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Cardiovascular Magnetic Resonance for Rejection Surveillance After Cardiac Transplantation

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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 currently 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 to determine CMR cutoff values between rejection grades. A prospective randomized noninferiority pilot study was then undertaken in adult orthotopic heart transplant recipients who were randomized at 4 weeks after orthotopic heart transplantation to either CMR- or EMB-based rejection surveillance. Clinical end points were assessed at 52 weeks.

RESULTS: Four hundred one CMR studies and 354 EMB procedures were performed in 106 participants. Forty heart transplant recipients were randomized. CMR-based multiparametric assessment was highly reproducible and reliable at detecting ACAR (area under the curve, 0.92; sensitivity, 93%; specificity, 92%; negative predictive value, 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 of 20 [45%] versus 18 of 20 [90%]; odds ratio, 0.091; *P*=0.006) and infection (7 of 20 [35%] versus 14 of 20 [70%]; odds ratio, 0.192; *P*=0,019) were lower in the CMR group. On 15 occasions (6%), patients who 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 noninvasive CMR-based surveillance strategy for ACAR in the first year after orthotopic heart transplantation is feasible compared with EMB-based surveillance.

REGISTRATION: HREC/13/SVH/66 and HREC/17/SVH/80. Australian New Zealand Clinical Trials Registry: ACTRN12618000672257.

Key Words: heart transplantation ■ magnetic resonance imaging

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Clinical Perspective

What Is New?

- In this exploratory randomized trial, which assessed the feasibility of cardiac magnetic resonance (CMR)–based surveillance for the management of orthotopic cardiac transplant recipients in the first year after transplantation, multiparametric tissue mapping by CMR reliably graded acute cardiac allograft rejection compared with endomyocardial biopsy–based surveillance, resulting in equivalent immunosuppression exposure without increased risk of infections, hospitalizations, cardiomyopathy, or kidney injury.
- CMR-based surveillance of acute cardiac allograft rejection significantly reduced the requirement for invasive endomyocardial biopsy 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 endomyocardial biopsy in the first year after transplantation.
- Prospective multicenter studies are warranted to further explore the safety, efficacy, and cost-effectiveness of CMR for cardiac allograft rejection surveillance after transplantation.

Nonstandard Abbreviations and Acronyms

AMR	antibody-mediated rejection
AUC	area under the curve
CMR	cardiovascular magnetic resonance
EMB	endomyocardial biopsy
GEP	gene expression profiling
hs-TnT	high-sensitivity troponin T
ISHLT	International Society of Heart and Lung Transplantation
NT-proBNP	N-terminal probrain natriuretic peptide
OHT	orthotopic heart transplantation

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 noninvasive detection of cardiac allograft rejection,[4] endomyocardial biopsy (EMB) remains the primary method of surveillance for rejection.[5] However, EMB is invasive and subject to both sampling error and significant interreporter 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 noninvasive 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 cutoff 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 with EMB-based monitoring in the first year after cardiac transplantation.

METHODS

The data, analytical methods, and study materials will not be made publicly available to other researchers for purposes of reproducing the results or replicating the procedure because of logistic and ethical constraints; however, interested parties are welcome to visit onsite 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, enrollment, and randomization are summarized here and detailed in Figure S1.

The initial observational phase involved a prospective cross-sectional study in which 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 (hs-TnT) and NT-proBNP (N-terminal probrain natriuretic peptide) were also measured within 24 hours of cardiac biopsies with electrochemiluminescent immunoassay methods on a Roche e-Module analyzer (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. Patients not undergoing transplantation without a history of cardiac pathology and with a normal CMR, as determined by an independent specialist at our center, were used as healthy nontransplantation control subjects (Table 1).

Table 1. Baseline Patient Characteristics

Recipient characteristics	Validation phase		Randomization phase		*P* value
	CMR healthy control group (n=33)	CMR/EMB validation group (n=33)	CMR group (n=20)	EMB group (n=20)	
Age (95% CI), y	50 (40–59)	38 (34–42)	53 (47–60)	50 (44–57)	0.41
Sex, n (%)					0.71
Female	25 (49)	13 (41)	4 (20)	5 (25)	
Male	26 (51)	20 (59)	16 (80)	15 (75)	
Cause, n (%)					0.19
Congenital heart disease	…	1 (3)	0 (0)	3 (15)	
Dilated cardiomyopathy	…	21 (64)	8 (40)	6 (30)	
Hypertrophic cardiomyopathy	…	2 (6)	0 (0)	1 (5)	
Ischemic heart disease	…	6 (18)	9 (45)	6 (30)	
Infiltrative cardiomyopathy, other	…	3 (9)	1 (5)	1 (5)	
Infiltrative cardiomyopathy, amyloidosis	…	0 (0)	1 (5)	3 (15)	
Postpartum cardiomyopathy	…	0 (0)	1 (5)	0 (0)	
Ischemic time (95% CI), min	…	196(131–262)	226 (196–256)	227 (203–250)	0.978
CMV positive at time of enrollment, n (%)	…	7 (21)	12 (60)	13 (65)	0.744
CMR (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 regimen at time of enrollment, n (%)					
Mycophenolate/tacrolimus/prednisolone	…	16 (49)	20 (100)	20 (100)	
Mycophenolate/everolimus/tacrolimus/prednisolone	…	12(36)	0 (0)	0 (0)	
Mycophenolate/cyclosporine/prednisolone	…	2 (6)	0 (0)	0 (0)	
Mycophenolate/cyclosporine/everolimus/prednisolone	…	3 (9)	0 (0)	0 (0)	
Creatinine (95% CI), mmol/L	…	103 (89–118)	104 (89–120)	133 (91–175)	0.34

Baseline patient characteristics at enrollment. *P* values represent comparisons between phase 2 groups. CMR indicates cardiac magnetic resonance; CMV, cytomegalovirus; EMB, endomyocardial biopsy; IQR, interquartile range; LVEF, left ventricular ejection fraction; and RVEF, right ventricular ejection fraction.

The data from the observational phase were used to plan a randomized, prospective, noninferiority pilot study, which was conducted from February 1, 2018, through March 10, 2020. Patients ≥18 years of age undergoing orthotopic heart transplantation (OHT) were screened and randomized at 4 weeks after transplantation to EMB- or CMR-based surveillance (Table 1). All participants were followed up until 52 weeks after transplantation. Patients were randomly assigned in 2×2 blocks with an online randomizer[23] by an independent adjudicator. Both patients and clinicians were unblinded. Patients randomized to the EMB group underwent EMB at weeks 4, 6, 8, 10, 12, 16, 20, 24, and 32 after 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.[24] Antibody-mediated rejection (AMR) was interpreted according to the 2011 ISHLT consensus classification system.[24,25] Further details on the biopsy protocol and analysis are provided in the Supplemental Material. Patients in the EMB group also underwent a baseline CMR at the time of enrollment and at 52 weeks after 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 was performed to ensure recovery. Additional CMR scans and transthoracic echocardiograms 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 that the patient may come to harm without a biopsy. This occurred infrequently, as detailed later.

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

Imaging

Noncontrast CMR studies were performed at 1.5 T (Achieva, Philips Medical Systems, Best, the Netherlands) with a 32-channel coil. The CMR protocol is described in detail in the Supplemental Material. In brief, steady-state free precession 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 sequence was used to acquire T1 maps in a single midventricular short-axis plane, as described previously.[13,14,26] The T2 maps were acquired with a respiratory-navigated black-blood, turbo-spin-echo 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 with commercially available software (CVI42, Circle Cardiovascular Imaging, Inc, Calgary, AB, Canada). Regions of interest were carefully drawn along the interventricular septum, as well as circumferentially on the midventricular short-axis slice, to acquire septal and global T1 and T2 values, as described previously.[11,13,14,26–28]

Left and right ventricular ejection fractions were derived from short-axis cine images, involving semiautomated analysis after manual definition of endocardial and epicardial borders (excluding papillary muscles and trabeculae) and the mitral, tricuspid, and pulmonary valve annular planes with cvi42 (Circle Cardiovascular Imaging).

Stratification and Treatment

Allograft rejection events were defined with ISHLT criteria: for no rejection, grade 0; low-grade rejection, grade 1R; and high-grade rejection, grade 2R, 3R or 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, multiparametric T1- and T2-mapping cutoff values. Details on the methodology for rejection grading are described in the Supplemental Material and Table S1.

All transplant recipients received induction therapy with 20 mg basiliximab, a monoclonal antibody against CD25, at day 0 and day 4 after transplantation. Time of initiation and doses of immunosuppressants used after transplantation were according to the cardiac transplantation 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 (greater than grade 2R) rejection. Secondary outcomes included frequency and cumulative freedom from low-grade (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-operating characteristic curve (AUC). Statistically optimal CMR criteria for discrimination of ISHLT rejection grades were determined by the Youden index. Receiver-operating characteristic curves were compared according to the methodology of Hanley and MacNeil.[29]

The sample size for the primary outcome was based on a noninferiority analysis using the Cohen weighted κ coefficient with linear weights to calculate the interobserver agreement between independent histologists in grading significant rejection from biopsy specimens observed in the validation phase of the trial (indicated in Table S2).[30,31] We calculated that a target of 40 patients (20 per group) would provide 80% power at a 1-sided α of 0.05 to test the primary outcome and that CMR did not miss significant rejection (greater than grade 2R) compared with EMB (was noninferior), with 11 repeated measures (longitudinal biopsies or CMR scans) for each subject, assuming a first-order autoregressive correlation structure with a base correlation of 0.1 and a noninferiority margin of time-averaged difference in ISHLT greater than grade 2R rejection proportions of −9% between the CMR and EMB groups (assuming an ISHLT grade 2R or greater rejection rate of 13% in both groups if not different from the validation phase), that is a relative risk margin of 0.23 comparing CMR with EMB.[32] We considered this stringent choice of a −9% margin (actual interrater disagreement, 8.8%; Supplemental Material gives derivation) a clinically meaningful index to refute noninferiority for ISHLT grade 2R or greater rejection on the basis of an interrater agreement of 91.2% between 2 independent histologists (Cohen $\kappa = 0.473$, $P<0.001$; Table S2).

Continuous data are described as mean and 95% CIs. Categorical data were displayed as event frequency (percent). Interobserver variability of CMR analyses was assessed with the intraclass correlation coefficient method and Bland-Altman plots. Receiver-operating characteristics curves were used to investigate the ability of CMR to detect low-grade and high-grade rejection events.

Patient-level comparisons of categorical or continuous variables between groups for baseline characteristics, rates of infection, hospitalization, and allograft rejection over the study duration were performed with logistic regression without random effects, Mann-Whitney *U* test, or Kruskal-Wallis test, as appropriate. For event-level comparisons, the generalized estimating equations log-link binomial generalized linear model was applied to compare ISHLT grade 2R or greater rejection and other binary outcomes with repeated measures, and the first-order autoregressive 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, as well as hospitalization attributable to rejection, during the 1-year follow-up period. Kaplan-Meier survival analysis with the log-rank test was used to compare survival curves between the groups, with Cox regression used to derive hazard ratios. The proportional hazard assumption was tested with Schoenfeld residuals and was found to be valid. A 2-tailed value of $p < 0.05$ was considered statistically significant.

Statistical analyses were performed with IBM SPSS statistics version 25 (IBM Corp, Armonk, NY), R (R Core Team, Vienna, Austria, 2012), and lme4.[33]

RESULTS

Patients

Four hundred one CMR studies and 354 EMB procedures were performed in 106 participants (73 heart transplant recipients and 33 nontransplantation healthy control subjects). Patient assignment to groups across each trial phase is summarized in Table 1 and in the Consolidated Standards of Reporting Trials diagram in Figure S1.

In the validation phase, a total of 108 EMBs were performed with simultaneous CMR in 33 OHT recipients. In addition, 33 CMR scans were performed in 33 non-OHT healthy control subjects. 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% grade 2R or 3R, 3.7% clinically diagnosed rejection, and 3.7% AMR). Of 33 patients, 8 patients had clinically significant rejection (Table 1).

In the subsequent randomized phase, a total of 238 CMR scans and 15 EMBs (11 EMBs performed in conjunction with CMR) were performed on 20 OHT recipients randomized to the CMR group, and a total of 235 EMBs were performed in 20 OHT recipients randomized to the EMB group. The baseline characteristics of the patients between groups were well matched (Table 1). Only 2 patients did not complete the study; 1 patient in the CMR arm died of kidney failure, and 1 patient in the EMB arm had 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 interobserver agreement and correlation were observed for both T1 (coefficient of variation, 1.3%; r=0.94, P<0.001) and T2 mapping (coefficient of variation, 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 (r=0.97, P<0.001) mapping (Figure S2), findings that formed the basis for selection of the interventricular septum as the region of interest for all subsequent analyses.

Receiver-operating characteristics curve analysis for detecting high-grade rejection (ISHLT grades 2R, 3R, and AMR and clinically diagnosed) by CMR yielded an AUC of 0.897 for T1 and 0.938 for T2 (Table 2 and Figure 1A and 1B). On the basis of the Youden score, optimal cutoffs values for detecting high-grade rejection with T1 and T2 mapping were 1029 and 59.5 milliseconds, respectively. Reliability in detecting high-grade rejection with these CMR parameters was consistent with significant differences in T1 and T2 values observed between low-grade and high-grade rejection events (Figure 1C and 1D). Furthermore, with a multiparametric approach, an AUC of 0.92 was achieved with greater sensitivity (93%), specificity (92%), and negative predictive value (99%) compared with single-parameter approaches (Table 2 and Figure 1B). Inclusively, minimum T1 and T2 (mean) values for AMR remained consistently above the defined threshold for high-grade rejection (T1, 1110 milliseconds [range 1030-1152 milliseconds]; T2, 71.5 milliseconds [range, 65-76 milliseconds]; Figure 1C and 1D).

The sensitivity for detecting low-grade (ISHLT grade 1R) rejection by CMR was more modest, yielding an AUC of 0.697 for native T1 and 0.689 for T2 parameters (Table 2 and Figure 1A). From the highest Youden score for the detection of clinically significant rejection, cutoff T1 and T2 mapping values of 996.5 and 56.5 milliseconds, 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 and 2F). 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).

The hematological biomarkers hs-TnT 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 a discriminatory capability similar to that of combined CMR markers for grade 1R rejection but performed less well for predicting grade 2R rejection (Table 2, Figure S3, and Table S3).

Multiparametric rejection risk stratification with all markers (T1, T2, steady-state–corrected hs-TnT [rel], and NT-proBNP) demonstrated a discriminatory capability similar to that of multiparametric CMR-only markers for grade 1R rejection. However, the discriminatory capability of all markers combined was lower for grade 2R rejection compared with combined or individual CMR-only markers (Table 2 and Figure S3).

Allograft Rejection Events

In the prospective randomization phase, the number of patients who experienced grade 2R or greater rejection events did not differ between the CMR and EMB groups

Table 2. ISHLT Grades, CMR Parameters, and Cardiac Biomarkers

ISHLT grade	Parameter	Optimal cutoff	Sensitivity, %	Specificity, %	PPV, %	NPV, %	AUC	95% CI
1R	hs-TnT	42.5 ng/L	51.4	76.9	58.%	79.0	0.666*	0.541–0.790
	hs-TnT baseline corrected (Δ)	14.5 ng/L	20.5	67.3	33.3	50.0	0.455	0.334–0.576
	hs-TnT baseline corrected (rel)	0.46	77.4	50.0	61.1	50.9	0.619	0.475–0.762
	hs-TnT steady state corrected (Δ)	11.5 ng/L	74.4	55.1	56.9	50.0	0.645*	0.528–0.763
	hs-TnT 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 hs-TnT, 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, hs-TnT	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, hs-TnT, NT-proBNP	Index=2.5	62.9	81.1	26.7	81.8	0.729†	0.609–0.848
2R, 3R, AMR, clinical rejection	hs-TnT	82.5 ng/L	40.0	84.7	40.0	84.8	0.591	0.428–0.754
	hs-TnT baseline corrected (Δ)	12.5 ng/L	6.3	62.5	3.6	81.8	0.398	0.270–0.527
	hs-TnT baseline corrected (rel)	0.98	50.0	71.1	35.0	75.9	0.613	0.436–0.789
	hs-TnT steady state corrected (Δ)	83.5 ng/L	31.3	94.4	55.6	81.8	0.586	0.413–0.759
	hs-TnT 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 hs-TnT, 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, hs-TnT	Index=1.5	80.0	88.1	60.0	94.6	0.820‡	0.679–0.962
	Combined T1, T2. steady state–corrected hs-TnT (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, hs-TnT, 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 hs-TnT (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. Index is the minimum number of parameters required to meet or exceed the threshold for that ISHLT grade. Corrected (Δ) is absolute difference from baseline or steady state. Corrected (rel) is relative change from baseline or steady state (ratio). AUC indicates area under the curve; CMR, cardiac magnetic resonance; hs-TnT, high-sensitivity troponin T; ISHLT, International Society of Heart and Lung Transplantation; NPV, negative predictive value; NT-proBNP, N-terminal probrain natriuretic peptide; PPV, positive predictive value; and rel, relative.

*$P \leq 0.05$.

†$P \leq 0.01$.

‡$P \leq 0.001$.

(7 versus 8; $P=0.744$; Figure5 and Figure 2A, 2C, and 2D). CMR did not miss grade 2R rejection episodes relative to EMB, noting our prespecified tolerance level as shown in Figure 1C and 1D. The odds ratio of grade 2R or greater rejection being diagnosed by CMR versus EMB is 2.06 (95% CI, 1.27–3.34; Figure 5 and Figure 2A, 2C, and 2D).

The observed cumulative freedom from all rejection grades was greater in the CMR group compared with the EMB group (hazard ratio, 0.383 [95% CI, 0.180–0.82]; $P=0.003$; Figure S4A). According to Cox regression, the cumulative freedom from grade 2R rejection episodes was not statistically different between the 2 groups (hazard ratio, 0.893 [95% CI, 0.32–2.46]; $P=0.82$; Figure 2A). In light of the lower incidence of grade 1R rejection events in the CMR group compared with the EMB group, there was a favorable cumulative freedom from grade 1R rejection episodes in the CMR group compared with the EMB group (hazard ratio, 0.354 [95% CI, 0.16–0.77]; $P=0.002$; Figure S4B).

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 of these 11 (82%) EMB results were identical to

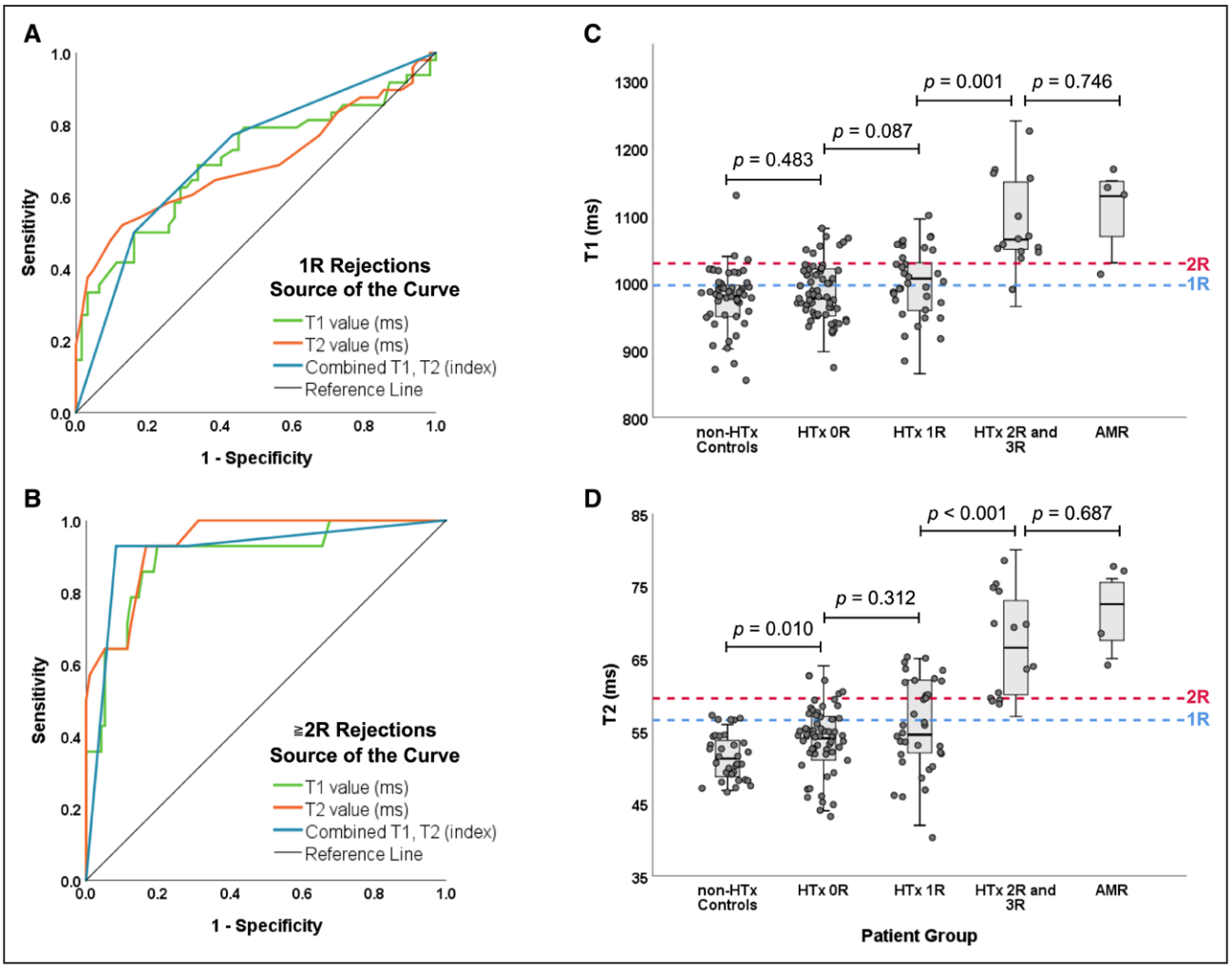

Figure 1. Validation of CMR-based classification of rejection events.
A and **B**, Receiver-operator characteristics curves for assessment of diagnostic performance in the detection of rejection events. **A**, International Society of Heart and Lung Transplantation (ISHLT) grade 1R events. **B**, ISHLT grade 2R or greater events, which include 2R, 3R, antibody-mediated rejection (AMR), or clinical rejection. **C** and **D**, Cardiac magnetic resonance (CMR) parameters stratified by group, partitioned by CMR detection threshold for ISHLT grade 1R and 2R rejection events. Native T1 (**C**) and T2 relaxation times (**D**) for each patient stratified by group. Normal control subjects are patients with normal cardiovascular function who have not received a heart transplant (HTx). Patients with HTx stratified into ISHLT grade 0R, 1R, 2R to 3R, and AMR are patients who have had a heart transplantation. Blue shows the CMR cutoff for grade 1R events; and red, CMR cutoff for 2R events.

the CMR results, and 2 (18%) suggested that the severity of rejection was 1 grade lower than the CMR result (Figure 2C and 2D). By 5 to 12 weeks, the average number of cumulative surveillance procedures increased slightly in the EMB arm (EMB, n=3.3±1.1; CMR, n=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 the 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 the CMR or EMB groups (Figure 5). There were fewer cytomegalovirus and noncytomegalovirus viral infection events in the CMR group compared with the EMB group (cytomegalovirus, 1 versus 8, *P*=0.04: noncytomegalovirus, 3 versus 13, *P*<0.06). The risk of infection episodes was decreased in the CMR group compared with the EMB group because of the increased incidence of viral infection in the latter (odds ratio, 0.373 [95% CI, 0.211–0.486]; *P*=0.02; Figure 5).

Hospitalization and Biopsy Complications

There were fewer unplanned hospitalization events (32 versus 46; *P*=0.03) and fewer individual patients hospitalized (9 versus 18; *P*<0.006) in the CMR group compared with the EMB group. However, there was no

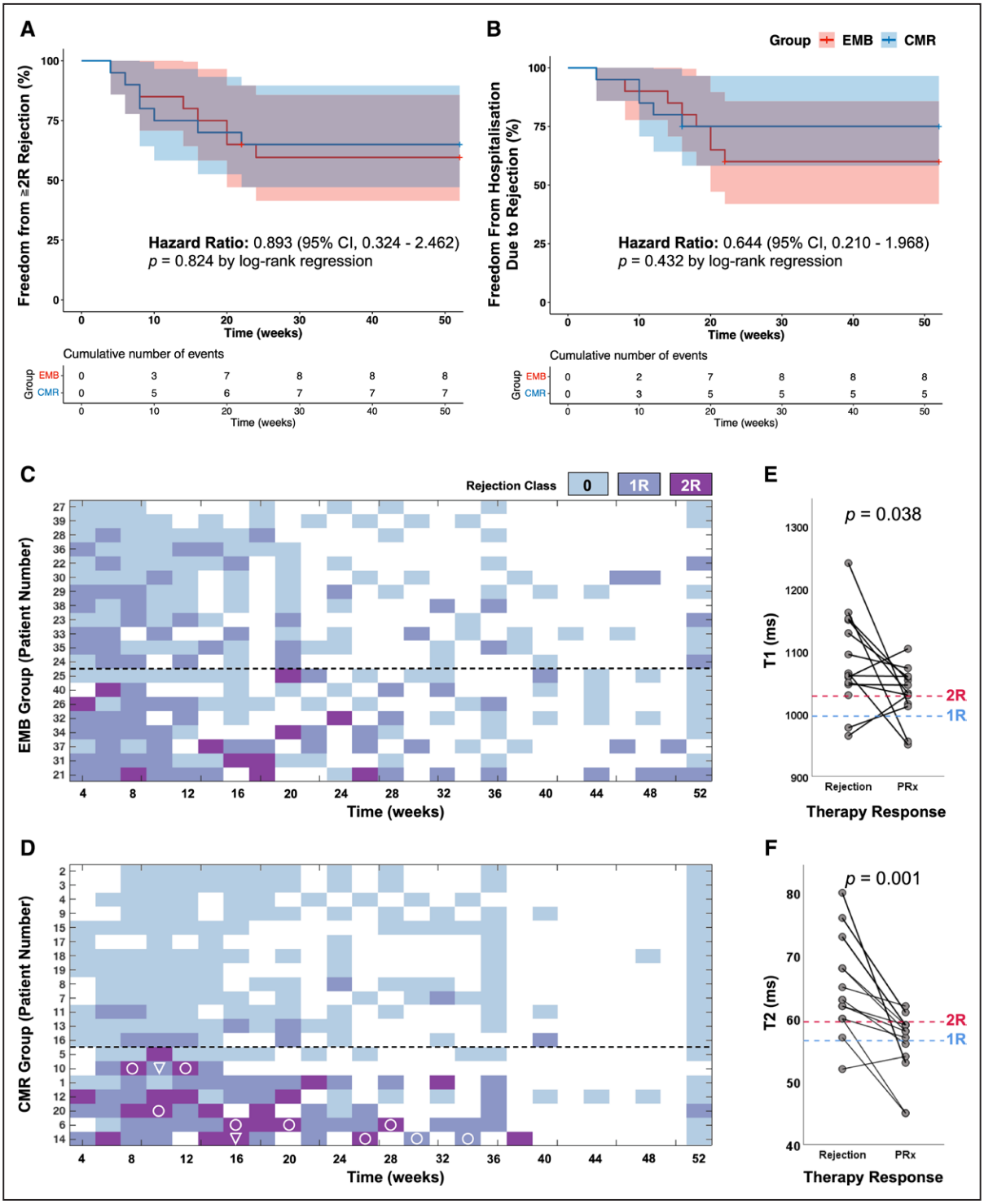

Figure 2. Prognostic distribution of cardiac rejection.

A and **B**, Prognosis of adverse events stratified by surveillance arm from week 0 to 52 after transplantation based on Kaplan-Meyer cumulative survival analysis. **A**, Time to first grade 2R or greater rejection event. **B**, Time to first hospitalization attributable to rejection event. Numbers below panels indicate the total number of patients who have experienced rejection events with respect to time. Shaded region represents 95% CI. **C** and **D**, Mosaic plots indicating the time and severity of rejection for each patient stratified into (**C**) endomyocardial biopsy (EMB) and (**D**) cardiac magnetic resonance (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 grade 2R rejection. Patients are ranked according to number of 2R events on the *y* axis. Histological confirmation: ○, no change in rejection class; and ▽, decrease change in rejection class by 1 grade. **E** and **F**, Response to pulse immunotherapy (PRx) across the 2 trial phases for significant rejection. All significant rejection events (International Society of Heart and Lung Transplantation [ISHLT] grade 2R, 3R, or antibody-mediated rejection) 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.

significant difference in the median length of hospitalization between the CMR and EMB groups (4.6 [3.1–6.1] days versus 5.4 [3.6–7.2] days; P=0.82; Figure 5). There were 3 biopsy-related complications in EMB group. There were 1 carotid artery puncture with no clinically significant sequelae and 2 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 (prednisolone dose, $P_{time \times study\ arm}$=0.437; tacrolimus level, $P_{time \times study\ arm}$=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 were reduced significantly to 8.2 (7.3–9.2) ng/mL by week 52 (P_{time}<0.001). Similar trends were observed for methylprednisolone dose and total corticosteroid dose used in pulse immunotherapy for significant rejection events (Figure 3).

Pulse immunotherapy resulted in marked attenuation of both T1 and T2 levels over the course of 2 weeks (Figure 2E and 2F), whereas time to resolution below grade 2R after corticosteroid administration was similar between the study arms (CMR, 3.6 weeks [mean] [95% CI, 2.6–4.6] versus EMB, 4.2 [95% CI, 2.4–5.9]; 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 2 groups (Figure 4A, 4B, and 4D). 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 to 40.8] µmol/L versus 19.8 [−1.7 to 41.2] µmol/L; P=0.381) or at week 52 (Figure 4C).

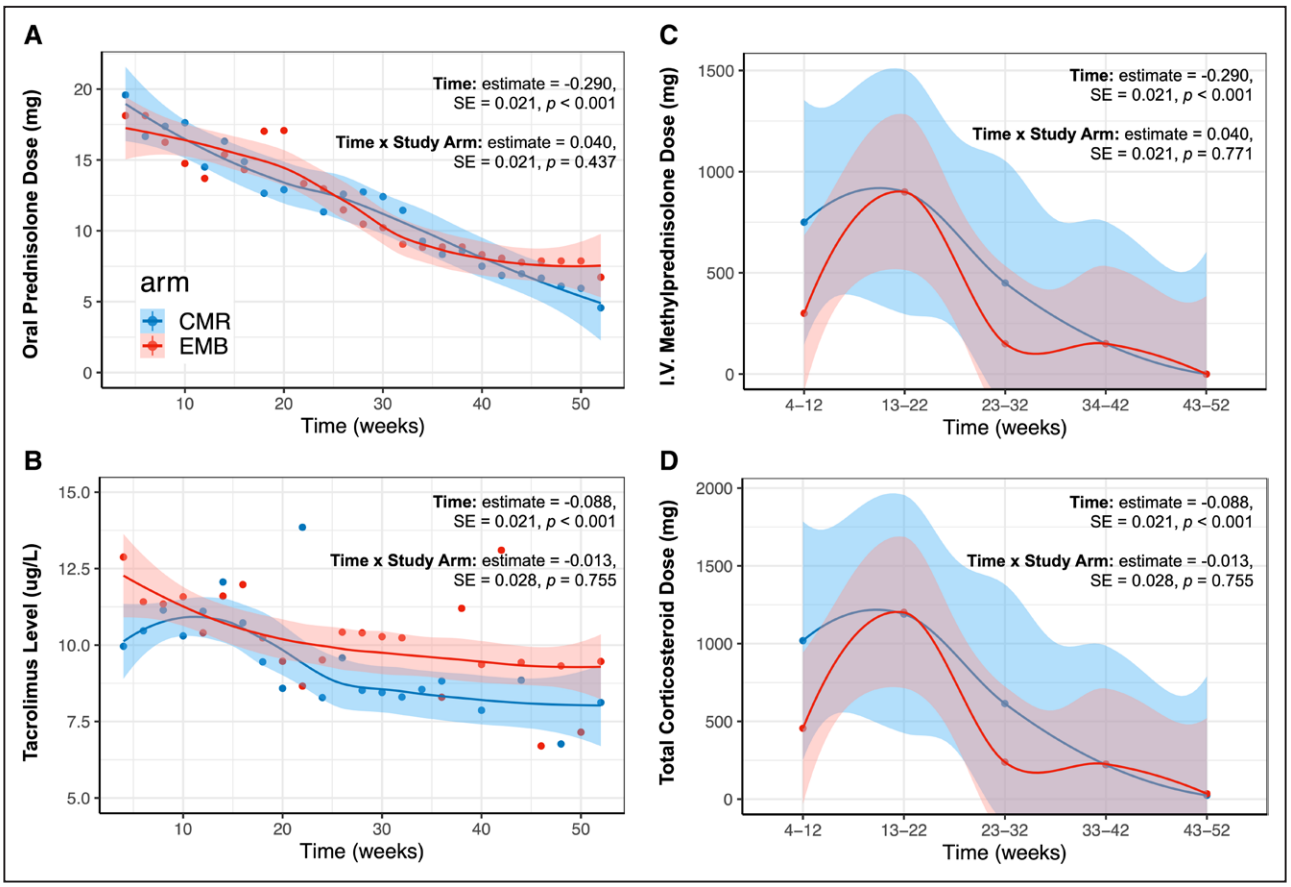

Figure 3. Corticosteroid dose and tacrolimus levels throughout duration of study.
Medical therapy given throughout the study duration. **A**, Prednisolone dose. **B**, Measured tacrolimus levels. **C**, Median intravenous methylprednisolone dose per 8-week period. **D**, Median total corticosteroid dose per 8-week period. A statistically significant time-dependent titrated reduction in prednisolone dose, resolution-of-rejection–dependent reduction in methylprednisolone and total corticosteroid dose, and titrated reduction in tacrolimus levels were observed, which were not significantly different between groups. Variance in loess spline line plots demonstrates the 95% CI. ●Shows actual temporal mean value. CMR indicates cardiac magnetic resonance; and EMB, endomyocardial biopsy.

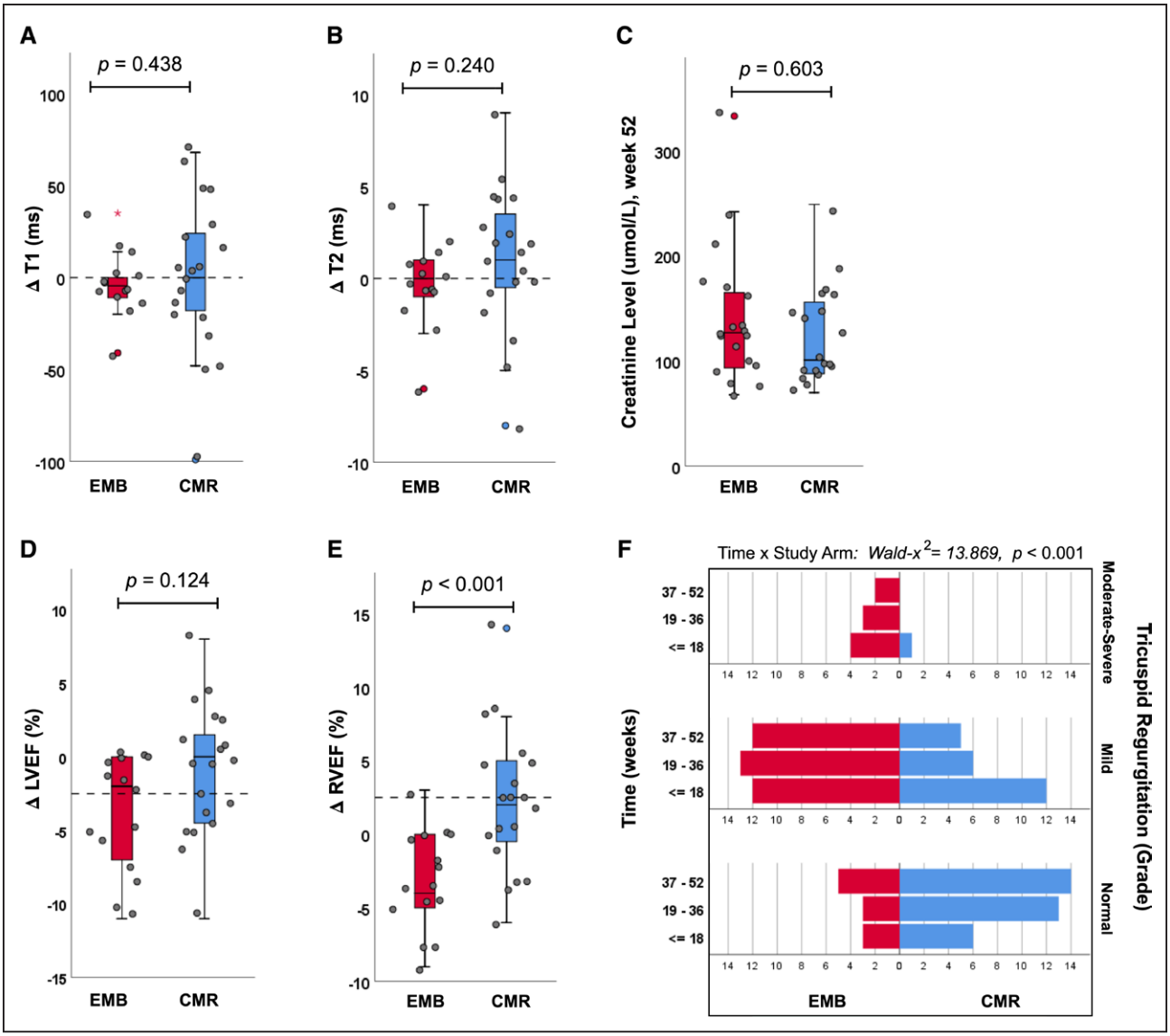

Figure 4. Change in structural and functional parameters throughout the study period.
A through **F**, Comparison of change (Δ) in cardiac magnetic resonance (CMR), kidney function, and tricuspid valve parameters across the study duration between study arms. **A**, Septal native T1 time. **B**, Septal native T2 time. **C**, Serum creatinine levels at week 52. **D**, Left ventricular ejection fraction (LVEF). **E**, Right ventricular ejection fraction (RVEF) demonstrating significant attenuation in the biopsy arm. **F**, Number of patients across each study period who exhibited tricuspid valve regurgitation on the basis of qualitative visual assessment of color Doppler jet area on transthoracic echocardiography. EMB indicates endomyocardial biopsy.

Right ventricular ejection fraction fell subtly but significantly in the EMB group compared with the CMR group over the study period (−3.06% [−4.9% to −1.2%] versus 1.35% [−1.3% to 4.0%]; $P<0.001$). In addition, there was a significant ($P<0.0.001$) trend toward increased tricuspid regurgitation in the EMB arm compared with the CMR arm (Figure 4F). By 18 weeks after transplantation, the prevalence of mild (EMB, n=12; CMR, n=12) and moderate to severe (EMB, n=4; CMR, n=1) tricuspid regurgitation was similar between groups. After 36 weeks after transplantation, the prevalence of mild tricuspid regurgitation was greatest in the EMB arm (EMB, n=12; CMR, n=5). However, there was no significant difference in the incidence of moderate to severe tricuspid regurgitation (EMB, n=2; CMR, n=0; odds ratio, 0.18 [95% CI, 0.001–2.41]; $P=0.290$).

DISCUSSION

In this pilot study of stable cardiac transplant recipients randomized 4 weeks after transplantation, CMR-based rejection surveillance compared with 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 4 weeks after transplantation, during an early high-risk period for allograft rejection. To the best of our knowledge, this is

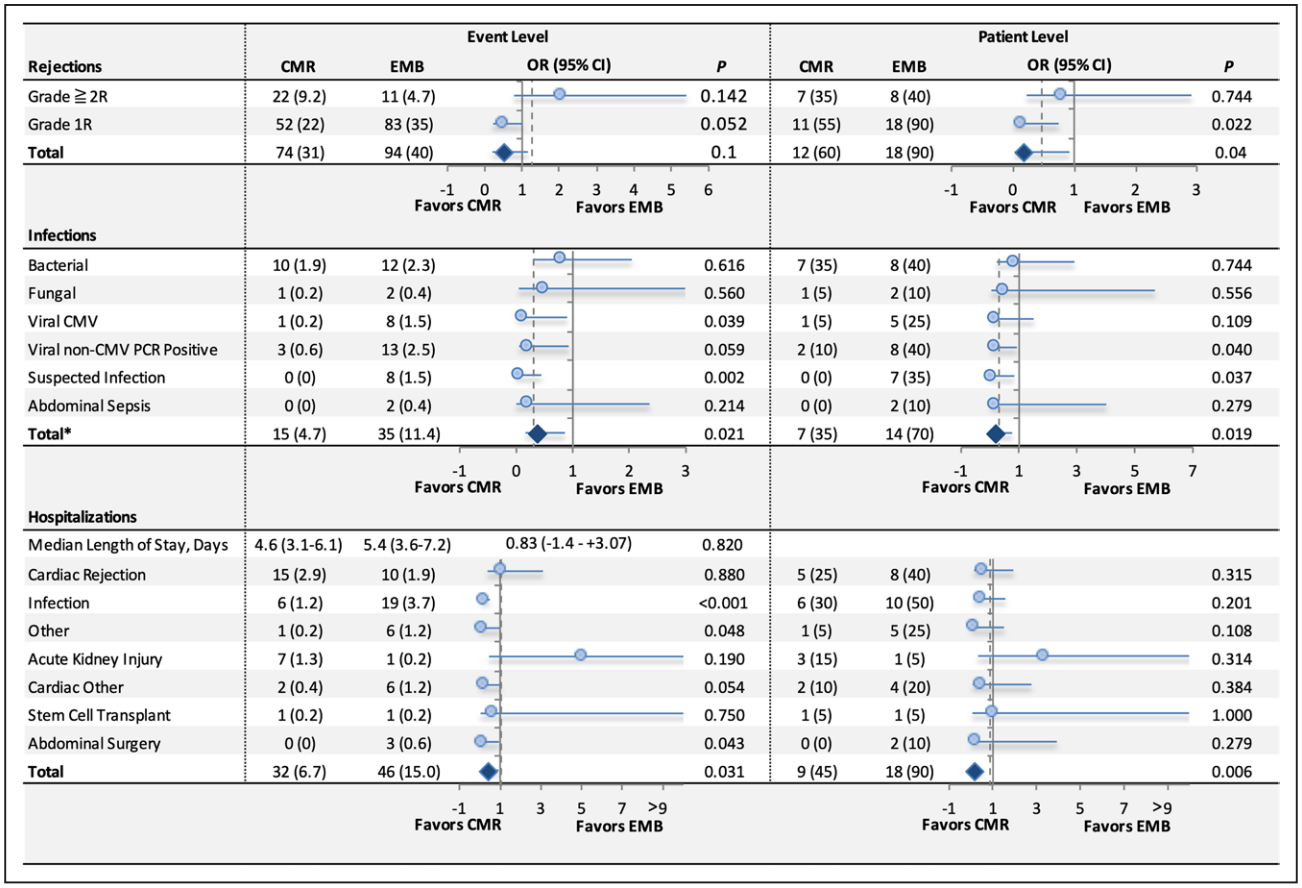

Figure 5. Clinical outcomes.

Event-level and patient-level comparison of clinical factors between groups. Suspected infection events were clinically diagnosed infection events that were culture or polymerase chain reaction (PCR) negative. Values reported as number of events or number of patients and proportions (percent). Line in forest plot shows the mean odds ratio (OR). Median length of stay reported as mean (95% CI) and effect size difference (95% CI). CMR indicates cardiac magnetic resonance; CMV, cytomegalovirus; and EMB, endomyocardial biopsy. *Total infections excluding suspected infections plus abdominal sepsis.

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 because 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 noninvasive surveillance strategy in some centers. This strategy was validated in the IMAGE trial (A Comparison of AlloMap Molecular Testing and Traditional Biopsy-Based Surveillance for Heart Transplant Rejection), which randomized low-risk patients >6 months (although mostly >1 year) after transplantation to GEP or EMB.[4] The subsequent EIMAGE trial (Early Invasive Monitoring Attenuation Through Gene Expression) randomized patients earlier but excluded patients with previous rejection or donor-specific antibodies.[34] 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 is likewise similarly reliable (AUC, 0.92) at detecting allograft rejection compared with multiparametric CMR imaging used in our study.[35] The biomarkers hs-TnT and NT-proBNP have been demonstrated to correlate to native T1 mapping in cardiac transplant recipients.[11] Our initial observations in this regard suggest that further exploration of the role of steady-state–corrected hs-TnT 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, hs-TNT, NT-proBNP, donor-derived cell-free DNA, and GEP in rejection surveillance.

Key findings of our study are that the number of patients who experienced grade 2R rejection events was similar between the CMR and EMB groups and that CMR was noninferior to EMB at diagnosing clinically relevant grade 2R rejection events. Although there was a trend for patients in the CMR arm with grade 2R rejection to have a higher grade 2R recurrence rate compared with 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 (Figures 4 and 5). This finding is not simply attributable to our CMR

rejection classification criteria; we observed an excellent (n=9 of 11) concordance rate between EMB and CMR grading of grade 2R events when physicians requested a confirmatory biopsy (Figure 2D). Notably, there was no difference between groups in the time to first grade 2R rejection. Furthermore, patients who never experienced grade 2R events in the CMR group showed no difference in immunosuppression exposure, hospitalization, or infection rates compared with the EMB arm.

Although the greater detection rate of grade 1R rejection in the CMR group may be attributable to the lower negative predictive value of CMR for that grade, the distribution of grade 1R rejection in that arm was also highly associated with grade 2R or greater rejections (Figure 2D). We demonstrated low interobserver variability for CMR surveillance, in contrast to moderate interobserver agreement for histological surveillance (Figure S2B1 and S2B2 and Table S3). 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, hs-TnT, and NT-proBNP are not ideal for monitoring AMR, the molecular examination of EMB samples with markers such as donor-derived cell-free DNA shows promise in improving the precision and accuracy of rejection diagnosis and classification.[36] As described later, 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.[37] Both the 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 grade 2R rejection in the CMR surveillance group, an immediate reduction in rejection grade severity was noted in 19 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 was 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 has 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 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.[17,38]

Our studies used a well-validated T1 mapping sequence[13,14] in combination with a T2 mapping sequence that we initially validated in healthy control subjects 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 and 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.[17,39] Our study demonstrates proof of concept of the feasibility of CMR for rejection surveillance with 2 specific sequences. Although cross-vendor, cross–field strength, multicenter studies are warranted, many transplantation 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 transplantation populations.[39,40]

In our study, we observed biopsy-related complications requiring hospitalization in the EMB arm and increased mild-tricuspid regurgitation. The risk of persistent, moderate to severe tricuspid regurgitation was numerically greater but not statistically significant at 52 weeks after transplantation in the EMB arm (EMB, n=2, CMR, n=0; P=0.290). Together, these findings suggest another potential benefit of a noninvasive rejection surveillance strategy that 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.[41] Moreover, EMB is often performed under general anesthesia in children, which adds to procedural risk and invasiveness.[42] Myocardial tissue characterization by CMR is feasible and informative in the pediatric setting.[43–45] The CMR surveillance protocol we used requires no intravenous cannulation or 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 centers would require infrastructure-specific validation against EMB. Although similar numbers of patients in each arm experienced grade 2R rejection, there appears to be a trend toward an excess of recurrent grade 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 grade 2R rejection rates between groups; however, CMR may potentially overcall grade 2R rejection events, and further investigation of this possibility is required. A reassuring finding was that immunosuppression exposure, rates of hospitalization, and infection rates in the smaller subset who experienced grade 2R rejection were also similar between arms (Table S4). Extracellular volume values derived from T1 mapping are similar at 1.5 and 3.0 T and thus may be preferred for comparisons across field strengths.[39] We chose not to perform extracellular volume 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 used noninvasive method for rejection surveillance. Future studies should attempt to determine whether CMR provides incremental data to GEP and donor-derived cell-free DNA. Furthermore, we did not routinely perform coronary angiography at 1 year; thus, the implications of CMR rejection surveillance on coronary artery vasculopathy cannot be determined from our data. As mentioned, our study was not designed to detect AMR, and we hypothesize that molecular analysis of EMB samples combined with CMR data will play a role in the diagnosis and management of AMR. Last, because the study concluded at 1 year after transplantation, implications for long-term allograft function and coronary artery vasculopathy cannot be made.

Conclusions

CMR-based rejection surveillance compared with EMB-based surveillance of stable cardiac transplant recipients randomized 4 weeks after transplantation was feasible in the first year after transplantation and reduced the number of invasive biopsy procedures by 93.7% during this period. A multicenter clinical trial is warranted to confirm the efficacy and safety of these findings.

ARTICLE INFORMATION

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Supplemental Material

Expanded Methods

Figures S1–S6

Tables S1–S4

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