Background—Extracellular matrix expansion is a key element of ventricular remodeling and a potential therapeutic target. Cardiovascular magnetic resonance (CMR) T1-mapping techniques are increasingly used to evaluate myocardial extracellular volume (ECV); however, the most widely applied methods are without histological validation. Our aim was to perform comprehensive validation of (1) dynamic-equilibrium CMR (DynEq-CMR), where ECV is quantified using hematocrit-adjusted myocardial and blood T1 values measured before and after gadolinium bolus; and (2) isolated measurement of myocardial T1, used as an ECV surrogate.

Methods and Results—Whole-heart histological validation was performed using 96 tissue samples, analyzed for picrosirius red collagen volume fraction, obtained from each of 16 segments of the explanted hearts of 6 patients undergoing heart transplantation who had prospectively undergone CMR before transplantation (median interval between CMR and transplantation, 29 days). DynEq-CMR–derived ECV was calculated from T1 measurements made using a modified Look-Locker inversion recovery sequence before and 10 and 15 minutes post contrast. In addition, ECV was measured 2 to 20 minutes post contrast in 30 healthy volunteers. There was a strong linear relationship between DynEq-CMR–derived ECV and histological collagen volume fraction (P<0.001; within-subject: r=0.745; P<0.001; r2=0.555 and between-subject: r=0.945; P<0.01; r2=0.893; for ECV calculated using 15-minute postcontrast T1). Correlation was maintained throughout the entire heart. Isolated postcontrast T1 measurement showed significant within-subject correlation with histological collagen volume fraction (r=−0.741; P<0.001; r2=0.550 for 15-minute postcontrast T1), but between-subject correlations were not significant. DynEq-CMR–derived ECV varied significantly according to contrast dose, myocardial region, and sex.

Conclusions—DynEq-CMR–derived ECV shows a good correlation with histological collagen volume fraction throughout the whole heart. Isolated postcontrast T1 measurement is insufficient for ECV assessment. (Circ Cardiovasc Imaging. 2013;6:373-383.)

Key Words: collagen ■ histopathology ■ MRI ■ myocardial fibrosis

Expansion of the myocardial interstitial space is a feature common to a range of cardiac pathologies and seems fundamental to the process of adverse left ventricular (LV) remodeling.1-4 Interstitial expansion, in part attributable to accumulation of collagen, is associated with changes in mechanical and electric properties of the myocardium and as such may represent a sentinel phenotype, transitional between healthy myocardium and diseased myocardium associated with increased mortality risk.5-9 Furthermore, interstitial expansion is reversible and a potential therapeutic target.10-12 Quantification of the myocardial interstitial space, or extracellular volume (ECV), may therefore represent an important diagnostic and prognostic biomarker.

Clinical perspective on p 383
Endomyocardial biopsy represents the current gold standard for assessment of the myocardial interstitium; however, its invasive nature and lack of whole heart coverage restrict its clinical use for this purpose. Cardiovascular magnetic resonance (CMR) techniques that potentially allow noninvasive evaluation of the interstitial space have generated considerable recent interest.

Gadolinium chelates are standard extracellular contrast agents that potently shorten T1 relaxation time. Gadolinium concentration is directly related to change in the relaxation rate R1 (where R1 is the reciprocal of T1). Therefore, T1 measurement (mapping) before and after contrast administration can be used to calculate gadolinium concentration in myocardium and blood. At contrast agent equilibrium, gadolinium concentration in blood and myocardium, and because the volume of distribution of gadolinium (or ECV) in blood is known from the hematocrit, myocardial ECV can be calculated.

This approach requires a steady state to exist between blood and myocardial contrast agent for the effect of contrast agent kinetics to be removed. Using a primed gadolinium infusion to achieve contrast agent equilibrium (equilibrium contrast CMR), Flett et al13 demonstrated a strong correlation between CMR measurement of myocardial ECV and histological collagen volume fraction. However, because of the potentially cumbersome nature of this technique, 2 alternative methods of assessing ECV have become more widely adopted.

The first, which involves a contrast agent bolus (ie, without a subsequent infusion), assumes contrast agent kinetic effects to be negligible because of a dynamic equilibrium between blood and myocardium (dynamic-equilibrium CMR [DynEq-CMR]).9,14-19 In the second method, an isolated myocardial T1 measurement is made at a fixed time point after a bolus of contrast agent (ie, without precontrast or blood T1 measurements) and is used as a surrogate measure of ECV (isolated-T1).20-23 However, despite these techniques being applied to a rapidly expanding number of pathologies, both have potential shortcomings; the assumed dynamic equilibrium proposed in the first method may not always hold true in reality, and the second technique may be confounded by factors, such as body fat percentage, renal function, and hematocrit. Systematic in vivo histological validation of these techniques in humans is lacking.24-26

The aims of the current study were to first provide comprehensive, whole-heart, histological validation of the DynEq-CMR technique for measurement of myocardial ECV. This, at the same time, allowed assessment of the validity of isolated-T1 technique. Second, we aimed to assess the effect of contrast agent dose, postcontrast acquisition time, myocardial regionality, cardiac cycle, and sex on DynEq-CMR ECV measurement.

Methods

Study Design

All research was performed at University Hospital of South Manchester National Health Service (NHS) Trust, United Kingdom. An ethics committee of the UK National Research Ethics Service approved the study, and written informed consent was obtained from all participants. The work was conducted according to the Helsinki Declaration.

The study comprises 3 parts: (1) phantom studies; performed to validate the accuracy of the T1 mapping sequence used and to calculate a T1 heart-rate correction algorithm (see the online-only Data Supplement); (2) histological validation. CMR was performed prospectively in patients awaiting heart transplantation. When these patients subsequently underwent transplantation, the explanted hearts were used to provide whole-heart histological validation of the DynEq-CMR and isolated-T1 techniques; and (3) the effect of contrast dose, postcontrast acquisition time, myocardial regionality, cardiac cycle, and sex on DynEq-CMR was assessed in healthy volunteers.

CMR Imaging

All CMR imaging was performed with the same 1.5 Tesla scanner (Magnemot Avanto, Siemens Healthcare Sector, Erlangen, Germany) equipped with a 32-element phased-array coil.

T1 measurements were made using an ECG-gated single-shot modified Look-Locker inversion recovery (MOLLI) sequence as described by Messroghli et al.27 Typical parameters were field of view 340×255 mm, matrix 192×138, 8-mm slice thickness, flip angle 35°, parallel imaging factor 2 with 24 reference lines, 6/8 partial Fourier k-space sampling, acquisition time 201 ms for a single image, and initial effective inversion time (T1eff) 100 ms with a T1eff increment of 80 ms. To sample T1 recovery, serial single-shot diastolic images were acquired every heart beat after 3 nonelective adiabatic inversion pulses (ie, 3, 3, and 5 images after each inversion pulse, totaling 11 images), with 3 dummy heart beats before the second and third inversion pulses to allow recovery (17 heart beat total acquisition duration).

The gadolinium contrast agent used throughout the study was gadodiamide dimeglumine (Gd-DTPA; Magnevist; Bayer Healthcare, Leverkusen, Germany), administered as a single bolus of 2 mL/s followed by a 30-mL saline chaser bolus delivered at the same flow rate using a power injector.

Histological Validation

All patients on the heart transplant waiting list at University Hospital of South Manchester NHS Trust, United Kingdom (1 of 6 UK adult heart transplant centers), between January 1, 2011 and July 1, 2012, were screened for study eligibility. Of the 54 patients on the waiting list during this period, 41 had an intracardiac device that prohibited CMR, and 2 of the 13 patients without devices were deemed too unwell for CMR by the supervising medical team. Eleven patients were, therefore, invited for CMR, of which 2 refused consent. The 9 remaining patients underwent CMR, of which 6 underwent heart transplantation. As prespecified, no patients with acute myocardial inflammation (known or suspected myocarditis, acute myocardial infarction) or cardiac amyloidosis were included.

The CMR protocol included LV short-axis MOLLI sequence acquisition at basal, mid, and apical ventricular levels before, and 10 and 15 minutes after a bolus of 0.20 mmol/kg Gd-DTPA contrast agent (Figure 1). Standard long- and short-axis steady-state-free precession cine imaging was performed to assess LV mass and volumetric parameters. Standard late gadolinium enhancement (LGE) imaging was performed ≥10 minutes after contrast agent administration using spoiled gradient echo segmented inversion recovery, and phase-sensitive inversion recovery segmented gradient echo, sequences.28 Blood samples were taken at the time of CMR to measure hematocrit.

At the time of transplantation, the explanted hearts were immediately fixed in 10% buffered formalin. The hearts were cut at basal, mid, and apical LV levels using the MOLLI slice positions as guidance (Figure 1), and 16 tissue blocks were taken from the LV of each heart (96 samples in total) according to the American Heart Association/American College of Cardiology 16-segment model29 before being embedded in paraffin and stained with picrosirius red. High-power magnification (>200) digital images, excluding perivascular areas, underwent automated image analysis (macro written in ImageJ Bethesda, MD).30 As described previously,13 a combination of SD from mean signal and isodata automatic thresholding derived the collagen area, expressed as a percentage of total myocardial area, excluding fixation artifact. Twelve high-power fields were assessed.
To determine whether CVF measurements were affected by the degree of magnification used to acquire the histological images, CVF measurements were repeated at 2 additional magnifications (×50 and ×100) in 2 randomly selected tissue blocks from each patient (12 samples [13%] in total, ie, an additional 288 histological images).

Effect of Contrast Agent Dose, Acquisition Time, Myocardial Regionality, Cardiac Cycle, and Sex

Thirty completely asymptomatic healthy volunteers, with no known risk factors or history of cardiac disease, normal physical examination, and normal ECG, underwent CMR (subjects were not patients who had been referred for CMR that was subsequently found to be normal). CMR (cine and LGE imaging) was normal in all cases.

Volunteers were prospectively split into 3 age- and sex-matched groups and received Gd-DTPA contrast agent at the following doses: group A, 0.10 mmol/kg; group B, 0.15 mmol/kg; and group C, 0.20 mmol/kg.

The CMR protocol included a MOLLI sequence acquisition in short-axis at midventricular level before and at 2-, 4-, 6-, 8-, 10-, 12-, 14-, 15-, 16-, 18-, and 20-minute postcontrast administration. In addition, MOLLI imaging was also performed in early systole (150 ms after the R wave) precontrast and at 10- and 15-minute postcontrast administration. Standard long- and short-axis steady-state–free precession cine imaging was performed to assess LV mass and volumetric parameters. LGE imaging of the entire heart in short-axis was performed using single-shot phase sensitive inversion recovery imaging. Blood samples were taken at the time of CMR to measure the hematocrit.

CMR Image Analysis

For T₁ relaxation time measurements, endocardial and epicardial contours were drawn on the MOLLI images using Osirix Imaging Software (Pixmeo; Switzerland; version 4.0). An additional region of interest was drawn in the blood pool, avoiding papillary muscles and trabeculae, and the anterior right ventricular septal insertion point was marked. Regions of interest were manually translated on each T1eff time image to allow motion compensation. The same regions of interest were used on corresponding pre- and postcontrast images. To obtain voxel-wise T₁ relaxation times, a 3-parameter fit to the signal intensity, S as a function of T₁eff, was performed according to $S(T_{1eff}) = A - Be^{-\lambda(T_{1eff})}$ and $T_1$ was calculated as $T_1 = \frac{\ln(2)}{\lambda}$. Fitting was performed using MatLab (MathWorks, Natick, MA; vR2009a). Using the anterior septal insertion point as reference, T₁ maps were segmented according to the American Heart Association/American College of Cardiology model. Mean voxel T₁ relaxation time in each segment before and after contrast was then used to calculate segmental myocardial ECV according the following formula:

$\text{Extracellular volume fraction (ECV)} = \lambda \times (1 - \text{hematocrit}),$

where the partition coefficient, $\lambda = \frac{AR_1(\text{myocardium})}{AR_1(\text{blood})}$, is proportional to contrast agent concentration. $AR_1 = R_1(\text{postcontrast}) - R_1(\text{precontrast}).$

Separate ECV calculations were performed using the 10- and 15-minute postcontrast T₁ values acquired in patients awaiting transplantation, and using the 2- to 20-minute postcontrast T₁ values acquired in healthy subjects.

Precontrast and 15-minute postcontrast MOLLI image analysis was independently repeated in 50% (3 patients, 48 segments) of patients who had CMR before undergoing transplantation, and 33% of the healthy subjects (10 patients, 60 segments) by a second observer to assess interobserver variability of ECV measurement. Patients were randomly selected.

LV mass, end-diastolic volume, end-systolic volume, and ejection fraction were quantified from steady-state–free precession images using CMRtools (Cardiovascular Imaging Solutions, London, United Kingdom). LGE images were reported visually by 2 experienced operators (C.A.M. and M.S.). Segments were recorded as containing no LGE, infarct-typical LGE, or infarct-atypical LGE.

Statistical Analysis

All data were analyzed in a blinded fashion, with independent analysis of CMR (C.A.M. and M.S.) and histology (P.B.) data. Statistical analysis was performed using SPSS (IBM, version 19, IBM, Armonk, NY). Continuous variables are expressed as means±SD. For the histological validation, regression analysis using generalized estimating equations (GEEs) to adjust for repeated measurements within each subject was used to assess the relationship between DynEq-CMR–derived ECV and histological CVF. Within-subject and between-subject correlations were calculated using the methods described by Bland et al.2,13 The same analyses were used to assess the relationship between isolated postcontrast T₁ measurements and histological CVF. As prespecified, analyses were repeated after excluding segments containing infarct-typical LGE and after excluding segments containing any LGE (infarct-typical or infarct-atypical). Characteristics of healthy volunteers were compared across the 3 groups using 1-way ANOVA, as were precontrast...
T1 measurements. The effect of contrast agent dose on repeated postcontrast T1 measurements and the effect of contrast agent dose, myocardial region, and sex on repeated ECV measurements were assessed using a repeated measures ANOVA model. To assess the change in ECV measurement over time, ECV values calculated at 2 and 20 minutes post contrast were compared within each contrast agent dose group using a paired t test. Diastolic and systolic ECV measurements were compared within each contrast dose group using a paired t test. Interobserver agreement was evaluated using the repeatability coefficient, which calculates the range within which measurements by 2 different observers are expected to lie for 95% of subjects, and intraclass correlation coefficient (ICC).

## Results

### Histological Validation

Characteristics of each of the 6 patients who received a heart transplant after CMR are summarized in Table 1. Median time between CMR and transplantation was 29 days (in 5 patients the interval was ≤40 days, but in 1 patient the interval was 276 days). Mean histological CVF was 21.6±12.4% (range, 3.3%–55.2%; Figure 2). Mean DynEq-CMR–derived ECV calculated using the 10-minute postcontrast T1 values was 43.8±7.0% (range, 31.1%–65.1%; median within-subject range, 20.2%), and using the 15-minute postcontrast T1 values was 43.9±6.7% (range, 30.9%–68.4%; median within-subject range, 18.2%).

There was a significant linear relationship between ECV measured by DynEq-CMR, using both the 10- or 15-minute postcontrast T1 values and histological CVF. The correlation between mean ECV and CVF on a per individual basis was r=0.945; P=0.004; and r²=0.893 (Figure 4).

There was a significant linear relationship between isolated postcontrast T1 measurements made at 10 and 15 minutes post contrast and histological CVF (P<0.001 for both using GEE; Figure 3); however, this was largely driven by the within-subject correlations (isolated 10-minute postcontrast T1; r=−0.690; P=0.001; r²=0.475 and isolated 15-minute postcontrast T1; r=−0.741; P<0.001; r²=0.550). The between-subject correlations were not significant (isolated 10-minute postcontrast T1; r=−0.028; P=0.96 and isolated 15-minute postcontrast T1; r=−0.207; P=0.69). There was no correlation between precontrast T1 values and histological CVF (P=0.437 using GEE; within-subject: r=0.138; P=0.192 and between-subject: r=0.199; P=0.71).

In light of these findings, all subsequent analyses were performed using ECV calculated with DynEq-CMR using the 15-minute postcontrast T1 values.

There was a significant linear relationship between ECV and histological CVF in septal and nonseptal segments (P<0.001 using GEE for both), with minimal difference in within-subject and between-subject correlations (septum: within-subject, r=0.750; P<0.001; r²=0.560 and between-subject, r=0.940; P<0.01; r²=0.884; and nonseptum: within-subject, r=0.722; P=0.001; r²=0.521 and between-subject, r=0.941; P<0.01; r²=0.885). The significant linear relationship between ECV and histological CVF was also maintained across ventricular levels (P<0.001 using GEE for both basal and midventricular segments, and apical segments), with minimal difference in within-subject and between-subject correlations (basal and midventricle: within-subject, r=0.727; P<0.001; r²=0.529;
and between-subject, $r = 0.960; P < 0.01; r^2 = 0.922$; and apical ventricle: within-subject, $r = 0.748; P < 0.001; r^2 = 0.559$ and between-subject, $r = 0.892; P < 0.01; r^2 = 0.796$).

Infarct-typical LGE was present in 32 segments and infarct-atypical LGE was present in a further 10 segments. Only 1 segment displayed LGE throughout its entirety. When segments containing infarct-typical LGE were excluded (analysis performed on 64 segments), the linear relationship between ECV and histological CVF was maintained ($P < 0.001$ using GEE; within-subject: $r = 0.682; P < 0.001; r^2 = 0.465$ and between-subject: $r = 0.912; P < 0.01; r^2 = 0.832$; Figure 5). Likewise, the linear relationship between ECV and histological CVF remained when segments containing any LGE (infarct-typical and infarct-atypical patterns) were excluded (analysis performed on 54 segments; $P < 0.001$ using GEE; within-subject: $r = 0.652; P < 0.001; r^2 = 0.426$ and between-subject: $r = 0.843; P < 0.02; r^2 = 0.711$; Figure 5).

**Effect of Contrast Agent Dose, Myocardial Regionality, Cardiac Cycle, and Sex on ECV**

There were no significant differences in subject characteristics between contrast agent-dose groups (Table 2).

Precontrast myocardial (group A, 1051±49 ms; group B, 1045±49 ms; and group C, 1040±49 ms; $P=0.87$) and blood (group A, 1678±98 ms; group B, 1645±118 ms; and group C, 1686±101 ms; $P=0.66$) T1 relaxation times were not significantly different between groups. Mean myocardial (group

![Figure 3. Dynamic-equilibrium cardiovascular magnetic resonance (CMR)–measured myocardial extracellular volume, calculated using 10-min (A) and 15-min (B) postcontrast T1 values, plotted against histological collagen volume fraction. Isolated postcontrast T1 measurements made at 10-min (C) and 15-min (D) postcontrast plotted against histological collagen volume fraction. Symbols correspond to different patients, as set out in the legends.](http://circimaging.ahajournals.org/)

![Figure 4. Mean individual dynamic-equilibrium (cardiovascular magnetic resonance [CMR])–measured myocardial extracellular volume, calculated using 15-min postcontrast T1 values, plotted against mean individual histological collagen volume fraction.](http://circimaging.ahajournals.org/)
Table 2. Characteristics of Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=30)</th>
<th>Group A (0.10 mmol/kg; n=10)</th>
<th>Group B (0.15 mmol/kg; n=10)</th>
<th>Group C (0.20 mmol/kg; n=10)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0.98</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>45±13 (22–65)</td>
<td>44±14 (23–65)</td>
<td>45±14 (22–64)</td>
<td>46±13 (28–64)</td>
<td>0.75</td>
</tr>
<tr>
<td>Women</td>
<td>45±15 (22–65)</td>
<td>44±15 (27–65)</td>
<td>46±17 (22–64)</td>
<td>44±15 (30–60)</td>
<td>0.67</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.1±13.8</td>
<td>74.8±12.9</td>
<td>70.3±16.0</td>
<td>73.1±13.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.69±0.10</td>
<td>1.71±0.09</td>
<td>1.67±0.10</td>
<td>1.70±0.11</td>
<td>0.67</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.83±0.20</td>
<td>1.87±0.19</td>
<td>1.78±2.4</td>
<td>1.85±0.18</td>
<td>0.65</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68±9</td>
<td>67±9</td>
<td>70±11</td>
<td>66±8</td>
<td>0.67</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>114±11</td>
<td>116±11</td>
<td>114±11</td>
<td>112±12</td>
<td>0.63</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>67±11</td>
<td>71±11</td>
<td>65±13</td>
<td>66±7</td>
<td>0.41</td>
</tr>
<tr>
<td>eGFR, mL/min per m²</td>
<td>95±17</td>
<td>94±11</td>
<td>100±20</td>
<td>91±19</td>
<td>0.50</td>
</tr>
<tr>
<td>Indexed EDV, mL/m²</td>
<td>79±8</td>
<td>78±8</td>
<td>79±7</td>
<td>80±8</td>
<td>0.83</td>
</tr>
<tr>
<td>Indexed ESV, mL/m²</td>
<td>26±5</td>
<td>27±5</td>
<td>27±5</td>
<td>26±5</td>
<td>0.78</td>
</tr>
<tr>
<td>EF, %</td>
<td>67±5</td>
<td>66±4</td>
<td>66±4</td>
<td>68±5</td>
<td>0.45</td>
</tr>
<tr>
<td>BSA corrected mass, g/m²</td>
<td>45±8</td>
<td>44±9</td>
<td>43±8</td>
<td>47±7</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Indexed refers to indexed to body surface area. Values are mean±SD. BP indicates blood pressure; BSA, body surface area; EDV, end-diastolic volume; EF, ejection fraction; eGFR, estimated glomerular filtration rate; and ESV, end-systolic volume.

ECV varied significantly between myocardial regions. In each group ECV was highest in the septum and lowest in the lateral wall, as exemplified by group C (results for other groups were similar): anterior 24.9±2.5%, septal 27.0±2.5%, inferior 25.2±2.1%, and lateral 24.2±2.2% (P<0.001). ECV did not differ significantly between diastole and systole in any group (group A: diastolic ECV 28.5±3.2%, systolic ECV 28.1±3.4%, P=0.26; group B: diastolic ECV 26.0±3.4%, systolic ECV 25.9±3.3%, P=0.74; and group C: diastolic ECV 25.7±2.0%, systolic ECV 25.5±2.6%, P=0.56).

Mean ECV was significantly higher in women than in men in each group (group A: women 29.6±3.0%, men 25.4±3.0%, P<0.001; group B: women 27.4±2.7%, men 23.6±2.9%, P<0.001; and group C: women 26.1±2.8%, men 24.7±2.5%, P=0.027).

A, 542±65 ms; group B, 465±69 ms; group C, 407±55 ms; P<0.001) and blood (group A, 407±73 ms; group B, 307±67 ms; and group C, 252±48 ms; P<0.001) T₁ relaxation times averaged over all time points post contrast shortened significantly as contrast dose increased (Figure 6). Measurements of mean ECV averaged over all time points were significantly higher in group A compared with groups B and C (group A, 27.7±3.7%; group B, 25.8±3.4%; and group C, 25.8±2.8%; P<0.001), but the difference between groups B and C was not significant (Figure 6). Mean measured ECV increased linearly over time in each group; between the 2- and the 20-minute postcontrast acquisitions mean ECV increased from 27.2±2.7% to 28.8±3.4% (P=0.020) in group A; 25.3±2.8% to 26.5±3.2% (P=0.004) in group B; and 25.2±1.7% to 26.2±2.1% (P=0.068) in group C.

Figure 5. Dynamic-equilibrium cardiovascular magnetic resonance (CMR)–measured myocardial extracellular volume fraction plotted against histological collagen volume fraction, split according to the presence (red symbols) or absence (black symbols) of infarct-typical late gadolinium enhancement (LGE; A), and split according to the presence (red symbols) or absence (black symbols) of any LGE (infarct-typical or infarct-atypical patterns; B). Symbols correspond to different patients, as set out in the legends.
In healthy subjects receiving 0.20 mmol/kg contrast agent (ie, group C), mean segmental ECV calculated using 15-minute postcontrast T1 values was 25.5±2.6%. In patients before transplantation (who received the same type and dose of contrast agent), mean ECV, also calculated using 15-minute postcontrast T1 values, in segments without LGE was 41.4±5.0% \((P<0.001\) when compared with healthy subjects), and in segments with LGE was 47.0±7.4% \((P<0.001\) when compared with healthy subjects and when compared with segments without LGE; Figure 7). In patients before transplantation, there was a significant difference in mean ECV between segments without LGE, segments with infarct-atypical LGE (45.8±4.7%), and segments with infarct-typical LGE (47.4±8.1%; \(P<0.001\)). On post hoc analysis the difference in ECV between segments with infarct-typical LGE and segments without LGE was significant \((P<0.001)\), but the difference between segments with infarct-atypical LGE and segments without LGE was not \((P=0.125;\) Figure 7).

Mean precontrast myocardial T1 relaxation time was significantly higher in patients before transplantation (who received the same type and dose of contrast agent), mean ECV, also calculated using 15-minute postcontrast T1 values, in segments without LGE was 41.4±5.0% \((P<0.001\) when compared with healthy subjects), and in segments with LGE was 47.0±7.4% \((P<0.001\) when compared with healthy subjects and when compared with segments without LGE; Figure 7). In patients before transplantation, there was a significant difference in mean ECV between segments without LGE, segments with infarct-atypical LGE (45.8±4.7%), and segments with infarct-typical LGE (47.4±8.1%; \(P<0.001\)). On post hoc analysis the difference in ECV between segments with infarct-typical LGE and segments without LGE was significant \((P<0.001)\), but the difference between segments with infarct-atypical LGE and segments without LGE was not \((P=0.125;\) Figure 7).

**Comparison of ECV in Health and Disease**

In healthy subjects receiving 0.20 mmol/kg contrast agent (ie, group C), mean segmental ECV calculated using 15-minute postcontrast T1 values was 25.5±2.6%. In patients before transplantation (who received the same type and dose of contrast agent), mean ECV, also calculated using 15-minute postcontrast T1 values, in segments without LGE was 41.4±5.0% \((P<0.001\) when compared with healthy subjects), and in segments with LGE was 47.0±7.4% \((P<0.001\) when compared with healthy subjects and when compared with segments without LGE; Figure 7). In patients before transplantation, there was a significant difference in mean ECV between segments without LGE, segments with infarct-atypical LGE (45.8±4.7%), and segments with infarct-typical LGE (47.4±8.1%; \(P<0.001\)). On post hoc analysis the difference in ECV between segments with infarct-typical LGE and segments without LGE was significant \((P<0.001)\), but the difference between segments with infarct-atypical LGE and segments without LGE was not \((P=0.125;\) Figure 7).

Mean precontrast myocardial T1 relaxation time was significantly higher in patients before transplantation than in healthy subjects \((1187±163\) versus \(1045±46\) ms; \(P<0.001)\).

**Repeatability**

Interobserver variability for DynEq-CMR measurement of ECV in healthy subjects was 2.3% (ICC, 0.932), ranging
from 1.4% (ICC, 0.969) for septal segments to 2.7% (ICC, 0.914) for nonseptal segments. Interobserver variability for DynEq-CMR measurement of ECV in patients before transplantation was 5.1% (ICC, 0.883), ranging from 2.9% (ICC, 0.944) for septal segments to 5.9% (ICC, 0.850) for nonseptal segments, and ranging from 4.5% (ICC, 0.907) for basal and midventricular segments to 6.7% (ICC, 0.798) for apical segments. For histological CVF assessment, interobserver variability was 2.8% (ICC, 0.994); however, within the same tissue sample, CVF was not uniform between high-powered fields, with a mean SD of 49% normalized to CVF%. Mean CVF measurements did not vary significantly according to histological magnification (×200, 14.0±11.2%; ×50, 14.4±12.5%; ×100, 14.0±11.7%; P=0.507).

Discussion
This is the first human study to provide comprehensive histological validation of the DynEq-CMR technique for quantification of myocardial ECV, and the first to provide LV histological validation of the isolated postcontrast T1 measurement technique. Indeed, this is the largest histological validation, in terms of number of tissue samples, of any CMR ECV quantification method, and the only to provide whole heart tissue corroboration.

The DynEq-CMR technique relies on the assumption of a 2-compartment model, whereby a steady state is assumed to exist between the intravascular and interstitial compartments, with equal contrast agent concentrations in each, because of rapid exchange of contrast agent between the compartments. The small increase in ECV over time seen here (Figure 6), which is in keeping with the findings of Kawel et al16 and Schelbert et al,14 suggests that the 2-compartment model may be limited and an incomplete dynamic equilibrium between blood and myocardium is achieved. The lack of equilibrium may be because of penetration of gadolinium into other compartments, such as bone and synovial fluid, and because of faster renal clearance than exchange rate between compartments. Indeed, the latter reason, in particular, may explain the significantly higher ECV values and greatest increase in ECV over time, seen in the healthy subjects receiving the lowest contrast dose.

As a result of the incomplete dynamic equilibrium, the correlation between DynEq-CMR–derived ECV and histological CVF changed over time. Nevertheless, the relationship between DynEq-CMR–derived ECV and histological CVF in the current study is comparable with that found in the study by Flett et al13 using the EQ-CMR technique (r²=0.80), in which basal septal ECV was compared with histological CVF of tissue obtained from the basal septum at surgical biopsy in patients undergoing valve replacement for aortic stenosis (18 patients) or myectomy for hypertrophic cardiomyopathy (8 patients). The DynEq-CMR technique used here, however, offers advantages compared with the EQ-CMR technique in terms of being substantially simpler to perform and less time consuming and could easily be incorporated into routine scanning protocols.

There was a strong linear relationship between CMR-derived ECV and histological CVF across the whole spectrum of ECV and CVF, which is an important finding as it means that ECV is suitable for stratifying patients based on CVF. Nevertheless, the intercept of the linear regression equation for the relationship between ECV and CVF was not zero, and the slope was different from 1 (unity), findings which are in keeping with those of Flett et al13 and Messroghli et al.25 As discussed by Di Carli et al,35 the y-intercept represents a surrogate for all of the multiple components of the myocardial interstitium, which as well as collagen include non-collagenous proteins, fibroblasts, endothelial cells, and vessels. In addition, although we excluded patients with specific myocardial inflammatory conditions per se, all subjects had end-stage heart failure and as such myocardial inflammation, and hence edematous expansion of the interstitial space without collagen deposition, may have been present, which may be one explanation for the higher mean precontrast T1 seen in pretransplant patients compared with healthy volunteers. As such it is logical to assume that CMR-derived ECV does not only reflect CVF; however, it seems that excess ECV beyond the baseline is explained by increases in CVF, provided other causes of ECV expansion are excluded. The y-intercept and slope of the equation may also have been influenced by changes in the extracellular compartment relating to the tissue processing itself, and by the CVF quantification technique.

Flett et al13 excluded patients who demonstrated any LGE in the region of the biopsy. Wong et al,9 who demonstrated an association between DynEq-CMR–derived ECV and short-term all-cause mortality (but without histological corroboration), excluded regions of myocardium in the vicinity of infarct-typical LGE but included myocardium displaying infarct-atypical LGE. Others have quantified ECV in myocardium exhibiting infarct-atypical LGE (also without histological corroboration).16,36 Expansion of the myocardial interstitial space seems to occur as a continuous spectrum. As such we included all tissue samples in our analysis, regardless of LGE status, and simply quantified ECV in each. Nevertheless, our study demonstrated that the correlation between DynEq-CMR–derived ECV and histological CVF remained strong when segments containing any LGE were excluded and when segments containing infarct-atypical LGE only were excluded, as well as when segments containing LGE were included.

Indeed, this study serves to highlight the potential shortcomings of the LGE technique relating to its relative signal intensity nature. Although the LGE technique is very well established for infarction detection and quantification, the ability of LGE to detect and quantify myocardial fibrosis in nonischemic cardiomyopathies is much less well validated. Myocardial fibrosis exists as a spectrum from diffuse to focal. Such homogeneity, or lack of heterogeneity, is problematic for the LGE technique because it relies on normal regions of myocardium to serve as reference for nulling. As a result, LGE quantification (and hence quantification of fibrosis via the LGE technique) in nonischemic cardiomyopathies is dependent on the signal intensity threshold used.37 As demonstrated in Figure 5, in the current study, there was considerable overlap in histological CVF between segments...
that displayed LGE and those that did not (although it should be recognized that CVF and ECV values in the current study represent mean values for entire segments, with only 1 segment displaying LGE throughout). Furthermore, the difference in mean ECV between segments without LGE and those with atypical LGE was not significant, although overall mean differences in ECV may not be reflective of differences in individual patients. Nevertheless, in keeping with other work, this study suggests that myocardial fibrosis in nonischemic cardiomyopathies may be better assessed using ECV techniques rather than LGE.38

The correlation between DynEq-CMR–derived ECV and histological CVF was maintained throughout the LV, although there was greater ECV measurement variability in nonseptal compared with septal regions and in apical compared with basal and midventricular myocardium, likely reflecting the comparatively thinner ventricular walls in these regions. The degree of variability in the midventricular slice was comparable with that reported in other studies.40,38 Variability at other ventricular levels has not previously been reported.

Mean DynEq-CMR–derived ECV in healthy subjects was in keeping with that found in other studies.9,14–16,18,19,39 Interestingly, ECV was significantly higher in the septum compared with other myocardial regions in healthy subjects. This is in keeping with histological data from healthy myocardium, which demonstrate higher collagen content in the septum compared with other regions,40 and with DynEq-CMR data reported by Kawel et al.19 This finding, which may be secondary to the septum being exposed to mechanical strain from both ventricles, has important implications both in terms of CMR ECV measurement and histological assessment of CVF, as septal sampling seems not necessarily representative of other myocardial regions.

In addition, ECV was seen to vary significantly between sexes. This is in keeping with the findings of Sado et al39 who, using EQ-CMR in healthy subjects with a similar age range to here, found sex was an independent predictor of ECV in multivariate analysis (myocardial mass, hematocrit, and patient height were not); indeed, the absolute difference in ECV between sexes reported by Sado et al39 is very similar to that found here. The reasons for the sex difference are not clear. However, given that the correlation between ECV and histological CVF was maintained throughout the heart, with low observer variability particularly in the midventricular slice, this finding seems genuine (ie, it does not seem to be related to potentially greater partial voluming effects in theoretically thinner female LV walls). As Sado et al39 discuss, it may be that this sex difference in ECV contributes to known differences in cardiovascular disease expression between men and women. Further investigation of this finding is required.

Mean DynEq-CMR–derived ECV in segments containing LGE was similar to that reported by Ugander et al.16 who assessed ECV in patients with cardiac disease exhibiting infarct-typical or infarct-atypical LGE undergoing clinical CMR. However, mean ECV in myocardium remote from segments containing LGE in the pretransplant patients in the current study was substantially higher than ECV in myocardium remote from segments containing LGE in the study by Ugander et al.16 This is likely to reflect the end-stage nature of cardiac disease in the cohort studied here. As a result, although there was considerable overlap between ECV in healthy subjects and ECV in myocardium in patients with cardiac disease but remote from LGE in the study by Ugander et al16 (and in other studies),38,39 there was very little overlap in the current study.

Iles et al41 found a significant correlation (r=−0.7; P=0.03) between LV midventricular isolated 15-minute postcontrast T1 values and histological CVF in 9 heart transplant recipients, although tissue was of right ventricular origin and of small volume (obtained via transvenous endomyocardial biopsy), and this technique has been increasingly applied as a surrogate for ECV. However, isolated postcontrast T1 measurements are confounded by several factors, such as renal function, hematocrit, body fat, and myocardial steatosis. In keeping with this, although the within-subject correlation between isolated postcontrast T1 measurements and histological CVF was high in the current study, there was no significant correlation between subjects.

Limitations
Although the number of tissue samples included in this study is large, the total number of patients is relatively small. However, this reflects the difficulty in obtaining whole-heart histological data from patients who have recently undergone CMR, and who do not have an acute inflammatory myocardial condition. A greater number of patients could potentially have been recruited by performing the study using postmortem tissue obtained from patients who had undergone CMR for other reasons and subsequently died. However, immediate formalin fixation would not have been possible, which may have introduced error in histological CVF quantification, as may cause of death. In the current study, hearts remained in vivo until explantation when they were immediately fixed in formalin, minimizing postmortem changes. The interval between CMR and transplantation could have resulted in changes in myocardial collagen content. However in 5 of the 6 patients, the interval was minimal (≤40 days). There was a small improvement in correlation between DynEq-CMR–derived ECV and histological CVF when ECV was calculated using the 15-minute postcontrast T1 values compared with when the 10-minute postcontrast values were used. As such, the optimal postcontrast time for T1 acquisition was not ascertained, and it is possible that the correlation may have improved further with later postcontrast T1 measurements. However, the clinical status of the patients involved, that is, end-stage heart failure, necessitated relatively short scan duration. Finally, an alternative CMR method of quantifying ECV that has been less commonly applied than the methods assessed here, in which contrast agent kinetics are deconvoluted using mathematical modeling, was not investigated.41

Conclusions
This study provides comprehensive validation of the DynEq-CMR method for measurement of myocardial ECV. Isolated postcontrast measurement of myocardial T1 is insufficient for ECV assessment.
Acknowledgments

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Disclosures

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References


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CLINICAL PERSPECTIVE

Expansion of the myocardial interstitial space seems fundamental to the process of left ventricular remodeling, is an independent predictor of mortality, and is a potential therapeutic target. Quantification of the interstitial space, or extracellular volume (ECV), may therefore represent an important biomarker. Cardiovascular magnetic resonance techniques that allow the noninvasive evaluation of ECV have generated considerable recent interest. One such technique is the dynamic equilibrium technique, in which ECV is quantified using hematocrit-adjusted myocardial and blood T1 relaxation times, a fundamental magnetic property, measured before and after standard gadolinium contrast agent bolus. This article demonstrates that although the dynamic equilibrium of contrast agent between blood and myocardium, which the technique assumes, may be incomplete with a small change in measured ECV seen over time, dynamic equilibrium-cardiovascular magnetic resonance-derived ECV has a strong linear correlation with histological collagen, maintained throughout the entire heart. As such it seems that this technique, which can easily be incorporated into routine clinical cardiovascular magnetic resonance scanning, allows accurate evaluation of the interstitial space. Measured ECV varies with contrast dose and, therefore, a consistent protocol is required. In addition, ECV seems to differ between myocardial regions and according to sex. Finally, because of factors that confound postcontrast myocardial T1, such as renal function and body habitus, isolated postcontrast measurement of myocardial T1 seems insufficient for the assessment of ECV.
Comprehensive Validation of Cardiovascular Magnetic Resonance Techniques for the Assessment of Myocardial Extracellular Volume
Christopher A. Miller, Josephine H. Naish, Paul Bishop, Glyn Coutts, David Clark, Sha Zhao, Simon G. Ray, Nizar Yonan, Simon G. Williams, Andrew S. Flett, James C. Moon, Andreas Greiser, Geoffrey J.M. Parker and Matthias Schmitt

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SUPPLEMENTAL MATERIAL

Phantom studies

Supplemental methods
Fifteen agarose and CuSO₄ phantoms with T₁ values of 175 – 1775ms (i.e. a range encompassing physiological pre- and post-contrast myocardial and blood T1 values), and a constant physiological T₂ value (55±8ms), were scanned using the MOLLI sequence with simulated heart rates (50 to 110 beats per minute, 10 beats increments). Reference T₁ relaxation times were measured using an inversion recovery spin echo sequence with 10 inversion times ranging from 25 to 5000ms (TR 10s, TE 8.1ms); reference T2 relaxation times were measured using a spin echo sequence with 10 echo times ranging from 10 to 200ms (TR 10s). Phantom imaging was repeated in order to assess inter-scan reproducibility of MOLLI-derived T₁ measurements.

Supplemental results
As has been demonstrated previously,¹ the accuracy of the MOLLI sequence for measurement of T₁ relaxation time was found to be dependent on T₁ and heart-rate, with progressive underestimation of T₁ relaxation time, as compared with the inversion recovery spin echo-derived T₁ measurements, as T₁ and heart rate increased (Supplemental Figure 1). Inter-scan reproducibility of phantom T₁ measurements made using the MOLLI sequence was 2.1ms, (intraclass correlation coefficient 1.0).

Heart rate correction algorithm
Using the above phantom data, MOLLI-derived T₁ relaxation times at different heart rates were fitted to the inversion recovery spin echo-derived T₁ relaxation times using second order polynomial fitting, in order to derive the following correction algorithm,² which was subsequently applied to all in-vivo MOLLI-derived T₁ measurements:
\[ T_{1}\text{-IR} = A(T_{1}\text{-MOLLI})^2 + B(T_{1}\text{-MOLLI}) + C \]

Where \( T_{1}\text{-IR} \) = inversion recovery spin echo-derived \( T_1 \) relaxation time; \( T_{1}\text{-MOLLI} \) = MOLLI-derived \( T_1 \) relaxation time; and \( A, B \) and \( C \) are as displayed in Supplemental Table 1. For the in vivo data, linear interpolation of the coefficients between adjacent simulated heart rates was used.

**Impact of heart rate correction algorithm**

Application of the heart rate correction algorithm significantly increased mean pre-contrast myocardial \( T_1 \) relaxation times (for example in patients prior to transplantation, uncorrected \( T_1 \) 1086±129ms; corrected \( T_1 \) 1187±163ms; \( p<0.001 \)). However the heart rate correction algorithm did not lead to a significant difference in mean DynEq-CMR-derived ECV (ECV calculated using uncorrected \( T_1 \) values 43.8±6.8\% versus ECV calculated using corrected \( T_1 \) values 43.9±6.7\%, \( p=0.903 \)). Furthermore, the linear regression equation for the relationship between DynEq-CMR-derived ECV and histological CVF using uncorrected \( T_1 \) values (histological CVF = 1.445 x ECV – 41.588) was almost identical to that when corrected \( T_1 \) values were used (histological CVF = 1.451 x ECV – 42.023). As such, for future work using DynEq-CMR-derived ECV calculated using the MOLLI sequence applied here, a heart rate correction algorithm (which is cumbersome to apply) may not be necessary. Nevertheless, for this validatory paper the heart rate correction algorithm was applied throughout.
## Supplemental Table 1

<table>
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<th>Heart rate</th>
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Supplemental Figure 1.

Black line represents the identity line.
Supplemental references
