

Native T1 Mapping in the Diagnosis of Cardiac Allograft Rejection - a Prospective Histologically-Validated Study

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Abstract:

Objectives: This study aimed to determine the role of T1 mapping in identifying cardiac allograft rejection.

Background: Endomyocardial biopsy (EMBx), the current gold standard to diagnose cardiac allograft rejection, is associated with potentially serious complications. Cardiovascular magnetic resonance (CMR)-based T1 mapping detects interstitial oedema and fibrosis, which are important markers of acute and chronic rejection. Therefore, T1 mapping can potentially diagnose cardiac allograft rejection non-invasively.

Methods: Patients underwent CMR within 24 hours of EMBx. T1 maps were acquired at 1.5T. EMBx-determined rejection was graded according to International Society of Heart and Lung Transplant (ISHLT) criteria.

Results: Of 112 biopsies with simultaneous CMR, 60 were classified as group 0 (ISHLT grade 0), 35 group 1 (ISHLT grade 1R), and 17 group 2 (2R, 3R, clinically diagnosed rejection, antibody-mediated rejection (AMR)). Native T1 values in patients with grade 0 biopsies and LV ejection fraction >60% (983 ± 42 ms (95% CI 972-994)) were comparable to non-transplant healthy controls (974 ± 45 ms, 95% CI 962-987). T1 values were significantly higher in group 2 (1066 ± 78 ms) vs. Group 0 (984 ± 42 ms; $p=0.0001$) and vs. Group 1 (1001 ± 54 ms; $p=0.001$). After excluding patients with an eGFR <50 ml/min/m², there was a moderate correlation of log transformed native T1 with hsTNT ($r=0.54$, $p<0.0001$) and pro-BNP ($r=0.67$, $p<0.0001$). Using a T1 cut off value of 1029ms, the sensitivity, specificity and negative predictive value were 93%, 79% and 99% respectively.

Conclusions: Myocardial tissue characterisation with T1-mapping displays excellent negative predictive capacity for the non-invasive detection of cardiac allograft rejection and holds promise to substantially reduce EMBx requirement in cardiac transplant rejection surveillance.

Key Words: Cardiac MRI (CMR), T1-mapping, endomyocardial biopsy (EMBx), Cardiac transplantation

Abbreviations:

CMR: Cardiovascular Magnetic Resonance

EMBx: Endomyocardial Biopsy

hsTNT: High sensitive troponin T

Pro-BNP: Pro-brain type natriuretic peptide

AMR: Antibody mediated rejection

ECV: Extracellular volume

eGFR: Estimated glomerular filtration rate.

Introduction: Acute allograft rejection remains one of the commonest complications in the first year after transplantation (1) with 20% of patients experiencing at least one episode of acute cellular rejection (ACR) in this period (1,2) and 10% of patients experiencing antibody mediated rejection (AMR) (3). Acute allograft rejection often results in allograft dysfunction and is one of the main determinants of mortality and morbidity in early post-transplant period (1). Moreover, recurrent and chronic rejection leads to allograft vasculopathy, one of the commonest indications for redo-transplantation (4-7). Therefore, accurate and timely diagnosis of allograft rejection is paramount to enable early and effective treatment.

Histological analysis of an endomyocardial biopsy (EMBx) is the current gold standard for the diagnosis of cardiac allograft rejection. Many transplant centres have intense surveillance programs with recipients undergoing 12-15 biopsies in first 12 months. An EMBx is an invasive procedure associated with a 3-6% risk of serious complications including carotid artery puncture, pneumothorax, tricuspid regurgitation, cardiac arrhythmias and cardiac tamponade (8-11). Furthermore, EMBx and histological analysis are neither very accurate nor reproducible with high false positive and false negative results reported because of sampling error and inter-observer variability (12-14). Therefore, an alternative diagnostic strategy is required which is less invasive and more accurate.

Cardiovascular magnetic resonance imaging (CMR) is non-invasive and has the capacity to assess regions of myocardium not accessible to EMBx. T1- and T2-mapping imaging sequences enable accurate and reproducible detection of myocardial interstitial oedema and fibrosis (15-19). Their role in the detection and monitoring of acute myocarditis and myocardial ischemia is well established (15-18). Studies have also demonstrated the role of T1 mapping in the detection of interstitial myocardial fibrosis in scleroderma, rheumatoid arthritis and in the post-myocardial-

infarction setting (20-22). Interstitial edema is also an important marker of acute allograft rejection (23,24), whilst interstitial fibrosis is a hallmark of low-grade chronic rejection (25). We hypothesized that CMR would detect acute cardiac allograft rejection and studied the role of T1 mapping in the diagnosis and management of allograft rejection in heart transplant recipients.

Methods:

Patients and study design:

In this observational, prospective cross-sectional study, all patients undergoing heart transplantation from 1st April 2014 to 31st December 2015 at a single centre were screened. Exclusion criteria were standard contraindications to CMR including claustrophobia and inability to lie supine. The study was approved by the St. Vincent's Hospital Human Research Ethics Committee, Sydney, New South Wales, Australia (HREC/13/SVH/66). All patients gave written informed consent. All CMRs were performed within 24 hours of routine surveillance cardiac biopsies at 6, 8, 10, 12, 20, 24, 32 and 52 weeks after transplantation. If patients had clinically significant rejection as determined by histology, CMR was repeated along with the repeat biopsy after a course of treatment with pulse immunosuppressive therapy. Six patients in the cohort also underwent a non-routine cardiac biopsy based on clinical suspicion of rejection by an independent transplant physician and were also recruited into the study.

Serum highly sensitive troponin T (hsTNT) and pro-brain type natriuretic peptide (pro-BNP) were also measured within 24 hours of cardiac biopsies.

Non-transplant patients without any background cardiac history and a low pre-test probability of cardiomyopathy who were referred to our centre for CMR for non-specific symptoms and were reported to have a normal CMR by an independent specialist were used for comparison.

CMR acquisition: CMR studies were performed on a 1.5 T scanner (Achieva, Philips Medical Systems, Best, Netherlands) equipped with a 32-channel coil. Steady-state free precession (SSFP) cine images in standard long-axis (4, 3 and 2 chamber) and short axis views were performed. (26-28). An SSFP, single breath-hold, Modified Look-Locker Inversion recovery (MOLLI) sequence was used to acquire T1 maps in a single mid-ventricular short axis plane (FA 50°, voxel size 1.8 x 1.8 x 8.0mm, 8 images from two adiabatic pre-pulse induced inversions (3,(2),5))(29,30). Intravenous gadobutrol (Bayer Healthcare, Leverkusen, Germany) was administered in dose of 0.1 mmol/kg per body weight, as per the local clinical protocol only to those patients whose estimated glomerular filtration rate (eGFR) was greater than 40ml/min/1.73 m². T1 maps were also acquired twenty minutes after contrast administration. The hematocrit was measured within 24 hours of CMR to calculate extracellular volume (ECV) as previously described (31,32).

CMR analysis: All CMR images were analysed using commercially available software (CMR⁴², Circle Cardiovascular Imaging, Inc., Calgary, Canada). Regions of interest were drawn along the interventricular septum as well as circumferentially in mid-ventricular short axis slice to acquire septal and global T1 values (26-28,33) (Figure 1a). Care was taken to avoid the endo- and epicardium. After manual motion correction of each individual image, septal and global T1 values were calculated by fitting an exponential model to data from eight images at different inversion times (26-28,30,33). Myocardial partition coefficients (λ) and extracellular volume fractions (ECV) were calculated as previously described ($\lambda = \Delta R1_{\text{myocardium}}/\Delta R1_{\text{blood}}$; $ECV = \lambda (1 - \text{haematocrit})$) (32).

Cardiac biopsies: The endomyocardial cardiac biopsies were performed from a right internal jugular approach. Biopsies were stained with haematoxylin and eosin and were reported by an

anatomical pathologist blinded to CMR results. The biopsies were graded as 0, 1R, 2R or 3R in accordance with International Society of Heart & Lung Transplantation (ISHLT) criteria (24). Patients who were diagnosed with antibody mediated rejection (AMR) based on a combination of clinical suspicion, allograft dysfunction, serological and tissue markers (C4d staining on immunohistochemistry and elevated donor-specific antibodies) were also included. Patients who were treated for clinically-diagnosed rejection by an independent physician because of a high clinical index of suspicion based on signs and symptoms of rejection including chest pain, palpitations, dyspnoea, weight gain, ankle edema, along with decline in echocardiography-derived LV ejection fraction despite bland biopsies were also included.

In asymptomatic patients, pulse immunosuppressive therapy is generally indicated for ISHLT grade 2R, 3R or AMR (34), therefore, the cohort was grouped into three pre-specified groups, Group 0= ISHLT grade 0, Group 1= ISHLT grade 1R, Group 2= grade 2R, 3R, AMR and clinically diagnosed rejections.

Cardiac biopsies were also stained with Masson's trichrome stain to quantify fibrosis using semi-automated purpose-designed local software (Figure 1b & 1c).

Statistical analysis: Statistical analysis was performed using GraphPad Prism® 7.00 (GraphPad Software, Inc., La Jolla, CA, USA) and Microsoft Excel. Categorical data is expressed as number and percentage while continuous variables as mean \pm SD. A *p* value of < 0.05 was considered significant. For the comparison of normally distributed variables, unpaired student t-test, paired t-test, analysis of variance-ANOVA and Tukey's multiple comparisons tests were used as appropriate. Correlation was measured using Pearson's coefficient. Nineteen randomly selected CMRs were used for inter-observer variability using Bland & Altman plots and coefficients of variance. A Receiver Operator Curve (ROC) was used to measure sensitivity and specificity.

RESULTS:

A total of 169 scans were performed (118 biopsy-matched CMRs in 34 heart transplant recipients and 51 CMRs in non-transplant healthy controls; Table 1). Logistic limitations (scanner access) prevented some patients having concurrent CMRs. Six scans were excluded, one due to significant motion artefact on CMR, one due to inadequate tissue sampling on biopsy, one due to the presence of severe mitral regurgitation and three because patients could not lie flat long enough to perform T1 mapping. Eighty-nine percent of the scans were performed within the first-year after transplantation. Twelve scans (11%) were performed more than 12 months post transplantation along with clinically-indicated biopsies.

Of 112 biopsies with simultaneous CMR, 60 (54%) demonstrated no rejection (Group 0 (ISHLT grade 0)), 35 (31%) demonstrated only mild rejection (Group 1 (ISHLT grade 1R)), and 17 (15%) demonstrated clinically-significant rejection requiring pulse immunosuppression (Group 2 (2R, 3R, clinically diagnosed rejection, AMR, and 2R on a repeat biopsy after recent pulse immunosuppression)). Out of 34 patients, seven patients had clinically significant rejections. The majority of rejections occurred in first 12 months (n=12), while two occurred in one patient in the second year after transplantation (3R & 2R) and three rejection episodes (two AMR and one clinically diagnosed) in another patient more than 5 years post-transplantation (Patients 5 & 1 respectively in supplementary material).

Normal T1 values in Heart Transplantation:

To establish normal native T1 values in heart transplant patients, patients with both ISHLT grade 0 biopsies and a LV ejection fraction >60% were selected (Table 2). Their mean T1 value was marginally higher compared to non-transplant healthy controls ($983 \pm 42\text{ms}$ vs $974 \pm 45\text{ms}$; $p=0.30$) with no statistically significant difference (Figure 2a). Their septal T1 values were

higher than global T1 values ($983 \pm 42\text{ms}$ vs $969 \pm 39\text{ms}$; $p < 0.0001$); the difference was small, though statistically significant (Figure 2b). There was no significant correlation between native T1 values and donor age, sex, time post-transplantation or transplant-procedure ischemic time (Figure 2c, 2d).

Native T1 Across Rejection Groups:

Comparing across various rejection groups, the native T1 values were significantly higher in patients with clinically significant rejection: Group 2 ($1066 \pm 78\text{ms}$) vs. Group 0 ($984 \pm 42\text{ms}$; $p = 0.0001$) and vs. Group 1 ($1001 \pm 54\text{ms}$; $p = 0.001$) (Figure 3a, Table 3). There was no statistically significant difference between group 0 and group 1. On comparison of individual rejection grades and rejection types, T1 values were significantly higher in both AMR ($1137 \pm 25\text{ms}$) and grade 2R/3R ($1091 \pm 97\text{ms}$) compared to grade 0 ($984 \pm 42\text{ms}$; $p = 0.0001$) and 1R ($1001 \pm 54\text{ms}$; $p = 0.001$) (Figure 3b). The values were also significantly higher in patients with clinically diagnosed rejection ($1052 \pm 11\text{ms}$) compared to grade 0 ($P = 0.01$) but this difference was not statistically significant compared to grade 1R. The patients who still had grade 2R rejection on biopsy within 1-3 weeks of treatment with pulse immunosuppressive therapy for grade 2R/3R had lower T1 values ($969 \pm 10\text{ms}$) compared to grade 2R/3R ($p = 0.001$) and AMR ($p = 0.0001$).

Comparing septal and global T1 values within each individual rejection group, there was no statistically significant difference between measurement techniques (Figure 3c).

Analysis of repeated-observations have been made in the small subset of patients who have experienced significant rejection. To account for this, we have also analysed the data by selecting only the first episode of each rejection grade within individual patients. This reduced the total

number of biopsies to 55 in 34 patients with simultaneous CMR. Twenty-seven (49%) biopsies demonstrated no rejection (Group 0 (ISHLT grade 0)), 18 (33%) demonstrated only mild rejection (Group 1 (ISHLT grade 1R)), and 10 (18%) demonstrated clinically-significant rejection requiring pulse immunosuppression (Group 2 (2R, 3R, clinically diagnosed rejection, AMR, and 2R on a repeat biopsy after recent pulse immunosuppression)). The native T1 values were still significantly higher in patients with clinically significant rejection: Group 2 ($1088 \pm 87\text{ms}$) vs. Group 0 ($977 \pm 46\text{ms}$; $p < 0.0001$) and vs. Group 1 ($1007 \pm 56\text{ms}$; $p = 0.003$) (Supplementary Figure 3).

Tracking response to pulse immunosuppression with T1 mapping:

Six patients with significant rejection underwent serial studies. Native T1 mapping values were increased during acute rejection episodes (1093 ± 76) and reduced significantly after pulse immunosuppressive therapy in all but one patient (996 ± 43) ($p = 0.02$, paired T-test; Figure 3d, supplementary Figures 1 & 2).

Comparison of Extracellular volume (ECV) fraction with rejection groups and interstitial fibrosis

Intravenous contrast was administered in 21 patients with 51 CMRs to measure extracellular volume fraction. Numbers were significantly smaller than the cohort size due to renal impairment, which occurs commonly after heart transplantation (albeit often transiently due to medication side-effects). There was no statistical difference between rejection groups, however, interpretation is significantly limited by the small sample size (only four CMRs were performed with contrast during periods of significant rejection).

The degree of fibrosis was measured in tissue sample using Masson's trichrome stain and semi-automated software. There was no significant correlation between ECV and degree of fibrosis

($r=0.20$, $P=0.22$) (Figure 4a). Comparing native T1 values with degree of fibrosis, there was weak, non statistically-significant correlation ($r=0.21$, $P=0.051$) (Figure 4b).

Comparison of native T1 with different parameters:

Both left ventricular ejection fraction (LVEF) and mass were weakly correlated with native T1 (LVEF vs T1: $r=0.22$, $P=0.02$; LV mass vs T1: $r=0.22$, $P=0.02$). There was a modest correlation between log transformed native T1 and hsTNT ($r=0.34$, $p=0.001$) as well as pro-BNP ($r=0.61$, $p<0.0001$) (Figure 4c, 4e). After excluding patients with an eGFR <50 ml/min/m², this correlation improved (log T1 vs log hsTNT: $r=0.54$, $p<0.0001$; logT1 vs log pro-BNP: $r=0.67$, $p<0.0001$) (Figure 4d, 4f).

Native T1 Specificity and Sensitivity:

After plotting native T1 values on a Receiver Operator Curve (ROC), the area under the curve (AUC) was 0.89. Using a T1 value of 1029ms as a cut off, the sensitivity for detecting clinically significant rejection was 93%, with a specificity of 79% and negative predictive value of 99% (Figure 5a). Nineteen CMRs were randomly selected and native T1 mapping values were measured by two independent CMR physicians. The inter-observer variability was measured using Bland-Altman method of comparison (Figure 5b). The coefficient of variance was 1.3%.

Discussion:

This study explores the role of CMR-based myocardial T1 mapping in heart transplantation and demonstrates that T1 mapping is highly sensitive for the diagnosis of clinically significant cardiac allograft rejection and is able to track recovery after pulse immunosuppressive therapy. Most studies to date focussing on the role of CMR in heart transplantation have used less reproducible sequences to define edema and fibrosis including STIR (Short Tau Inversion Recovery sequence), T1-weighted spin-echo sequence for global relative enhancement (gRE)

and LGE (late gadolinium enhancement (35) or recruited patients more than six months after transplantation, missing the most critical period to diagnose rejection(36). A recent study by Miller et al. using T1 mapping demonstrated a trend towards increased T1 values in patients with rejection but did not achieve statistical significance (37). Our study utilised a well-validated T1 mapping sequence (17) and recruited patients from 6 weeks post transplantation (average time from transplant date to 1st CMR was 10 weeks); and majority of the scans (n=61, 55%) were performed in first 6 months after transplantation.

This study establishes a normal T1 mapping range for heart transplant recipients using a widely available MOLLI sequence. We found that the normal native T1 value in heart transplant recipients with histological grade 0 rejection (and normal LVEF) was 983 ± 45 ms which is comparable to our non-transplant healthy controls (974 ± 42 ms) and established normal values in the literature (30).

Native T1 values were significantly higher in patients with clinically significant rejection compared to those with non-clinically-significant or no rejection. T1 mapping values in the non-transplant population are elevated in conditions with known extracellular space expansion secondary to both oedema and interstitial fibrosis (15-18,21,22). Myocardial edema is an important pathological marker of acute allograft rejection (23). To help determine whether the native T1 value represented edema or fibrosis, we quantified the degree of fibrosis using a Masson's trichrome stain. Only a weak correlation was observed between T1 values and histologically quantified fibrosis (non-statistically significant), suggesting the majority of T1 change was attributable to interstitial edema rather than fibrosis. It is also important to note that patients can develop scar tissue due to repetitive biopsy from the same site reducing the accuracy of the association.

A subset of patients with ISHLT grade 1R rejection (mild) had elevated markers of myocardial injury (hsTNT and pro- BNP). These markers correlated fairly well with T1 values but not with ISHLT rejection grade. This suggests that some patients are experiencing low-grade active myocardial damage and edema, potentially missed by histological grading. A prospective randomized outcome study comparing T1 mapping and cardiac biopsies would help determine the clinical relevance of this discrepancy.

Using a T1 cut-off 1029 ms, we demonstrate high sensitivity (93%) and adequate specificity (79%) with an excellent negative predictive value (99%). In our study we missed only one significant episode of ISHLT Grade 2R rejection. In contrast to the other patients with clinically significant rejection, no change in T1 value or LVEF was observed after the administration of pulse corticosteroids in this patient, bringing into question the accuracy of the histological diagnosis. Comparison to the current gold standard, EMBx, was employed in this study, however, future prospective randomised studies with outcome endpoints will better determine the best way to diagnose and track cardiac allograft rejection. Notwithstanding, the majority of biopsies in our cohort (85%) displayed no clinically significant rejection and T1 mapping has promise to dramatically reduce the number of surveillance cardiac biopsies in the first year after transplantation.

All patients with AMR or ISHLT Grade 3R rejection had values higher than 1100ms. All patients with grade 2R or clinically-diagnosed rejection had values in the range of 1030-1070ms with the exception of the one patient mentioned above. This suggests that future larger studies may be able to determine T1 based cut-off values that prompt further investigation for AMR, a condition which is notoriously difficult to diagnose and requires tailored immunosuppression.

Although there was no statistically significant difference in ECV values between groups, the sample size was too small to draw any conclusions. Due to the high prevalence of renal impairment (>50%), it is unlikely that ECV measurement, which requires gadolinium contrast administration, is a practical method for determining myocardial expansion in this cohort.

Our study also demonstrates that native T1 mapping tracks recovery from clinically-significant rejection after pulse immunosuppressive therapy. T1 values fell from elevated levels in patients with clinically significant rejection after therapy. Furthermore, a hypothesis-generating finding of our study was that several patients had normalised their T1 values after pulse immunosuppressive therapy despite a persistent ISHLT Grade 2R on their serial biopsy. This suggests that perhaps the histopathological recovery may lag behind the reduction in CMR-determined myocardial edema.

Limitations:

One hundred and two scans (93%) were performed within 24 hours of cardiac biopsy whilst 8 CMRs were performed just outside the pre-specified 24 hr period due to logistic reasons. None of these biopsies revealed significant rejection and none of these patients had any pulse immunosuppression or change in their immunosuppressive therapy between cardiac biopsy and CMR. The prospective, observational study was conducted over a relatively short period of 12 months. The sample size is relatively small, although not unreasonable for a transplant population. Cardiac biopsies were used as the gold standard comparator for T1 mapping; however, it is well recognized that this has poor reproducibility with high inter-observer variability. Cardiac biopsies were taken from the right ventricular septal endocardium, whereas, T1 mapping regions of interest were placed in the mid-wall to avoid blood-pool artefact. A prospective randomized study with morbidity and mortality endpoints is required to further

evaluate the safety of replacing cardiac biopsy-diagnosed rejection with a CMR-based diagnosis.

Conclusion:

T1 mapping is highly sensitive for the diagnosis of clinically significant cardiac allograft rejection and tracks recovery after pulse immunosuppressive therapy. The technique demonstrates excellent interobserver reproducibility and holds promise to be a routine non-invasive method of cardiac allograft rejection surveillance in the first year after heart transplantation.

Clinical Perspectives:**Competency in Patient Care and Procedural Skills:**

Our research demonstrates that the use of non-invasive and highly reproducible native T1-mapping is able to substantially reduce the number of routine surveillance cardiac biopsies after cardiac transplantation and therefore, can improve overall patient care.

Translational Outlook:

This study warrants a prospective randomised control trial using mortality and morbidity endpoints before native T1 mapping may replace the current gold standard.

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Figure Legends:

Figure 1: T1 mapping and cardiac biopsy with Masson's trichrome stain. 1a. Mid-ventricular short axis slice demonstrating calculation of septal (orange) and global (blue) T1 values. **1b.** Cardiac biopsy with Masson's Trichrome stain for fibrosis. **1c.** Processed image in semi-automated purpose designed software to quantify fibrosis.

Figure 2: Scatter plots for T1 values in normal heart transplant population (ISHLT grade 0 with left ventricular ejection fraction > 60%). 2a. Heart transplant grade 0 vs non-transplant healthy controls ($p=0.30$). **2b.** Septal T1 vs global T1 ($p<0.0001$). **2c.** Correlation of T1 with time post-transplantation ($p=0.64$). **2d.** Correlation of T1 with transplant-procedure ischaemia time ($p=0.75$). HTx grade 0: Heart transplant patients with grade 0. ISHLT: International Society of Heart and Lung Transplantation.

Figure 3: T1 values across various rejection types. 3a. Comparison of T1 in group 2 ($1066 \pm 78\text{msec}$) with group 0 ($984 \pm 42\text{msec}$; $p=0.0001$) and group 1 ($1001 \pm 54\text{msec}$; $p=0.001$). Group 0= ISHLT Grade 0; group 1= ISHLT grade 1R; group 2 (clinically significant rejection) = Grade 2R, 3R, AMR and clinically diagnosed rejections). **3b.** Comparison of T1 across various rejection grades and types; AMR= antibody mediated rejection, ($1137 \pm 25\text{msec}$) and ISHLT grade 2R/3R ($1091 \pm 97\text{msec}$) had significantly high T1 values compared to ISHLT grade 0 ($984 \pm 42\text{msec}$; $p=0.0001$) and ISHLT grade 1R ($1001 \pm 54\text{msec}$; $p=0.001$). Clin= clinically diagnosed rejection ($1052 \pm 11\text{msec}$) had significantly higher T1 values compared to ISHLT grade 0 ($p=0.01$). 2R/PRx= Patients with grade 2R rejection on biopsy within 1-3 weeks of treatment for grade 2R/3R, ($969 \pm 10\text{msec}$). **3c.** Comparison of septal and global T1 values within each rejection group. **3d.** Comparison of native T1 mapping values during acute rejection episodes (1093 ± 76) and post pulse immunosuppressive therapy (PRx= 996 ± 43 ; $p=0.02$) using paired T-test.

Figure 4: Correlation of T1 with fibrosis, troponin and Pro-BNP. 4a. Degree of fibrosis vs ECV ($r=0.20$, $P=0.22$). **4b.** Degree of fibrosis vs T1 ($r=0.21$, $P=0.051$). **4c.** log T1 vs log hsTNT in all patients ($r=0.34$, $p=0.001$). **4d.** log T1 vs log hsTNT in patients with $\text{eGFR} \geq 50 \text{ ml/min/m}^2$ ($r=0.54$, $p<0.0001$). **4e.** log T1 vs log pro-BNP in all patients ($r=0.61$, $p<0.0001$). **4f.** log T1 vs log pro-BNP in patients with $\text{eGFR} \geq 50 \text{ ml/min/m}^2$ ($r=0.67$, $p<0.0001$). ECV= extracellular volume; hsTNT= High sensitive troponin T; pro-BNP= Pro-brain type natriuretic peptide.

Figure 5: Sensitivity, specificity and inter-observer variability of T1 mapping. 5a. A Receiver Operator Curve (ROC) for T1 mapping (using 1029msec as a cut off, the sensitivity =93%; specificity =79%; negative predictive value of 99%, AUC 0.89). **5b.** Bland & Altman plots for inter-observer variability of T1 mapping.

Table 1: Patients' characteristics

	Healthy Controls (n= 51)	Transplant Group (n=34)	P Values
No. of scans (113)	51	112	
Sex (%)			
Male	51	59	
Female	49	41	
Age (years)	46 ± 14	36 ± 14	
Height (m)	1.72 ± 0.10	1.75 ± 0.6	
Weight (Kg)	78 ± 13	86 ± 14	
Body Mass Index (BMI)		28 ± 5	
Left ventricular ejection fraction (EF %)	58 ± 18	68 ± 9	
Left ventricular mass (g)	166 ± 75	144 ± 30	
Ischaemia time (mins)		205± 100	0.2
CMRs within first year post-transplantation (n=100)		89%	
Mean time to recruitment (Months post-transplantation)		2.5 ± 1.3	<0.0001
CMRs > 12 months post- transplantation (n=12)		11%	
Mean time between CMR and EMBx (hours)		19 ± 68	<0.0001
No. of scans per patient		3 ± 2	0.01
CMV status			
• Positive		8 (7%)	
• Negative		40 (36%)	

<ul style="list-style-type: none"> Unknown 		64 (57%)	
eGFR (ml/min/1.73m ²)		60 ± 19	>0.100
Rejection grades			
<ul style="list-style-type: none"> Grade 0 Grade 1R Grade 2R/3R AMR Clinically diagnosed rejections 2R post treatment (PRx)[†] 		60 (54%) 35 (31%) 6 (5%) 3 (2.5%) 5 (4.5%) 3 (3%)	
Immunosuppression at time of EMBx:			
<ul style="list-style-type: none"> MMF/TAC/Prednisolone MMF/RAD/TAC/Prednisolone MMF/Cyclosporine/Prednisolone MMF/Cyclosporine/RAD/prednisolone 		64 (57%) 42 (38%) 2 (2%) 4 (3%)	

EMBx=endomyocardial biopsy; CMV= Cytomegalovirus; eGFR=estimated glomerular filtration rate;

AMR= antibody mediated rejection; MMF= Mycophenolate; TAC= Tacrolimus; RAD= Everolimus

[†]Biopsies with grade 2R rejection within 1-3 weeks of pulse immunosuppressive therapy

Table 2: Normal T1 Values in Heart Transplant Patients

	Heart transplant grade 0 with normal EF (n=56)	Non-transplant healthy controls (n=51)	P value
	983 ± 42	974 ± 45	0.30
Septal (msec)	983 ± 42		<0.0001
Global (msec)	969 ± 39		
Male	981 ± 45 (n=36)*		0.65
Female	986 ± 36 (n=20) [†]		
[‡] 0-3 Months (n=16)	988 ± 50		0.64
3-6 Months (n=19)	980 ± 35		

6-12 Months (n=13)	976 ± 26		
> 12 Months (n=5)	959 ± 50		

*Male donors; †Female donors; ‡Time post transplantation EF= Left ventricular ejection fraction

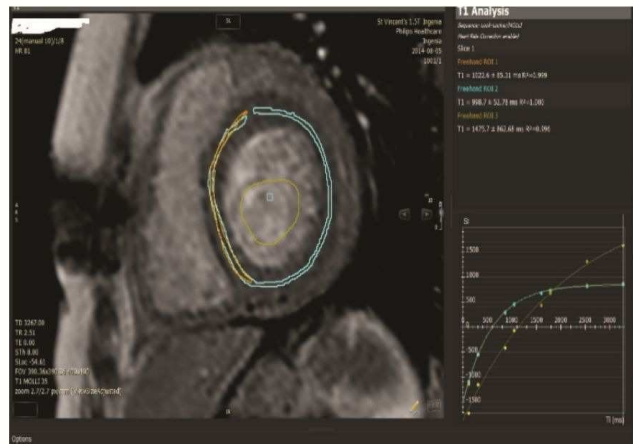
Table 3: Comparison of different parameters across various rejection groups

	Group 0 n= 60	Group 1 N= 35	Group 2 N= 17	P value
Septal T1 (msec)	984 ± 42	1001 ± 54	1066 ± 78	0.0001
Global T1 (msec)	970 ± 41	993 ± 50	1052 ± 69	0.0001
LVEF (%)	67 ± 10	72 ± 6	65 ± 12	0.01
LV mass (gm)	145 ± 24	141 ± 31	154 ± 40	0.30
ECV	0.35 ± 0.05 (n=31)	0.39 ± 0.10 (n=16)	0.38 ± 0.03 (n=4)	0.22
hsTNT	41 ± 51 (n=38)	61 ± 47 (n=20)	63 ± 50 (n=15)	0.23

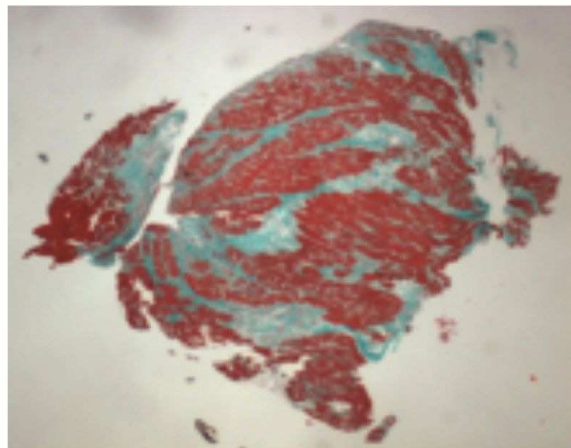
Group 0= ISHLT Grade 0; group 1= ISHLT grade 1R; group 2= Grade 2R, 3R, antibody mediated rejection (AMR) and clinically diagnosed rejections. ECV= extracellular volume; LVEF= left ventricular ejection fraction; LV mass= left ventricular mass; hsTNT= high sensitive troponin T.

Figure 1

1a



1b



1c

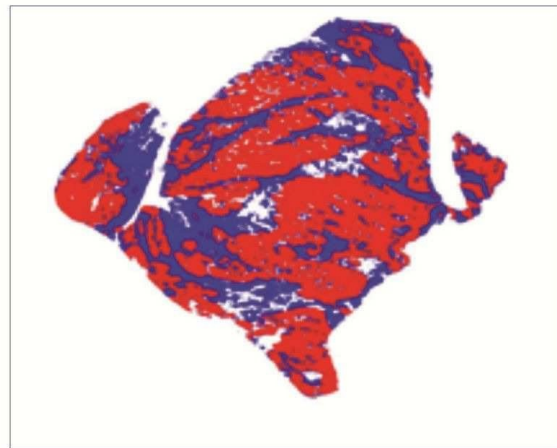
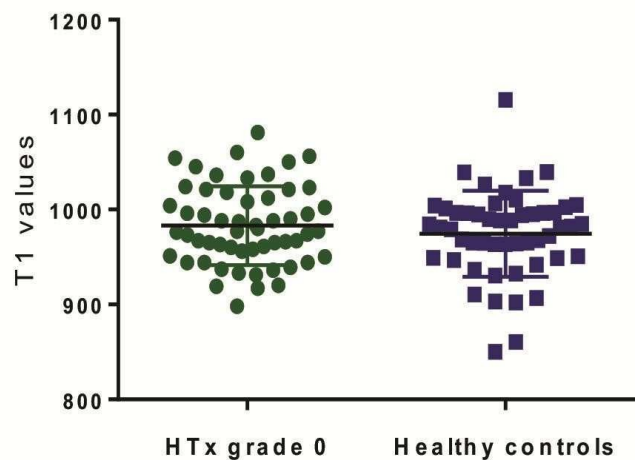
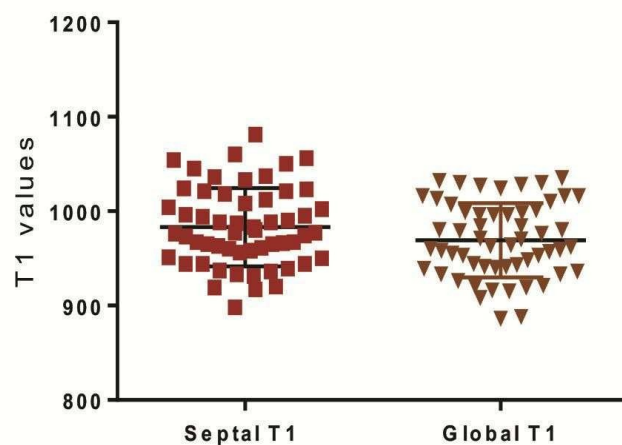


Figure 2

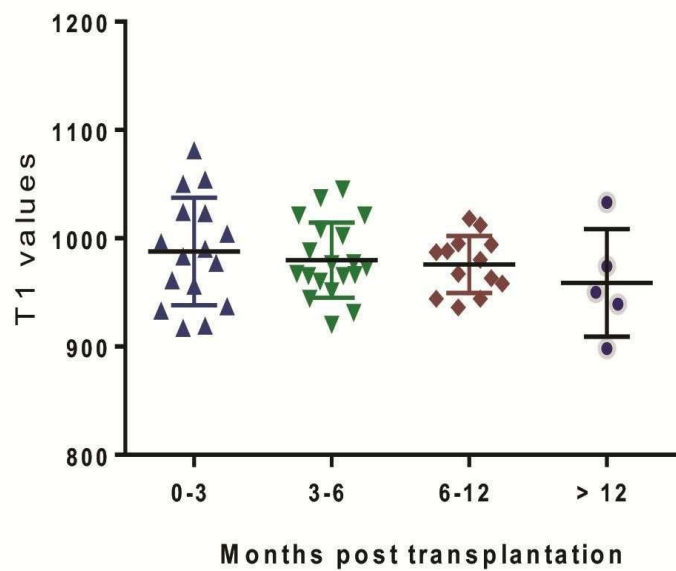
2a



2b



2c



2d

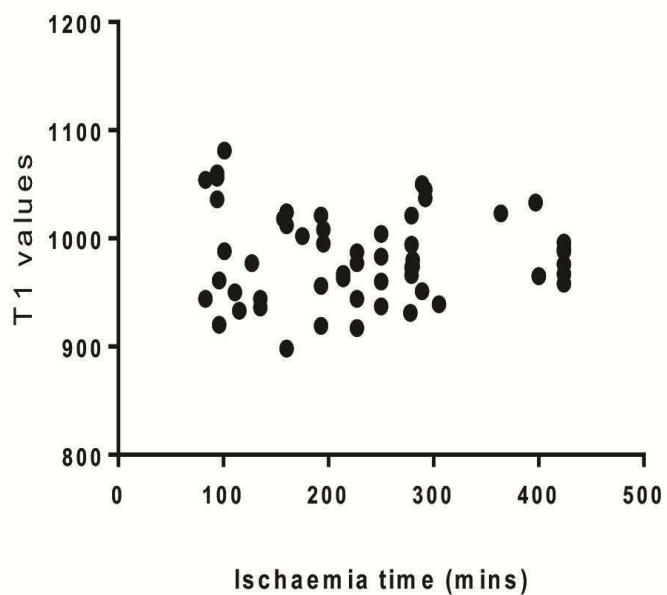
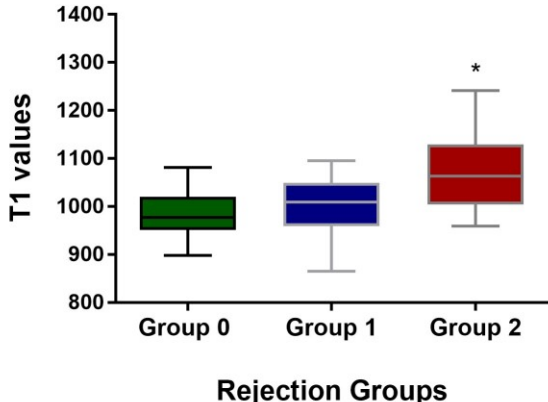
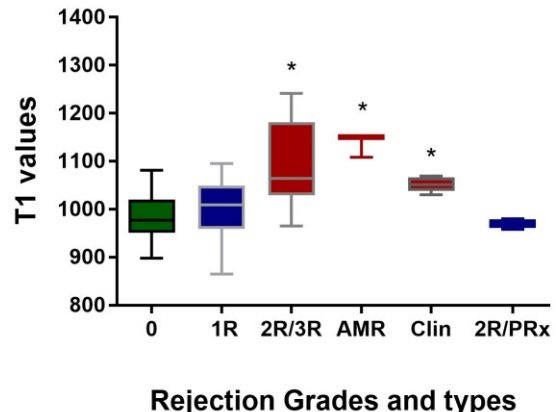


Figure 3

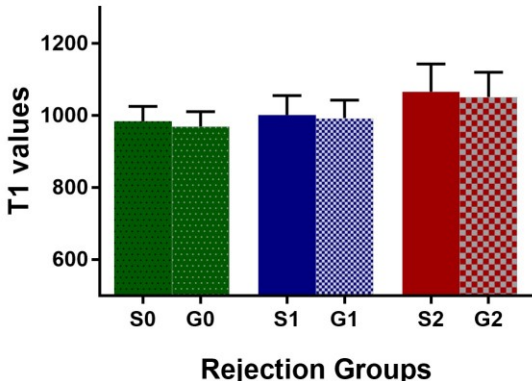
3a



3b



3c



3d

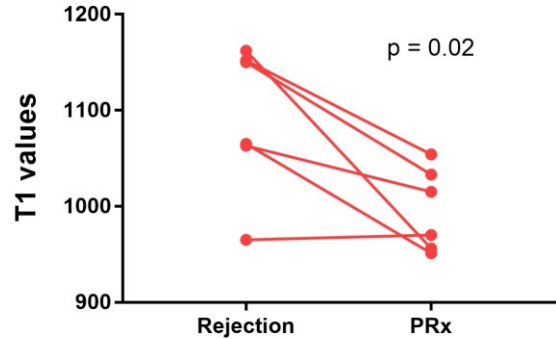


Figure 4

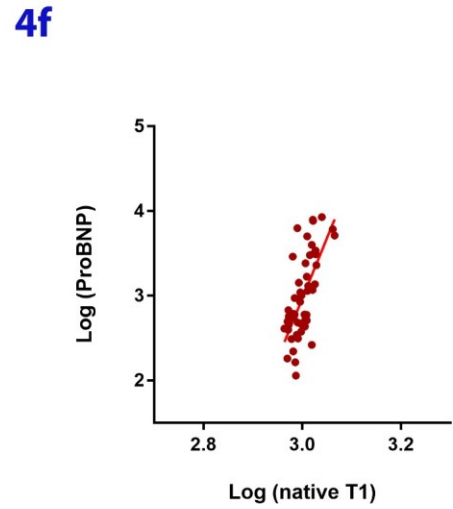
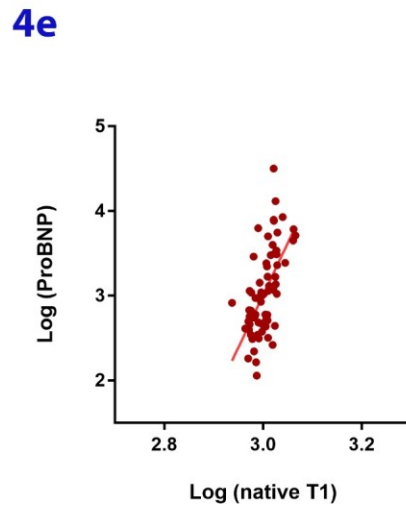
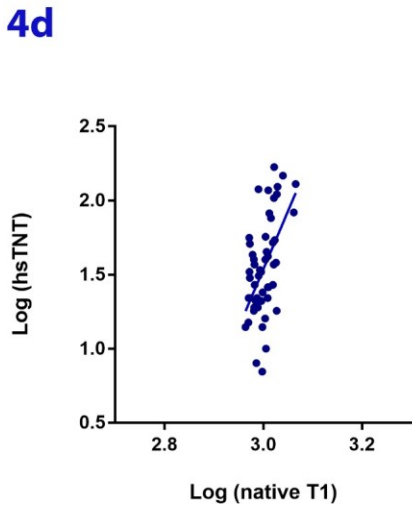
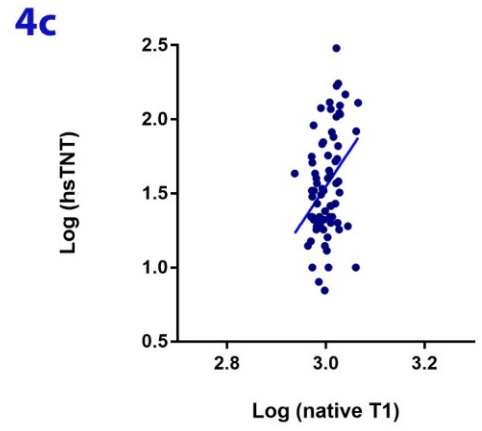
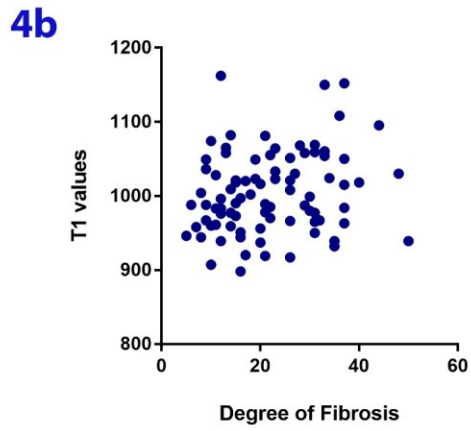
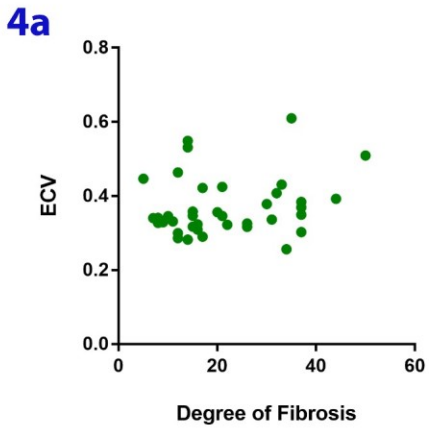
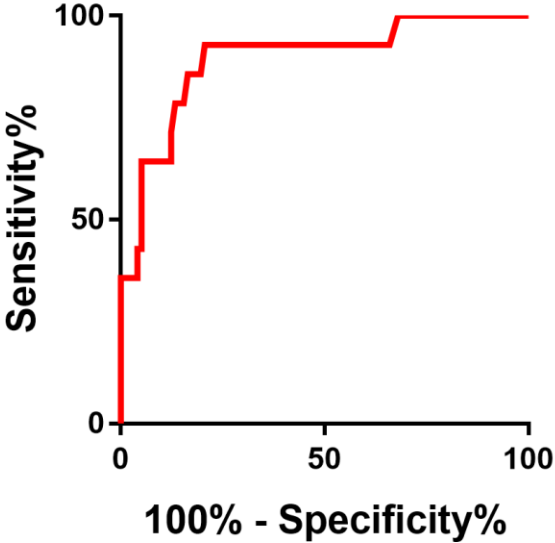
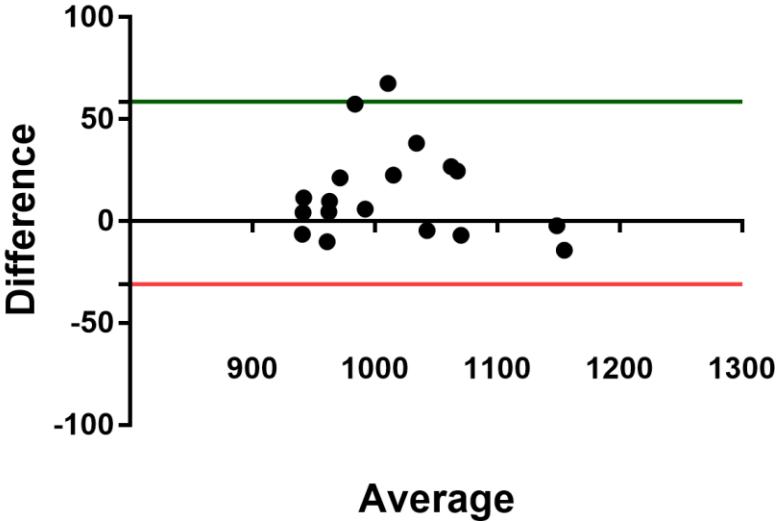


Figure 5

5a

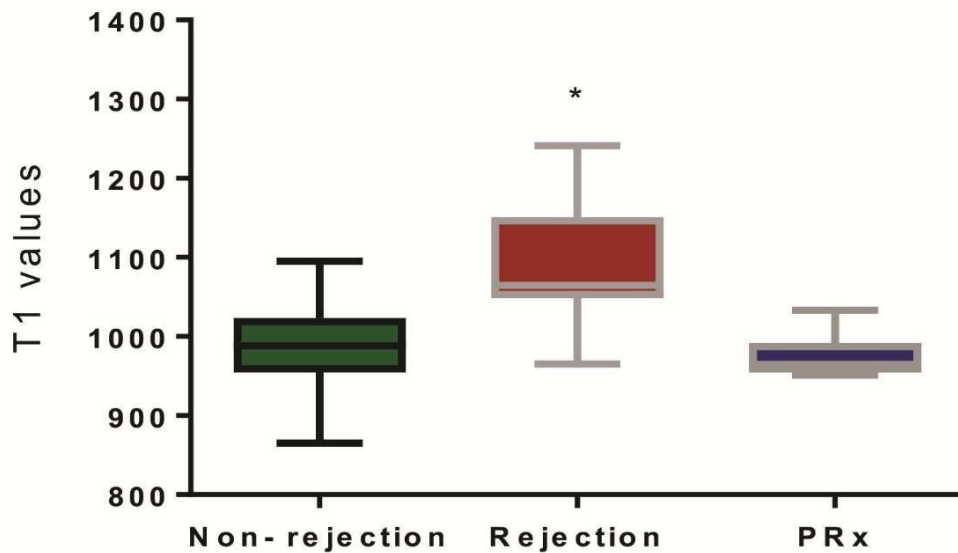


5b

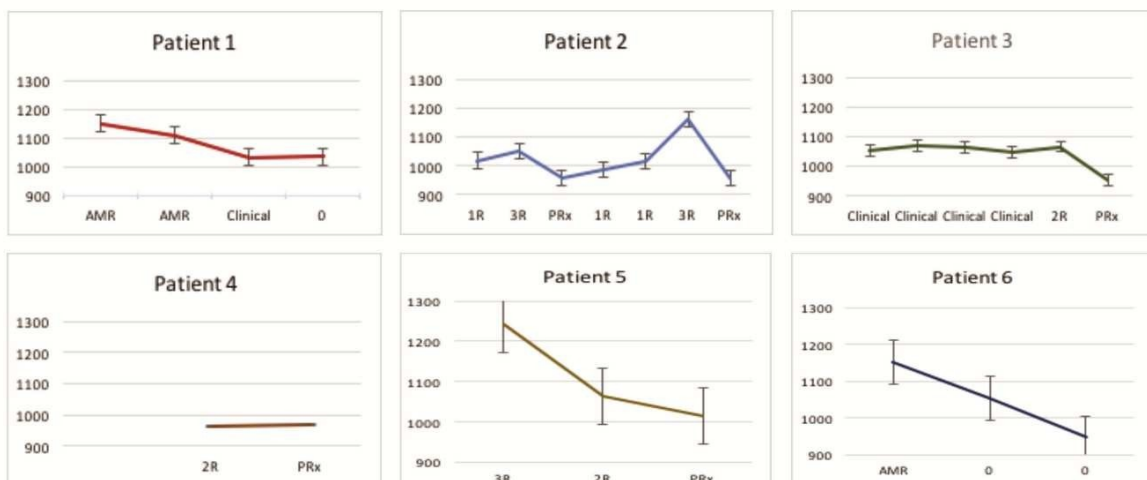


Native T1 Mapping in the Diagnosis of Cardiac Allograft Rejection - a Prospective Histologically-Validated Study

Supplementary Data:



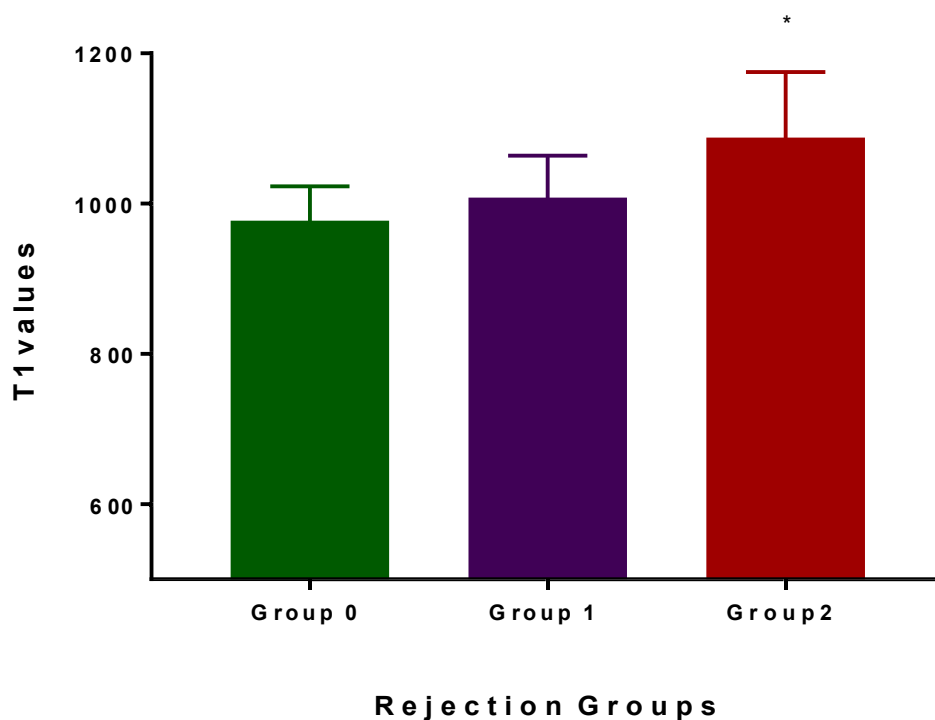
Supplementary Figure 1: Role of T1 mapping in tracking response to pulse immunosuppressive therapy. Comparison of T1 values in post-treatment group (PRx=975 msec \pm 30) with non-rejection (990 \pm 48ms; $p > 0.05$) and rejection groups (1087 \pm 69ms; $p = 0.0001$).



Supplementary Figure 2. Serial T1 values in individual patients with significant rejection.

Native T1 values in Individual Patients with Clinically Significant Rejection:

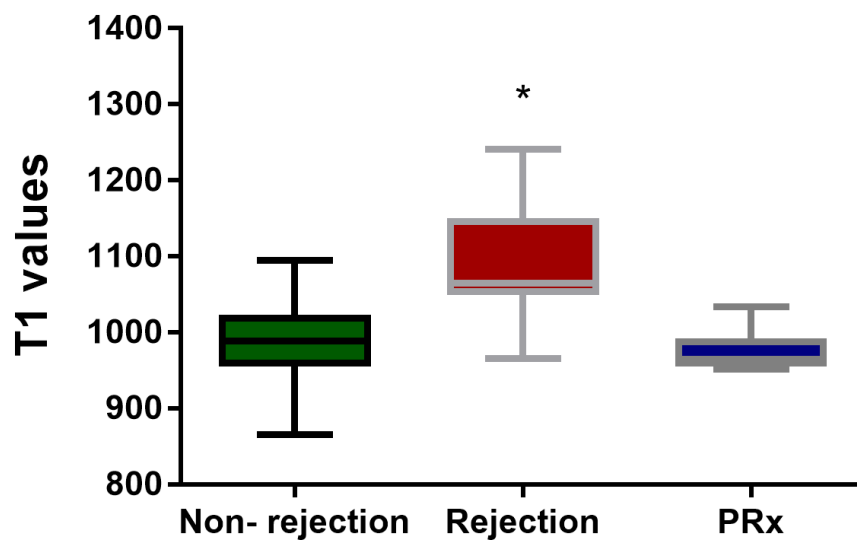
Patient 1 had a normal T1 value (965ms) with grade 2R histological rejection. After pulse immunosuppressive therapy, the histological grade improved to 1R while T1 value remained normal (970ms). Similarly, patient 3 had a decline in left ventricular ejection fraction, and was diagnosed to have rejection on a clinical basis without histological evidence. This patient was managed more conservatively with an increase in immunosuppression (without pulse steroids) until cardiac biopsy finally revealed grade 2R rejection. At this stage the patient was pulsed with steroids and the T1 values decreased directly thereafter.



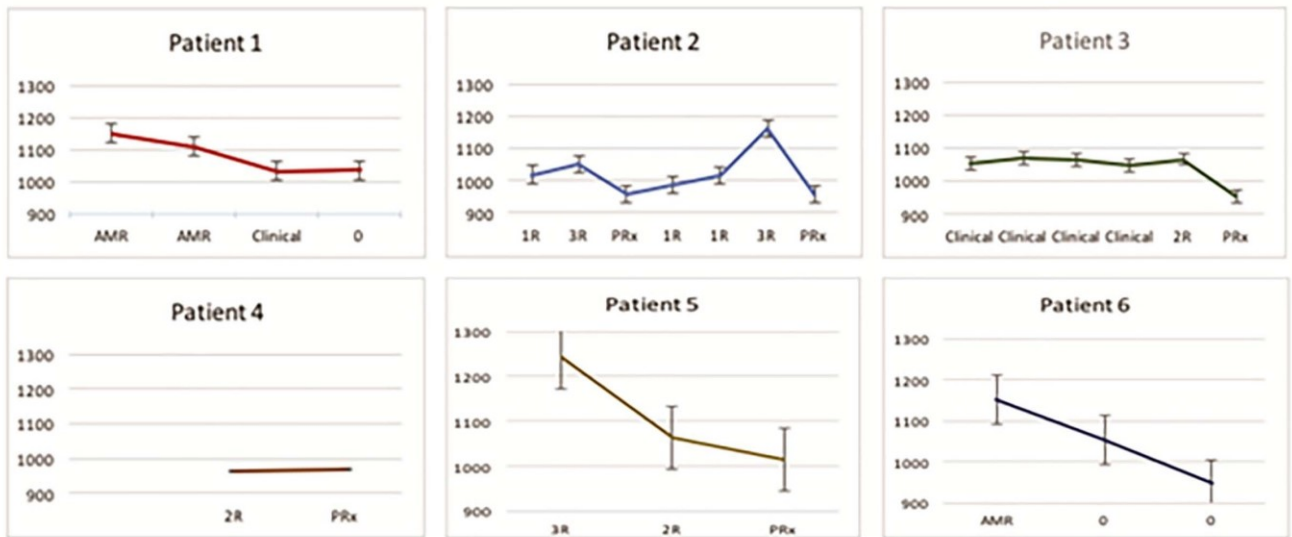
Supplementary Figure 3: T1 values across various rejection types by selecting only first episode of each rejection type within individual patients. Group 2 had high native T1 values (1088 ± 87ms) compared to Group 0 (977 ± 46ms; p<0.0001) and Group 1 (1007 ± 56ms; p=0.003). Group 0= ISHLT

Grade 0; group 1= ISHLT grade 1R; group 2 (clinically significant rejection)= Grade 2R, 3R, AMR and clinically diagnosed rejections).

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

