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ORIGINAL ARTICLE

Diagnostic and prognostic value of MRI T2 quantification in heart transplant patients

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Summary

This study was designed retrospectively to assess the value of myocardial T2 to detect or predict ongoing acute heart rejection, in heart transplant patients, with a 1.5-T MRI magnet. One hundred and ninety-six myocardial T2 quantifications were performed on sixty consecutive heart transplant patients during routine follow-up. T2 values were assessed (i) with regard to the results of concomitant biopsies and (ii) with a Cox multivariate model for the prediction of subsequent rejections, defined by a ≥ grade 2 at biopsy or highly suspected in the absence of biopsy (>10% drop in ejection fraction with subsequent reversibility under treatment). T2 values were proposed as main covariate, after logit transformation and adjustment for other confounding parameters such as delay since graft surgery and delay before biopsy. T2 values were strongly linked (i) to the presence of rejection on concomitant biopsy ($P < 0.0001$) and (ii) to the risk of subsequent rejection on Cox multivariate model ($P < 0.001$). T2 values above 60 ms were associated with relative risk of rejection higher than 2.0 and rapidly increasing. In conclusion, myocardial T2 yields a high diagnostic and prognostic value for graft rejection in heart transplant patients.

Introduction

Cardiac transplantation offers improved quality of life and better survival for patients with advanced heart failure [1]. Nevertheless, this improvement is balanced by the risk of serious adverse events and especially of acute rejection during the first year [2,3]. Efficient monitoring of this risk is therefore mandatory to adapt immunosuppressive therapy. Clinical features of acute rejection are unreliable, with patients often remaining asymptomatic. Therefore, the gold standard for the diagnosis of acute cellular rejection is endomyocardial biopsy (EMB) [2–4]. This procedure is occasionally responsible for complications, however [4,5], and there are conflicting data on both its variability and accuracy [6–8]. The EMB pathological classification proposed by the International Society for Heart and Lung Transplantation (ISHLT) has been modified in 2005, but still remains controversial [8,9]. According to Subherwal *et al.* [10], acute rejection with apparently normal EMB could occur in up to 20% of patients. Hence, there is a need for a noninvasive and more accurate identification of allograft rejection [3,8,11–14]. Eleven years ago, we presented a first study using myocardial T2 determination provided by a black-blood MRI sequence on a low-field 0.5 T magnet to detect acute heart transplant rejection [11]. This T2 determination was able to identify most of the acute rejections defined by the presence of damaged myocytes within EMB (grade ≥ 2) and to predict the

subsequent occurrence of acute rejection. Since this study, however, many changes have occurred in MRI technology and in transplanted patient care [15]. First, immunosuppressive treatments have markedly improved, leading to enhanced survival and to lowered risk of acute rejection [16,17]. Furthermore, acute rejections are now more silent and more difficult to detect [18]. Second, MRI technology has considerably changed with the diffusion of higher field magnets.

This study was therefore designed to assess the diagnostic and prognostic value of myocardial T2 quantification determined with a 1.5 T MRI magnet in currently monitored transplanted patients.

Materials and methods

This retrospective monocentric noninterventional study complies with the Declaration of Helsinki regarding medical research on human subjects and was approved by a local ethical committee.

Population

Sixty different and consecutive heart transplant patients benefited between January 2005 and April 2012 of a routine monitoring based on EMB and T2 quantification in MRI. Two hundred and twelve consecutive MRI examinations for heart graft monitoring were thus performed (on average: 3.5 ± 5.0 examinations per patient; maximum: 27). Two to four examinations per week could be dedicated to this indication. Therefore, patients were referred to MRI according to the magnet availability and to the likelihood of acute rejection (based on clinical or echographic signs and delay from transplantation).

Transvenous EMB was performed only as clinically indicated for systematic control or in cases of suspected rejection. The systematic controls were performed at the following rates: at 2-week intervals for the first 4 months following transplantation and then at monthly intervals until the end of the first year. Biopsies were obtained from the right side of the interventricular septum after the introduction of a dedicated catheter through the internal jugular vein. At least four specimens of right ventricular tissue were obtained with the same catheter. Biopsies were interpreted by two experienced pathologists, blinded to the T2 values, and graded according to ISHLT [9]: Grade 0 Revised (0R) = no evidence of rejection; Grade 1 Revised (1R) = mild rejection corresponding to Grades 1A, 1B and 2 of the 1990 ISHLT classification; Grade 2 Revised (2R) = moderate rejection corresponding to Grade 3A of the 1990 classification; and Grade 3 Revised (3R) = severe rejection corresponding to Grades 3B and 4 of the 1990 classification.

T2 quantifications

Magnetic resonance imaging was conducted using a 1.5-T magnet (Signa Excite; GE Medical Systems, Milwaukee, WI, USA) and a body coil for transmission and reception of the MRI signal. The imaging protocol included localization sequences followed by the selection of a single mid-ventricular short axis slice for T2 measurement. Slice thickness was 10 mm. This slice was acquired ten times with different effective echo times (TE) ranging evenly from 9 to 80 ms. The sequence was a black-blood double-inversion fast-spin echo/turbo-spin echo, with an echo train length/turbo factor of 16. The first inversion was nonselective and immediately followed by a selective slice inversion. The mean interecho time was 4.71 ms (±0.30). The repetition time (TR) was equal to two heart beats. As TR was relatively short, complete recovery of longitudinal magnetization was not obtained. An inversion time of 485 ms was chosen to withdraw most of the blood-related signal [19]. The main acquisition parameters were as follows: field of view 42 × 42 cm, acquisition matrix 256 × 192, half Fourier space, and number of excitations equal to one. Images were obtained at the same phase of the cardiac cycle aiming for mid-diastole on sequential heart beats using prospective ECG gating. Each image was recorded during an end-expiratory breath-hold of no more than fifteen seconds.

All postprocessing computations were performed with a dedicated software developed with MATLAB (The Math Works Inc., Natick, MA, USA). At first, studies were anonymously submitted to one senior observer (JME) experienced in cardiac MRI and blinded to all patient data. Successive images obtained for each TE were corrected for heart displacement (translation and rotation) using an automatic feature-based image registration technique. The registration consisted in minimizing the distance between features extracted from each image by a Sobel edge detector using gradient descent optimization. The registration could be assessed by the observer through a movie presenting the ten images in a loop. When necessary, a manual correction of heart displacement was subsequently carried out. Thereafter, two regions of interest (ROI) were drawn by the observer covering the septum of one image and corresponding to the anteroseptal and inferoseptal segments according to AHA recommendations [20]. These two ROI were copied/pasted onto each image of the multiple TE series. Inferior, anterior, and lateral walls were not analyzed in this study, as previously described, to avoid the confusing influence of signal from surrounding fat [11]. An example of the positioning of these ROI is provided in Fig. 1a. T2 calculations were performed for each ROI with a nonlinear two-parameter Levenberg–Marquardt exponential fit applied to the myocardial signal measured on the echo images and based upon the equation

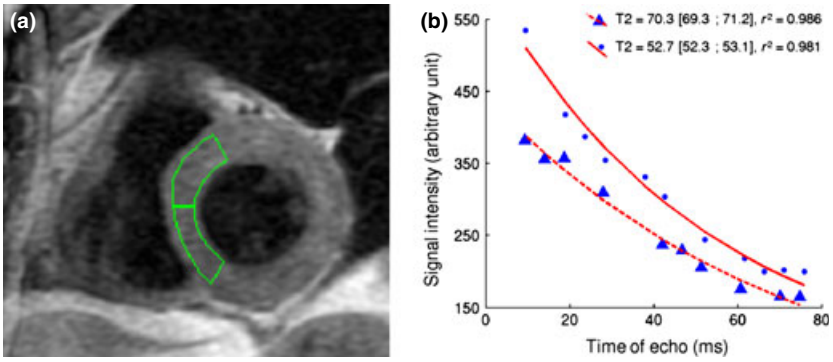

Figure 1 Illustration of our method to quantify myocardial T2. (a) Example of the positioning of the regions of interest for both septal segments. The physician chose to avoid the border of the myocardium to avoid error due to imperfect registration between the ten images. (b) Examples of exponential fits for normal and high T2. Both curves comprise 10 points corresponding to the signal intensity within a given septal regions of interest of images acquired with 10 different echo times (TE). Myocardium with higher T2 needs more time to decrease its signal intensity.

$M(\mathrm{TE}) = M_0 \times e^{-\frac{TE}{T2}}$, TE being the specific effective echo time obtained during acquisition and M(TE) the myocardial signal. Examples of this fit are presented in Fig. 1b. Confidence intervals for T2 measurements and r^2 values were computed from the exponential fit. Segments with $r^2 < 0.97$ were discarded from the final analysis. When both septal segments were associated with $r^2 \geq 0.97$, the mean of the two T2 values was used. This procedure was applied a second time by another experienced observer (LB) to assess interobserver reproducibility in a small subgroup, composed of the last thirty MRI examinations of the overall study.

Prognostic value of myocardial T2

To assess the predictive value of myocardial T2 measurements, patients were observed after each T2 quantification, and the time separating the examination from the nearest subsequent episode of significant rejection was collected. Observations were censored at the date of the last medical consultation or at the time of the next MRI T2 quantification. Observations were also censored whenever a rejection was related to an evident discontinuation of immunosuppressive treatment. Significant rejection was defined as follows: (i) acute rejection documented by the presence of damaged myocytes in EMB (former grade 2 plus grades 2R and 3R) or (ii) marked decrease in left ventricle ejection fraction (>10%), reversible after subsequent increase in immunosuppressive treatment, whenever EMB could not be performed because of technical difficulties for catheterization, or unstable clinical state.

Statistics

Descriptive data are reported as mean ± standard deviation for continuous variables and proportion for qualitative variables. A Bland and Altman analysis [21] was performed within a subgroup of 30 MRI examinations to assess T2 measurement reproducibility and compute interobserver 95% limits of agreement. The subgroup of T2 quantifications performed within 48 h of biopsy was analyzed (i) to compute the mean T2 value corresponding to grade 0 EMB and (ii) to compare T2 and biopsy grades with nonparametric Kruskal–Wallis tests and (iii) with receiver operating characteristics (ROC) curves.

To explore the predictive value of T2 quantification, the relationship with subsequent significant rejection was assessed by multivariate Cox analysis. The covariates chosen for adjustment were delay since graft surgery (which obviously influences the instantaneous risk of graft rejection) and delay between MRI and biopsy. To cope with the influence of the patient (mandatory as several MRIs were performed for each patient), a mixed model was used. For the Cox analysis, T2 was encoded with a logit transformation because the influence of T2 measurements was expected not to comply with the implicit log-linearity hypothesis of the Cox model (i.e., proportionality between myocardial T2 and risk). The parameters of this logit transformation and the parameters of the mixed Cox model are detailed in Appendix 1. Instantaneous relative risk factor for T2 was deduced from the model.

All statistical analyses were performed using R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) [22]. A $P < 0.05$ was considered to be statistically significant.

Results

Population

Our population comprised 60 patients (47 men and 13 women). The main characteristics of the population are summarized in Table 1. Twenty-seven patients (four females and twenty-three males) presented one to three moderate-to-severe acute rejections documented by

Table 1. Population characteristics with biopsy and MRI examination results.

Population	*n* = 60
Sex (Male, Female)	M = 47, F = 13
Age at transplantation, years (SD)	41 years (14.9) min = 14 years, max = 67 years
Donor age, years (SD)*	36 years (12.3) min = 12 years, max = 62 years
Body mass index, kg/m^2 (SD)	23.7 (4.2)
Follow-up duration, years (SD)	4.2 years (2.3)
Endomyocardial biopsies	*n* = 714
grade 0R	*n* = 256
grade 1R	*n* = 406
grade 2R	*n* = 47
grade 3R	*n* = 5
Biopsies, per patient	11.9 (6.2)
MRI examinations	*n* = 212
Time from transplant to MRI	2 years (2.3) min = 13 days, max = 12 years
T2 quantifications	*n* = 196
T2, ms (SD)	60.7 ms (11)
When EMB grade 0R (*n* = 14)	55.0 ms (2.3)
When EMB grade 1R (*n* = 42)	64.1 ms (11)
When EMB grades 2R and 3R (*n* = 19)	72.1 ms (9)
Time from T2 quantifications to EMB, days	
Whole population (*n* = 196)	Median=40 days
When T2 < 55 ms (*n* = 52)	Median=28 days
When T2 ≥ 55 ms and ≤65 ms (*n* = 94)	Median=77 days
When T2 > 65 ms (*n* = 50)	Median=20 days

EMB, endomyocardial biopsy.
*Data available for 50 donors only.

EMB (47 grade 2R and 5 grade 3R), with 46% of the documented rejections occurring during the first year after transplantation.

T2 quantifications

Two hundred and twelve MRI examinations were performed. No adverse event was reported. Sixteen of these examinations were discarded for inadequate precision of T2 measurement (two exponential fits with r^2 under 0.97). Therefore, the final analysis incorporated the results of 196 T2 quantifications. Within this population, the mean T2 value was 60.7 ± 11 ms, and thresholds were 55 ms for the upper limit of the first quartile ($T2_{25\%}$) and 65 ms for the lower limit of the last quartile ($T2_{75\%}$). The 95% confidence interval of the T2 calculated within a given ROI had a raw width of 0.80 ± 0.23 ms. The interobserver 95% limits of agreement of the T2 measurements within a subset of 30 examinations were 2.4 ± 0.37 ms. Seventy-five myocardial T2 quantifications were performed within 48 h of a biopsy, and in this subgroup, (i) the confidence interval of the myocardial T2 in the subset of patients with grade 0 EMB was 55 ± 2.3 ms; (ii) there was a strong link ($P < 0.0001$) between T2 values and EMB as detailed in Fig. 2; and (iii) there was indeed a clear separation with no overlap between patients with grade 0 and those with grade ≥2 at EMB,

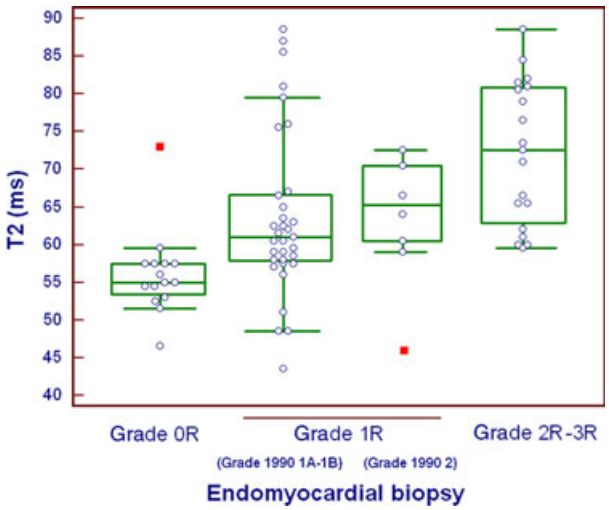

Figure 2 Box and whisker plots of T2 values according to the pathological grading of endomyocardial biopsies. The comparison was performed in a subset of patients when no more than 48 h of separate MRI and biopsy (75 cases). The dark squares correspond to outliers: the grade 0R one is a biopsy false negative (presence of many calcifications in the biopsy due to primary hyperoxaluria and reversible drop of ejection fraction: case number 8 in Table 2).

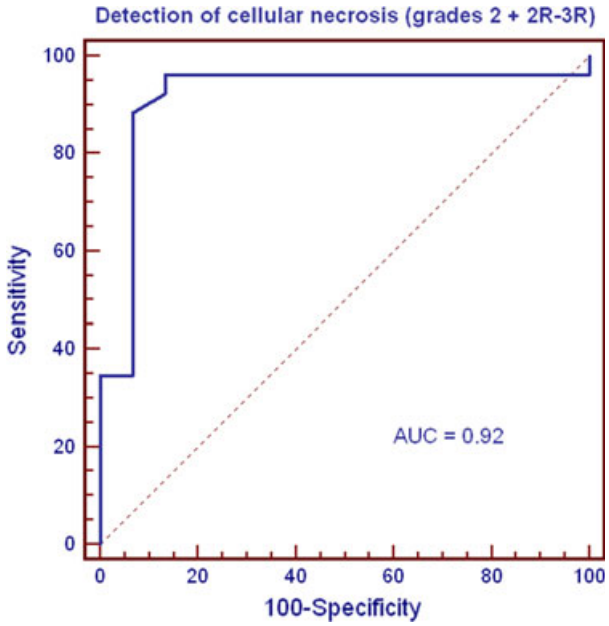

Figure 3 Receiving operator characteristics curve of T2 quantification used to separate normal biopsies (grade 0) from biopsies with damaged myocytes (former grade 2 plus grades 2R–3R).

Table 2. Acute rejection diagnosed on a reversible alteration of ejection fraction.

	Before rejection		During rejection			After rejection	
	Clinic	EF (%)	Clinic	EF (%)	Treatment	Clinic	EF (%)
1	I	55	II	35	Cb	I	55
2	I	65	III	35	Cb	II-	55
3	I	65	II	40	Aug(C + T)	II	55
4	II-	60	II+	40	Aug(T)	II	55
5	I	60	II	45	Cb	I	60
6	I	60	II	45	Cb	I	60
7	I	60	II	35	Cb	II	50
8	I	60	III	35	Cb	I	50
9	I	60	II+	40	Cb	I	60
10	I	50	III	30	Cb + Gb	II, death 2 m	50
11	I	60	I	45	Aug(C)	I	60
12	I	60	II	40	Sw(Y,TSR) + Cb + aug(M)	I	50
13	I	60	II	40	Cb	I	50
14	I	50	III	25	Cb	I	50
15	I	65	II	45	Cb	II-	50
16	I	65	III	20	E	II	30
17	I	60	II	30	Cb	II	45
18	II	45	II	30	Cb	I	45
19	I	60	I	30	Cb	I	50
20	I	50	II	40	Cb	I	50
21	I	65	II	45	Cb	I	55

Treatment: Xb, bolus of X; aug(X), augmentation of X; sw(X,Y), switch from X to Y; C, corticoid; T, tacrolimus; TSR, tacrolimus sustained release; E, everolimus; Y, cyclosporine; G, antithymocyte globulin; M, mycophenolate.
Clinic : Roman figures represent NYHA class.

Table 3. Relative risk (RR) of ongoing rejection and delay before next rejection for different myocardial T2.

T2 (ms)	RR	T2 (ms)	Delay before rejection* Mean (Median)
55	1.0	<55	102 days (28 days)
58.5	1.5	≥55 and ≤65	24 days (6 days)
60	1.8		
61	2.0		
64	3.0		
68	4.0	>65	11 days (0 days)
75	5.0		

The relative risk of rejection remains low, for T2 below 60 ms, but rapidly increases for higher T2 values.
*The observations without rejection were discarded for the computation.

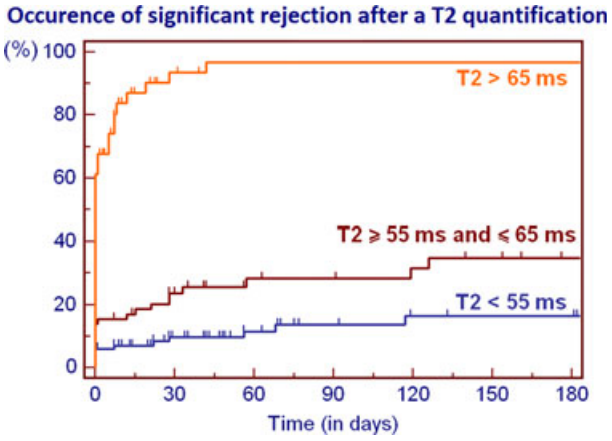

Figure 4 Prognostic value of T2 quantification during the follow-up of heart transplant patients: The three lines represent the prevalence of significant rejection in a given delay (in days) after a T2 quantification according to the measured T2 values: low T2 (<55 ms: 52 examinations), intermediate T2 (≥55 and ≤65 ms: 94 examinations), and high T2 (>65 ms: 50 examinations). The observations of patients were censored at the next T2 quantification or rejection or major change of treatment. This categorical variable (low, intermediate, or high T2) was significant within the Cox model ($P = 0.04$ for intermediate, $P < 0.0001$ for high T2).

grade 1 being mostly associated with intermediate T2 values. This separation between patients with grade 0 and those with grade ≥2 at EMB is illustrated in Fig. 3 with an area under the ROC curve of 0.92.

Prognostic analysis

Sixty-five episodes of significant rejections were identified after suppression of the censored events: 44 because of EMB and 21 because of a reversible alteration of ejection fraction. These 21 cases are described in Table 2. The overall fit of the Cox model was excellent for predicting the

occurrence of definite or highly probable acute rejection ($P < 0.0001$). After adjustment for the other covariates, there was a significant association between the risk of such rejection and T2 variations ($P = 0.0007$). The relative risks of rejection associated with different values of T2 above 55 ms are presented in Table 3. The relative risk for a T2 above 60 ms is 2.0 and raises to 4.0 above 65 ms. To illustrate the influence of T2 value on event-free survival, survival curves were drawn (Fig. 4) for patients with low (<55 ms), medium (≥55 ms and ≤65 ms), and high (>65 ms) T2. Low T2 values correspond to the first quartile of T2 values (<25%), and high T2 values correspond to the fourth quartile of T2 values (>75%).

Discussion

Heart transplant patients have to cope with the permanent threat of acute graft rejection [10]. This rejection may be life-threatening, and many discussions have been held to try to optimize the monitoring of these patients [3,23,24]. EMB remains the gold standard for diagnosis of cellular acute graft rejection, even though less invasive diagnostic methods such as MRI T2 quantification have been proposed [3,11,24]. Our method for T2 quantification was already published several years ago [11] and is mentioned in the "ISHLT Guidelines for the care of Heart Transplant Recipients" [24]. Hence, for the past several years, two to four MRI examinations per week have been dedicated to the monitoring of heart transplant patients in our institution, with a priority to patients with a higher likelihood of rejection. The present retrospective analysis of these MRI examinations gave evidence that myocardial T2 quantification still yielded high diagnostic and prognostic values in this population monitored with a modern 1.5-T MRI magnet in replacement of an older low-field system.

Indeed, in a first instance, our findings show that this technique provides robust results with a low rate of inconclusive examinations (7.5%) along with a high interobserver reproducibility of the T2 measurements. Limits of agreement between the two observers were only of 2.4 ms and corresponded to less than 5% of the mean myocardial T2 values.

Second, the high diagnostic value of the T2 measurement could be confirmed in a subgroup where MRI had been performed within 48 h of EMB (see Fig. 2). In this subgroup, moderate-to-severe rejections (≥grade 2R at EMB) were associated with high T2 values (mean = 72.5 ms), whereas biopsies with no rejection (grade 0R) were associated with low T2 values (mean = 55 ms). By contrast, the mild forms of rejections (grade 1R) were associated with the whole range of T2 values, although the 1R biopsies showing damaged myocytes (former ISHLT grade 2) were associated with higher T2 values (mean = 65 ms). This is in perfect accordance with our previous report [11] and justifies our choice to define rejection by the presence of damaged myocytes within the biopsy (former grade 2 plus grades 2R and 3R) for the prognostic study.

Third, the high prognostic value of the T2 measurement could also be confirmed with regard to the risk of certain or highly probable rejection. This composite end point was used because, in clinical practice, several patients were treated for acute rejection, whereas EMB was not performed because it was not considered technically possible or safe (clinical instability). In twenty-one among our sixty-five cases of acute rejection treatment, this treatment was decided in the absence of EMB result. However, the high probability of acute rejection could be confirmed by clinical and echographic monitoring, as detailed in Table 2. The curves displayed in Fig. 4 indicate that the rate of definite or highly probable rejection during follow-up is dramatically higher in the group with high myocardial T2 (>65 ms) than in the remaining groups. However, there are several potential confounding factors in this analysis because of the retrospective nature of this study, as patients with a higher risk of rejection had a higher priority for referral to MRI and because the delay between EMB and MRI was variable, according to current recommendations and to the delay from transplantation. This is why a Cox analysis was conducted with multivariate adjustment to cope with these confounding factors. The variables proposed for adjustment were chosen from the literature and based on our experience. Variable dependence was verified using a univariate model before entering the said variables in multivariate analysis. To the best of our knowledge, this study is the first to study the prognostic value of myocardial T2 with adjustments in a multivariate model. We found a strong relationship between the relative risk of rejection and increase in T2 values. The relative risk of rejection remains low, for T2 below 60 ms, but rapidly increases for higher T2 values.

Our findings constitute a strong argument in favor of a more extensive use of T2 quantification for risk stratification in the current monitoring of heart transplant patients. This could lead to recommending a decrease in the frequency of monitoring after a low T2 quantification, given the low probability of major event in this instance. However, this particular point should be confirmed prospectively in a multicentric and randomized study.

Limitations

Because this study was retrospective, the intervals between T2 measurements and EMB were variables, and no systematic T2 follow-up was conducted after treated rejections.

The authors acknowledge the existence of emerging methods for T2 quantifications that were not available at the beginning of this study. We used a conventional black-blood spin-echo MRI sequence, which requires several breath-hold recordings for a single slice, whereas myocardial T2 may now be measured with only one breath-hold per slice. However, these T2 mapping methods are not available on every MRI scanner and are dependent on the manufacturer. Moreover, they are much more sensitive to various artifacts and especially to field and pulse inhomogeneities. Our procedure remains relatively short (≤15 min) with the major advantage of being readily available, easy to perform, and highly robust. No specific dedicated cardiac coil or sequence is required, and only a conventional MRI scanner with an ECG-triggering system is needed.

Conclusion

Myocardial T2 measurements yield a high diagnostic and prognostic value for graft rejection in heart transplant patients. This simple MRI measurement could be very useful in the monitoring of these patients and could lead to a reduced need for EMB.

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Authorship

LB, TV, and PYM: wrote the manuscript. LB, TV, and JME: collected data. FO and JF: programmed the software. FV: managed the patients' follow-up. LB and GH: performed the statistical analysis. All authors contributed to the manuscript critical review.

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Appendix 1

The logit transformation used the following function: $\text{logit}(T2) = \frac{e^{(T_2-M)/s}}{1+e^{(T_2-M)/s}}$ with M being the value of T2 optimally separating patients with and without rejection and S being a factor coding for the slope of the logit function. We chose M = 60 ms from the results of the 75 couples MRI/EMB performed simultaneously (Fig. 2). S was chosen, among values equally distributed in a plausible interval (from 1 to 10 ms), to optimize the Akaike information criterion (AIC) of the model. The best S value was 4.0 ms.

The proportional hazard assumption of the model was verified with the function "cox.zph()". The log-linearity assumption was verified by testing square and higher polynomials of each covariate. The mixed model version of the Cox analysis was adapted from the "coxme" R package and used in a stepwise manner with usual parameters (variable entered if $P < 0.05$ and removed if $P > 0.1$).

