Cardiac Magnetic Resonance T2 Mapping in the Monitoring and Follow-up of Acute Cardiac Transplant Rejection: A Pilot Study

Usman et al: T2 Mapping in Cardiac Transplantation

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Abstract

Background—Acute rejection is a major factor impacting survival in the first 12 months after cardiac transplantation. Transplant monitoring requires invasive techniques. Cardiac magnetic resonance (CMR), non-invasive testing, has been used in monitoring heart transplants. Prolonged T2 relaxation has been related to transplant edema and possibly rejection. We hypothesize that prolonged T2 reflects transplant rejection and that quantitative T2 mapping will concure with the pathological and clinical findings of acute rejection.

Methods and Results—Patients were recruited within the first year after transplantation. Biopsies were graded according to the International Society for Heart Lung Transplant system for cellular rejection with immunohistochemistry for humoral rejection. Rejection was also considered if patients presented with signs and symptoms of hemodynamic compromise without biopsy evidence of rejection who subsequently improved with treatment. Patients underwent a novel single-shot T2-prepared SSFP four-chamber and three short axis sequences and regions of interest were drawn overlying T2 maps by two independent blinded reviewers. A total of 74 (68 analyzable) CMRs T2 maps in 53 patients were performed. There were four cellular, two humoral, and two hemodynamic rejection cases. The average T2 relaxation time for grade 0R (n=46) and grade 1R (n=17) was 52.5 ± 2.2 ms and 53.1 ± 3.3 ms (mean ± standard deviation) respectively. The average T2 relaxation for grade 2R (n=3) was 59.6 ± 3.1 ms and 3R (n=1) was 60.3 ms (all p-value < 0.05 compared to controls). The T2 average in humoral rejection cases (n=2) was 59.2 ± 3.3 ms and the hemodynamic rejection (n=2) was 61.1 ms ± 1.8 ms (p< 0.05 versus controls). The average T2 relaxation time for all-cause rejection versus no-rejection is 60.1 ± 2.1 versus 52.8 ± 2.7 ms (p-value < 0.05). All rejection cases were rescanned 2.5 months after treatment and demonstrated T2 normalization with average of 51.4 ± 1.6 ms. No difference was found in ventricular function between non-rejection and rejection patients, except in ventricular mass 107.8 ±10.3 gm versus 127.5 ± 10.4 gm (p < 0.05).

Conclusions—Quantitative T2 mapping offers a novel non-invasive tool for transplant monitoring, and these initial findings suggest potential utility in characterizing rejections. Given the limited numbers a larger multi-institution study may help elucidate the benefits of T2 mapping as an adjunctive tool in routine monitoring of cardiac transplants.

Key Words: diagnosis, heart failure, magnetic resonance imaging, transplantation, rejection, edema
Approximately 5000 heart transplants occur worldwide each year. Nearly half are performed in North America. Cardiac transplantation is currently the only definitive treatment for end stage heart failure.\(^1\) One of the primary concerns post heart transplant is acute transplant rejection. Approximately 21% of cardiac transplant patients experience at least one episode of acute allograft rejection in the first year. Furthermore, rejection is the cause of 12% of deaths between one and twelve months post transplantation.\(^1\) Therefore, patients undergo intensive and often invasive surveillance regimens to detect this potentially catastrophic complication.\(^2\) Despite the frequency of acute rejection, survival after transplantation has improved over the last twenty years. This is mainly due to the introduction of novel therapeutic drugs such as anti-lymphocyte inhibitors, calcineurin inhibitors, and anti-proliferation agents, which rapidly prevent complications, further highlighting the importance of early detection.\(^1\)

Acute transplant rejection is caused either by cellular or antibody mediated mechanisms.\(^3\) These processes precipitate inflammatory responses of varying intensity within the myocardium producing myocardial edema, cellular infiltration, and eventually cell death. Clinically, this may manifests as coronary injury, ventricular tachyarrhythmia, often leading to acute heart failure, and ultimately allograft failure or death.\(^3\) Repeated episodes of rejection may result in myocardial fibrosis with development of left ventricular dysfunction over time.\(^4\) The diagnosis of acute cellular rejection (ACR) is established by endomyocardial biopsy (EMB) at the time of routine surveillance. EMB is performed if patients are symptomatic or if there is high clinical suspicion. Cellular rejection is mediated by T-lymphocytes and is identified by unique pathological markers under light microscopy.\(^5,6\) Antibody-mediated rejection (AMR), caused by B lymphocytes, is diagnosed by detection of specific humoral antibodies identified by special immunohistochemical (IHC) stains within the tissue matrix.\(^7,8\) Studies have shown that an average of 3-5% of patients have one episode of ACR with hemodynamic compromise. Alternatively, it has been demonstrated that 10-20% of patients have had hemodynamic changes without evidence of ACR on pathology. In this ACR negative population it has been found that AMR markers are present in only 15% of patients.\(^9\) Therefore, although cellular and antibody mediated rejection is involved in the majority of
acute transplant rejection; there are patients who have symptomatic rejection with no evidence of ACR or AMR. This small minority of patients experience hemodynamic compromise with improvement after empiric immunomodulatory treatment. Thus, EMB is not definitive in detecting rejection, but is currently considered the gold standard.

Current cardiac transplant monitoring with EMB is performed frequently in the first year post transplant. These monitoring regimens, while comprehensive in nature, are invasive, costly and time consuming, with many potential side effects. EMB has potential complications such as ventricular perforation, precipitiation of ventricular tachyarrhythmia and access site hematoma. Furthermore, biopsies are prone to sampling error leading to occasional false negatives and the need for re-biopsy in case of inadequate tissue sampling.11 With the advent of improved immunosuppression and lower incidence of ACR and AMR invasive monitoring has become less favorable.1 Over the last decade various researchers have developed non-invasive techniques to monitor for acute cardiac transplant rejection.9-12,13 Thus far, these surveillance modalities such as biomarkers, gene expression, and anti-myosin scintigraphy have been met with limited success.1

Magnetic resonance imaging (MRI) is frequently used to image edema in different parts of the body.14-16 Since there is evidence that myocardial edema correlates with acute transplant rejection, it stands that CMR may also have some utility in diagnosing this condition.17-31 CMR T2 mapping has the added benefit of being able to quantify numerically the tissue T2 relaxation values.32 T2 values are known to be prolonged in tissues with high water content due to edema, for example in acute myocardial infarction and myocarditis.33 Previous literature using animal and human models have supported detection and prediction of acute heart transplant rejection using T2 weighted imaging (T2W).19,22,34-36 These studies were limited by older imaging techniques such as spin-echo and turbo-spin-echo (TSE), poor magnet strength, and more poor temporal resolution in comparison to what is currently available. These protocols have long scan times with limited image quality which caused unreliable T2 measurements; moreover;
these older techniques report qualitative data. More recently, T2 quantification using a pulse sequence based on steady state free precession (SSFP) has shown promising results for detecting myocardial edema associated acute myocardial infarction. The purpose of this study was to assess whether left ventricular myocardial T2 quantification using a balanced SSFP approach can be used to quantitatively diagnose acute transplant rejection.

Methods

Patient Selection and Study Design

This prospective transplant study was conducted from September 2009 through December 2011. Patients underwent CMR within one year of transplantation or if admitted for suspected transplant rejection. The study was approved by the institutional review board and written informed consent was obtained from all patients. All CMR scans were performed within a 48 hour window of EMB, however priority was given to administration of immunosuppression medications and treatment planning over research CMR scanning. Prior to the commencement of the study CMR scans on a control cohort of normal volunteers was performed to determine the average T2 relaxation across the normal myocardium. Healthy, non-cardiac controls were recruited from volunteers at the Center for Advanced Magnetic Resonance Imaging (CAMRI) core facility at Northwestern Memorial Hospital.

Inclusion criteria for the study were all heart transplant patients at our center. After obtaining consent, CMR was performed for those patients receiving EMB in within their first year post transplant or for those patients at their routine surveillance visits at one year. Patients with rejection were matched by age, gender, time with transplant 1:4 with non-rejection patients. CMR was also carried out on any patient with clinical suspicion of cardiac rejection, (i.e. tachycardia, arrhythmia, chest pain, dyspnea, tachypnea, fluid overload, EKG changes), positive cellular rejection by EMB at routine surveillance, or positive IHC staining at routine surveillance within 6 hours of treatment initiation. If the cardiologist ordered a non-routine EMB based on clinical evidence the patient was enrolled for CMR, minimizing the difference in
threshold for ordering diagnostic testing biases for planned statistical comparison. Patients were excluded if there was any suspicion or clinical evidence of ongoing myocarditis or acute myocardial infarction. Myocarditis was excluded by biopsy and 2 out of 3 classic MRI criteria. Since gadolinium was not administered late gadolinium enhancement (LGE) was not ascertained. MI was excluded by EKG findings and troponin levels. Patients were also excluded if they were claustrophobic, had a pacemaker, or implantable cardiac defibrillator in place prohibiting entry into scanner. Patients who were deemed to have positive rejection by any means; either, clinical presentation of hemodynamic compromise, those who were referred to inpatient immunosuppression treatment, EMB with ACR 2R or greater, or positive AMR by immunofluorescence, were rescanned in a follow-up protocol with T2 mapping for potential rejection resolution. Finally, patients with cardiac transplant greater than 2 years of transplanting age were excluded from the study.

MRI Protocol

All studies were carried out on a 1.5T MRI scanner (Espree, Avanto, and Siemens Medical Solutions). The MRI protocol consisted of cine SSFP and T2 quantification. No contrast was administered. Breath hold segmented cine SSFP of the entire heart was carried out in multiple orientations including 2-chamber, 4-chamber, 3-chamber and stack of short axis views of the left ventricle using the following scan parameters: TR/TE: 3.2/1.6 msec, flip angle 70, field of view: 250 x 350, matrix: 150 x 192, slice thickness 6 mm, GRAPPA x 2, acquisition time per slice 4-5 sec. The T2 mapping technique was based on the approach described by Giri et al. Three T2-prepared SSFP images with varying T2-prep times (0, 24 and 55 ms) were acquired in a breath hold fashion in a 4-chamber and 3 short-axis orientations through the left ventricle. Integrated image registration was utilized to eliminate subtle respiratory and cardiac motion. Thereafter, automated pixel-wise fit was carried out to generate a T2 color map and analysis by drawing overlying regions of interest (ROI) was performed.

T2 Analysis Technique
T2 relaxation measurements were obtained from the left ventricle during diastole. The American Heart Association segmented model of the left ventricle was used to divide the area of the left ventricle into 17 ROI. The mean regional T2 values were calculated by visually drawing ventricular borders in 16 short axis and 4 long axis areas. Data was recorded by two separate image analyzers per patient and entered by a third member of the team into the database. Image analyzers were blinded to pathology results from the biopsy and to the clinical scenario of presentation. Furthermore, a reader also performed volumetric analysis of the left ventricle using Siemens software (ARGUS, Siemens Medical Solutions, Erlanger Germany) to determine ejection fraction, end-systolic volume, end-diastolic volume, and myocardial mass using a semi-automated technique.

*Endomyocardial Biopsy – Cellular and Humoral Rejection Grading*

Right ventricular EMB was performed in the cardiac catheterization lab by board certified interventional cardiologists. Histopathology of endomyocardial tissue was carried out and graded according to the International Society of Heart Lung Transplantation (ISHLT) criteria for defining the grade of acute cellular allograft rejection. Briefly, the grading system is as follows: grade 0R indicated no cellular rejection, grade 1R indicated mild cellular rejection determined by interstitial or peri-vascular infiltrate with up to one focus of myocyte damage, grade 2R indicated moderate cellular rejection defined as two or more foci of infiltrate with associated myocyte damage, and grade 3R indicated severe cellular rejection demonstrated by diffuse infiltration with multifocal myocyte damage with possible hemorrhage or vasculitis. In asymptomatic patients with no echocardiography evidence of heart failure and grade 0R or 1R cellular rejection there were no changes in immunosuppression medication regimens. Both grade 2R and 3R cases received immediate immunosuppressant therapy with treatment modalities such as intravenous immunoglobulin, plasmapharesis, steroids, or change in long term medications.

EMB specimens were also stained for immunoglobulin deposition and complement detection by immunofluorescence for potential detection of antibody mediated humoral rejection. All transplant biopsy specimens were stained for interstitial reactivity to anti-IgG, IgA, IgM, C1q, C3, C4d. Grading
was rated from 0 to 3 plus (+) using standard ISHLT nomenclature. This was defined as either immunofluorescence staining with immunoglobulin staining or complement fixation, or CD68 positivity for macrophages in capillaries identified using CD31, CD34, C4d staining by paraffin immunohistochemistry, or fibrin in myocardium. Pathology data was interpreted by expert cardiac pathologists at Northwestern Memorial hospital and images were obtained of all acute transplant rejection cases.

**Statistical Analysis**

Patient baseline characteristics were obtained through retrospective chart review. Descriptive statistics of baseline characteristics were performed on Stata 12 (Statacorp, College Station, TX) or on Microsoft Excel (Microsoft 2007, Seattle WA). Values are reported at mean ± standard deviation. \( \chi^2 \) testing for categorical variables and student t-testing for continuous variables were used for comparative analysis, \( p < 0.05 \) was considered statistically significant. Receiver-Operator-Curves (ROC) were used to identify the cutoff values in comparison to the biopsy results. Power analysis was performed to determine a minimum detectable difference in the mean T2 values between the control and transplant groups in this pilot study. Prior analysis by Marie et al. was used to determine a clinically significant T2 relaxation cutoff of 60 ms for rejection analysis, and control volunteer data was used to obtain an estimate of the standard deviation of differences between control and transplant patients of 3 ms.\(^{21,22,34} \) In order to obtain 80% power to detect a clinically significant difference of 5ms, a sample size of at least 7 rejection and 28 non-rejection patients were required (a 1:4 ratio). Power analysis was performed to determine the minimal cutoff for pilot study analysis and to project possibility of future studies. Two independent blinded reviewers (MW and JM) drew ROIs for T2 mapping analysis on the left ventricle. Inter-rater agreement was calculated through a Cohen’s kappa. One reviewer drew ROIs twice and intra-rater reliability was also calculated and reported.
Results

Controls

A control cohort study with 14 health volunteers demonstrated average myocardium T2 of 52.2 ± 3.4 ms with no statistically significant differences across the 16 segments of LV in the AHA model.

Total Transplant Population

A total of 74 scans were performed in 53 heart transplant patients. They were recruited from a total 141 post transplant patients receiving follow up care representing 37.6% of all cardiac transplant patients at our institution. An average of 12.2 EMB was performed per patient in the first year. A total of 57.1% were grade 0R, 39.2% grade 1R, 3.0% grade 2R, and 0.4% 3R. A total of 517 IHC stains were performed with an average of 9% of cases with any type of antibody marker positive for humoral reactivity.

Study cohort

The 53 patients scanned were equally distributed in males and female patients (31 male, 22 female, average age: 55 years ± 6.2, range: 20-69 years). (Figure 1) The average BMI in the all patients undergoing CMR at the time of transplant was 25.4, 35% had hypertension, 29% had diabetes mellitus, and 91% of recipients were transplanted with a NYHA class 4 status. All demographic variables between the study cohort and the transplantation population were statistically similar, in terms of gender, age, NYHA class at time of transplant, etc therefore the scanning group represented a statistically indifferent group from the overall transplant population at our center. At the time of CMR the average blood urea nitrogen level 26.9 ± 16.5 mg/dL, creatinine of 1.4 ± 0.6 mg. (Table 1) At the time of CMR 97.3% of patients were immunosuppressed with tacrolimus with an average tacrolimus level of 10.3 ± 4.5 μg/L. 93.2% of patients were using mycophenolate mofitil and 66.2% patients had steroid immunosuppression at the time of CMR. 10.8% of patients were on beta blockers, 1.4% on diltiazem, and 25.7% on amlodipine which all modify CMR ECG gating.
**T2 Mapping for Rejection**

Of the total 74 CMR performed there were 74 matched EMB, however 6 scans did not have requisite T2 mapping. (Figure 1) 4 of the 6 missing T2 mapping was due to protocol error and failure to record T2 mapping. In addition there were 2 scan failures, first due to retained pacing wires which altered the magnetic field preventing from accurate image acquisition and second due to claustrophobia after entering scanner. A total of 68 CMRs were available for analysis. The average EMB occurred 1.6 days prior to CMR scanning. There were a total of 46 grade 0R, 17 grade 1R, 3 grade 2R, and 1 grade 3R ACR found by EMB (Figure 2). There were 2 cases of AMR, and 2 cases of clinical rejection with negative EMB for ACR and AMR however admitted for treatment. The first patient presented with shortness of breath and acute diastolic failure on echo which was classified as hemodynamic compromise while the other patient presented with orthopnea, dyspnea on exertion and depressed ejection fraction without troponin leak or EKG changes. Both patients had negative EMB for ACR and AMR but improved after immnosuppressive therapy with intravenous immunoglobin and plasmapheresis.

The average T2 time for grade 0R, 1R, 2R, 3R ACR was 52.5 ± 2.2, 53.1 ± 3.3, 59.6 ± 3.1, 60.3 ms respectively (p-value of Control, 0R &1R versus ≥2R < 0.05). (Figure 2) The T2 average in the two AMR patients was 59.2 ± 3.3 and two clinical rejection patients 61.1 ± 1.8 ms (p <0.05 compared to controls). The average T2 relaxation time for all-cause rejection; ACR, AMR, and hemodynamic compromise, versus no rejection is 60.1 ± 2.1 versus 52.8 ± 2.7 ms (p-value < 0.05). (Figure 2) All assumed rejection cases were followed up on average 2.5 months later for follow up after acute transplant rejection with CMR with average T2 relaxation time of 51.4 ± 1.6 ms with p-value <0.05 when compared to T2 relaxation at the time of rejection. (Figure 2)

**Testing Sensitivity and Specificity**

A preliminary receiver operator curve was built based on limited cohort size and sensitivity and specificity was calculated for cutoff T2 values from this pilot data. (Figure 3) The optimal T2 time cutoff,
maximizing both sensitivity and specificity, was 56.4 ms with sensitivity and specificity of 86.5%/94.6%.
However, a cutoff based on pre-determined T2 relaxation time of 60 ms produced a sensitivity and specificity of 72% and 96%. Pathological correlation, based on IHC and cellular grading were obtained for all patients with rejection. Figure 4 illustrates two cases, first with T2 mapping mid segment view analysis above (panel a) and corresponding IHC AMR staining below (panel c) and also with T2 four chamber view above (panel b) and cellular infiltration demonstrating 2R rejection below (panel d). Additionally, their spatial correlation between biopsy site and T2 mappings assessed global elevations while EMB reflects local pathological infiltration from the biopsy site.

**Ventricular Function and Reliability**

Ventricular function for both no rejection cases and all cause rejection was obtained by ARGUS (Siemens medical) post processing. The average ejection fraction was 59.8 ± 10.3% versus 49.3 ± 12.5%, end diastolic volume of 110.5 ± 28.2 ml versus 117.6 ± 18.9 ml, end systolic volume of 45.6 ± 20.7 ml versus 60.4 ± 20.7, cardiac output 5.9 ± 1.6 versus 5.9 ±1.6 l/min, respectively. (Table 2) All values were statistically not significantly different, p-value > 0.05, except myocardial mass. Myocardial mass was determined to be 107.8 ±10.3 versus 127.5 ± 10.4 for no rejection versus all cause rejection, (p<0.05). A Cohen’s kappa for inter-rater agreement demonstrated 97.3% reliability in T2 relaxation estimates per AHA region for all 68 T2 maps. A 98.5% intra-rater reliability was determined for reader M.W. averaged across all 68 T2 maps.

**Discussion**

There is a need for non-invasive monitoring for acute transplant rejection after cardiac transplantation. This report highlights the use of myocardial edema T2 relaxation measurement for acute cardiac transplant rejection in a clinical setting. Our study is performed in a sizable cohort from the total cardiac transplant patient population in our center. Results from this study suggest that quantification of T2
relaxation can help augment the currently available tools in identifying and diagnosing acute cardiac transplant rejection. T2 mapping provides a gradient of scores across the myocardial wall that may reflect the underlying edema which may be missed in random sampled EMB. Example imaging (4) demonstrates T2 elevation maps in both ACR and AMR cases. In this study, we found that T2 values were not statistically significantly different between grade 0R and grade 1R rejections; however T2 values did increase at grade 2R and higher. This relationship was also noted in cases of AMR and those who presented with hemodynamic compromise with negative ACR and AMR but those who were responders to immunosuppression. Additionally, we found that prolonged T2 values returned to normal several weeks after a treated episode of acute transplant rejection which may illustrate acute transplant edema resolution after treatment.

A recent review has been published highlighting the utility of CMR in cardiac transplantation rejection reigniting interest in edema imaging.9 There have been several animal and human trials evaluating T2 relaxation time in cardiac transplant rejection. Unfortunately, these animal trials are based on dog and rat models and use older CMR imaging techniques, older transplant immunosuppression (cyclosporine) which is used only in limited circumstances, and used older qualitative T2 assessments.40-42 Nonetheless, prior research has demonstrated that T2 relaxation times increase with rejection.19, 25-27, 29, 42-44 There have been eight human trials comparing T2 relaxation times to transplant rejection as determined by EMB.5, 18, 21-24, 30, 45 Four of these trials showed significant correlation between T2 relaxation times and transplant rejection.9 Two of the trials which failed to demonstrate correlation between T2 relaxation and acute transplant rejection had image acquisitions during ventricular systole which is now known to result in severe signal loss and poor image quality.18, 24 The other two studies had long time lapses between EMB and CMR. Furthermore, one study did not report T2 relaxation in rejection.46 T2 relaxation times depend on magnetic field strength and vary based on the pulse sequences used. Here we use a unique SSFP sequence that allows for improved insensitivity to myocardial motion, higher sub-endocardial signal, and objective measurements from T2 quantification mapping.32 Due to this reason this novel SSFP based T2
mapping technique makes rejection detection more feasible. More recently, findings by Taylor et al. used T2 qualitative techniques to demonstrate elevated T2 in cardiac transplant rejection. They found that patients with confirmed rejection had elevated T2 enhancement patterns and contrast patterns. This finding corroborates our findings; however our study adds quantitative evidence to the level of edema formation in acute cardiac transplant rejection. Given that the current literature for both quantitative and qualitative T2 analysis, in addition to our pilot study which demonstrates elevated T2 relaxation time in cardiac transplant rejection, a complete investigational trial with prospective enrollment may be useful to delineate the usefulness of T2 mapping in cardiac transplant rejection monitoring. This study will require multi-institutional collaboration due to the fact that rejection episodes are now a rarer clinical entity.

We found that prolonged T2 times resolved in patients after immunosuppression treatment. The current threshold for initiation of changes in immunosuppression treatment is ACR 2R or greater, the presence of AMR, or if hemodynamic compromise is ongoing. This study demonstrated that the prolongation of T2 relaxation times observed in transplant rejection normalized after immunosuppression treatment within a period of two months. Marie et al. published results of T2 resolution after acute rejection and also found normalization of T2 values from average of 60 ms to 49 ms. Therefore SSFP T2 mapping not only demonstrates predictability of elevated T2 values to rejection we believe that without resolution of T2 prolongation patients may remain at higher risk of future rejection. Fortunately, since the year 2001 immunosuppression treatments has improved and caused a decrease the rates of acute rejection. This decrease in rejection cases has limited our study in capturing patients with rejection. This limits the study in determining if T2 mapping is predictive of the future risk of rejection with prior T2 elevation; we believe this relationship may hold true for a limited period of time, tapering off over time. Ostensibly, T2 mapping can be obtain for a patient over the course of the year and used as a surrogate marker of rejection infiltration. Based on a patient’s prior T2 mapping average we may be able to titrate medications similar to how we currently monitor tacrolimus levels.
Another added advantage of CMR is the ability to survey the entire myocardial wall. EMB can result in false negatives secondary to sampling error. It is plausible that during EMB an area of normal ventricle is sampled or insufficient myocardial material is obtained posing a difficulty in characterizing pathology specimens. As discussed by Fishbein et al. often patients with grade 0R or 1R ACR present with hemodynamic compromise and respond to treatment. We illustrate a case of two patients with both ACR and AMR negative biopsy, who presented with hemodynamic compromise and with elevated T2 values. These patients were also noted to have abnormal ventricular function measured by echocardiography.

Patients similar to these two often improve with augmented immunosuppression in spite of a negative biopsy results. Clinically, grade 2R ACR is used as the cut-off for treatment changes. However, with a numerical T2 relaxation score, improved stratification may help target patients who are candidates for immunosuppression treatment changes prior to the development of hemodynamic compromise. There may be correlation between tacrolimus levels and T2 relaxation scores; however this is an area under investigation. Monitoring elevations in T2 values may initiate trigger points for immunosuppression medication changes earlier rather than waiting for rejection episodes to manifest. CMR can also provide ventricular function data similar to echocardiography data collected on routine surveillance. We were able to note a statistically significantly elevation in myocardial mass, possibly reflecting increased water weight during rejection. Although myocardial mass obtained from CMR is a calculated value based on normal values further studies assessing ventricular function, pressure, and flow may further entrench the role of CMR in acute transplant rejection monitoring.

As the ROC curve demonstrates, different T2 value cutoffs produce different sensitivities and specificities for the detection of rejection. In this study, if treatment decisions were based on T2 relaxation cutoff values at 60 milliseconds this would have resulted in seven out of eight true rejection patients properly receiving immunosuppression. In this scenario only one additional patient would have inadvertently received treatment and one patient with acute rejection would not have meet criteria for immunosuppression treatment based on a T2 cutoff of 60ms. This conservative cutoff did not reflect the
maximized sensitivity and specificity point on the ROC curve. The ROC curve point at the 45 degree angle, which maximizes both sensitivity and specificity, would be at 56 milliseconds. This cutoff has been used in the in earlier animal and human research models for myocardial edema and rejection.\textsuperscript{21, 22, 34, 49, 50} In a scenario where 56 ms is used as the T2 threshold for treatment all eight true rejection cases would have been captured by the T2 mapping results; however, three non-rejecting patients would be included as false positives by T2 mapping and inadvertently receive treatment. Therefore, a more conservative T2 cutoff at 60 milliseconds, maximizes specificity at 97%, and would enable T2 mapping to augment EMB pathology. However, a lower threshold does not rule out rejection since EMB is fraught with sampling error and those three patients with elevated T2 scores may have in fact had subclinical rejection ongoing.

During the study, patients found to be positive for rejection by standard surveillance EMB were immediately started on immunosuppression medication. This limits the true measurement of T2 relaxation at the time of rejection, T2 values may be higher if scanning was initiated prior to treatment. Another limitation is inability to perform a pure randomized trial. Rejection episodes, in the era of excellent immunosuppression occur in a limited number of patients. In this case, as a pilot study, we were only able to capture eight rejection episodes over two and a half years. Patients were often targeted for scanning when they became symptomatic which may result in some selection error; however, there were a handful of patients that our research team was not alerted to. A ROC based on 8 positive patients is limited, however the statistically significant differences in the means points to two different groups of T2 average values; those above or below 56 or 60 ms. A larger multi-institutional trial is necessary to bolster numbers and find true test sensitivity and specificity. Finally, there are complications associated with EMB such as ventricular perforation and precipitation of tachyarrhythmia.\textsuperscript{45, 51, 52} We found no complications with the use of EMB; however, we did experience scanning difficulty in two patients secondary to pacing wires and claustrophobia. Therefore, CMR is only available for a subset of all transplanted patients, excluding many with pacemakers or remnant wires.
Conclusion

Improvements in T2 imaging with improved magnet strength, better pulse sequences, and improved fat and blood suppression techniques will likely strengthen the association between T2 relaxation times and transplant rejection. Quantitative T2 mapping is a useful adjunct to the diagnostic tools available in cardiac transplant monitoring, and warrants further consideration for acute transplant rejection follow-up.

Larger trials need to be carried out to validate these early but promising results.

Disclosures

None.

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Table 1. Cardiac transplant patient Characteristics for individuals from study who underwent Cardiac Magnetic Resonance Imaging for suspected acute cardiac transplant rejection within year 1 of transplantation

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Non Rejection Patients</th>
<th>Rejection Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Scans</td>
<td>88.2% (n=60)</td>
<td>11.8% (n=8)</td>
</tr>
<tr>
<td>Average Age</td>
<td>53.2 ± 4.2</td>
<td>56.9 ± 3.2</td>
</tr>
<tr>
<td>% Female</td>
<td>28.3% (n=17)</td>
<td>62.5% (n=5)</td>
</tr>
<tr>
<td>Average BMI</td>
<td>25.1</td>
<td>26.0</td>
</tr>
<tr>
<td>% with baseline HTN</td>
<td>83.3% (n=50)</td>
<td>66% (n=8)</td>
</tr>
<tr>
<td>% with baseline DM</td>
<td>33% (n=20)</td>
<td>12.5% (n=1)</td>
</tr>
<tr>
<td>NYHA at time to TXP</td>
<td>3.9</td>
<td>4</td>
</tr>
</tbody>
</table>

Baseline data on all scanned patients from transplant group - No co-morbid, sex-based or racial/ethnic-based differences were found between the rejection and non-rejection cases, demonstrating equal and statistically appropriate groups for comparison.
Table 2. Semi-automatic determination of Cardiac Volumetric analysis as determined by cardiac magnetic resonance imaging comparing all cause rejection versus no rejection patients

<table>
<thead>
<tr>
<th>CMR Ventricular Function</th>
<th>No Rejection Patients</th>
<th>Rejection Patients</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection Fraction (%)</td>
<td>59.8 ± 10.3%</td>
<td>49.3 ± 12.5%</td>
<td>ns</td>
</tr>
<tr>
<td>End Diastolic Volume (ml)</td>
<td>110.5 ± 28.2</td>
<td>117.6 ± 18.9</td>
<td>ns</td>
</tr>
<tr>
<td>End Systolic Volume (ml)</td>
<td>45.6 ± 20.7</td>
<td>60.4 ± 20.7</td>
<td>ns</td>
</tr>
<tr>
<td>Stroke Volume (ml)</td>
<td>64.9 ± 17.5</td>
<td>57.1 ± 13.3</td>
<td>ns</td>
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<tr>
<td>Cardiac Output (l/min)</td>
<td>5.9 ± 1.6</td>
<td>5.9 ± 1.6</td>
<td>ns</td>
</tr>
<tr>
<td>Myocardial Mass (g)</td>
<td>107.8 ±10.3</td>
<td>127.5 ± 10.4</td>
<td>P=0.049</td>
</tr>
</tbody>
</table>

CMR Ventricular function data comparing all cause rejection versus no rejection. No differences were found in all major functional parameters as determined by CMR for all-cause rejection versus no rejection groups; however, statistically different differences were found in calculated myocardial mass.
Figure Legends

**Figure 1.** Sequential numeric ordering of all CMR T2 relaxation measurements demonstrate the overall data spread and lack of outliers within the data. Here each individual scan, both transplant rejectors and non-rejector cases are intermixed. The graph leads to the identification of two distinct groups of patients, with the majority of rejection cases after the 56-60 milliseconds mark with a distinct upswing in T2 relaxation times. An asterisk * indicates clinically evident rejection based on either EMB or clinical evaluation.

**Figure 2.** Acute Transplant Rejection grouped by ISHLT grading EMB for ACR and/or IHC for AMR and/or clinical versus no rejection versus resolution. Control group data obtained from data published by Giri et al.32 Average T2 relaxation time for controls, 0R, 1R compared to 2R, 3R ACR, AMR, and clinical rejection. Follow up imaging after rejection was obtained and indicated in the last column. 0R and 1R grade cellular rejection do not alter immunosuppression treatment plan, however; grade 2R, AMR and hemodynamic changes warrant treatment changes. An * asterisk is placed over bars that represents statistically significance compared to controls.

**Figure 3.** Receiver Operator Curve of All-Cause rejection to determine the sensitivity and specificity of T2 mapping versus gold standard biopsy diagnosis. Maximizing both sensitivity and specificity at a cutoff of 56.2 ms demonstrates a sensitivity and specificity of 86.5%/94.6%. A T2 relaxation time cutoff based on pre-validated time of 60 ms by Marie et al. produces a sensitivity and specificity of 72% and 96%, respectively.21, 22, 34
**Figure 4.** T2 Mapping example of short axis and 4 chamber views. These T2 rejection cases highlight the division of the myocardium into the conventional 17 segment model. This also demonstrates the regions of interest (ROIs) that were drawn on the ventricular wall to determine the T2 areas for averaging. 16 of the 17 segments in the short axis were used (Apical tip excluded) in addition to 4 sections from the 4 chamber view. T2 maps were acquired in breath-held cardiac gated images. The figure shows a case example of T2 mapping of a patient with AMR (Panel A and Panel C) with elevated T2 relaxation times and a case example of T2 mapping of patient with ACR (Panel B and Panel C) also with elevated T2 relaxation times. Images obtained from pathology cross section slides at the time of EMB from Northwestern Memorial Hospital Department of Pathology (Chicago, IL).
T2 Relaxation Time for ACR, AMR, & Clinical Rejection versus Controls and No Rejection

- Control (n=14)
- 0R (n=46)
- 1R (n=17)
- 2R (n=3)
- 3R (n=1)
- AMR (n=2)
- Clinical Rejection (n=2)
- All Cause Rejection (n=8)
- Follow Up (n=8)
Area Under Curve = 0.89
Cardiac Magnetic Resonance T2 Mapping in the Monitoring and Follow-Up of Acute Cardiac Transplant Rejection: A Pilot Study

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