parameter that best discriminated between HFpEF (n = 62) and hypertensive (n = 22) heart disease subjects (29). The independent associations between iECV with LAVImax and serum creatinine in the present study are also similar to results from the PARAMOUNT study of HFpEF (30). In that trial, ST2, galectin-3, matrix metalloproteinase-2, and collagen type III N-terminal propeptide as surrogate plasma markers of fibrosis (and the extracellular matrix) also correlated strongly with left atrial volume and renal

TABLE 4 Univariate and Multivariate Linear Regression Models for the Associations With iECV

	Univariate Analysis		Multivariate Analysis	
	Standardized Coefficients (Beta)	p Value	Standardized Coefficients (Beta)	p Value
Clinical				
Heart rate, beats/min	−0.188	0.072	−0.173	0.025
Males	0.504	<0.0001	-	NS
Systolic blood pressure, mm Hg	0.254	0.015	0.230	0.001
BMI, kg/m^2	−0.230	0.028	-	NS
Diabetes	0.181	0.084	0.205	0.005
Hypertension	0.179	0.088	0.162	0.028
Sera values				
Creatinine, mmol/l	0.271	0.009	0.196	<0.008
Hemoglobin, g/l	−0.188	0.072	-	NS
BNP, ng/l	0.449	<0.0001	-	NS
CMR				
LVEDVI	0.578	<0.0001	-	-
LVEDMI	0.888	<0.0001	-	-
LV mass/volume	0.348	0.001	0.541	<0.0001
*RVEDVI, ml/m^2	0.456	<0.0001	0.538	<0.0001
*RVESVI, ml/m^2	0.300	0.004	-	-
LAVImax, ml/m^2	0.269	0.009	0.264	0.001

Overall multivariate model R^2 = 0.657. *Variables which exhibited significant collinearity; of these variables, RVEDVI (ml/m^2) was entered into multivariate analysis. LVEDVI and LVEDMI were not entered into multivariate analysis.

BMI = body mass index; NS = not significant; other abbreviations as in Tables 1 and 2.

dysfunction (estimated glomerular filtration rate). Interestingly, unlike the aforementioned study, our data shows that iECV did not correlate with E/E′ or natriuretic peptides, which may partly be explained by the differing fluid status of subjects, most of whom invariably had a period of offloading with diuretics prior to CMR as part of routine clinical care (24,31).

iECV appears to detect diseased myocardium not readily apparent with LGE, which was not associated with outcome. Unlike irreversible replacement fibrosis identified by LGE, diffuse fibrosis may be reversible and therefore a potential therapeutic target (22). The present work lends further support to a growing body of evidence highlighting ECV (and iECV) as promising biomarkers across a spectrum of cardiac pathologies. Furthermore, their association with outcomes appears stronger than conventional LGE assessment, which has historically been more extensively studied (9,22).

In the present cohort, iECV was a more powerful predictor of outcomes, even compared to ECV. This is most likely because iECV depends upon LV mass for calculation, which in turn is closely linked to LV volumes, all of which have been linked previously with outcomes (26).

STUDY LIMITATIONS. Although the authors' definition of HFpEF was not in accordance with the latest European Society of Cardiology guidelines (32), a pragmatic approach was taken to reflect a real-world setting. In particular, diastolic dysfunction was not a prerequisite for study entry because it is reportedly absent in nearly one-third of contemporary HFpEF clinical trials and conversely also identified in a significant proportion of asymptomatic elderly subjects (33). As the iECV data have been acquired only once, causality between causes of increased iECV cannot be inferred. The full-width half-maximum technique for quantification of LGE was used, which may allow comparison with other studies using this technique, but it should be borne in mind that significant variability arises from drawing LV contours (34). A proportion of consecutive trial subjects (nearly 24%) who underwent CMR did not undergo MOLLI imaging due to the sequence not being available (i.e., not performed). This may have introduced potential bias. However, a comparison between the HFpEF group who underwent MOLLI imaging and those who did not (Supplemental Table 5) revealed no major differences in baseline clinical characteristics, providing strong support that our results are likely representative across the whole cohort.

CONCLUSIONS

Focal and diffuse fibrosis are more prevalent in HFpEF subjects than in healthy control subjects of similar age and sex. Diffuse fibrosis as assessed by iECV in HFpEF correlates with markers of biventricular and left atrial remodeling as well as renal dysfunction and is an independent predictor of adverse outcomes in HFpEF.

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ADDRESS FOR CORRESPONDENCE: Dr. Prathap Kanagala, Department of Cardiovascular Sciences, Glenfield Hospital, Groby Road, Leicester LE3 9QP, United Kingdom. E-mail: pkk12@leicester.ac.uk.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: HFpEF is seen in up to 50% of patients hospitalized for heart failure and is associated with a poor prognosis but no definite treatments. Using CMR, the present study shows that patients with HFpEF have increased levels of diffuse and focal fibrosis associated with concentric remodeling and diastolic dysfunction. In HFpEF, diffuse fibrosis assessed using a new marker (indexed extracellular volume) was independently associated with adverse prognosis and may be useful in future risk stratification of these patients.

TRANSLATIONAL OUTLOOK: As diffuse fibrosis is reversible, randomized controlled trials of interventions to reduce diffuse fibrosis should be considered to prevent and potentially treat HFpEF.

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APPENDIX For supplemental tables and a figure, please see the online version of this paper.