Cardiovascular Magnetic Resonance for Rejection Surveillance after Cardiac Transplantation

Running Title: CMR surveillance after cardiac transplantation

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Abstract

Background:

Endomyocardial biopsy (EMB) is the gold standard method for surveillance of acute cardiac allograft rejection (ACAR) despite its invasive nature. Cardiovascular magnetic resonance (CMR)-based myocardial tissue characterization allows detection of myocarditis. The feasibility of CMR-based surveillance for ACAR induced myocarditis in the first year after heart transplantation is hitherto undescribed.

Methods:

CMR-based multiparametric mapping was initially assessed in a prospective cross-sectional fashion to establish agreement between CMR- and EMB-based ACAR and determine CMR cut-off values between rejection grades. A prospective randomized non-inferiority pilot study was then undertaken in adult orthotopic heart transplant (OHT) recipients who were randomized at four weeks post-OHT to either CMR- or EMB-based rejection surveillance. Clinical endpoints were assessed at 52 weeks.

Results:

Four hundred and one CMR studies and 354 EMB procedures were performed in 106 participants. Forty HT recipients were randomized. CMR-based multi-parametric assessment was highly reproducible and reliable at detecting ACAR (AUC=0.92; sensitivity=93%; specificity=92%; NPV=99%) with greater specificity and negative predictive value than either T1 or T2 parametric CMR-mapping alone. High-grade rejection occurred in similar numbers of patients in each randomized group (CMR n=7; EMB n=8, p=0.74). Despite similarities in immunosuppression requirements, kidney function and mortality between groups, the rates of hospitalization (9/20 (45%) vs 18/20 (90%), OR=0.091, *p*=0.006), and infection (7/20 (35%) vs 14/20 (70%), OR=0.192, *p*=0,019) were lower in the CMR group. On 15 occasions (6%), patients that were randomized to the CMR arm underwent EMB for clarification or logistic reasons representing a 94% reduction in the requirement for EMB-based surveillance.

Conclusions:

A non-invasive CMR-based surveillance strategy for ACAR in the first year after OHT is feasible compared to EMB-based surveillance.

Clinical Trial Registration:

HREC/13/SVH/66 and HREC/17/SVH/80

Australian New Zealand Clinical Trials Registry: ACTRN12618000672257

Keywords

Orthotopic Heart Transplantation (OHT), Cardiac Magnetic Resonance Imaging (CMR), Acute Cardiac Allograft Rejection (ACAR), Immunosuppression, Surveillance.

Non-Standard Abbreviations and Acronyms

ACAR – Acute Cardiac Allograft Rejection

AMR – Antibody Mediated Rejection

AUC – Area Under the Curve

CAV – Coronary Artery Vasculopathy

CMR – Cardiovascular Magnetic Resonance Imaging

CMV - Cytomegalovirus

CV - Coefficient of Variation

CI - Confidence Interval

ddcfDNA – Donor Derived Cell-Free DNA

ECV – Extracellular Volume

EMB – Endomyocardial Biopsy

GEE – Generalized Estimating Equations

GEP – Gene Expression Profiling

HR – Hazard Ratio

hsTnT – High Sensitivity Troponin T

ISHLT – International Society of Heart and Lung Transplantation

LVEF – Left Ventricular Ejection Fraction

MOLLI – Modified Look-Locker inversion recovery

NPV – Negative Predictive Value

NT-proBNP – N-Terminal Pro-B-Type Natriuretic Peptide

OHT – Orthotopic Heart Transplantation

OR – Odds Ratio

PPV – Positive Predictive Value

PRx – Pulse Immunotherapy

RR – Relative Risk

RVEF – Right Ventricular Ejection Fraction

ROC – Receiver Operator Characteristics Curve

SSFP – Steady-State free procession

TTE – Trans-Thoracic Echocardiography

Clinical Perspective

What's new?

- In this exploratory randomised trial, which assessed the feasibility of CMR-based surveillance for the management of orthotopic cardiac transplant recipients in the first-year post-transplantation, multi-parametric tissue mapping by CMR reliably graded acute cardiac allograft rejection compared to EMB-based surveillance resulting in equivalent immunosuppression exposure, without increased risk of infections, hospitalisations, cardiomyopathy, or kidney injury.
- CMR-based surveillance of ACAR significantly reduced the requirement for invasive EMB procedures in the first year after transplantation.

What are the clinical implications?

- CMR accurately diagnoses cardiac allograft rejection in the first year after transplantation demonstrating feasibility to guide immunosuppression management.
- CMR imaging has the potential to yield substantial benefits to patients by reducing potential complications associated with EMB in the first year after transplantation.
- Prospective multi-center studies are warranted to further explore the safety, efficacy, and cost-effectiveness of CMR for cardiac allograft rejection surveillance after transplantation.

Introduction

Cardiac transplantation remains the most effective treatment for end-stage heart failure with excellent short and long-term survival rates.[1] However, cardiac allograft rejection remains a major complication in the first year after transplantation.[2] Rejection episodes are associated with an increased risk of graft dysfunction and morbidity.[3] Despite advances in the non-invasive detection of cardiac allograft rejection,[4] endomyocardial biopsy (EMB) remains the primary method of surveillance for rejection.[5] Notably, EMB is invasive and subject to both sampling error and significant inter-reporter variability, potentially leading to misdiagnosis and inappropriate treatment.[2, 6-8]

Cardiovascular magnetic resonance (CMR) imaging-based myocardial tissue characterization with T1 and T2 mapping has emerged as a non-invasive and highly sensitive method of detecting cardiac allograft rejection, with numerous studies demonstrating good correlation between CMR-based mapping and histopathology-determined rejection.[9-22]

We assessed the diagnostic performance of CMR for rejection surveillance in the first year after cardiac transplantation. An initial validation diagnostic performance study was undertaken with the aim of determining the sensitivity and specificity of CMR multiparametric mapping for the detection of allograft rejection. These data were used to determine cut-off values for rejection detection and the methodology for a prospective randomized pilot study. We tested the hypothesis that CMR-based monitoring for cardiac allograft rejection was feasible compared to EMB-based monitoring in the first year after cardiac transplantation.

Methods

The data, analytic methods, and study materials will not be made publicly available to other researchers for purposes of reproducing the results or replicating the procedure due to logistic and ethical constraints; however, interested parties are welcome to visit on-site to review the data and methodology.

Study Design

The research was conducted at St. Vincent's Hospital, Sydney, and was approved by the St. Vincent's Hospital Human Research Ethics Committee, Sydney, New South Wales, Australia (validation stage: HREC/13/SVH/66; randomization stage: HREC/17/SVH/80). All patients provided written informed consent. Patient eligibility, enrolment, and randomization are summarized below and detailed in figure S1 in the supplement.

The initial observational phase involved a prospective cross-sectional study where all patients who underwent cardiac transplantation from April 1, 2014 to December 31, 2015 were screened. All CMR studies were performed within 24 hours of routine surveillance cardiac biopsies undertaken at 6, 8, 10, 12, 20, 24, 32, and 52 weeks after transplantation. Serum high-sensitivity troponin T (hsTnT) and N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) were also measured within 24 h of cardiac biopsies using electrochemiluminescent immunoassay methods on a Roche e-Module analyser (Roche Diagnostics, GmbH). If patients had clinically significant EMB-determined rejection, CMR was also performed alongside the routine repeat biopsy after a course of pulse immunosuppressive therapy to ensure recovery. Non-transplant patients without a history of cardiac pathology and with a normal CMR, as determined by an independent specialist at our center were used as healthy non-transplant controls (Table 1).

The data from the observational phase were used to plan a randomized, prospective non-inferiority pilot study, which was conducted from February 1st, 2018 through March 10th, 2020. Patients aged 18 years, or older undergoing orthotopic heart transplantation were screened and randomized at 4 weeks after transplantation to EMB- or CMR-based surveillance (Table 1). All participants were followed until 52 weeks post-transplantation. Patients were randomly assigned in 2x2 blocks using the online randomizer (http://www.randomizer.org) by an independent adjudicator. Patients and clinicians were both unblinded. Patients randomized to the EMB group underwent EMB at weeks 4, 6, 8, 10, 12, 16, 20, 24, and 32 post-transplantation, as per an established protocol at our center. The biopsies were interpreted according to 2005 International Society of Heart and Lung Transplantation (ISHLT) criteria by experienced histopathologists.[23] Antibody mediated rejection (AMR) was interpreted according to the 2011 ISHLT consensus classification system.[23, 24] Further details regarding the

biopsy protocol and analysis are provided in the supplement. Patients in the EMB group also underwent a baseline CMR at time of enrolment and at 52-weeks post-transplantation.

Patients randomized to the CMR group underwent CMR imaging at the same time-points. In either group, whenever significant rejection was diagnosed and pulse immunosuppression administered, follow-up EMB in the EMB group or follow-up CMR in the CMR-guided group were performed to ensure recovery. Additional CMR scans and transthoracic echocardiograms (TTE) were also performed if requested by the treating physician, according to clinical state. Given that this was the first study of its kind and the potentially serious consequences of missing significant rejection, independent treating physicians were allowed to request an EMB at their discretion if they felt the patient may come to harm without a biopsy. This occurred infrequently, as detailed below.

Exclusion criteria included hemodynamic instability at 3 weeks after transplantation or at the time of screening, severe uncontrolled rejection prior to screening (defined as ≥2 consecutive ISHLT Grade 2R rejection events, or a single 3R rejection event in the first 3 weeks after transplantation), ongoing sepsis at 3 weeks post-transplantation, ongoing wound dehiscence or infection 3 weeks post-transplantation, kidney failure requiring dialysis at 3 weeks after transplantation or any standard contraindications to CMR scanning.

Imaging

Non-contrast CMR studies were performed at 1.5-Tesla (Achieva, Philips Medical Systems, Best, the Netherlands) with a 32-channel coil. The CMR protocol is described in detail in the supplement. In brief, steady-state free precession (SSFP) cine images in standard long- and short-axis views were performed for analysis of global ventricular function. A single breath-hold, modified Look-Locker inversion recovery (MOLLI) sequence was used to acquire T1 maps in a single mid-ventricular short-axis plane, as previously described.[13, 14, 25] The T2-maps were acquired using a respiratory-navigated black-blood, turbo-spin-echo (TSE) sequence on a midventricular short-axis slice that was sampled at different echo-times to enable reconstruction of a pixel-wise T2 map. All CMR images were analyzed using commercially available software (CVI42, Circle Cardiovascular Imaging, Inc., Calgary, Alberta, Canada). Regions of interest were carefully drawn along the interventricular septum, as well as circumferentially on the mid ventricular short-axis slice to acquire septal and global T1 and T2 values, as previously described.[11, 13, 14, 25-27]

Left ventricular ejection fraction (LVEF) and right ventricular ejection fraction (RVEF) were derived from short-axis cine images, involving semi-automated analysis following manual definition of endocardial and epicardial borders (excluding papillary muscles and trabeculae), and the mitral,

tricuspid, and pulmonary valve annular planes using cvi42 (Circle Cardiovascular Imaging, Calgary, Canada).

Stratification and Treatment

Allograft rejection events were defined utilizing ISHLT criteria: for no rejection "class 0", low-grade rejection "class 1R", and high-grade rejection "class 2R, 3R or Antibody-mediated rejection (AMR)". For patients randomized to the EMB group, stratification was defined histologically. For patients randomized to the CMR group, stratification was defined according to the initially validated, multi-parametric T1- and T2-mapping cut-off values. Details regarding the methodology for rejection grading are described in the supplement and table S1.

All transplant recipients received induction therapy with 20mg of basiliximab, a monoclonal antibody against CD25, at day 0, and day 4 post-transplantation. Time of initiation and doses of immunosuppressants used post-transplantation were as per the cardiac transplant protocol of St. Vincent's Hospital. All patients received tacrolimus, mycophenolate, and corticosteroids concurrently with or without everolimus at the treating physician's discretion. Likewise, corticosteroid dosing was at physician discretion. Rejection episodes were treated with a pulse of high-dose intravenous or oral corticosteroids, with or without T-cell depleting antibodies, depending on the severity of rejection.

Trial Outcomes

The randomized controlled pilot trial was designed to compare clinical outcomes of CMR-guided surveillance of transplant rejection versus EMB-based surveillance. The primary outcome was frequency, and cumulative freedom from significant (≥2R) rejection. Secondary outcomes included frequency and cumulative freedom from low-grade (1R) rejection, infection, hospitalization, length of hospital stay, death, immunosuppression exposure, kidney function, myocardial function, and the incidence of biopsy-related complications.

Statistical Analysis

The performance characteristics of CMR and cardiac biomarkers to detect biopsy confirmed rejection were determined by the area under the receiver operator characteristic curve (AUC). Statistically optimal CMR criteria for discrimination of ISHLT rejection grades was determined by the Youden index. Receiver operator characteristic curves were compared based on methodology of Hanley and MacNeil.[28]

The sample size for the primary outcome was based on a non-inferiority analysis using Cohen's weighted kappa coefficient with linear weights to calculate the inter-observer agreement between independent histologists in grading significant rejection from biopsy specimens observed in the validation phase of the trial (indicated in table S2).[29, 30] We calculated that a target of 40 patients (20

per group) would provide 80% power at a one-sided α of 0.05 to test the primary outcome, that CMR did not miss significant rejection (≥2R) compared to EMB (was non-inferior), with 11 repeated measures (longitudinal biopsies or CMR scans) for each subject, assuming a first-order autoregressive AR(1) correlation structure with base correlation of 0.1, and a non-inferiority margin of time-averaged difference in ISHLT≥2R rejection proportions of -9% between CMR and EMB groups (assuming a ISHLT≥2R rejection rate 13% in both groups if not different to the validation phase), that is a relative risk (RR) margin of 0.23 comparing CMR to EMB.[31] We considered this stringent choice of -9% margin (actual interrater disagreement = 8.8%, see supplement for derivation) a clinically meaningful index to refute non-inferiority for ISHLT≥2R rejection based on an interrater agreement of 91.2% between two independent histologists (Cohen's Kappa = 0.473, p<0.001) (table S2).

Continuous data are described as mean ($\bar{x}$) and 95% confidence intervals (95% CI). Categorical data were displayed as event frequency (%). Inter-observer variability of CMR analyses were assessed using Intraclass Correlation Coefficient (ICC) method and Bland-Altman plots. Receiver Operator Characteristics (ROC) Curves were used to investigate the ability of CMR to detect low-grade and high-grade rejection events.

Patient level comparison of categorical or continuous variables between groups for baseline characteristics, rates of infection, hospitalization and allograft rejection over the study duration were performed using either logistic regression without random effects, Mann-Whitney U or Kruskall-Wallis tests, as appropriate. For event level comparisons, the generalized estimating equations (GEE) log-link binomial generalised linear model was applied to compare ISHLT ≥2R rejection and other binary outcomes with repeated measures, and the AR(1) working correlation structure was used to account for the within subject correlation. Firth logistic regression for rate events was used to deal with the complete separation problem.

The linear mixed effects models were used to assess the within-subject association of the effects of rejection surveillance method on immunosuppression therapy (oral prednisolone, intravenous methylprednisolone (stratified into quintiles), total corticosteroid dose (stratified into quintiles), and tacrolimus levels in plasma as well as the severity of tricuspid regurgitation grade over each follow-up period. In the model, individuals were random effects, surveillance method and time were fixed effects, and the interaction term was surveillance method multiplied by time.

Time to event analysis was applied to assess the cumulative freedom from significant rejection, and hospitalization due to rejection during the 1-year follow-up period. Kaplan–Meier survival analysis with log-rank test was used to compare survival curves between the groups, and with Cox regression

used to derive hazard ratios. The proportional hazard assumption was tested using Schoenfeld residuals and was found to be valid. A two tailed *p* value less than 0.05 was considered statistically significant.

Statistical analyses were performed using IBM SPSS Statistics Version 25 (IBM Corporation, Armonk, NY, USA), R (R Core Team, 2012), and lme4.[32]

Results

Patients

Four hundred and one CMR studies and 354 EMB procedures were performed in 106 participants (73 HT recipients and 33 non-HT healthy controls). Patient assignment to groups across each trial phase is summarized in Table 1 herein, and in the CONSORT diagram, figure S1.

In the validation phase, a total of 108 EMB were performed with simultaneous CMR in 33 OHT recipients. In addition, 33 CMR scans were performed in 33 non-OHT healthy controls. In OHT recipients, 60 (56%) CMR studies were classified as group 0 (ISHLT grade 0), 34 (31%) in group 1 (ISHLT grade 1R), and 14 (13%) in group 2 (5.5% 2R or 3R, 3.7% clinically diagnosed rejection, and 3.7% AMR). Out of 33 patients, eight patients had clinically significant rejection (Table 1).

In the subsequent randomized phase, a total of 238 CMR scans and 15 EMB (11 EMB performed in conjunction with CMR) were performed on 20 OHT recipients that were randomized to the CMR group, while a total of 235 EMB were performed in 20 OHT recipients that were randomized to the EMB group. The baseline characteristics of the patients between groups were well matched (Table 1). Only two patients did not complete the study; one patient in the CMR arm died from kidney failure, and one patient in the EMB arm died due to sudden cardiac death.

CMR Validation

Building on our published experience with T1 mapping validation,[11] here we report expanded T1 mapping data and the incremental value of T2 mapping in rejection detection. Excellent inter-observer agreement and correlation were observed for both T1 (CV=1.3%, r=0.94, $p<0.001$) and T2 mapping (CV=4.6%, r=0.99, $p<0.001$) (Figure S2). Likewise, excellent correlations were observed between interventricular septal and left ventricular global values for both T1 (r=0.95, $p<0.001$) and T2 mapping (r=0.97, $p<0.001$) (Figure S2); findings that formed the basis for selection of the interventricular septum as the region-of-interest (ROI) for all subsequent analyses.

Receiver operator characteristics curve analysis for detecting high-grade rejection (ISHLT 2R,3R, AMR and clinically diagnosed) by CMR yielded an AUC of 0.897 for T1 and 0.938 for T2 (Table 2 and figure 1A-B). Based on the Youden score, optimal cut-offs values for detecting high-grade

rejection using T1 and T2 mapping were 1029 ms and 59.5 ms, respectively. Reliability in detecting high grade rejection using these CMR parameters was consistent with significant differences in T1 and T2 values observed between low grade and high-grade rejection events (Figure 1C-D). Furthermore, by employing a multi-parametric approach, an AUC of 0.92 was achieved with greater sensitivity (93%), specificity (92%), and negative predictive value (99%) compared to single parameter approaches (Table 2, and Figure 1B). Inclusively, minimum T1 and T2 values for AMR remained consistently above the defined threshold for high-grade rejection (mean, range: T1; 1030-1152ms, T2: 71.5, 65-76ms), (figure 1C-D).

The sensitivity for detecting low grade (ISHLT 1R) rejection by CMR was more modest, yielding an AUC of 0.697 for native T1 and 0.689 for T2 parameters respectively (Table 2 and figure 1A). Based on the highest Youden score for the detection of clinically significant rejection, cut off T1 and T2 mapping values of 996.5ms and 56.5ms, respectively, were selected. T1 and T2 mapping values decreased significantly (T1, p=0.038; T2, p=0.001) after pulse immunosuppressive therapy for significant rejection (Figure 2E-F). Furthermore, duration to convalescence was similar between groups (CMR 3.6 (2.6-4.6) weeks, EMB 4.1 (2.4-5.9) weeks, p=0.663) (Figure S5).

Hematological biomarkers, hsTnT and NT-proBNP demonstrated modest AUC values even when corrected for baseline or steady-state values on an individual basis (Table 2, Figure S3 and Table S3). Hematological biomarkers, in combination, had similar discriminatory capability as combined CMR markers for 1R rejection, however, performed less well for predicting 2R rejection (Table 2, Figure S3 and Table S3).

Multiparametric rejection risk stratification using all markers (T1, T2, steady-state-corrected hsTnT (rel) and NT-proBNP), demonstrated similar discriminatory capability to multiparametric CMR-only markers for 1R rejection, however the discriminatory capability of all markers combined were lower for 2R rejection compared to combined or individual CMR-only markers (Table 2, Figure S3).

Allograft Rejection Events

In the prospective randomization phase, the number of patients that experienced ≥2R rejection events did not differ between the CMR and EMB groups (7 vs 8, p = 0.744). (Table 3, Figures 2A and 2C-D)). CMR did not miss 2R rejection episodes relative to EMB noting our prespecified tolerance level as shown in Table 3, the odds ratio (OR) of ≥2R rejection being diagnosed by CMR vs. EMB is 2.06 with 95% CI (1.27, 3.34). (Table 3, Figure 2A, Figure 2C-D).

The observed cumulative freedom from all rejection grades was greater in the CMR group compared to the EMB group (HR 0.383, CI 0.180-0.82, p =0.003 (Figure S4 A). Based on Cox regression the cumulative freedom from 2R rejection episodes was not statistically different between the two groups (HR 0.893, CI 0.32-2.46, p = 0.82) (Figure 2A). In light of the lower incidence of 1R rejection events

in the CMR group compared to the EMB group, there was a favorable cumulative freedom from grade 1R rejection episodes in the CMR group compared to the EMB group, (HR 0.354, CI 0.16- 0.77, p = 0.002) (Figure S4 B).

Given the potential risks of missing high-grade rejection events, treating physicians were allowed to order an EMB at their clinical discretion. Eleven such 'confirmatory' EMB procedures were performed in the CMR group. Nine out of these eleven (82%) EMB results were identical to the CMR result, and two (18%) suggested the severity of rejection was 1 grade lower than the CMR result (Figure 2C-D). By 5-12 weeks, the average number of cumulative surveillance procedures increased slightly in the EMB arm (*n*, EMB 3.3 ± 1.1; CMR 2.8 ± 1.3; p = 0.018), however there were no significant differences in the cumulative number of procedures performed at any other time point between EMB and CMR arms throughout the study duration (Figure S6).

Infection

There was no significant difference in the frequency of infection events or in the number of patients who experienced bacterial (patients, p = 0.74; events p = 0.62) or fungal (patients p = 0.56; events p = 0.56) infection between CMR or EMB groups (Table 3). There were fewer CMV and non-CMV viral infection events in the CMR group compared to the EMB group, (CMV 1 vs 8, p= 0.04: non-CMV 3 vs 13, p < 0.06). The risk of infection episodes was decreased in the CMR group compared to the EMB group because of the increased incidence of viral infection in the latter (OR 0.373; CI 0.211-0.486, p = 0.02, Table 3).

Hospitalization and Biopsy Complications

There were fewer unplanned hospitalization events (32 vs 46, p= 0.03) and fewer individual patients hospitalized (9 vs 18, p < 0.006) in the CMR group compared to the EMB group. However, there was no significant difference in the median length of hospitalization between the CMR and EMB groups (4.6 (3.1-6.1) vs 5.4 (3.6-7.2) days, p=0.82) (Table 3). There were three biopsy-related complications in EMB group. There was one carotid artery puncture with no clinically significant sequelae, and two internal jugular vein access site thrombi necessitating temporary oral anticoagulation but with no consequent bleeding events.

Immunosuppression exposure

Both the CMR and EMB groups were exposed to similar immunosuppression throughout the study; specifically, similar oral prednisolone doses, and tacrolimus levels over time were observed ($p_{\text{time x study arm}}$: prednisolone dose = 0.437; tacrolimus level = 0.755) (Figure 3). Across the groups, prednisolone dose was 15.9 (14.0-17.6) mg at the 4-week post-OHT entry point and was reduced significantly over the course of the study to 6.8 (5.1-8.5) mg by week 52 (p_{time} < 0.001). Across the groups, tacrolimus

levels were 11.7 (10.7-12.7) ng/ml at the 4-week post-OHT entry point and was reduced significantly to 8.2 (7.3-9.2) ng/ml by week 52 ($p_{time} < 0.001$). Similar trends were observed for methylprednisolone dosage and total corticosteroid dosage utilised in pulse immunotherapy (PRx) for significant rejection events (Figure 3).

Pulse immunotherapy resulted in marked attenuation of both T1 and T2 levels over the course of two weeks (figure 2E-F), whereas time to sub-2R resolution following corticosteroid administration were similar between arms of the study (mean, 95%CI: CMR 3.6, 2.6-4.6 weeks; vs EMB 4.2, 2.4-5.9 weeks, p=0.663) (Figure S5).

Myocardial and Kidney Function

Changes in Left ventricular and interventricular septal CMR-derived functional and structural parameters across the 52 week study period were similar between the two groups (Figure 4A,B,D). There was no significant difference in the change in creatinine levels throughout the study duration between the EMB and CMR groups (4.2 (-32.5 - +40.8) vs 19.8 (-1.7 - +41.2) μmol/L, p = 0.381) or at week 52 (Figure 4C).

Right ventricular ejection fraction fell subtly but significantly in the EMB group compared to the CMR group over the study period (–3.06 (-4.9 - -1.2) % vs +1.35 (-1.3 – +4.0) %, $p < 0.001$). In addition, there was a significant (p < 0.0.001) trend towards increased tricuspid regurgitation in the EMB arm compared to the CMR arm (Figure 4F). By 18 weeks post-transplantation, the prevalence of mild (n; EMB 12, CMR 12) and moderate-severe (n; EMB 4, CMR 1) tricuspid regurgitation was similar between groups. After 36 weeks post-transplantation, the prevalence of mild tricuspid regurgitation was greatest in the EMB arm (n; EMB 12, CMR 5), however there was no significant difference in the incidence of moderate-severe tricuspid regurgitation (n; EMB 2, CMR 0, OR = 0.18, 0.001-2.41, p = 0.290).

Discussion

In this pilot study of stable cardiac transplant recipients randomized four weeks after transplantation, CMR-based rejection surveillance as compared to EMB-based surveillance was feasible in the first year after transplantation and effectively reduced the number of invasive EMB procedures by 94% during this period.

Randomization was well tolerated at four weeks after transplantation; during an early high-risk period for allograft rejection. To the best of our knowledge, this is the first study to randomize patients to CMR or EMB surveillance. Few studies have sought to challenge the role of EMB in this early high-risk period as undetected rejection may lead to sudden death, long-term graft dysfunction, accelerated allograft vasculopathy and fibrosis.[3]

Gene expression profiling (GEP) has become a routine non-invasive surveillance strategy in some centers. This strategy was validated in the IMAGE trial that randomized low-risk patients >6 months (although mostly >1 year) post-transplant to GEP or EMB.[4] The subsequent EIMAGE trial randomized patients earlier but excluded patients with previous rejection or donor specific antibodies.[33] Although very unstable patients were excluded from our study, those with previous rejection or donor specific antibodies were included with adequate tolerability and key rejection outcomes were no different at 1 year between groups. Donor-derived cell-free DNA (ddcfDNA) is likewise similarly reliable (AUC=0.92) at detecting allograft rejection compared to multiparametric CMR imaging used in our study.[34] The biomarkers hsTnT and NT-proBNP have been demonstrated to correlate to native T1 mapping in cardiac transplant recipients.[11] Our initial observations in this regard, suggests that further exploration of the role of steady-state-corrected hsTnT and NT-proBNP, which circulate in response to cardiac rejection, is warranted. We envisage that future studies will explore the potentially complementary roles of CMR, hsTNT, NT-proBNP, ddcfDNA, and GEP in rejection surveillance.

Key findings of our study are that the number of patients that experienced 2R rejection events were similar between the CMR and EMB groups and CMR was non-inferior to EMB at diagnosing clinically relevant 2R rejection events. Although there was a trend for patients in the CMR arm with 2R rejection to have a higher 2R recurrence rate compared to those under EMB surveillance, this did not result in higher overall immunosuppression, infection, or hospitalization, nor did it result in adverse left ventricular functional or structural change at 12 months (Table 3, Figure 4). This finding is not simply due to our CMR rejection classification criteria, as we observed an excellent (n=9 out of 11) concordance rate between EMB and CMR grading of 2R events when physicians requested a

confirmatory biopsy (figure 2D). Notably, there was no difference between groups in the time to first 2R rejection. Furthermore, patients that never experienced 2R events in the CMR group showed no difference in immunosuppression exposure, hospitalisation or infection rates compared to the EMB arm.

While the greater detection rate of 1R rejection in the CMR group may be due to the lower negative predictive value (NPP) of CMR for that grade, the distribution of 1R rejection in that arm was also highly associated with ζ2R rejections (figure 2D). We demonstrated low interobserver variability for CMR surveillance, in contrast to moderate interobserver agreement for histological surveillance (Figure S2, B1-B2, and table S3). Notwithstanding, future studies will help refine CMR classification of rejection beyond the current ISHLT ordinal grading system.

There were insufficient cases of AMR in our cohort to inform the role of CMR in the detection of AMR. Although it is likely that patients with significant AMR would have myocardial edema or dysfunction detectable by CMR given that T1 and T2 values exceeded the threshold for significant rejection, such patients would likely then require an EMB to confirm a diagnosis of AMR. Accordingly, although GEP, hsTnT or NT-proBNP are not ideal for monitoring AMR, the molecular examination of EMB samples, utilizing markers including ddcfDNA shows promise in improving the precision and accuracy of rejection diagnosis and classification.[35] As described below, CMR may play a role in tracking response to immunosuppressive therapy for AMR.

EMB-guided immunosuppression weaning is standard practice in the first year after heart transplantation and is well validated.[36] Both CMR and EMB groups were administered similar immunosuppression doses over the course of the study and displayed similar weaning trends up to 52 weeks of follow-up (Figure 3). Likewise, CMR accurately tracked responses to pulse immunosuppressive treatment, both in the initial validation phase and in the subsequent randomized trial. In patients with 2R rejection in the CMR surveillance group, an immediate reduction in rejection grade severity was noted in 19 out of 22 episodes (86%) after pulse immunosuppression therapy. Equivalent time to quiescence of rejection episodes was noted between groups across both phases of the study (Figure S5). Given that immunosuppression exposure was similar between groups, the finding that the total number of infections and associated hospitalizations were higher in the EMB arm is judiciously interpreted as warranting further confirmation in larger multicenter studies.

Good correlation between CMR-based rejection measures and traditional EMB-based rejection grading have been demonstrated by numerous groups.[9-11, 17, 20, 21] Myocardial native T1 time reflects both intracellular and extracellular signals and is elevated with fibrosis and inflammation. T1-

mapping-based extracellular volume fraction (ECV) is more representative of the extracellular space alone but requires gadolinium administration. T2-mapping-based myocardial T2 time is reflective of myocardial edema and inflammation and is well validated in cardiac allograft rejection.[17, 37]

Our studies utilized a well-validated T1 mapping sequence [13, 14] in combination with a T2 mapping sequence that we initially validated in healthy controls and transplant recipients, and then revalidated successfully in a prospective-randomized study with clinical outcome data. We demonstrated that the described CMR approach is clinically robust against operator dependent variance as well as region selection indicating that multiple imaging slices may not be required for analysis of findings. T2 mapping improved the diagnostic performance of CMR over T1 mapping alone. T1 and T2 mapping sequences are vendor and field-strength specific, each with their merits and limitations.[17, 38] Our study demonstrates proof-of-concept in the feasibility of CMR for rejection surveillance with two specific sequences. Although cross-vendor, cross-field-strength, multicenter studies are warranted, many transplant centers already have access to this technology and could readily derive institution-specific reference ranges for native T1, T2, and extracellular volume fraction in their transplant populations.[38, 39]

In our study, we observed biopsy-related complications requiring hospitalization in the EMB arm as well as increased mild-tricuspid regurgitation. The risk of persistent, moderate-severe tricuspid regurgitation was numerically greater but not statistically significant at 52 weeks post-transplantation in the EMB arm (n; EMB 2, CMR 0, $p = 0.290$). Together these suggest another potential benefit of a non-invasive rejection surveillance strategy which requires further investigation.

Surveillance EMB is important for cardiac rejection surveillance in pediatric populations because signs of allograft rejection may be more difficult to appreciate in this cohort.[40] Moreover, EMB is often performed under general anesthesia in children which adds to procedural risk and invasiveness.[41] Myocardial tissue characterisation by CMR is feasible and informative in the pediatric setting.[42-44] The CMR surveillance protocol we used requires no intravenous cannulation, nor any gadolinium administration and the T2 mapping sequence does not require breath-holding. CMR holds promise to substantially improve cardiac allograft rejection surveillance in the pediatric setting.

Limitations

The results of our trial must be interpreted in the context of several important limitations. This study was conducted at a single center. Generalization at other centres would require infrastructure specific validation against EMB. Although similar numbers of patients in each arm experienced 2R rejection, there appears to be a trend towards an excess of recurrent 2R rejection events in the CMR-treated arm, this did not reach statistical significance. The small sample size in this pilot trial may have contributed

to an imbalance in the true 2R rejection rates between groups; however, CMR may potentially overcall 2R rejection events and further investigation of this possibility is required. Reassuringly, immunosuppression exposure, rates of hospitalisation, and infection in the smaller subset that experienced 2R rejection were also similar between each arm (Table S4). ECV values derived from T1 mapping are similar at 1.5 T and 3.0 T and thus may be preferred for comparisons across field strengths.[38] We chose not to perform ECV mapping or late gadolinium enhancement, to avoid the need for repeated gadolinium administration over the course of 1 year. In addition, we did not compare the CMR and EMB against cell free DNA, an increasingly utilised non-invasive method for rejection surveillance. Future studies should attempt to determine if CMR provides incremental data to GEP and ddcfDNA. Furthermore, we did not routinely perform coronary angiography at 1 year and as such the implications of CMR rejection surveillance on coronary artery vasculopathy (CAV) cannot be determined from our data. As mentioned earlier, our study was not designed to detect AMR and we hypothesise that molecular analysis of EMB samples combined with CMR data will play a role in the diagnosis and management of AMR. Finally, as the study concluded at 1 year after transplantation, implications for long-term allograft function and CAV cannot be drawn.

Conclusion

Cardiovascular magnetic resonance-based rejection surveillance as compared to EMB-based surveillance of stable cardiac transplant recipients, randomized four weeks after transplantation, was feasible in the first year after transplantation, reduced the number of invasive biopsy procedures by 93.7% percent during this period. A multi-center clinical trial is warranted to confirm the efficacy and safety of these findings.

Acknowledgments:

First Authorship: C.A. and M.I. contributed equally to this work.

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Funding Acquisition: A.J., C.A.

Supervision. A.J.

Illustration: J.P.

Sources of funding:

National Heart Foundation of Australia – PhD student scholarship (ID#1015420).

Australian Department of Education – PhD student scholarship.

St Vincent's Clinic Foundation, Australia – Study funds for imaging.

Disclosures:

Dr Keogh has conducted clinical trial research for Actelion, Pfizer, United Therapeutics, Arena, Acceleron, Bayer, Respira, GlaxoSmithKline, and Gilead. Dr Macdonald has received an institutional research grant from Novartis; has been on the advisory boards of Novartis and AstraZeneca. All other authors report no relationships relevant to the contents of this paper.

Supplemental Materials:

Expanded Methods

Supplemental Figures S1 – S6

Supplemental Tables S1 – S4

Tables

Table 1. Baseline Patient Characteristics

Recipient Characteristics	Validation Phase		Randomization Phase		
	CMR Healthy Control Group (n = 33)	CMR/EMB Validation Group (n=33)	CMR Group (n=20)	EMB Group (n=20)	*p* Value
Age, $\bar{x}$ Years (95%CI)	50 (40-59)	38 (34-42)	53 (47-60)	50 (44-57)	0.41
Sex, n patients (%)					0.71
Female	25 (49)	13 (41)	4 (20)	5 (25)	
Male	26 (51)	20 (59)	16 (80)	15 (75)	
Etiology, n patients (%)					0.19
Congenital Heart Disease	-	1 (3)	0 (0)	3 (15)	
Dilated CM	-	21 (64)	8 (40)	6 (30)	
Hypertrophic CM	-	2 (6)	0 (0)	1 (5)	
Ischemic Heart Disease	-	6 (18)	9 (45)	6 (30)	
Infiltrative CM other	-	3 (9)	1 (5)	1 (5)	
Infiltrative CM Amyloidosis	-	0 (0)	1 (5)	3 (15)	
Postpartum CM	-	0 (0)	1 (5)	0 (0)	
Ischemic Time, $\bar{x}$ mins (95%CI)	-	196(131-262)	226 (196-256)	227 (203-250)	0.978
CMV positive at time of Enrolment, n patients (%)	-	7 (21)	12 (60)	13 (65)	0.744
CMR, $\bar{x}$ value (95%CI)					
LVEF (%)	65 (61-70)	68 (66-70)	70 (68-72)	66 (65-67)	<0.01
RVEF (%)	-	-	65 (61-68)	62 (61-64)	0.25
Septal T1 (ms)	974 962-987)	956 (935-977)	984 (965-1002)	985 (969-1001)	0.20
Septal T2 (ms)	51.1 (50.1-52.1)	51.5 (47.9-55.1)	54.0 (52.2-55.8)	54.6 (53.0-56.2)	0.76
Immunosuppression Regime at time of enrolment, n patients (%)					
MMF/TAC/PRED	-	16 (49)	20 (100)	20 (100)	
MMF/RAD/TAC/PRED	-	12(36)	0 (0)	0 (0)	
MMF/CYC/PRED	-	2 (6)	0 (0)	0 (0)	
MMF/CYC/RAD/PRED	-	3 (9)	0 (0)	0 (0)	
Creatinine, $\bar{x}$ mmol/L (95%CI)	-	103 (89-118)	104 (89-120)	133 (91-175)	0.34

Baseline patient characteristics at enrolment. *P*-values represent comparisons between phase-2 groups. CM, cardiomyopathy; MMF, mycophenolate; TAC, tacrolimus, RAD, everolimus; CYC, cyclosporine; PRED, prednisolone; IQR, interquartile range.

Table 2: ISHLT grades, CMR parameters and cardiac biomarkers

ISHLT Grade	Parameter	Optimal Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	95%CI
1R	hsTnT	42.5 ng/L	51.4%	76.9%	58.3%	79.0%	0.666*	0.541-0.790
	hsTnT baseline corrected (Δ)	14.5 ng/L	20.5%	67.3%	33.3%	50.0%	0.455	0.334-0.576
	hsTnT baseline corrected (rel)	0.46	77.4%	50.0%	61.1%	50.9%	0.619	0.475-0.762
	hsTnT steady-state corrected (Δ)	11.5 ng/L	74.4%	55.1%	56.9%	50.0%	0.645*	0.528-0.763
	hsTnT steady-state corrected (rel)	1.88	64.1%	63.8%	58.5%	50.0%	0.625*	0.504-0.745
	NT-proBNP	519 ng/L	86.1%	45.9%	50.0%	87.8%	0.662*	0.536-0.788
	Combined hsTnT, NT-proBNP	Index = 1.5	51.4%	81.1%	30.0%	84.2%	0.710†	0.589-0.830
	Native T1	996.5 ms	68.8%	66.1%	61.1%	73.2%	0.697‡	0.593-0.800
	T2 Relaxation	56.5 ms	58.3%	74.2%	63.6%	69.7%	0.689†	0.583-0.796
	Combined T1, T2	Index = 1.5	50.0%	83.9%	70.1%	68.4%	0.714‡	0.616.0.813
	Combined T1, T2, hsTnT	Index = 1.5	62.9%	74.4%	27.8%	73.2%	0.701†	0.582-0.827
	Combined T1, T2, NT-proBNP	Index = 2.5	55.6%	89.2%	35.3%	83.3%	0.723†	0.605-0.842
	Combined T1, T2, hsTnT, NT-proBNP	Index = 2.5	62.9%	81.1%	26.7%	81.8%	0.729†	0.609-0.848
2R, 3R, AMR, Clinical Rejection	hsTnT	82.5 ng/L	40.0%	84.7%	40.0%	84.8%	0.591	0.428-0.754
	hsTnT baseline corrected (Δ)	12.5 ng/L	6.3%	62.5%	3.6%	81.8%	0.398	0.270-0.527
	hsTnT baseline corrected (rel)	0.98	50.0%	71.1%	35.0%	75.9%	0.613	0.436-0.789
	hsTnT steady-state corrected (Δ)	83.5 ng/L	31.3%	94.4%	55.6%	81.8%	0.586	0.413-0.759
	hsTnT steady-state corrected (rel)	5.3	37.5%	94.3%	60.0%	81.0%	0.622	0.448-0.796
	NT-proBNP	2434 ng/L	66.7%	84.5%	52.6%	90.7%	0.740†	0.588-0.893
	Combined hsTnT, NT-proBNP	Index = 0.5	66.7%	78.9%	50.0%	96.2%	0.735†	0.581-0.889
	Native T1	1029.0 ms	92.9%	80.2%	40.6%	98.7%	0.897‡	0.803-0.990
	T2 Relaxation	59.5 ms	92.9%	83.3%	44.8%	98.8%	0.938‡	0.885-0.990
	Combined T1, T2	Index = 1.5	92.9%	91.7%	61.9%	98.9%	0.916‡	0.823 – 1.00
	Combined T1, T2, hsTnT	Index = 1.5	80.0%	88.1%	60.0%	94.6%	0.820‡	0.679-0.962
	Combined T1, T2. steady-state corrected hsTnT (rel)	Index = 1.5	80.0%	91.2%	63.6%	94.6%	0.870‡	0.748-0.992
	Combined T1, T2, NT-proBNP	Index = 1.5	80.0%	87.9%	40.0%	94.4%	0.830‡	0.688-0.972
	Combined T1, T2, hsTnT, NT-proBNP	Index = 1.5	80.0%	84.2%	50.0%	94.1%	0.815‡	0.671-0.959
	Combined T1, T2, steady state corrected hsTnT (rel), NT-proBNP	Index = 1.5	80.0%	87.3%	40.0%	94.4%	0.859‡	0.733-0.986

Diagnostic accuracy of CMR and cardiac biomarkers for the ISHLT grading of cardiac rejection episodes. Asymptotic significance: symbols; * p < 0.05, † p < 0.01, ‡ p < 0.001. Index = minimum number of parameters that are required to meet or exceed the threshold for that ISHLT grade. Corrected (Δ) = absolute difference from baseline or steady-state. Corrected (rel) = relative change from baseline or steady state (ratio).

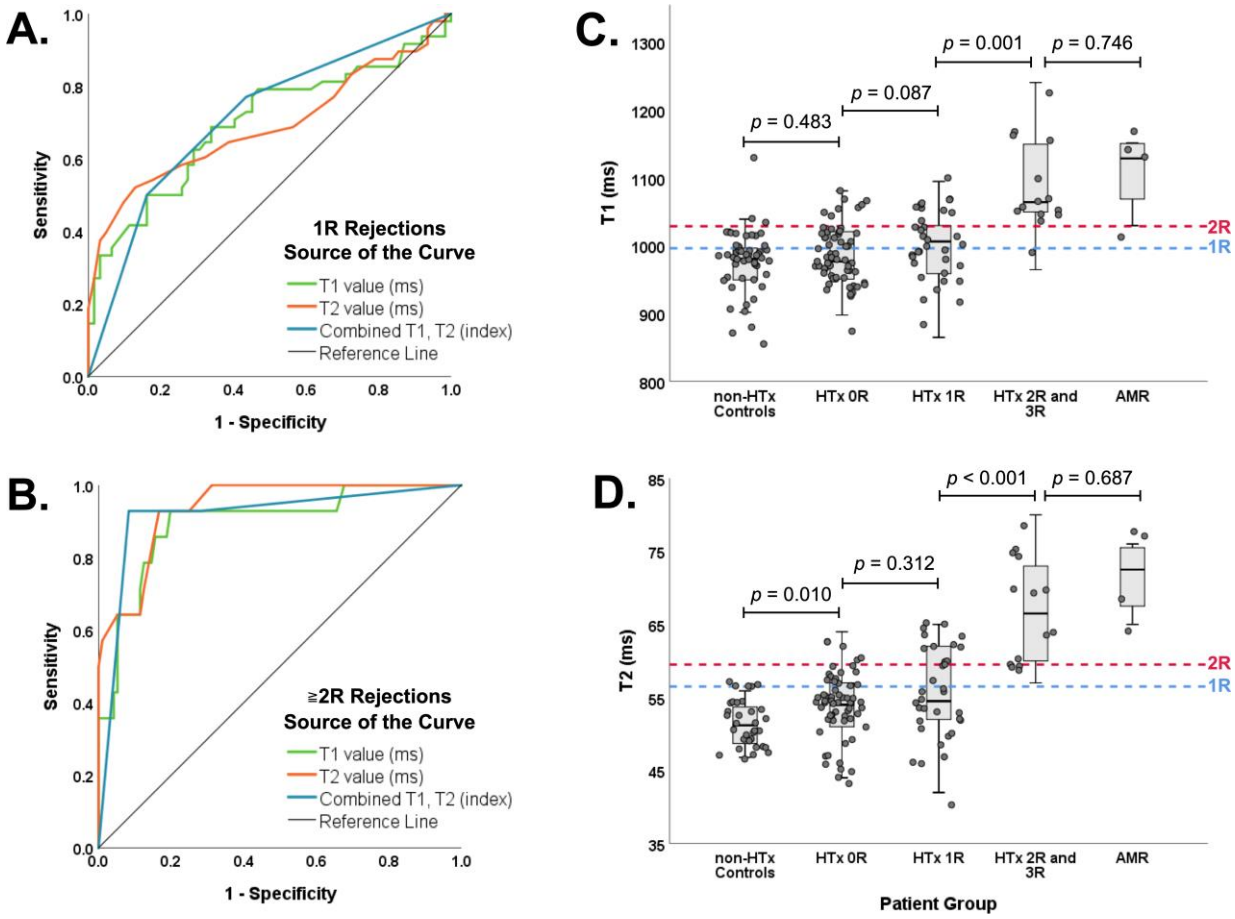
Table 3. Clinical Outcomes

	Event Level				Patient Level			
Rejections	**CMR**	**EMB**	**OR (95% CI)**	***p***	**CMR**	**EMB**	**OR (95% CI)**	***p***
Grade ≧ 2R	22 (9.2)	11 (4.7)		0.142	7 (35)	8 (40)		0.744
Grade 1R	52 (22)	83 (35)		0.052	11 (55)	18 (90)		0.022
Total	74 (31)	94 (40)		0.1	12 (60)	18 (90)		0.04
			-1 0 1 2 3 4 5 6 Favours CMR Favours EMB				-1 0 1 2 3 Favours CMR Favours EMB	
Infections								
Bacterial	10 (1.9)	12 (2.3)		0.616	7 (35)	8 (40)		0.744
Fungal	1 (0.2)	2 (0.4)		0.560	1 (5)	2 (10)		0.556
Viral CMV	1 (0.2)	8 (1.5)		0.039	1 (5)	5 (25)		0.109
Viral non-CMV PCR Positive	3 (0.6)	13 (2.5)		0.059	2 (10)	8 (40)		0.040
Suspected Infection	0 (0)	8 (1.5)		0.002	0 (0)	7 (35)		0.037
Abdominal Sepsis	0 (0)	2 (0.4)		0.214	0 (0)	2 (10)		0.279
Total*	15 (4.7)	35 (11.4)		0.021	7 (35)	14 (70)		0.019
			-1 0 1 2 3 Favours CMR Favours EMB				-1 1 3 5 7 Favours CMR Favours EMB	
Hospitalisations								
Median Length of Stay, Days	4.6 (3.1-6.1)	5.4 (3.6-7.2)	0.83 (-1.4 - +3.07)	0.820				
Cardiac Rejection	15 (2.9)	10 (1.9)		0.880	5 (25)	8 (40)		0.315
Infection	6 (1.2)	19 (3.7)		<0.001	6 (30)	10 (50)		0.201
Other	1 (0.2)	6 (1.2)		0.048	1 (5)	5 (25)		0.108
Acute Kidney Injury	7 (1.3)	1 (0.2)		0.190	3 (15)	1 (5)		0.314
Cardiac Other	2 (0.4)	6 (1.2)		0.054	2 (10)	4 (20)		0.384
Stem Cell Transplant	1 (0.2)	1 (0.2)		0.750	1 (5)	1 (5)		1.000
Abdominal Surgery	0 (0)	3 (0.6)		0.043	0 (0)	2 (10)		0.279
Total	32 (6.7)	46 (15.0)		0.031	9 (45)	18 (90)		0.006
			-1 1 3 5 7 9 Favours CMR Favours EMB				-1 1 3 5 7 9 Favours CMR Favours EMB	

Event level, and patient level comparison of clinical factors between groups. "Suspected Infection" events were clinically diagnosed infection events that were culture or PCR negative. Values reported as number of events or number of patients, and proportions (%). OR = Odds Ratio. "---" in forrest plot = mean OR. Median length of stay reported as mean (95% CI), and effect size difference (95% CI). Total* = Total infections excluding suspected infections, and abdominal sepsis.

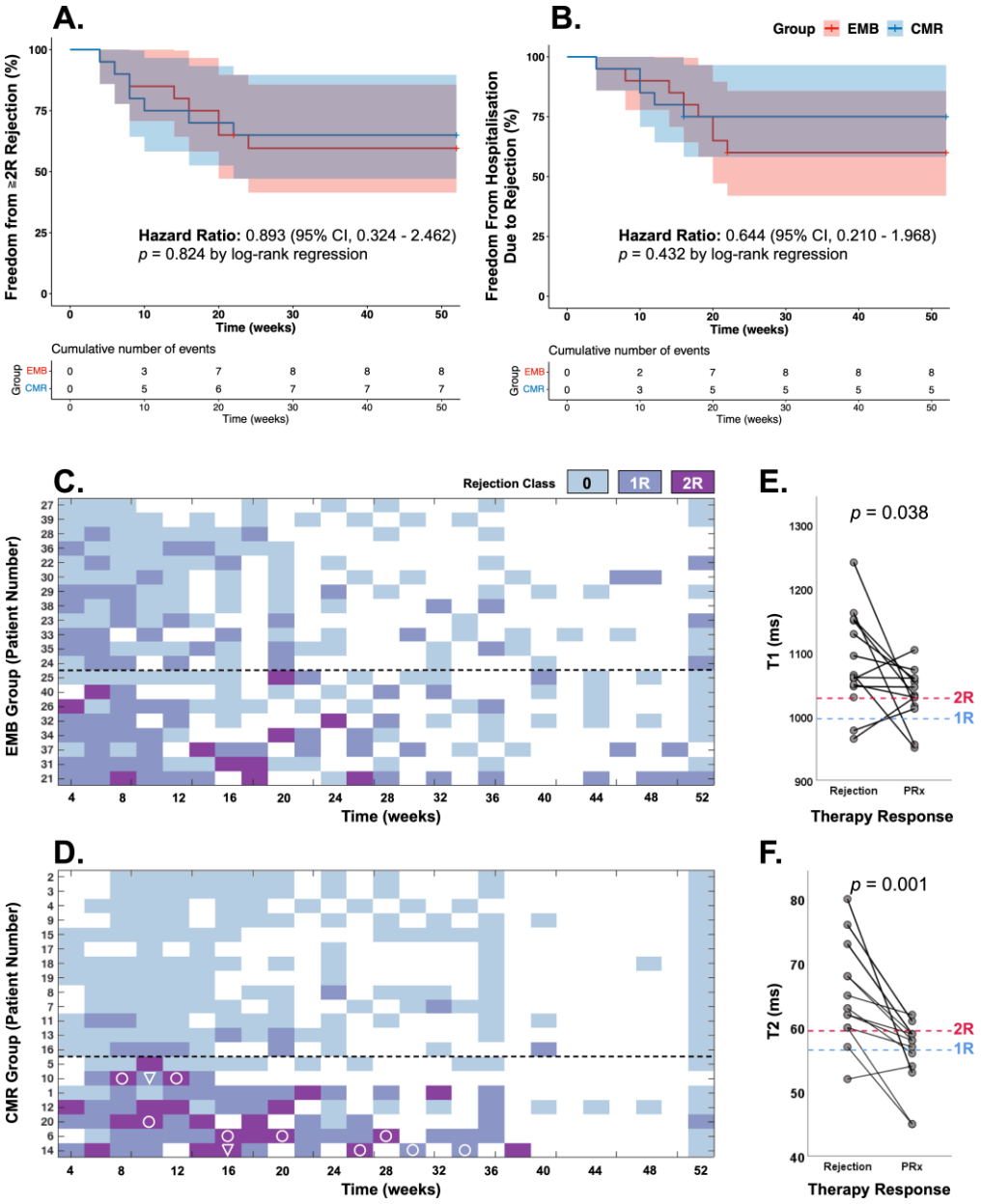
Figures

Figure 1. Validation of CMR-based classification of rejection events.



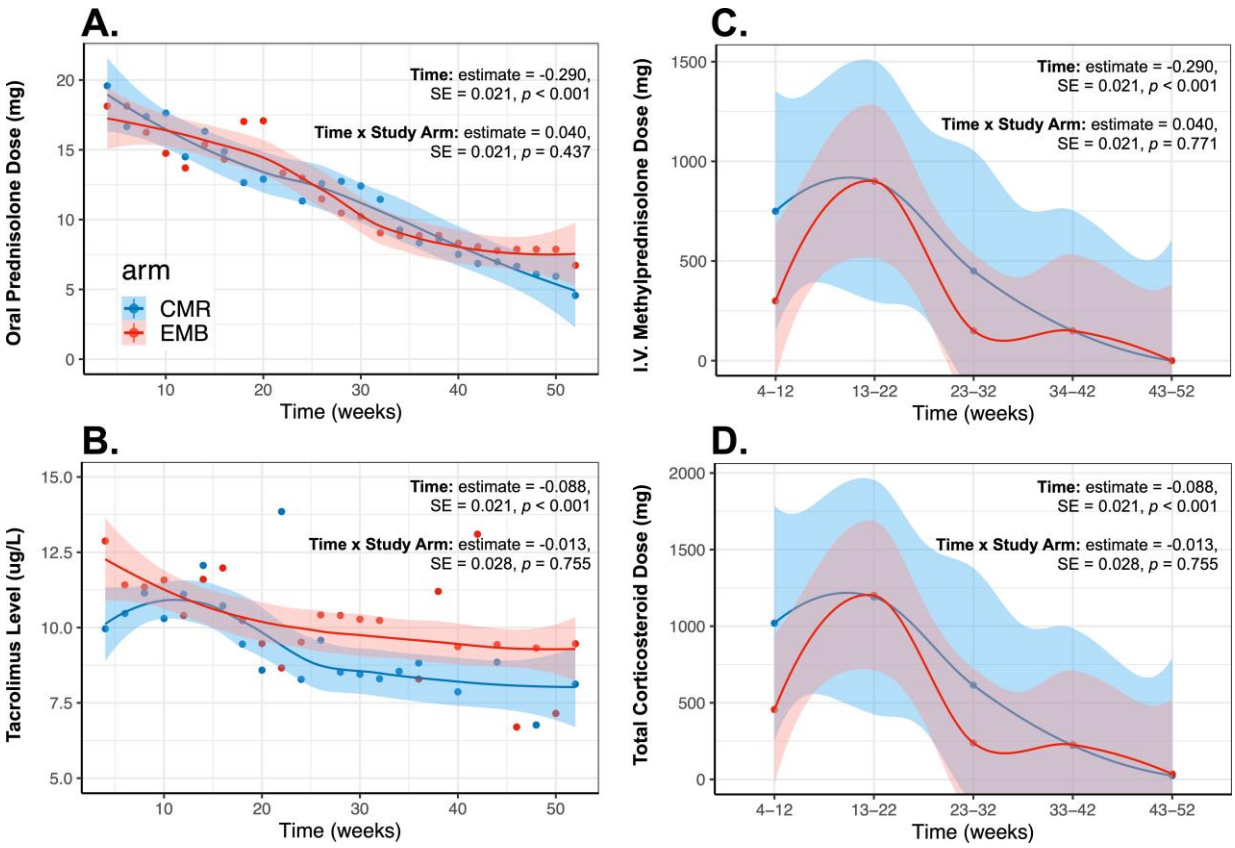
A-B. Receiver Operator Characteristics Curves for assessment of diagnostic performance in the detection of rejection events **A.** ISHLT grade 1R events. **B.** ISHLT grade ≧ 2R events which includes 2R, 3R, AMR (Antibody Mediated Rejection), or Clinical Rejection. **C-D.** CMR parameters stratified by group, partitioned by CMR detection threshold for ISHLT 1R and 2R rejection events. **C.** Native T1 and **D.** T2 relaxation times for each patient stratified by group. Normal controls are patients with normal cardiovascular function which have not received a heart transplant (HTx). HTx patients stratified into ISHLT grade 0R, 1R, 2R to 3R, and AMR (Antibody Mediated Rejection) are patients who have received a heart transplant. Blue --- = CMR cutoff for 1R events. Red --- = CMR cutoff for 2R events.

Figure 2. Prognostic distribution of cardiac rejection



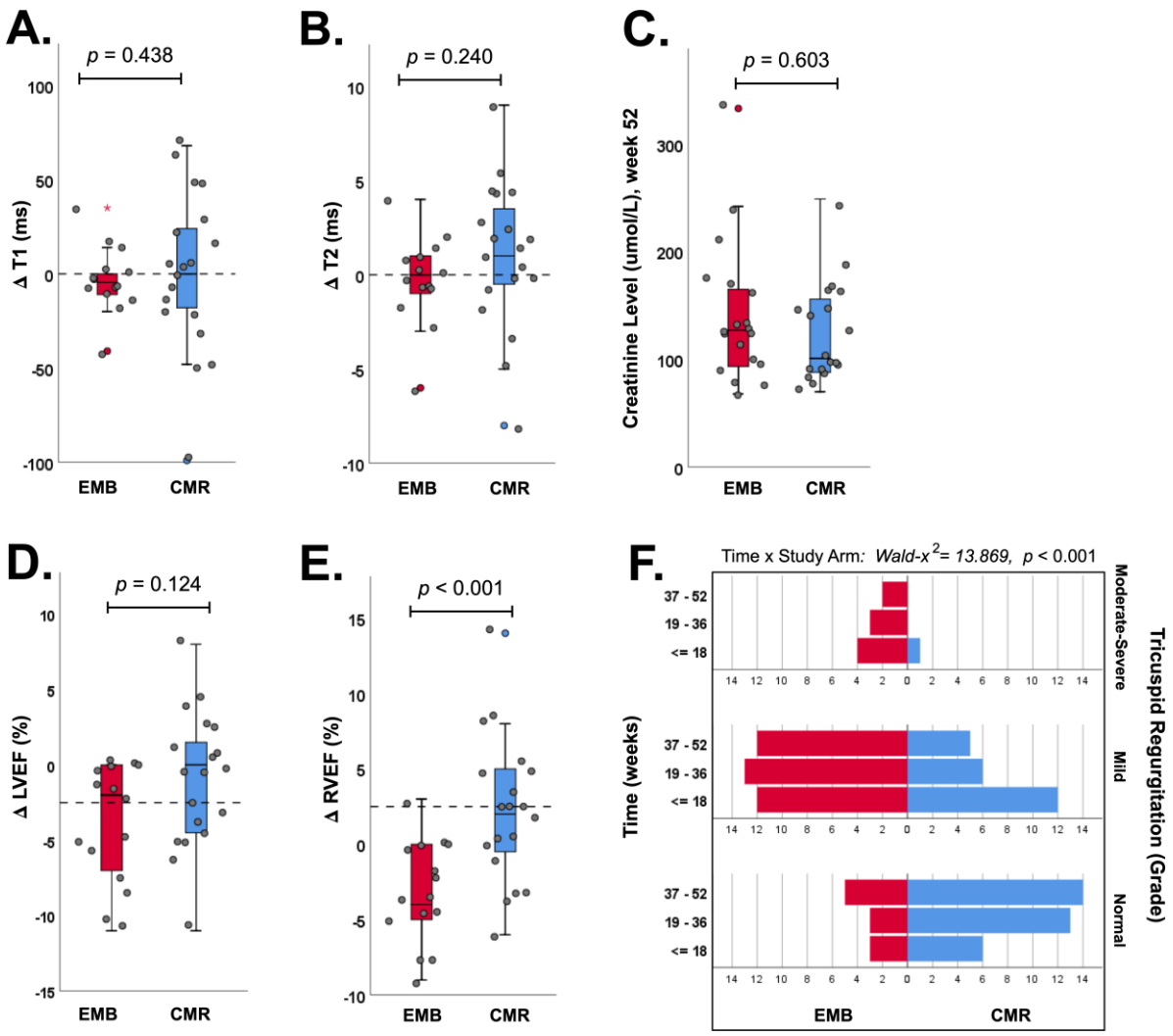
A-B. Prognosis of adverse events stratified by surveillance arm from week 0 to week 52 post-transplantation based on Kaplan-Meyer cumulative survival analysis. **A.** Time to first grade 2R or greater rejection event. **B.** Time to first hospitalisation due to rejection event. Numbers below panels indicate the total number of patients that have experienced rejection events with respect to time. Shaded region represents 95% confidence interval. **C-D.** Mosaic plots indicating the time and severity of rejection for each patient stratified into **C.** EMB and **D.** CMR groups. Individual patient numbers listed on the y-axis are sorted by most (top) to least (bottom) total number of rejection events. Lines (---) indicate partitioning of patients with 2R rejection. **Y-axis**: Patients are ranked according to number of 2R events. Histological confirmation, symbols: ○ = no change in rejection class; ▼ = decrease change in rejection class by one grade. **E-F.** Response to pulse immunotherapy (PRx) across the two trial phases for significant rejection. All significant rejection events (ISHLT grade 2R, 3R or AMR) and subsequent resolved rejection events (ISHLT grade 0R or 1R) after PRx were confirmed histologically. **E.** Effect of PRx on T1 attenuation in confirmed resolution of significant rejection. **F.** Effect of PRx on T2 attenuation in confirmed resolution of significant rejection.

Figure 3. Corticosteroid dose and Tacrolimus levels throughout duration of study



Medical therapy given throughout the study duration. **A.** Prednisolone dose; **B.** Measured tacrolimus levels; **C.** Median I.V. methyl-prednisolone dose per 8-week period; and **D.** Median Total corticosteroid dose per 8-week period. A statistically significant time dependent titrated reduction in prednisolone dose, resolution-of-rejection dependent reduction in methyl-prednisolone and total corticosteroid dose, and titrated reduction in tacrolimus levels was observed, which were not significantly different between groups. Variance in loess spline line plots demonstrate the 95% CI. ● = actual temporal mean value.

Figure 4. Change in structural and functional parameters throughout the study period.



A-F. Comparison of change (1′) in CMR, kidney function, and tricuspid valve parameters across the study duration between study arms. **A.** Septal Native T1 time. **B.** Septal Native T2 time. **C.** Serum creatinine levels at week 52. **D.** LVEF. **E.** RVEF, demonstrating significant attenuation in the biopsy arm. **F.** Number of patients across each study period that exhibited tricuspid valve regurgitation based on qualitative visual assessment of colour doppler jet area on trans transthoracic echocardiography.

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