Equilibrium Contrast Cardiovascular Magnetic Resonance for the Measurement of Diffuse Myocardial Fibrosis
Preliminary Validation in Humans

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Background—Diffuse myocardial fibrosis is a final end point in most cardiac diseases. It is missed by the cardiovascular magnetic resonance (CMR) late gadolinium enhancement technique. Currently, quantifying diffuse myocardial fibrosis requires invasive biopsy, with inherent risk and sampling error. We have developed a robust and noninvasive technique, equilibrium contrast CMR (EQ–CMR) to quantify diffuse fibrosis and have validated it against the current gold standard of surgical myocardial biopsy.

Methods and Results—The 3 principles of EQ–CMR are a bolus of extracellular gadolinium contrast followed by continuous infusion to achieve equilibrium; a blood sample to measure blood volume of distribution (1−hematocrit); and CMR to measure pre- and postequilibrium T1 (with heart rate correction). The myocardial volume of distribution is calculated, reflecting diffuse myocardial fibrosis. Clinical validation occurred in patients undergoing aortic valve replacement for aortic stenosis or myectomy in hypertrophic cardiomyopathy (n=18 and n=8, respectively). Surgical biopsies were analyzed for picrosirius red fibrosis quantification on histology. The mean histological fibrosis was 20.5±11% in aortic stenosis and 17.1±7.4% in hypertrophic cardiomyopathy. EQ–CMR correlated strongly with biopsy histological fibrosis: aortic stenosis, \( r^2 = 0.86 \), Kendall Tau coefficient (T) = 0.71, \( P < 0.001 \); hypertrophic cardiomyopathy, \( r^2 = 0.62 \), T = 0.52, \( P = 0.08 \); combined \( r^2 = 0.80 \), T = 0.67, \( P < 0.001 \).

Conclusions—We have developed and validated a new technique, EQ–CMR, to measure diffuse myocardial fibrosis as an add-on to a standard CMR scan, which allows for the noninvasive quantification of the diffuse fibrosis burden in myocardial diseases. (Circulation. 2010;122:138-144.)

Key Words: magnetic resonance imaging ■ cardiomyopathy ■ imaging ■ endomyocardial fibrosis ■ collagen

Fibrosis is a final common end point in virtually all pathological processes in human organs and tissues. In the heart, focal fibrosis (scar) as a result of myocardial infarction is the leading cause of death and heart failure in the world.1 Cardiovascular magnetic resonance (CMR) using the late gadolinium enhancement (LGE) contrast technique is the gold standard method for its assessment.2,3 Focal fibrosis also occurs in other diseases, including cardiomyopathy,4 myocarditis,5 and infiltrative diseases.6 Scar leads to an increased volume of distribution for gadolinium and slower contrast kinetics,7 leading to late enhancement of scar by CMR.

Clinical Perspective on p 144

Diffuse myocardial fibrosis is a covert process that occurs as a part of normal aging.8–10 It is accelerated in diseases such as hypertension, aortic stenosis, and cardiomyopathy,11–13 where it contributes to breathlessness, heart failure, and arrhythmia.14–16 Unlike scar, however, it may be reversible and is a treatment target.17,18 Currently, the only method to quantify diffuse fibrosis is invasive biopsy, which carries significant morbidity and is prone to sampling error.19 The LGE technique cannot be used to visualize diffuse fibrosis because “normal” myocardium with diffuse fibrosis is “nulled” to highlight focal scar, thereby losing all information of any background interstitial expansion. Recently, attempts to quantify diffuse fibrosis with CMR have been made, but they have required complex kinetic modeling and have not definitively excluded confounding factors such as heart rate, body composition, and renal clearance variability. Histological validation, where present, has

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been limited and predominantly performed in animals\textsuperscript{20} and ex vivo tissue.\textsuperscript{21–23}

In this study, we describe a potentially clinically applicable, robust, noninvasive method to quantify diffuse myocardial fibrosis, equilibrium contrast CMR (EQ–CMR), which we have prospectively validated against the current gold standard of surgical myocardial biopsy collagen volume fraction (CVF) quantification in patients with aortic stenosis and hypertrophic cardiomyopathy undergoing surgery. This method is based on 3 elements: a bolus of the extracellular contrast agent gadolinium (Gd-DTPA) followed by continuous infusion to achieve blood:myocardial contrast equilibrium; a blood test to measure blood contrast volume of distribution; and CMR before and after contrast equilibrium to measure changes in tissue signal (T1 measurement with heart rate correction; see Methods and online-only Data Supplement). This allows for the calculation of the myocardial contrast volume of distribution ($V_d(m)$), which closely reflects the amount of fibrosis because collagen is aqueous and Gd-DTPA is an extracellular tracer that can occupy this space freely.\textsuperscript{23}

**Methods**

**Technique: Theory**

Gd-DTPA is a magnetic resonance extracellular contrast agent that potently shortens T1. We measured the change in T1 before and after a steady-state infusion of Gd-DTPA. This is given by the following equation:

\[
\frac{1}{T_1}_{\text{post}} = \frac{1}{T_1}_{\text{pre}} + \frac{1}{T_1}_{\text{Gd-DTPA}}
\]

where

\[
\frac{1}{T_1}_{\text{Gd-DTPA}} = R1[\text{Gd-DTPA}]
\]

\[
\text{post} = \text{measured T1 after contrast, pre} = \text{T1 before contrast, } R1 = \text{T1 relaxivity of Gd-DTPA (known)}.
\]

At contrast equilibrium, extracellular [Gd-DTPA] is the same in blood and myocardium. The volume of distribution in blood ($V_d(b)$) is known, so $V_d(m)$ is as follows:\textsuperscript{23,24}:

\[
V_d(m) = (1 - \text{hematocrit}) \times \frac{\left(\frac{1}{T_1}_{\text{myo,post}} - \frac{1}{T_1}_{\text{myo,pre}}\right)}{\left(\frac{1}{T_1}_{\text{blood,post}} - \frac{1}{T_1}_{\text{blood,pre}}\right)}
\]

**Achieving Contrast Equilibrium**

Contrast equilibrium was achieved by primed infusion (a loading bolus followed by a slow continuous infusion). The induction of an early steady state was optimized in preliminary work by iteration of infusion rate and timing, with confirmation in 8 study patients and 8 normal volunteers, who remained in the scanner for the duration of the infusion. For rapid within-subject T1 measurement, a T1 (time to inversion) scout sequence (steady-state free precession, TR=3.1 ms, $TE=1.27$ ms, slice thickness 8 mm, Flip angle 30°, TI range [105 - the RR interval] phases >14, centric reordering of phase encoding) in the transverse plane was used to measure T1' (T1 prime, the T1 without heart rate or readout correction [ie, a reliable reflection of intrasubject T1 variation over time], but confounded by the heart rate, so unsuitable for intersubject comparison) repeated every 5 minutes. Steady state was defined as occurring when the variation in T1' on serial measurements within an individual was within 5% of the final 10 minutes of T1' measurements.

**Clinical Validation**

All research was carried out at Great Ormond Street Hospital for Children and University College London Hospitals, London, United Kingdom, between October 2008 and July 2009. An ethics committee of the UK National Research Ethics Service approved the study, and all patients and volunteers gave informed consent conforming to the declaration of Helsinki (V revision, 2000). We validated the in vivo measurements against human operative myocardial biopsy in patients with aortic stenosis. Consecutive preoperative patients were invited to participate, unless they had uncontrolled tachycardia, a contraindication to CMR, or severely impaired renal function (glomerular filtration rate <30 mL/min). A second disease model was also studied: hypertrophic cardiomyopathy with outflow tract obstruction requiring myectomy. The LGE images were reported by 2 experienced operators, and patients with LGE (by visual inspection) in an area that was likely to be involved in the myectomy, biopsy site, or T1 measurement region of interest (n=5) were excluded (as prespecified) from analysis. Blood samples were taken at the time of CMR to measure $V_d(b)$.

**CMR Protocol**

The CMR protocol is outlined in Figure 1. CMR was performed on a 1.5T magnet (Avanto, Siemens Medical Solutions). Within a standard clinical scan (Pilots, transverse black blood images, volumes, aortic valve view with flow maps, and LGE imaging), a T1 measurement sequence was performed precontrast (8 breath-holds, details below). After a bolus of Gadoteric acid (0.1 mmol/kg, Dotarem; Guerbet SA) and standard LGE imaging, the patient was removed from the scanner. We aimed to achieve contrast equilibrium within 1 hour using a 0.1 mmol/kg Gd-DTPA bolus (Dotarem; Guerbet), followed by a 15-minute pause, then an infusion at a rate of 0.0011 mmol/kg/min (equivalent to 0.1 mmol/kg over 90 minutes). At any time between 45 and 80 minutes after the bolus, the patient was returned to the scanner and the T1 measurement repeated (with replicating, a total of 9 breath-holds). Some patients (n=8; normal volunteers, n=8) remained in the scanner to test contrast equilibrium, and some (n=22) had the T1 measurements performed twice to assess repeatability.

**T1 Measurement**

The T1 measurement used a standard LGE sequence. This is a multi–breath-hold, spoiled gradient echo inversion recovery (IR) sequence with the following parameters: slice thickness 8 mm, TR 9.8 ms, TE=4.6 ms, $\alpha=21^\circ$, field of view 340×220 mm (transverse plane), sampled matrix size 256×105, 21 k-space lines acquired every other RR interval (21 segments with linear reordered phase encoding), spatial resolution 1.3×2.1×8 mm, no parallel imaging. T1 measurement was performed before and after equilibrium contrast in a single transverse slice in a single phase using increasing inversion times (TI) per breath-hold of 140 ms, then 200 to 800 ms in 100-ms increments (Figure 2). Regions of interest were drawn in the blood and in the septum at a point that was likely to encompass the biopsy/myectomy site. Mean signal intensities were plotted with restoration of phase (performed manually by making all points to the left of the magnitude minimum negative), and a curve-fitting technique was used to find the null point: T1'. A heart rate correction algorithm corrected for incomplete longitudinal recovery, and phantom work demonstrated that the combination of low flip angle, short readout, and no parallel imaging constrained readout-related deviation of the derived T1 compared with T1 relaxometry to <5%.
Key factors used in the methodology are as follows: (1) the use of null points, which are coil location and scanner image scaling independent; (2) pre- and postscanning with a single change: equilibrium contrast (no significant heart rate change pre- to postcontrast [mean RR interval 875 ± 198 vs 901 ± 220 ms], meticulous isocentering and reimporting of the prescan sequence), and (3) the use of the blood:myocardial ratio such that any systematic changes affecting both tissue signals may affect their signal ratio less.

Histology
For histological analysis, an intraoperative deep myocardial biopsy (Tru-Cut needle) was taken in aortic stenosis (from the basal septum in the outflow tract with guidance from author A.S.F.), and the myectomy specimen was used in hypertrophic cardiomyopathy. Samples were fixed immediately in 10% buffered formalin, embedded in paraffin, and stained with picrosirius red. High-power magnification (×200) digital images excluding subendocardial or perivascular areas underwent automated image analysis (macro written in ImageJ). 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. On average, 12 high-power fields were assessed per biopsy, and the mean percent fibrosis and fibrosis heterogeneity (coefficient of variation between fields) were obtained.

Data Analysis and Statistics
All CMR studies were performed before the intracardiac biopsy. All data were analyzed in a blinded fashion, with independent analysis of the CMR data (A.S.F., J.C.M.) and histology (M.A.). Interstudy repeatability for the CMR measurement of T1 and the CVF measurement was assessed by calculating the coefficient of variability (equal to the SD of the difference between 2 measurements over the mean of the 2 measurements, expressed as a percentage) as well as intraclass correlation coefficient (ICC). Correlation was assessed using Kendall’s test. Analyses were performed in SPSS version 15.

Results
Achieving Contrast Equilibrium
In the subset of patients (n=8; normal volunteers, n=8) who remained in the scanner during equilibration, contrast equilibrium (Figure 3) was achieved within 30 and 45 minutes of the contrast bolus in 90% and 100% of subjects, respectively. The mean time to contrast equilibrium for myocardium, blood, and their ratio were 19.8, 26.10 and 20.8 minutes, respectively. We incorporated a margin for error. The mean length of infusion was 40 ± 7.9 minutes, the time for the second scan acquisition (9 breath-holds) was 5 minutes ± 42 seconds, and the total Gd-DTPA contrast dose was 0.14 ± 0.009 mmol/kg.

Myocardial Biopsy
All biopsies were uneventful. The mean histological CVF was 20.5% in aortic stenosis (intersubject range 6% to 39.8%, SD=11.0, n=18), and 17.1% in hypertrophic cardiomyopathy (intersubject range 10.4% to 30.6%, SD=7.37, n=8) (Figure 4).

EQ–CMR Measurement of Fibrosis
In this validation study, fibrosis was quantified in the basal septum at a point that was likely to encompass the cardiac biopsy/myectomy. Of 31 patients, 19 (11 aortic stenosis and 8 hypertrophic cardiomyopathy) patients had focal fibrosis detected as LGE. In 5 patients, this was in the potential area of biopsy and, as prespecified, these patients were excluded from further analysis (Figure 5). In the remaining 26 patients, Vd(m) measured by EQ–CMR correlated strongly with CVF in aortic stenosis and the combined population: r²=0.86, Kendall’s Tau coefficient (T)=0.71, P<0.001, and r²=0.80,
T={0.67, P<0.001, respectively (Figure 6). In hypertrophic cardiomyopathy the correlation approached statistical significance: $r^2=0.62$, $T=0.52$, $P=0.08$. The linear correlation equation for this relation was $\text{CVF}\% = 197 \times \text{Vd(m)} - 36$.

Measurement Repeatability

In a subset of patients ($n=22$), CMR measurement and analysis were repeated. Intra-study CMR reproducibility was 1%, SD <1% (ICC=0.969). For CVF assessment, automated image analysis reproducibility was 5% (ICC=0.989); however, within the same biopsy, CVF extent was not uniform between high-power fields, with a mean normalized (to CVF%) SD of 37% in aortic stenosis and 41% in hypertrophic cardiomyopathy across an average of 12 high-power fields per biopsy.

Discussion

In this study, we have developed and validated a novel technique, EC–CMR, to measure diffuse myocardial fibrosis, an important parameter previously only quantifiable with cardiac biopsy. In aortic stenosis, there is a strong correlation between EQ–CMR and histological biopsy CVF, which directly reflects diffuse fibrosis. EQ–CMR is potentially clinically applicable, adding only 10 minutes of scan time to a conventional CMR, and uses existing technologies (standard clinical contrast dose [0.2 mmol/kg], standard sequences, and standard scanner). The patient spends additional time in the department (30 to 90 minutes) during the continuous infusion, but the flexibility in timing of the postinfusion scan means this can be easily incorporated into clinical workflow.

In aortic stenosis patients, the extent and range of diffuse fibrosis was broad: 6% to 40% of total myocardium. In this condition, as in other diseases, evidence suggests that the extent of fibrosis is an important determinant of outcome and may explain why valve stenosis severity and hemodynamic parameters alone only partially explain symptoms and outcome. Diffuse fibrosis is an important clinical parameter and is associated with worsening ventricular systolic and diastolic function and adverse remodeling. More extensive fibrosis is associated with advanced heart failure, regardless of its cause. It is also a potentially reversible phenomenon, and several therapies seem to exert their beneficial effects via myocardial fibrosis regulation. The correlation in aortic stenosis was stronger than that found in hypertrophic cardiomyopathy ($r^2 0.86$ versus 0.62). This was an expected finding, as fibrosis in hypertrophic cardiomyopathy is thought to be less diffuse in nature than fibrosis in our primary disease model, aortic stenosis.

Other noninvasive measures of diffuse fibrosis have limitations: Blood biomarkers are relatively nonspecific because collagen degradation products may arise in other tissues, and levels are influenced by clearance variability. Echocardiog-
raphy parameters that assess diastolic function are dependent not only on fibrosis, but also on filling pressures and active myocyte relaxation. An in vivo measure of diffuse fibrosis could be important across the whole spectrum of cardiac conditions, with applications including research, diagnostics, disease progression, prognostics, and therapeutics. Applications could include diseases of massive interstitial expansion, such as cardiac amyloidosis, or even simple ageing, where interstitial expansion may be modest and invasive biopsy not warranted. Furthermore, the principles of EQ–CMR may be more widely applicable. Equilibrium contrast magnetic resonance imaging (EQ–MRI) should be possible, indeed more straightforward, in other organs, such as the liver, ovary, bowel, arterial wall, or lymph nodes.

Over the last 5 years, LGE CMR has become routine for noninvasive clinical assessment of focal scar such as myocardial infarction. Despite the success of this technique, it is limited by its inability to detect diffuse fibrosis. Prior CMR studies have shown that diffuse fibrosis causes subtle ex vivo intrinsic tissue signal differences. Using a bolus of contrast, the presence of diffuse fibrosis has been inferred by detecting elevated peak tissue concentrations in cardiomyopathy compared with normals. Recently, mathematical modeling of this effect has been used to derive partition coefficient estimates. Contrast has been used as a continuous infusion instead of a bolus to image focal scar, to measure the scar partition coefficient, and in autopsy samples to measure the contrast volume of distribution of diffuse fibrosis, but it has not been used in vivo. In this study we used a primed continuous infusion (removing contrast kinetic effects), a direct blood volume of distribution measure (1/Hematocrit) to obtain V(d,m) rather than partition coefficient, and measured diffuse fibrosis in vivo. A robust but simple MRI technique and Microsoft Excel–based heart rate correction algorithm were used with human tissue correlation (not surrogate or animal model) in 2 different diseases.

Figure 5. Examples of excluded patients. Patients with LGE in the basal septum, the likely area of biopsy, were prespecified as not for analysis (n=5). The 2 shown here (upper, aortic stenosis; lower, hypertrophic cardiomyopathy), had a V(d,m) that was lower than expected from the biopsy CVF, suggesting the biopsy had sampled the area of late gadolinium enhancement (left).

Figure 6. MRI–measured myocardial volume of distribution against histological CVF. V(d,m) correlates with CVF in aortic stenosis (left, n=18), hypertrophic cardiomyopathy (middle, n=8), and the combined population (right, n=26).
Our preliminary investigations suggest EQ–CMR is reproducible and may be more reliable than the current biopsy gold standard. We attempted to optimize the gold standard by using intraoperative, deep sampling in a disease model with relative fibrosis homogeneity. However, the biopsy sample size of <0.1 g, less than a thousandth of the total myocardial volume, remains more prone to sampling error than our chosen CMR analysis volume, which is 30 to 50 times larger. With further refinement, EQ–CMR has the potential for whole-heart fibrosis quantification/mapping to truly reflect the global myocardial burden.

Limitations
Our data show that 80% of the between-subject differences in Vd(b) are explained by the measured CVF, but that this relationship is not one to one. A number of factors may influence this offset correlation. The measured CVF is the volume after fixation, not the in vivo volume, and it is assumed to be entirely available for Gd-DTPA distribution (as if no interstitial matrix components are present). Similarly, the measured Vd(b) assumes all plasma is available for Gd-DTPA distribution, yet proteins such as albumin are assumed to be entirely available for Gd-DTPA distribution a few percent. Finally, there are differences in the macro-environment between blood and tissue, potentially affecting the relaxivity of Gd-DTPA, although prior work suggests such differences are negligible. Finally, there was an up to 5% offset of our T1 measurements to actual T1. Despite all these factors, EQ–CMR is a potentially clinically applicable technique. Before it can be applied in routine practice, however, it would be necessary to establish the distribution in healthy individuals (especially as diffuse fibrosis is known to occur as part of aging). In addition, the sample size in this validation study was small.

Conclusions
We have developed a new technique, EC–CMR, that can quantify diffuse myocardial fibrosis with a strong correlation with the current gold standard of histological biopsy. The technique has the potential for wide applicability in disease characterization, monitoring, outcome prediction, and therapeutics. The principles described for this technique are likely to prove transferable to other organs.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Diffuse myocardial fibrosis is a final common end point in cardiovascular disease and is associated with symptoms, impaired ventricular function, and adverse prognosis. Even though it is thought to be a key “missing parameter” in clinical cardiology, and it is a target for many therapies (eg, angiotensin-converting enzyme inhibitors, aldosterone antagonists), it can only be quantified by histology on biopsy, with inherent risk and sampling error. As a result, it is not well understood in the clinical arena. Echocardiographic surrogates such as markers of diastolic dysfunction are unsatisfactory because they are influenced by multiple other factors. We developed and performed initial validation for a new technique, equilibrium contrast cardiovascular magnetic resonance (EQ–CMR), as a method to quantify diffuse myocardial fibrosis. We designed it to be easy to implement on any scanner. EQ–CMR involves standard MRI technology and contrast agent and can be integrated into a standard CMR protocol. We have shown that EQ–CMR correlates with histological fibrosis in 2 conditions, aortic stenosis and hypertrophic cardiomyopathy. This work is at an early stage, but it appears that, for the first time, it is possible to measure the diffuse myocardial fibrosis burden noninvasively. With further work, this technique could have widespread implications in disease characterization, monitoring, outcome prediction, and therapeutics.
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SUPPLEMENTAL MATERIAL

a) T1 measurement technique development

Phantom samples were manufactured using agar jelly (10 g/l) and pipetted drops of Gd-DTPA and MnCl$_2$ to produce phantoms with varying T1 and T2 properties. These were imaged using the standard gradient echo IR sequence (Slice thickness 8.0 mm, TR 9.8 ms, TE 4.6 ms) with varying heart rates, segmentation and repeat times with the gold standard being T1 relaxometry (We used a non-segmented IR GRE sequence, with TR 5000 ms, TE 2.67 ms, acquiring images at inversion times of 120ms, 200ms, 400ms, 800ms, 1600ms, 3200ms and no inversion. We performed a curve fit and calculated the x axis crossing as the null point and TI of the phantom).

b) Heart rate correction

The heart rate at the time of the T1 measurement sequence heavily influences the measured T1. We used a spoiled gradient echo inversion recovery technique and corrected the derived TI for incomplete longitudinal recovery using the following method:

After a 180° inversion pulse, $M_z$ (longitudinal magnetization) recovers exponentially from $-1$ to $+1$:

$$M_z = 1 - 2e^{-t/T1} \quad (1)$$

For breath-hold cardiac imaging the choice of TR is restrained to a multiple of the RR interval. During a segmented inversion recovery sequence, after a dummy pulse, $M_z$ reaches a ‘steady state’ somewhat less than 1, assuming the heart rate (TR) is regular. Call this $f$, the restoration factor. At steady state, magnetization recovers from $-f$ to $f$ during time TR.

$$f = 1 - (1+f)e^{-TR/T1} \quad (2)$$
Furthermore, when the image shows no signal ($M_z=0$), at time $T_{I'}$, magnetization has recovered from $-f$ to zero.

\begin{equation}
0 = 1 - (1+f)e^{-\frac{T_{I'}}{T_1}}
\end{equation}

We can isolate $T_1$ from Eq. 3:

\[ T_1 = \frac{T_{I'}}{\ln(1+f)} \]

Now if we substitute $T_1$ into Eq.2 we derive $f$:

\[ f = 1 - (1+f)^{1-\frac{TR}{T_{I'}}} \]

That gives us the equations for both $f$ and $T_1$, which can be solved for $0<f<1$ (see supplementary Excel file).

This method was proven in phantom experiments, (supplemental figure). At the limit, when $TR$ and $T_1$ are similar, imaging ghosting is present and the correction breaks down. In post-hoc analysis, when $T_1$ (corrected and uncorrected) were compared to CVF from histology removing the correction caused the $r^2$ to fall from 0.80 to 0.54.

c) Readout effect.

Multiple different readout combinations were tried iteratively. We found that a segmented readout reduced measured $T_1$ compared to relaxometry, but provided the segmentation and flip angle were relatively low (21 segments, $\alpha=21^\circ$), the difference from measured to true $T_1$ was kept to 5%.
Supplemental Figure Legend

Effects of mathematical heart rate correction

Left – Phantom experiment showing that HR correction restores measured T1 to actual T1 as TR shortens within reasonable limits. Right – a post-hoc analysis showing how important HR correction is – without it, the Vd(m):CVF correlation falls from 0.8 to 0.54.

Supplemental Figure