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Recurrent acute cellular rejection graded ISHLT 1R early after heart transplantation negatively affects long-term outcomes: The prognostic significance of 1990 ISHLT grades 1B and 2

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ABSTRACT

Purpose: We investigated the implications of early recurrent 1R rejections for long-term outcomes after heart transplantation (HT) and evaluated the prognostic significance of 1990 ISHLT grading 1B/2 versus 1A.

Methods: Data on all patients who underwent HT between 1992 and 2017 were reviewed. Patients with ≥2 endomyocardial biopsies graded 1R in the first 3 months were classified as "recurrent 1R." Those patients were further categorized according to 1A vs. 1B/2. Outcomes (>3 months) were long-term rejections and the combined endpoint of cardiac allograft vasculopathy (CAV) and cardiovascular (CV) mortality.

Results: Sixty-nine out of 228 patients were classified as recurrent grade 1R. In the recurrent 1R group, 2R rejection rate was significantly higher (2.6 ± 0.6 vs 1.2 ± 0.4, $p = 0.03$), while survival free of rejections was lower (5-year: 57.1% vs. 72.3%, $p = 0.022$). Multivariate analysis showed that early recurrent 1R rejection was associated with a 30% increased risk for subsequent major rejection. Among 28 patients classified as 1B/2 of the recurrent group, rejection scores were higher, while survival free of rejections was lower, compared to 37 patients of the recurrent group classified as 1A (5-year: 57.1% vs. 72.7%, $p = 0.013$). Kaplan-Meier analysis showed that CAV/CV mortality at 10 years of follow-up was significantly higher among the recurrent 1R group (38% vs. 18% $p < 0.05$). Multivariate analysis showed that early recurrent 1R rejections were associated with a 2.5-fold increased risk for CAV/CV mortality.

Conclusion: Early recurrent grade 1R rejections negatively affect long-term outcomes. The adverse outcomes are experienced mainly by 1R patients subcategorized as1B/2 and not 1A.

1. Introduction

Heart transplantation (HT) is currently the gold standard treatment for end-stage heart failure. Since the first human-to-human HT in 1967 [1], survival has improved considerably, with major advances in the survival of HT patients being intimately connected with the recognition and understanding of the rejection process. Simultaneously, the emphasis on the standardization of the pathology-based diagnosis of rejection [2] has established the foundations for the development of anti-rejection therapies and protocols [3]. Although rejection rates continue to decline, the risk of rejection still remains significant, particularly in the early period following HT, necessitating routine surveillance. In this regard, endomyocardial biopsies (EMBs) remain the method of choice for the diagnosis of allograft rejection, despite the introduction of new non-invasive modalities over the past decade [4].

As long ago as 1990, the International Society for Heart and Lung Transplantation (ISHLT) recognized the need to develop a working formula for grading cardiac allograft biopsies to resolve inconsistencies in pathology-based diagnoses between transplant centers and to address advances in the knowledge of antibody-mediated rejection. This working formula was subsequently upgraded in 2004, with the main change being the grouping together of low-grade acute cellular

Abbreviations: ACR, acute cellular rejection; CAV, cardiac allograft vasculopathy; CV, cardiovascular; EMB, endomyocardial biopsy; HT, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation

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rejection (ACR) categories such that 1990 ISHLT grades 1A, 1B and 2 were combined into a new, revised 2004 ISHLT Grade1R (mild, low-grade ACR) [2]. Grade 1R may be manifested in one of two ways: infiltration of perivascular and/or interstitial mononuclear cells without distortion of the normal architecture or with a single focus of mononuclear cells with associated myocyte damage [2]. Early data implied that graft function and 3-year survival were worse for patients with 1990 ISHLT grades 1B or 2 ACR on >20% of biopsies than for patients with at least 80% of grade 0 or 1A biopsies or for patients with only 1–2 episodes of grade 3A or higher rejection [5,6]. These data are in keeping with the findings of gene expression profiling of the peripheral blood of cardiac allograft recipients, which showed that the AlloMap scores of patients with biopsy grade 1B ACR were higher than those for recipients with grades 0 and 1A. In fact, the score for grade 1B ACR was comparable to that for grades ≥3A/2R [7].

In light of the paucity of studies on the suitability of the grade 1R classification as a guide to prognosis and treatment, we aimed to elucidate the implications of early recurrent 1R rejections on the long-term outcomes after HT and to evaluate whether the 1990 ISHLT ACR grade 1A carries the same prognostic significance as 1990 ISHLT grades 1B/2.

2. MATHERIALS and methods

2.1. Study population and registry design

Between 1992 and 2017, 285 HT patients were enrolled in the HT Registry of the Sheba tertiary medical center. The prospective follow-up of this cohort reported here excluded 57 of these patients for whom there was no EMB data (15 patients) or who exhibited rejection graded ISHLT 2R or higher on EMB in the first 3 months after HT (42 patients); the latter group was excluded because previous studies have shown that recurrent moderate or severe rejection (grade ≥ 2R) predicts worse long-term outcomes [8,9]. Institutional review board approval was obtained for the study.

2.2. Definitions and endpoints

2.2.1. Surveillance and classification of rejections

Rejections were diagnosed by routine or clinically indicated EMBs. Biopsies obtained before the introduction of the revised ISHLT rejection grading system [2] were reclassified according to the revised system, and biopsies obtained after the institution of the revised ISHLT grading system were also classified according to the 1990 ISHLT grading system [10]. For the primary analysis, patients with ≥2 EMBs graded 1R in the first 3 months were assigned to a group designated “recurrent 1R” group (69 patients), and those with <2 EMBs graded 1R in the first 3 months comprised a group classified as “non-recurrent 1R” (159 patients). To refine the classification of the recurrent 1R group, subjects were further categorized into 2 groups on the basis of the 1990 ISHLT grading system: recipients who exhibited only grade 1A rejection and those who exhibited only grade 1B or 2 rejections. Patients with combination of these rejections (1A and 1B or 2) were excluded from this sub-analysis. Routine EMBs were performed every week for the first 4 weeks post HT, twice a month during the second and third months, once a month for the following 3 months, and thereafter every 3 months until the end of the first year. From the end of the first year until the end of the fifth year, biopsies were carried out annually.

For each patient, two different rejection scores were calculated, as follows. 1) Total rejection score (TRS), as a measure of the severity of the rejection, was calculated according to the following weighting: 0R = 0, 1R = 1, 2R = 2, and 3R = 3. 2). Any rejection score (ARS), which reflected the total number of rejections, regardless of their severity, was calculated on the basis of 0R = 0, 1R = 1, 2R =1, and 3R = 1. Each score for each particular patient was normalized by dividing it by the cumulative scores for the total number of biopsy specimens taken during the study period for that patient [11].

Major rejection was defined as an event that led to acute augmentation of immunosuppression in conjunction with an ISHLT ≥2R right ventricular EMB result or non-cellular rejection (biopsy-negative rejection) with hemodynamic compromise (decrease in LVEF by >25%).

2.2.2. Immunosuppression

All patients were treated with a triple-drug regimen, with maintenance immunosuppression comprising a combination of prednisone, an antimetabolite, and a calcineurin inhibitor. In appropriately selected patients, everolimus was given. All patients received anti-thymocyte globulin induction therapy. Cyclosporine was commenced to achieve target trough blood levels of 250–300 ng/mL for the first 4 weeks, 200–250 ng/mL from 4 weeks to 6 months, 150–200 ng/mL from 6 months to 1 year, and 100–150 ng/mL after 1 year. During the 6 months post-operative tacrolimus target trough blood levels were 10–15 ng/mL, and later 5–10 ng/mL. Symptomatic acute cellular rejections were treated by steroid pulse therapy. Additional cytolytic immunosuppressive therapy with anti-thymocyte antibodies was administered if hemodynamic compromise was present or when no clinical improvement within 12–24 h of pulse therapy administration was observed. Asymptomatic acute cellular rejection grade 2R or 3R was also treated initially by high dose corticosteroids.

2.2.3. Primary graft dysfunction

Primary graft dysfunction was restricted to 24 h after surgery and was based on echocardiographic and/or hemodynamic considerations, according to the ISHLT consensus conference document [12].

2.2.4. Cardiac allograft vasculopathy

Cardiac allograft vasculopathy (CAV) was diagnosed by coronary angiography and invasive hemodynamic assessment performed annually, along with clinical assessment and echocardiography, combined according to the recommended nomenclature for CAV of the ISHLT consensus statement [13].

2.3. Outcome measures

The primary end point was taken as long-term rejections (>3 months) as assessed by TRS and ARS and by the first occurrence of a major rejection. Secondary end points (>3 months) were: 1) the combined endpoint of CAV and cardiovascular (CV) mortality and 2) the first occurrence of a major adverse event. Major adverse events included the development acute coronary syndrome/percutaneous coronary intervention, congestive heart failure, implantable pacemaker, and stroke.

2.4. Statistical analysis

Descriptive statistics were produced using means and standard deviations for continuous variables (e.g., age) and frequencies for categorical variables (e.g., gender). To examine differences in continuous variables between groups, Mann-Whitney procedures were used to avoid bias for non-normal distributions. To examine differences between groups and categorical variables, Chi-square tests were performed.

The Kaplan–Meier estimator was used to assess the time to the first occurrence of each endpoint by the recurrence of ISHLT 1R according to the above definitions, and groups were compared using the log-rank test. Multivariable Cox proportional hazard regression analysis was used to evaluate the association between the recurrence of 1R and the first occurrence of endpoints during follow-up. Covariates included in the multivariate models were identified by using the best subset procedure for variables that were predictive of the endpoint and were unbalanced between the two groups (candidate covariates are listed in Tables 1 and 2(. Sub-analysis of the recurrent 1R group according to the 1990 ISHLT classification was done similarly. To reduce the effects of

Table 1
Baseline characteristics of the two groups of patients with grade 1R rejection at 3 months.

	EMB grade 1R at 3 months		*P* value
	Non-recurrent 1R (N = 159)	Recurrent 1R (N = 69)	
Gender-recipient [male, %]	83	80	0.47
Gender-donor [male, %]	72	71	0.80
Age-recipient (years)	48 ± 15	49 ± 12	0.39
Age-donor (years)	33 ± 13	33 ± 13	0.87
BMI donor/BMI recipient	1.02 ± 3	1.01 ± 4	0.92
Etiology of heart failure (IHD %)	54	61	0.33
Hypertension (%)	36	32	0.58
Diabetes (%)	18	20	0.66
Dyslipidemia (%)	45	48	0.75
Past smoker (%)	40	39	0.82
Urgency status			
Status 1	72	71	0.82
Status 2	27	29	
Creatinine (mg/dL)	1.3 ± 0.9	1.3 ± 0.6	0.94
Bilirubin (mg/dL)	1.3 ± 1.9	1.0 ± 0.5	0.34
Systolic pulmonary artery pressure (mmHg)	52 ± 19	48 ± 18	0.23
Diastolic pulmonary artery pressure (mmHg)	25 ± 11	24 ± 11	0.61
Mean pulmonary artery pressure (mmHg)	35 ± 14	33 ± 13	0.61
Pulmonary capillary wedge pressure (mmHg)	25 ± 11	23 ± 11	0.29
Cardiac output (L/min)	3.6 ± 1.1	3.3 ± 1.5	0.38
Pulmonary vascular resistance (Wood)	2.9 ± 1.7	3.0 ± 1.7	0.99
ICD (%)	45	41	0.65
LVAD bridge to HT	19	12	0.16
PRA >30%	0.7	0.0	0.37
CMV mismatch	58	66	0.42
Recipient blood type			0.58
A	40	52	
AB	11	7	
B	22	19	
O	27	21	
Era (>2000)	55	62	0.31

Continuous variables and categorical variables are presented as means ± standard deviation and percentage, respectively.
[a]LDL - as measured at 3 months following HT.
BMI, body mass index; IHD, ischemic heart disease; ICD, implantable cardioverter defibrillator; LVAD, left ventricular assist device; HT, heart transplantation; PRA, panel of reactive antibodies; CMV, cytomegalovirus; LDL, low density lipoprotein.

confounding in regressions, propensity score analysis was conducted for multivariate regressions. This method attempts to reduce the bias due to confounding variables that could be found in estimates of the effect obtained from simply comparing outcomes among the groups.

The inter-rater variability in this single-center study is estimated to be lower than reported in an earlier multi-center study for the following reasons [14]. First, in our center, determination of major rejection is accepted only after discussion in a multi-participant panel. Second, approximately 70% of the early biopsies for the two study groups (see Section 3.2) were classified as 0R, a grading that is known to be associated with low inter- and intra-rater variability. Third, number of raters in our center is limited (and hence there is less likelihood for disagreement).

Data were analyzed with SPSS software version 23. A two-sided 0.05 significance level was used for hypothesis testing.

Table 2
Operative and post-operative data for the two groups of patients with grade 1R rejection at 3 months.

	Recurrent 1R in 3 months		P value
	Non-recurrent 1R (N = 159)	Recurrent 1R (N = 69)	
Operative Data			
Ischemic time (min)	165 ± 50	157 ± 44	0.34
Primary graft dysfunction (%)	30	16	**<0.05**
Length of hospitalization (days)	68 ± 18	43 ± 61	0.15
Post-operative hospitalization length (days)	19 ± 11	17 ± 10	0.73
Early complications	54	52	0.88
Post-Operative Data			
Statin after HT	89.5	92.8	0.449
Baseline LDL after HT[a]	106 ± 34	117 ± 37	**<0.05**
Hypertension after HT	76	70	0.29
Diabetes mellitus after HT	36	42	0.37
CMV disease	21	15	0.33
Immunosuppression			0.60
Cyclosporine-based	58.9	52.1	
Tacrolimus-based	40.3	45.8	
Everolimus-based	0.8	2.1	
End stage renal failure	12	17	0.33
Average follow up time (years)	8.0 ± 6.1	9.5 ± 6.8	0.084

Continuous variables and categorical variables are presented as means ± standard deviation and percentage, respectively. HT - heart transplantation, LDL - low density lipoprotein, CMV - cytomegalovirus.
[a] LDL - as measured at 3 months following HT.

3. Results

3.1. Clinical characteristics

The baseline characteristics of the study population by recurrence of 1R rejection are shown in Table 1. The recurrent 1R group included 69 patients, and the non-recurrent 1R group comprised 159. Baseline characteristics, including donor age, ratio of male to female recipients, body mass index, etiology of heart failure and metabolic risk profile, were similar for the two patient groups. All the recipients were non-sensitized, with low baseline panel reactive antibodies, and for both groups the percentage of patients bridged with a left ventricular assist device was low. There were no significant differences between the two groups in CMV mismatch status or in hemodynamic parameters.

Operative and postoperative data are summarized in Table 2. Recipients with recurrent 1R had lower primary graft dysfunction compared with the recipients with non-recurrent 1R (16% vs. 30%, $p < 0.05$). The vast majority of patients in both groups received statin therapy, but mean low density lipoprotein levels at 3 months following HT were higher in recipients with recurrent 1R than in those free of recurrent 1R (117 ± 37 vs. 106 ± 34, $p < 0.05$). Immunosuppression protocols were similar for the two groups. We note that due to significant differences between the two groups, the variables, primary graft dysfunction and low-density lipoprotein levels, were statistically controlled in further analyses.

3.2. Outcomes

3.2.1. Rejections

The total mean number of EMBs per patient at 3 months was similar for the two groups (Table 3). The mean number of EMBs graded 1R per patient in the recurrent group was 3.4 ± 2.0. In the recurrent 1R group, 2R rejection rate was significantly higher compared with the non-recurrent group (2.6 ± 0.6 vs 1.2 ± 0.4, $p = 0.03$).

Patients in the recurrent 1R group had higher TRS (reflecting the severity of the rejection) than patients in the non-recurrent 1R group

Table 3
Characterization of follow-up biopsies by 1R grade rejection at 3 months.

Time after HT		Non-recurrent 1R	Recurrent 1R	P value
0–3 months	Total biopsies	4.9 ± 2.0	5.4 ± 1.7	0.13
	1R per patient	0.89 ± 0.72	3.4 ± 2.0	0.012
> 3 months	Total biopsies	7.9 ± 4.0	9.3 ± 4.6	0.023
	1R per patient	1.5 ± 2.0	3.8 ± 1.8	< 0.001
	≥ 2R per patient	1.2 ± 0.4	2.6 ± 0.6	0.03

Values in the table are means ± standard deviation.

(0.82 ± 0.18 vs. 0.43 ± 0.30, $p < 0.001$, Table 4). In addition, recipients in the recurrent 1R group had higher ARS (representing the total number of rejections, regardless of severity) than those in the non-recurrent group (0.63 ± 0.24 vs. 0.30 ± 0.21, p < 0.001, Table 4).

Kaplan–Meier survival curves showed that survival free of major rejection beyond 3 months was lower for patients of the recurrent group than for those in the non-recurrent group (5-year survival free of rejections: 57.1% vs. 72.3%, $p = 0.022$; Fig. 1). Multivariate analysis showed that early recurrent 1R rejections in the first 3 months were associated with a 30% increased risk for subsequent major rejection. These results were further validated by using a propensity score adjusted Cox model (Table 5).

Analysis of the recurrent 1R group subdivided according to the 1990 ISHLT classification.

The recurrent 1R group was re-classified according to the 1990 ISHLT classification [10] for the 1A, 1B, and 2 grades of rejection. Of the 69 patients, 37 patients exhibited only grade 1A rejection and 28 had either grade 1B or grade 2. Four patients with a combination of rejection categories (1A + 1B or 2) were excluded from this analysis. No significant differences in baseline characteristics or operative and post-operative data were found between these two subgroups (Tables 1S and 2S).

TRS and ARS were found to be higher for patients with 1B or 2 rejection grades in comparison with patients with grade 1A alone (TRS: 0.89 ± 0.11 vs. 0.72 ± 0.13, $p = 0.012$; ARS: 0.75 ± 0.18 vs. 0.52 ± 0.13 $p = 0.001$, Table 4). Kaplan–Meier survival curves showed that survival free of major rejection after 3 months was lower for patients with grade 1B or 2 rejection types than for those with grade 1A rejection type (5-year rejections free survival: 57.1% vs. 72.7%, $p = 0.013$; Fig. 1B).

3.2.2. CAV/CV mortality

Kaplan-Meier survival analysis showed that CAV/CV mortality at 10 years of follow-up was significantly higher for the recurrent 1R group (38% vs. 18% $p < 0.05$, Fig. 2A), as further validated by a propensity score (Fig. 2B). CAV severity was evenly distributed: CAV1–33%; CAV2–36%; CAV3–31%. Multivariate analysis showed that early recurrent 1R rejections were associated with a 2.5-fold increased risk for CAV/CV mortality. These results were further validated by using a propensity score adjusted Cox model (Table 5).

In the analysis of the recurrent 1R group subdivided according to the 1990 ISHLT classification, Kaplan–Meier survival curves showed no differences between patients with grade 1B/2 rejection compared with patients with rejection type 1A (24.1% vs. 32.9%, $p = 0.587$; Fig. 3A), as further validated by a propensity score (Fig. 3B).

3.2.3. Major adverse events

In the primary analysis, survival free from major adverse events did not differ between patients in the recurrent 1R and non-recurrent 1R groups (10-year major adverse events free survival: 57.8% vs. 73.2%, $p = 0.213$; Fig. 4A). Survival free of major adverse events in the 're-classified' 1R group was higher for patients in the 1A rejection group than for those in the 1B/2 rejection group (10-year major adverse events free survival: 88.1% vs. 45.2%, $p = 0.02$; Fig. 4B).

4. Discussion

We report here the results of a study conducted to assess the implications of early recurrent 1R rejections on the long-term outcomes after HT. We showed that recurrent 1R in the first 3 months after HT is associated with higher rejection rates, with a 30% increased risk for subsequent major rejection. The combined outcomes of CAV/CV mortality were significantly higher for the patients experiencing early recurrent 1R rejections, with a 2.5-fold increased risk for combined CAV/CV mortality. Our data thus suggest that recurrent grade 1R is a clinically important observation that may be associated with worse late outcomes. We have further shown that for the group of recurrent 1R patients, the subgroup making the most marked contribution to the increased rejection risk and adverse outcomes comprised patients showing grade 1B or 2 rejections, as opposed to grade 1A rejections.

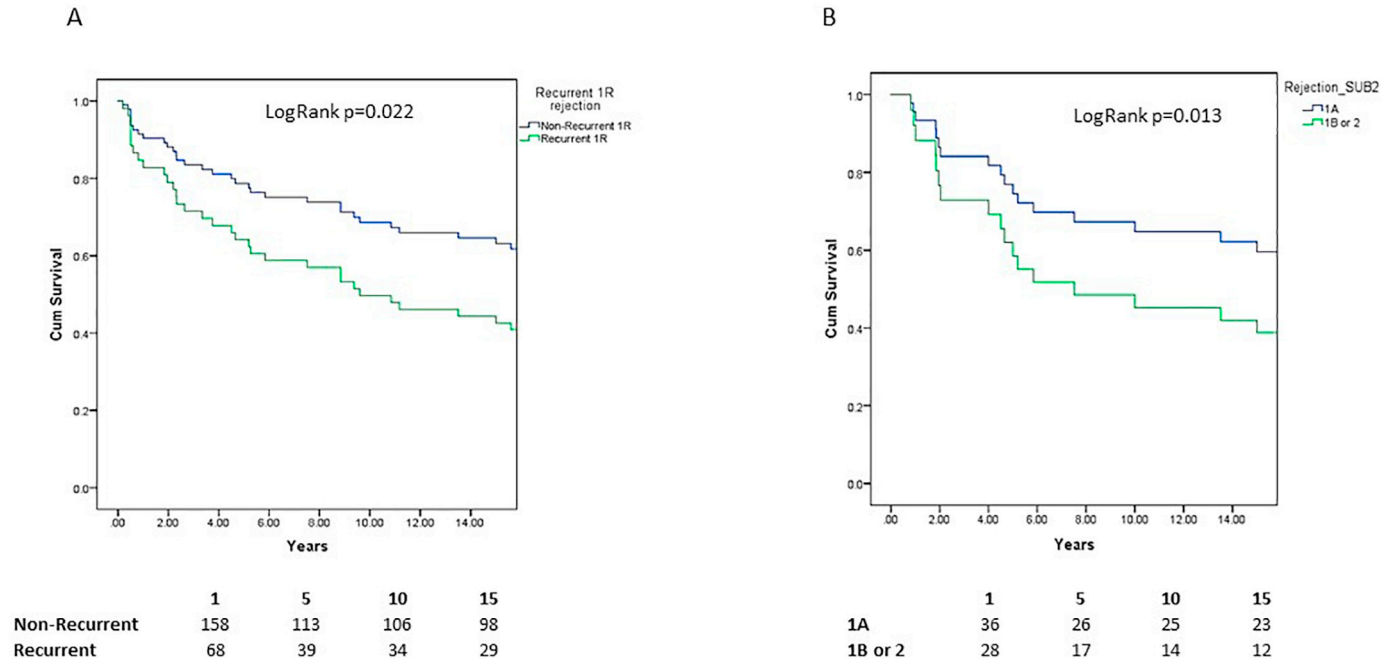
The hallmark of ACR is the presence of lymphocytes in the myocardium, with more severe rejection being associated with greater myocardial injury. The 2004 revised ISHLT classification for grading rejections is based on the same criteria of infiltration and myocyte damage as those used in the 1990 scoring system and reflects this continuum. However, one of the major differences between the two systems lies in the grouping the former low-grade rejection classes, 1A, 1B and 2, into the current grade 1R. According to the new grading system, only grades 2R and 3R require treatment, while the 'old' grade 2 biopsy specimens with focal infiltrates are now considered to indicate mild rejection for which treatment is not indicated [15].

It has traditionally been held that approximately 20% to 40% of patients experience moderate or severe ACR in the first year after transplantation [4,16]. However, a recent study (covering the years 2010–2014) has shown that histologic rejection has become less common, with only 24% of recipients experiencing any rejection between discharge from the hospital and 1 year after HT. [17] Similarly, there has been a decrease in the incidence of recipients experiencing treated rejection, as reported in the ISHLT registry: Between 2004 and 2006, 77% of recipients experiencing any rejection during the first year after discharge were treated, compared with only 52% between 2010 and 2014 [17]. However, since the ISHLT registry does not collect data on types of rejection, it is not clear whether these findings are secondary to the effect of the new classification described above or to the introduction of newer immunosuppressive regimens [18], among other factors. These considerations, combined with the asymptomatic nature of most histologic rejection episodes, exacerbate the complexity of the existing controversy regarding the need to augment immunosuppression based purely on histologic findings. The current study sheds some

Table 4
Rejection scores for primary and sub analysis.

	Primary analysis			Sub analysis		
	Non-recurrent 1R	Recurrent 1R	P value	1A grade	1B/2 grade	P value
Total Rejection Score	0.43 ± 0.30	0.82 ± 0.18	< 0.001	0.72 ± 0.13	0.89 ± 0.11	0.012
Any Rejection Score	0.30 ± 0.20	0.63 ± 0.24	< 0.001	0.52 ± 0.13	0.75 ± 0.18	0.001

Values in the table are mean rejection scores ± standard deviation.



	1	5	10	15
Non-Recurrent	158	113	106	98
Recurrent	68	39	34	29

	1	5	10	15
1A	36	26	25	23
1B or 2	28	17	14	12

Fig. 1. Kaplan-Meier estimates for survival free from major rejection* by recurrence of 1R (A) and for the recurrent 1R group categorized according to 1990 ISHLT classification (B) * > 3 months.

light on this controversy by demonstrating that the poorer long-term outcomes associated with recurrent histological 1R rejections in the first 3 months after HT are associated largely with recipients experiencing grades 1B/2 rejections.

While treatment is strongly recommended (class I indication (for symptomatic ACR irrespective of the ISHLT EMB grade and for asymptomatic grade 2R and 3R rejections, the therapeutic approach for asymptomatic grade 1R has not been well defined. The current recommendations indicate that asymptomatic mild cellular rejection (ISHLT 1R) does not require treatment in the vast majority of cases (class IIa indication). However, the controversy as to whether grade 2 (moderate) of the 1990 ISHLT classification represents a true entity is still to be resolved. Some transplant groups elect to treat grade 2 rejection, while other groups feel that for most, if not all, biopsy samples the pathology indicating a classification of grade 2 rejection may be attributed to Quilty lesions [4,19,20]. Furthermore, there are considerable clinical data indicating that grade 2 rejection does not progress, even without any change in the immunosuppressive regimen [21]. Thus, the therapeutic approach and clinical correlation for the new grading 1R has remained undefined to date.

The baseline patient characteristics in our series were similar in the recurrent and non-recurrent 1R groups, yet primary graft dysfunction was almost twice as frequent in the non-recurrent group. As recurrent 1R was associated with higher rates of CAV/CV mortality, our findings are in line with an earlier study showing that primary graft dysfunction does not lead to increased CAV in surviving patients [22]. This conclusion is supported by the multivariate modeling performed in this study demonstrating that primary graft dysfunction is not associated with an increased risk for CAV/CV mortality.

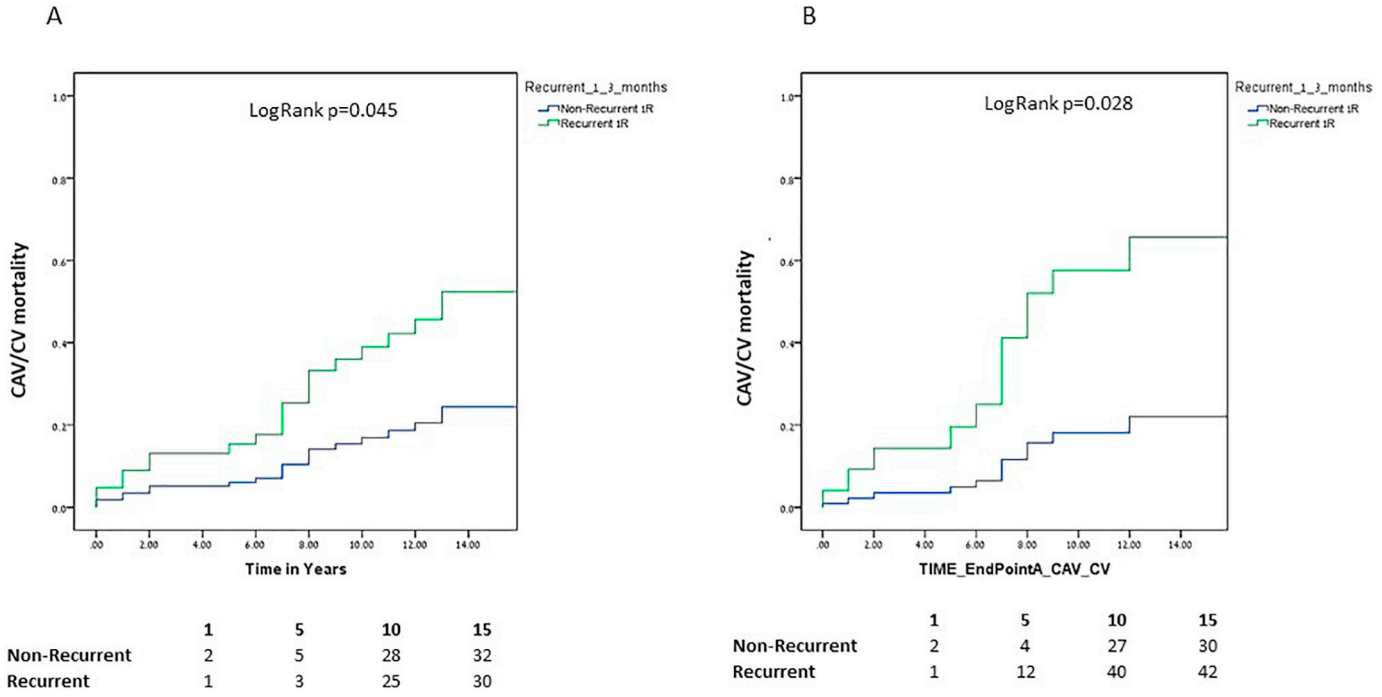
In the current study, we have shown that patients experiencing recurrent 1R rejections in the first 3 months after HT are at increased risk for subsequent major rejections. These findings support two earlier studies: The first – a retrospective analysis of a pediatric cohort – found that recurrent grade 1B ACR between 6 weeks and 1 year after HT is independently associated with decreased freedom from late ACR [5]. The second study, which pertained to adult HT recipients, showed that patients with grades 1B or 2 ACR in > 20% of biopsies had worse graft function and lower 3-year survival than patients with at least 80% grade 0 or 1A biopsies [6]. In keeping with these findings, we have also shown that grades 1B/2 rejection, as opposed to grade 1A rejection, is associated with worse outcomes.

We excluded from our current study patients with grade 2R rejection in the first 3 months after HT, since we were interested in the late effects of recurrent mild 1R rejections in the absence of an adverse

Table 5
Predicting major rejection and combined end point of CAV/CV mortality by recurrence of 1R: multivariate Cox proportional hazard model and propensity score analysis.

	Major rejection				CAV/CV mortality			
	Multivariate (Cox)		Propensity		Multivariate (Cox)		Propensity	
	HR	P	HR	P	HR	P	HR	P
Recurrent 1R	1.3 [1.2,1.8]	0.022	1.4 [1.01,1.80]	0.023	2.5 [1.6,3.3]	0.045	4.3 [2.7,6.0]	0.028
Primary graft dysfunction	0.9 [0.4,2.5]	0.431			1.3 [0.4,2,1]	0.636		
LDL after HT	1.01 [1.009,1.02]	0.038			1.01 [1.009,1.02]	0.038		

CAV, Cardiac allograft vasculopathy; CV, Cardiovascular; HR, hazard ratio; LDL, Low-density lipoprotein; HT, Heart transplantation.



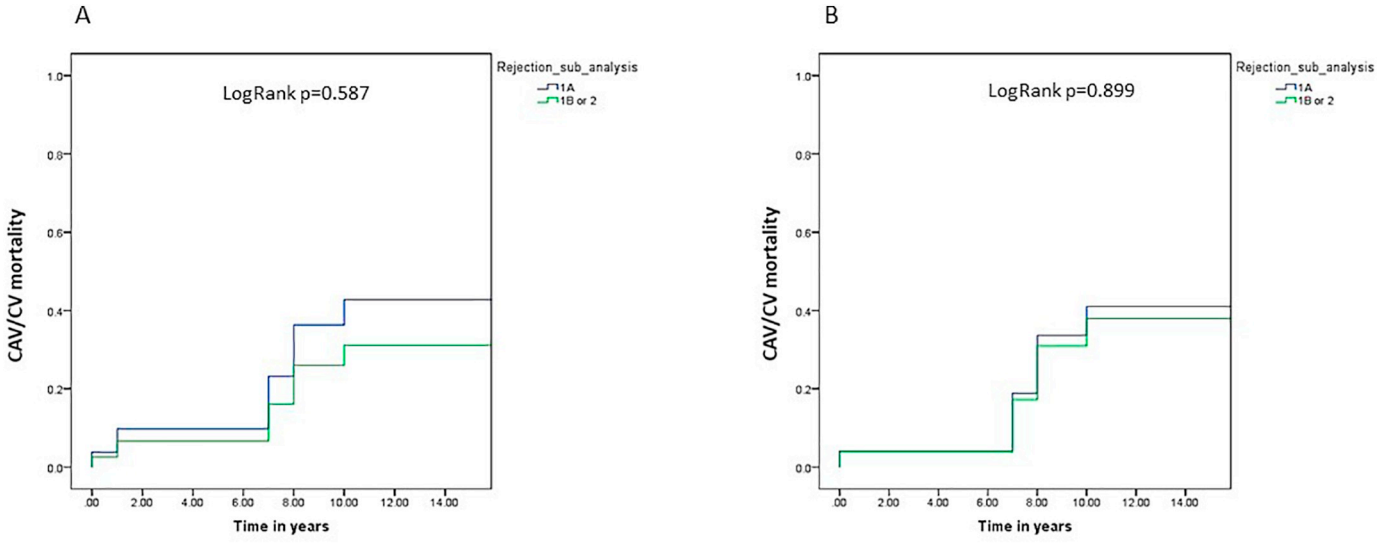
	1	5	10	15
Non-Recurrent	2	5	28	32
Recurrent	1	3	25	30

	1	5	10	15
Non-Recurrent	2	4	27	30
Recurrent	1	12	40	42

Fig. 2. Kaplan–Meier estimates of the combined end point of CAV/CV morality* by recurrence of 1R before (A) and after (B) propensity score.* > 3 months.

moderate rejection profile. We have shown that patients suffering from recurrent 1R have a 2.5-fold increased risk of CAV/CV mortality. It has been previously shown that acute rejection leads to CAV, one of the most significant obstacles to long-term survival, making surveillance and prompt treatment of acute rejection episodes even more crucial [23–29]. A previous study aimed to determine association of acute rejections and CAV and to differentiate between the effects of mild versus severe rejection episodes showed that moderate/severe acute rejection, but not mild rejection, has an independent cumulative effect on the onset of CAV. That study was based on the 1990 ISHLT classification, but we should be aware that in that classification the definition of 'mild rejection' included grades 1A, 1B and 2 untreated, while 'moderate/severe' rejection included both grade 2 treated on a clinical basis and grades 3A,3B and 4 [30]. As the new revised classification groups the 1990 ISHLT grade 2 rejection into the mild 1R category, both the above study and our current study strengthen the premise that recurrent 1R might negatively affect outcomes. The impact of recurrent 1R on CAV might be even more profound, since we have also shown that recurrent 1R rejections are associated with an elevated risk for both mild and moderate rejections during follow-up.

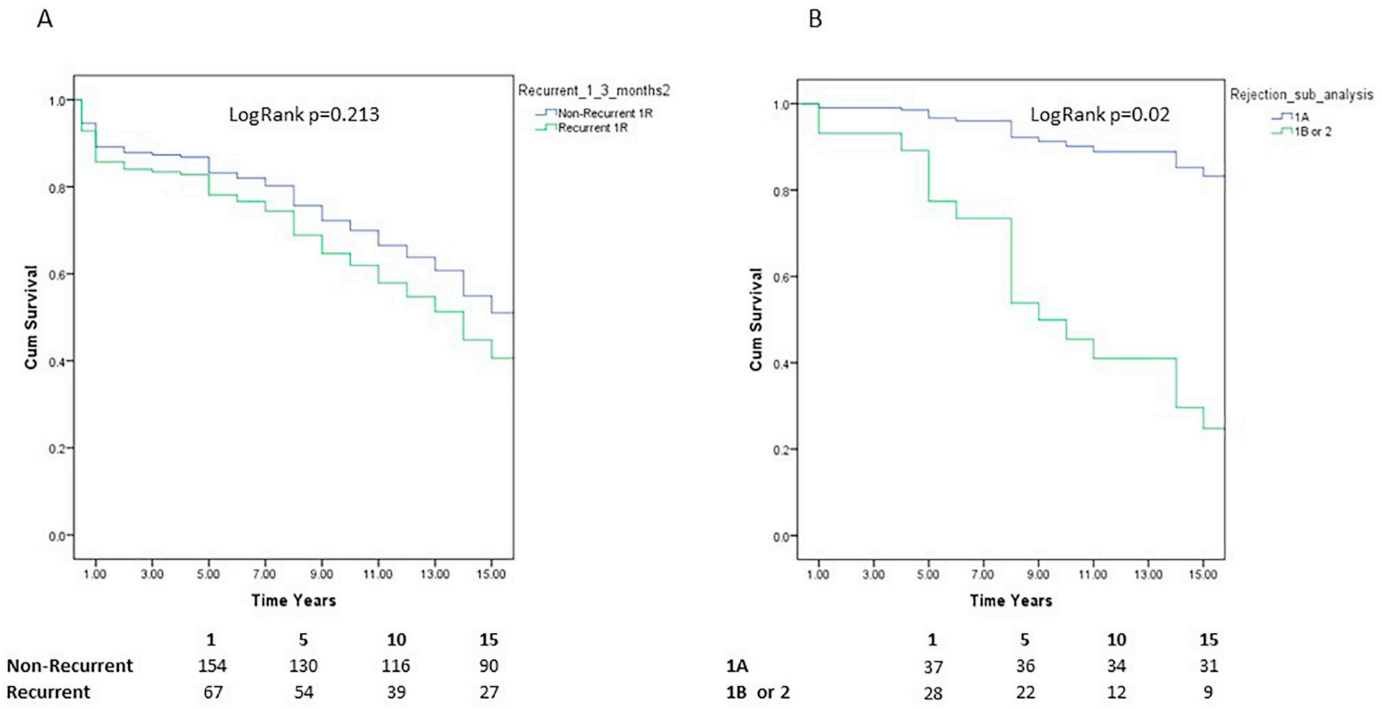
The findings of our study have important clinical implications: 1) Considering re-evaluation of immunomodulatory approaches for



	1	5	10	15
1A	36	26	25	23
1B or 2	28	17	14	12

	1	5	10	15
1A	1	4	12	13
1B or 2	0	2	6	6

Fig. 3. Kaplan–Meier estimates of the combined end point of CAV/CV morality* for the recurrent 1R group subcategorized according to the 1990 ISHLT classification before (A) and after (B) propensity score. * > 3 months.



	1	5	10	15
Non-Recurrent	154	130	116	90
Recurrent	67	54	39	27

	1	5	10	15
1A	37	36	34	31
1B or 2	28	22	12	9

Fig. 4. Kaplan–Meier estimates for survival free from major adverse events*: primary analysis (A) and sub analysis (B). * > 3 months.

patients experiencing 1R rejections, which are yet left to be determined. 2) We have shown that recurrent 1R rejections, and particularly those conforming to the 1990 ISHLT 1B/2 rejections categories, adversely affect outcomes. In light of these findings, together with an earlier study suggesting that the recurrent grade 1B ACR does not carry the same prognostic significance as lesser degrees of rejections [18], we suggest that any future grading system contain a separate category for a diffuse infiltrate with or without myocyte damage rather than focal perivascular infiltrates. 3) Patient risk stratification according to recurrence/non-recurrence of early mild rejection will help to facilitate the introduction of new rejection surveillance protocols. Such protocols should be designed to facilitate earlier, extensive, informed, and routine use of a combination of the new noninvasive modalities that have been introduced in recent years for low-risk patients, as to date there is no single technique that can replace the EMB.

5. Study limitations

There are some limitations inherent in our study that bear mention. First, like other studies in which rejection is a variable criterion, a universal limitation is inter-rater disagreement on EMBs grades, which cannot be completely eliminated, even though it has been estimated to be low. Nonetheless, the results of our study are further strengthened by the trend of a non-local expert panel to assign lower grades than local centres, which has been previously demonstrated [14]. Second, the investigation reported here was a retrospective observational single-center study, and not all possible confounders were recorded or adjusted for. Third, another limitation was the inability to collect all the variables that could have contributed to the mechanisms underlying our findings. Finally, the sample size of the sub-analysis was small, and thus any conclusions drawn from the data must be replicated with a larger sample and a prospective multicenter design.

6. Conclusions and clinical implications

Recurrent EMBs grade 1R occurring early in the post transplantation course is likely to be associated with more frequent and severe rejections and to negatively impact outcomes. Therefore, for patients falling into the grade 1R classification there is a necessity for closer follow-up and possibly revision of treatment parameters. We note that it is mainly the rejections that fit the 1990 ISHLT 1B/2 grading – and not those belonging to the 1990 ISHLT 1A grading – that are associated with the adverse outcomes of the revised grade 1R. It has recently been suggested that donor-derived cell free DNA correlates more closely with Intragraft mRNA transcripts (mRNAt) than pathology read biopsies [31]. A combination of these new and promising techniques will help in identifying those patients who apparently suffer a mild degree of rejection, leading to optimization of treatment and eventually to improved outcomes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trim.2019.03.003.

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