ORIGINAL ARTICLE

Human non-contrast T1 values and correlation with histology in diffuse fibrosis

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ABSTRACT

Background Aortic stenosis (AS) leads to diffuse fibrosis in the myocardium, which is linked to adverse outcome. Myocardial T1 values change with tissue composition.

Objective To test the hypothesis that our recently developed non-contrast cardiac magnetic resonance (CMR) T1 mapping sequence could identify myocardial fibrosis without contrast agent.

Design, setting and patients A prospective CMR non-contrast T1 mapping study of 109 patients with moderate and severe AS and 33 age- and gender-matched controls.

Methods CMR at 1.5 T, including non-contrast T1 mapping using a shortened modified Look–Locker inversion recovery sequence, was carried out. Biopsy samples for histological assessment of collagen volume fraction (CVF%) were obtained in 19 patients undergoing aortic valve replacement.

Results There was a significant correlation between T1 values and CVF% ($r=0.65$, $p=0.002$). Mean T1 values were significantly longer in all groups with severe AS (972 ± 33 ms in severe asymptomatic, 1014 ± 38 ms in severe symptomatic) than in normal controls (944 ± 16 ms) ($p<0.05$). The strongest associations with T1 values were for aortic valve area ($r=-0.40$, $p=0.001$) and left ventricular mass index (LVMI) ($r=0.36$, $p=0.008$), and these were the only independent predictors on multivariate analysis.

Conclusions Non-contrast T1 values are increased in patients with severe AS and further increase in symptomatic compared with asymptomatic patients. T1 values lengthened with greater LVMI and correlated with the degree of biopsy-quantified fibrosis. This may provide a useful clinical assessment of diffuse myocardial fibrosis in the future.

INTRODUCTION

The ability to quantify diffuse myocardial fibrosis (DMF) in patients non-invasively is of considerable clinical interest as the pathology of DMF is reversible with treatment and therefore a potential treatment target.[1 2] The final common end point of DMF is *irreversible focal fibrosis*, which is linked directly to prognosis in a variety of conditions, including aortic stenosis (AS), hypertrophic and dilated cardiomyopathy,[3–5] and can be measured with cardiovascular magnetic resonance (CMR) imaging using post-contrast late gadolinium enhancement.[6–8]

Measurement of DMF is, however, more complex. The current 'gold standard' method for quantification of DMF of the left ventricle is myocardial biopsy, which is invasive, associated with morbidity, subject to sampling error and cannot determine the fibrotic involvement of the whole left ventricle.[9] Focal fibrosis, such as infarction, changes the longitudinal relaxation properties (T1 values) of tissue during CMR,[10] and animal work suggests that the same occurs in diffuse fibrosis.[11] New CMR techniques have exploited these changes in T1 values to permit the measurement of DMF.[9 12] However, these methods measure *post-contrast* myocardial T1 values or the change in T1 after a contrast bolus or infusion,[13 14] and this is time consuming, demands complex processing and cannot be used in patients with severe renal impairment owing to the risk of nephrogenic systemic fibrosis.[9] A technique for measuring DMF without the need for contrast agent would therefore be attractive as it has the potential to reduce scan time (and cost), and can be applied in patients with renal disease without concern.

We therefore investigated the value of measuring *non-contrast* T1 values for assessment of DMF in humans using our recently developed shortened modified Look–Locker inversion recovery (ShMOLLI) T1 mapping sequence. Surgical biopsy specimens from patients with AS (a disease in which fibrosis is known to be diffuse and variable between patients) served as the gold standard disease model for DMF in this study.

ShMOLLI has already been used successfully in a clinical setting and showed increased non-contrast T1 values in patients with acute myocardial infarction.[15] Earlier studies in models of focal fibrosis, such as infarction, also showed increased myocardial non-contrast T1 values (eg, 1060 ± 61 ms compared with 987 ± 34 ms in remote myocardium).[10]

We therefore hypothesised that:
1. non-contrast T1 values would correlate well with the degree of fibrosis as measured in surgical biopsy specimens from patients with AS;
2. that T1 values would be increased in patients with AS compared with normal volunteers.

METHODS
Study population
Between July 2010 and March 2012, 24 symptomatic patients with severe AS awaiting aortic valve

replacement (AVR) were prospectively enrolled from the Heart Hospital Imaging Centre in London, UK; these patients underwent myocardial biopsy at the time of surgery. A second group of 85 asymptomatic patients with moderate or severe AS (based on Doppler echocardiographic demonstration of peak aortic valve gradient ≥36 mm Hg or valve area <1.5 cm^2, according to established criteria)[16] were recruited prospectively from cardiology clinics at the John Radcliffe Hospital in Oxford, UK. Exclusion criteria were contraindications to CMR (including defibrillators and pacemakers), more than mild aortic or mitral regurgitation, significant left ventricular (LV) dysfunction (LV ejection fraction <40%), uncontrolled hypertension or severe renal failure (serum creatinine >200μmol/l), which could in itself increase myocardial fibrosis.

Thirty-three age- and sex-matched normal volunteers were also recruited from both centres; comorbidities and symptoms of cardiac disease were excluded before inclusion in the study. The study was approved by the ethics committees at both institutions and all patients and normal volunteers gave written informed consent.

CMR protocol
All subjects underwent CMR with a 1.5 T clinical scanner (Avanto, Siemens Healthcare, Erlangen, Germany). In the AS population, standard LV volumes were acquired, together with additional short-axis images of the aortic valve, in line with standard cardiovascular MRI protocols.[17] In addition, a mid-ventricular short-axis slice was acquired using the ShMOLLI sequence for T1 mapping in all subjects.[18]

Image analysis
CMR image analysis was performed offline using Qmass software (Medis, Leiden, The Netherlands) according to the Society for Cardiovascular MR guidelines for reporting cardiovascular MR examinations.[19] LV volumes and function were analysed with all parameters indexed to body surface area. Aortic valve area was determined by direct valve planimetry.

T1 measurements
A region of interest in the septum was chosen to match the imaging and histological locations as closely as possible (biopsy samples were taken from the septum). Manual contouring was used to define the septum as the region of interest (ROI) on each image. ShMOLLI T1 maps sometimes demonstrated typical steady-state free precession susceptibilities to off-resonance artefacts and partial volume effects, and we assessed the images for areas with potentially compromised T1 values in three ways, as previously published[20 21]: we examined the T1 map itself, the raw T1 images, and also R^2 maps. In all cases, the myocardial T1 could be reliably measured within the septum on at least one large ROI. The T1 of the ROI was calculated using in-house software (MyoCardial Regions Of Interest (MC-ROI), programmed by author SKP in Interface Definition Language V.6.1, ITT Exelis, Mclean, Virginia, USA) as described by Piechnik et al.[18] See figure 1.

Histology
Histological sampling and analysis was performed as previously described by Flett et al.[14] In summary, an intraoperative deep myocardial biopsy specimen (Tru-cut type biopsy needle) was taken from the basal LV septum, stained with picrosirius red, and photographed at high-power magnification (×200), figure 2. The collagen volume fraction was automatically quantified over an average of 12 high-power fields using a purpose-written macro in

ImageJ.[22] All samples were analysed (by ASF) blinded to the CMR findings and T1 values. Samples were excluded if they were found to contain superficial (mainly endocardial) tissue or extremes of patchy fibrosis.

Exercise testing
All patients with asymptomatic AS underwent exercise treadmill testing. This was performed according to the Naughton protocol,[23] as this has a smoother profile of increasing speed/incline than other protocols, to allow a better assessment of maximum walk time.

Data analysis and statistics
Data analysis was performed with IBM SPSS Statistics, V.20.0 (Chicago, Illinois, USA). Values are expressed as means±SD unless otherwise stated. All continuous variables were normally distributed (Kolmogorov–Smirnov test). The χ^2 test was used to compare discrete data, as appropriate. Comparison of continuous variables between the four groups were performed using analysis of variance with post hoc Bonferroni correction. Correlations between variables were assessed using Pearson's method. Variables with a significant association with T1 from Pearson's analysis were entered into a multiple linear regression analysis to identify those with an independent association. Statistical tests were two tailed, and a p value <0.05 was considered to indicate a statistically significant difference.

RESULTS
### Baseline clinical and imaging data
One hundred and nine patients with AS and 33 normal volunteers were recruited in the study period. The demographic data are presented in table 1 and imaging data in table 2. There were no significant differences in gender or ages between the normal controls and patients with AS.

Correlation of histology and T1 values
There was a moderate correlation between myocardial T1 values and histological percentage of fibrosis (r=0.65, p=0.002), see figure 3. LV biopsy samples were obtained in 23 of the 24 patients with severe symptomatic AS. One patient had not yet undergone the planned operative procedure at the time of writing. Four biopsy specimens were excluded from further analysis according to the pre–specified exclusion criteria: (1) extremes of patchy fibrosis (n=2); (2) sample too superficial and included mainly endocardial tissue (n=2).

Non-contrast T1 values in patients with DMF and normal subjects
The mean T1 values in the AS group were significantly higher (971±39 ms) than in normal volunteers (944±16 ms) (p<0.001). T1 values lengthened with the severity of AS, however: mean±SD T1 values 955±30 ms in moderate AS, 972 ±33 ms in severe asymptomatic AS, 1014±38 ms in severe symptomatic AS, and were higher in both severe AS groups than in normal controls (p<0.05 for severe asymptomatic group; p<0.001 for severe symptomatic group; figure 4). T1 values in the group with severe symptomatic AS were also significantly higher than in the groups with severe asymptomatic AS and moderate AS (both p<0.001). Groups with moderate AS were not significantly different from normal controls. Non-contrast T1 values had a significant association with age, LV mass index, AV area, peak AV gradient and time walked on the treadmill (table 3). A stepwise multivariate linear regression analysis showed that only AV area (p=0.001) and LV mass index (p=0.008) were independently associated with non-contrast T1 values.
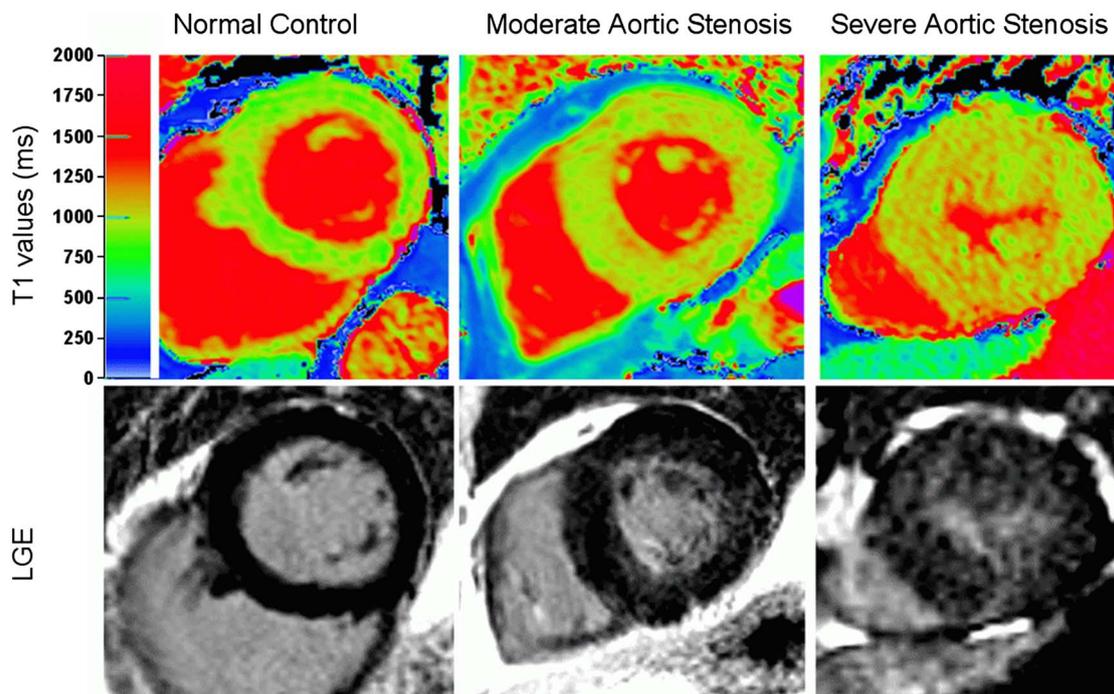
Figure 1 Top panel: colour maps of T1 values using shortened modified Look–Locker inversion in a mid-ventricular short-axis slice; bottom panel: the corresponding slice with late gadolinium enhancement (LGE) imaging. The left-hand panel shows a normal volunteer (T1=944 ms). The middle panels show moderate aortic stenosis (AS) with moderate left ventricular hypertrophy (T1=951 ms). The right-hand panel shows severe AS with severe left ventricular hypertrophy (T1=1020 ms).

DISCUSSION

We have shown that (1) non-contrast T1 values measured using the ShMOLLI CMR sequence[18] correlate well with percentage collagen volume fraction as measured from histology in patients with AS (a recognised model for DMF in humans); (2) non-contrast T1 values are increased in AS; and (3) non-contrast T1 values in AS increase with lesion severity.

To our knowledge, this is the first study in animals or humans to show a robust correlation between the degree of DMF and non-contrast T1 values measured using the ShMOLLI CMR sequence. T1 mapping sequences such as MOLLI (modified Look–Locker inversion recovery)[24] have been previously used to estimate DMF by determining the myocardial extracellular volume, but this requires the administration of the extracellular contrast agent gadolinium, to compare the ratio of signal change (T1 before and after contrast) in blood and myocardium[14] Breath-hold times for MOLLI sequences are also relatively long (15–20 s) and can be difficult in patients with cardiac disease.

ShMOLLI represents an exciting new development in the application of T1 mapping in clinical practice for a number of reasons. It enables myocardial T1 values to be measured robustly and in a single breath-hold (10 s), making this sequence easy to apply in a clinical setting even in patients who have difficulty in holding their breath. The generated values are substantially independent of heart rate, and data post-processing is
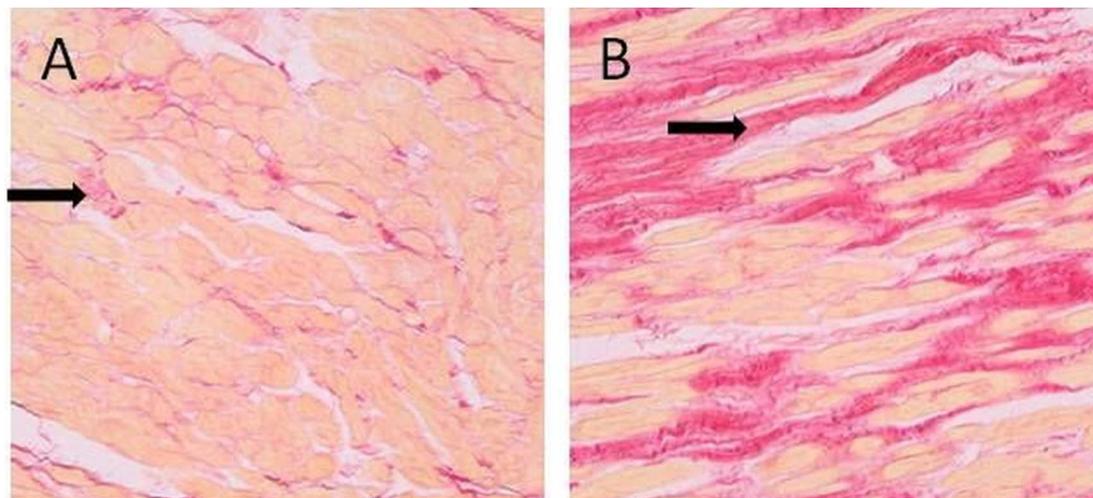
Figure 2 Histology of the myocardium in patients with aortic stenosis stained with picrosirius red. Black arrows show fibrosis. (A) A patient with mild fibrosis (collagen volume fraction (CVF)=8% and T1=952 ms). (B) A patient with heavy fibrosis (CVF=27% and T1=1063 ms).

Table 1 Baseline characteristics of study groups

Characteristics	Normal controls (n=33)	Moderate AS (n=63)	Severe asymptomatic AS (n=22)	Severe symptomatic AS (n=24)	p Value
Age, years	62.4±7	67.5±13.0	68.3±19.2	69.8±10.3	0.12
Male, n (%)	21 (64)	47 (75)	13 (59)	16 (67)	0.49
Body surface area, m²	1.86±0.21	1.98±0.19	1.86±0.22	1.88±0.19	0.05
Time on treadmill, s	–	1080±344	1042±350	–	0.69
Diabetes, n (%)	–	8 (13)	0 (0)	5 (21)	0.09
Current smokers, n (%)	–	5 (8)	3 (14)	2 (8)	0.72
Antihypertensive treatment, n (%)	–	10 (16)*	4 (18)*	14 (58)	<0.001
Statin treatment, n (%)	–	24 (38)	13 (59)	9 (38)	0.19

Values are means±SD unless otherwise stated.
p<0.05 considered significant.
*Statistically significant difference from group with severe symptomatic AS.
AS, aortic stenosis.

straightforward.[18] Most importantly, the T1 values measured are reproducible[20][21] and in our study were sufficiently accurate to detect changes in global myocardial T1 in patients with DMF *without the need for contrast*, and which correlated well with fibrosis burden measured in biopsy samples.

The simplicity of the method means that non-contrast mapping using SHMOLLI lends itself well to routine non-invasive assessment of DMF in cardiac disease; the technique is fast, simple to apply and the colour map generated in a single breath-hold may provide useful clinical information quickly. In addition, there are significant clinical advantages over non-contrast imaging; gadolinium contrast is a relative contraindication in patients with severe renal failure, carries a cost and increases scan time.

An interesting finding of this study was the range of T1 values measured in the patients with AS of mixed severity. The T1 values were longest in patients with severe symptomatic AS, and significant difference in T1 values were found between the groups with severe asymptomatic and symptomatic AS. The appearance of symptoms in patients with AS is not well explained by simple haemodynamics alone[25][26] and it has long been suspected that myocardial disease, particularly fibrosis, plays an important role; this study support that hypothesis. Recently, a large biopsy study confirmed that fibrosis in AS is linked to both clinical status and long-term survival after AVR.[27] CMR has shown an ability to detect focal fibrosis in patients

with AS using the late gadolinium enhancement technique, and this has been linked to prognosis.[3] The ability to measure DMF and focal fibrosis in AS non-invasively may thus be of significant clinical benefit, and be potentially useful for monitoring disease progression and antifibrotic treatments in both conventional and 'low-flow' AS.[6][28][29]

The overlap in T1 values between the group with moderate AS and normal volunteers in our study is noted. This may reflect normal myocardial tissue in this AS group with an excellent prognosis, and supports the concept that the myocardial response is an important part of the progression to more symptomatic disease. The negative correlation between increasing T1 values and time spent on the treadmill in the group as a whole would also support this. However, recent work shows an extremely wide range of DMF (8–42%) in symptomatic patients undergoing AVR[14] which suggests that the factors leading to symptom development in patients with AS are multiple, and may not be due to fibrosis alone. In the long term, an integration of several indicators may help to predict symptom progression, including fibrosis burden, pressure overload, degree of LV hypertrophy, filling pressure and perfusion reserve. However, the correlation of myocardial T1 with the degree of histological

Table 2 Baseline echocardiographic and cardiac MR parameters

| Parameters	Moderate AS (n=63)	Severe asymptomatic AS (n=22)	Severe symptomatic AS (n=24)
Echocardiography			
Peak AV velocity m/s	3.2±0.4	3.9±0.6*	4.3±0.6* **
Cardiac MRI			
LV mass (g)	159±39	162±41	185±48*
LV mass index (g/m²)	82±17	88±22	98±24*
LV ejection fraction (%)	69±8	75±9*	71±11.9
LVEDV (ml)	143±35	126±30	145±38
LVESV (ml)	46±21	32±16*	44±27
T1 values (ms)	955±30	972±33***	1014±38* ** ***

Values are means±SD.
*p<0.05 vs moderate AS, **p<0.05 vs severe asymptomatic AS, ***p<0.05 vs normal subjects.
AS, aortic stenosis; AV, aortic valve; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.
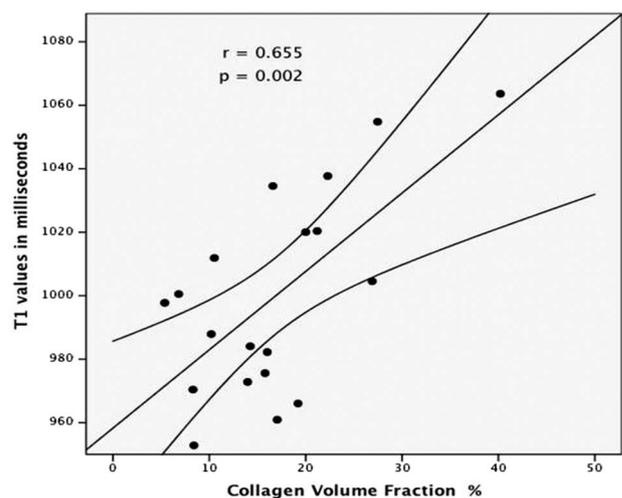
Figure 3 Correlation of myocardial collagen volume fraction from intraoperative myocardial biopsy specimens and non-contrast T1 times, in 19 subjects undergoing aortic valve replacement. The central line represents the regression line; the curved outside lines represent the 95% CI for this.
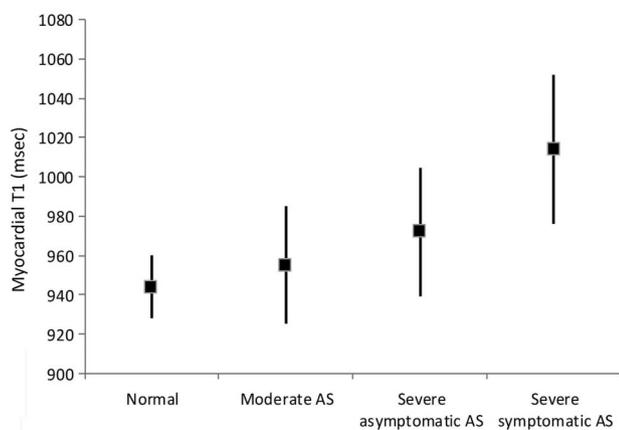
Figure 4 Whisker plot of mean myocardial T1 values (square symbols) with SDs (lines) in normal controls, and groups with moderate aortic stenosis (AS), severe asymptomatic AS and severe symptomatic AS.

fibrosis and the progression across AS groups suggests that this measure may have future potential as an indicator of myocardial dysfunction and/or prognosis.

Study limitations

The correlation between T1 values and fibrosis is based on a relatively small number of samples and might have been influenced by individuals with extreme levels of fibrosis. There is also a wide range of DMF for T1 values between 960 and 980 ms and overlap in T1 values between symptomatic and asymptomatic patients. This may limit its usefulness in individual patients.

Age has been shown to affect myocardial T1 values,[30] and we attempted to avoid this potentially confounding effect by matching our control subjects for age. The non-contrast T1 values for normal subjects in this study using ShMOLLI (944±16 ms) were marginally shorter than those published in the literature using the MOLLI sequence (977±33 ms).[10] Whether this is because our normal subjects were older than in previous studies (being age matched to the patients with AS), or because of differences in mean T1 values measured by different sequences, merits further investigation.[30] The fibrosis content of the ageing heart is unclear, however; a recent study showed no increase in myocardial extracellular volume as the heart ages,[31] whereas another small study showed an increase in interstitial fibrosis in the ageing heart.[32]

The only model of DMF investigated in this study was AS and it would be interesting to apply this method in other cardiac pathologies.

Table 3 Bivariate and multivariate analysis of associations with T1 values

	Bivariate analysis		Multivariate analysis	
	r	p Value	Standardised B	p Value
Age	0.21	0.009	–	–
LV mass index	0.36	<0.001	0.25	0.008
AV area	−0.40	<0.001	−0.32	0.001
Peak AV gradient	0.31	0.001	–	–
Time on treadmill	−0.25	0.033	–	–

p<0.001 was considered significant.
AV, aortic valve; LV, left ventricle.

CONCLUSION

We have shown that non-contrast T1 values measured using the ShMOLLI CMR sequence correlate with DMF burden measured by histology in patients with AS. The application of this technique is feasible in a clinical setting and it has the potential to be used as a simple, non-invasive, non-contrast assessment of DFM in cardiac disease. Further work will be required to determine its role in monitoring potential future antifibrotic treatments, and to aid risk stratification of cardiac patients.

REFERENCES

1. Diez J, Querejeta R, Lopez B, et al. Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chamber stiffness in hypertensive patients. Circulation 2002;105:2512–17.
2. Krayenbuehl HP, Hess OM, Monrad ES, et al. Left ventricular myocardial structure in aortic valve disease before, intermediate, and late after aortic valve replacement. Circulation 1989;79:744–55.
3. Dweck MR, Joshi S, Murigu T, et al. Midwall fibrosis is an independent predictor of mortality in patients with aortic stenosis. J Am Coll Cardiol 2011;58:1271–9.
4. Adabag AS, Maron BJ, Appelbaum E, et al. Occurrence and frequency of arrhythmias in hypertrophic cardiomyopathy in relation to delayed enhancement on cardiovascular magnetic resonance. J Am Coll Cardiol 2008;51:1369–74.
5. Assomull RG, Prasad SK, Lyne J, et al. Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy. J Am Coll Cardiol 2006;48:1977–85.
6. Azevedo CF, Nigri M, Higuchi ML, et al. Prognostic significance of myocardial fibrosis quantification by histopathology and magnetic resonance imaging in patients with severe aortic valve disease. J Am Coll Cardiol 2010;56:278–87.
7. Debl K, Djavidani B, Buchner S, et al. Delayed hyperenhancement in magnetic resonance imaging of left ventricular hypertrophy caused by aortic stenosis and hypertrophic cardiomyopathy: visualisation of focal fibrosis. Heart 2006;92:1447–51.
8. Rudolph A, Abdel-Aty H, Bohl S, et al. Noninvasive detection of fibrosis applying contrast-enhanced cardiac magnetic resonance in different forms of left ventricular hypertrophy relation to remodeling. J Am Coll Cardiol 2009;53:284–91.
9. Mewton N, Liu CY, Croisille P, et al. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. J Am Coll Cardiol 2011;57:891–903.
10. Messroghli DR, Niendorf T, Schulz-Menger J, et al. T1 mapping in patients with acute myocardial infarction. J Cardiovasc Magn Reson 2003;5:353–9.
11. Wisenberg G, Prato FS, Carroll SE, et al. Serial nuclear magnetic resonance imaging of acute myocardial infarction with and without reperfusion. Am Heart J 1988;115:510–18.
12. White SK, Sado DM, Flett AS, et al. Characterising the myocardial interstitial space: the clinical relevance of non-invasive imaging. Heart 2012;98:773–9.
13. Iles L, Pfluger H, Phrommintikul A, et al. Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. J Am Coll Cardiol 2008;52:1574–80.
14. Flett AS, Hayward MP, Ashworth MT, et al. Equilibrium contrast cardiovascular magnetic resonance for the measurement of diffuse myocardial fibrosis: preliminary validation in humans. Circulation 2010;122:138–44.

15 Dall'Armellina E, Piechnik SK, Ferreira VM, *et al*. Cardiovascular magnetic resonance by non contrast T1-mapping allows assessment of severity of injury in acute myocardial infarction. *J Cardiovasc Magn Reson* 2012;14:15.

16 Bonow RO, Carabello BA, Kanu C, *et al*. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (writing committee to revise the 1998 Guidelines for the Management of Patients With Valvular Heart Disease): developed in collaboration with the Society of Cardiovascular Anesthesiologists: endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. *Circulation* 2006;114:e84–231.

17 Kramer CM, Barkhausen J, Flamm SD, *et al*. Standardized cardiovascular magnetic resonance imaging (CMR) protocols, society for cardiovascular magnetic resonance: board of trustees task force on standardized protocols. *J Cardiovasc Magn Reson* 2008;10:35.

18 Piechnik SK, Ferreira VM, Dall'Armellina E, *et al*. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. *J Cardiovasc Magn Reson* 2010;12:69.

19 Hundley WG, Bluemke D, Bogaert JG, *et al*. Society for Cardiovascular Magnetic Resonance guidelines for reporting cardiovascular magnetic resonance examinations. *J Cardiovasc Magn Reson* 2009;11:5.

20 Dass S, Suttie JJ, Piechnik SK, *et al*. Myocardial tissue characterization using magnetic resonance noncontrast t1 mapping in hypertrophic and dilated cardiomyopathy. *Circ Cardiovasc Imaging* 2012;5:726–33.

21 Ferreira VM, Piechnik SK, Dall'Armellina E, *et al*. Non-contrast T1-mapping detects acute myocardial edema with high diagnostic accuracy: a comparison to T2-weighted cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2012;14:42.

22 Rasband W. *ImageJ*. Bethseda, MD: National Health of Health, 1997–2009; http://rsb.info.nih.gov./ij/.

23 Naughton J, Sevelius G, Balke B. Physiological responses of normal and pathological subjects to a modified work capacity test. *J Sports Med Phys Fitness* 1963;44:201–7.

24 Messroghli DR, Radjenovic A, Kozerke S, *et al*. Modified Look-Locker inversion recovery (MOLLI) for high-resolution T1 mapping of the heart. *Magn Reson Med* 2004;52:141–6.

25 Hachicha Z, Dumesnil JG, Bogaty P, *et al*. Paradoxical low-flow, low-gradient severe aortic stenosis despite preserved ejection fraction is associated with higher afterload and reduced survival. *Circulation* 2007;115:2856–64.

26 Minners J, Allgeier M, Gohlke-Baerwolf C, *et al*. Inconsistencies of echocardiographic criteria for the grading of aortic valve stenosis. *Eur Heart J* 2008;29:1043–8.

27 Milano AD, Faggian G, Dodonov M, *et al*. Prognostic value of myocardial fibrosis in patients with severe aortic valve stenosis. *J Thorac Cardiovasc Surg* 2012;144:830–7.

28 Herrmann S, Stork S, Niemann M, *et al*. Low-gradient aortic valve stenosis myocardial fibrosis and its influence on function and outcome. *J Am Coll Cardiol* 2011;58:402–12.

29 Lancellotti P, Magne J, Donal E, *et al*. Clinical outcome in asymptomatic severe aortic stenosis: insights from the new proposed aortic stenosis grading classification. *J Am Coll Cardiol* 2012;59:235–43.

30 Piechnik SK, Ferreira V, Lewandowski AJ, *et al*. Age and gender dependence of pre-contrast T1-relaxation times in normal human myocardium at 1.5T using ShMOLLI. *J Cardiovasc Magn Reson* 2012;14(Supp 1):P221.

31 Sado DM, Flett AS, Banypersad SM, *et al*. Cardiovascular magnetic resonance measurement of myocardial extracellular volume in health and disease. *Heart* 2012;98:1436–1.

32 Debessa Gazoti CR, Maifrino Mesiano LB, de Souza Rodrigues R. Age related changes of the collagen network of the human heart. *Mech Ageing Dev* 2001;122:1049–58.