ORIGINAL ARTICLE

Mild acute cellular rejection and development of cardiac allograft vasculopathy assessed by intravascular ultrasound and coronary angiography in heart transplant recipients—a SCHEDULE trial substudy

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SUMMARY

To evaluate the association between mild acute cellular rejection (ACR) and the development of cardiac allograft vasculopathy (CAV) after heart transplantation (HTx). Substudy of the SCHEDULE trial (n = 115), where de novo HTx recipients were randomized to (i) everolimus with early CNI elimination *or* (ii) CNI-based immunosuppression. Seventy-six patients (66%) were included based on matched intravascular ultrasound (IVUS) examinations at baseline and year 3 post-HTx. Biopsy-proven ACR within year 1 post-HTx was recorded and graded (1R, 2R, 3R). Development of CAV was assessed by IVUS and coronary angiography at year 3 post-HTx. Median age was 53 years (45–61), and 71% were male. ACR was recorded in 67%, and patients were grouped by rejection profile: no ACR (33%), only 1R (42%), and ≥2R (25%). Median ΔMIT (maximal intimal thickness)$_{BL\text{-}3Y}$ was not significantly different between groups (P = 0.84). The incidence of CAV was 49% by IVUS and 26% by coronary angiography with no significant differences between groups. No correlation was found between number of 1R and $\Delta MIT_{BL\text{-}3Y}$ (r = −0.025, P = 0.83). The number of 1R was not a significant predictor of $\Delta MIT_{BL\text{-}3Y}$ (P = 0.58), and no significant interaction with treatment was found (P = 0.98). The burden of mild ACR was not associated with CAV development.

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Key words
acute cellular rejection, cardiac allograft vasculopathy, coronary angiography, heart transplantation, intravascular ultrasound, mild rejection

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Introduction

Heart transplantation (HTx) is the best available therapy for eligible patients with end-stage heart failure. The major limitations to survival in the early post-transplant period are nonspecific graft failure, acute rejection, and infection. Beyond the first year cardiac allograft vasculopathy (CAV) and malignancy are the leading causes of mortality [1].

Cardiac allograft vasculopathy is characterized by diffuse, concentric, and longitudinal intimal thickening and luminal narrowing in the arteries of the allograft. It may also present as small vessel or microvascular disease [2,3]. The overall prevalence of CAV in survivors at 1, 5, and 10 years after HTx is 8%, 30%, and 50%, respectively [4].

The most common approach for disease diagnosis is yearly coronary angiography [5]. However, this method lacks sufficient sensitivity for detection of CAV and intravascular ultrasound (IVUS) is considered the gold-standard investigation for diagnosing CAV [6].

The mechanisms of CAV development are not fully elucidated, but the etiology appears to be multifactorial with both immunological and nonimmunological contributions [6]. Reflecting the immunological contribution, the number of episodes of moderate to severe acute cellular rejection (ACR), and the total number of cellular rejections has been reported to be associated with the development of CAV [7,8]. The mechanism is believed to relate to an increased inflammatory burden on the allograft predisposing to CAV development.

The association between mild ACR and CAV development is poorly understood. Given that mild rejection is far more frequent than moderate–severe rejection post-HTx, and less likely treated, further insight on this potential association is of clinical relevance and can lead to improved survival after HTx. Hence, the objective of the present study was to evaluate the association between mild rejection and the development of CAV in HTx recipients surviving three years.

Patients and methods

Study design and population

This is a substudy of the SCHEDULE trial (SCandinavian HEart transplant everolimus De novo stUdy with earLy calcineurin inhibitor avoidancE) which was a prospective, open-label, multicenter, randomized controlled study undertaken at five HTx centers in Scandinavia. Adult *de novo* HTx recipients ($n = 115$) were randomized in a 1:1 ratio to either (i) low-dose everolimus (EVR), low-dose cyclosporine (CsA), mycophenolate mofetil (MMF), and corticosteroids (CS) with withdrawal of CsA and step up to full dose EVR after 7–11 weeks *or* (ii) conventional treatment with CsA, MMF, and CS (Fig. 1). The purpose was to evaluate whether early initiation of EVR with early elimination of CsA compared with conventional CNI-based immunosuppression could improve long-term renal function and attenuate the progression of CAV. Detailed descriptions of the SCHEDULE trial have been published previously [9,10].

Of the 115 patients randomized in the SCHEDULE trial, 76 patients (66%) were included in the present study based on available matched IVUS examinations at baseline (7–11 weeks post-HTx) and 3-year follow-up post-HTx (Fig. 2). Comprehensive evaluations of the 1-year and 3-year IVUS population in the SCHEDULE trial have been reported previously [11,12].

The study was conducted in compliance with good clinical practice and in accordance with the Declaration of Helsinki and the Declaration of Istanbul 2008. The appropriate ethics committee for each participating center approved the study, and written informed consent was obtained from all study participants prior to inclusion. The SCHEDULE trial was registered with ClinicalTrials.gov (NCT01266148).

Hypothesis and endpoints

We hypothesized that the burden of mild rejection within the first year after HTx influences the development of CAV at 3-year follow-up after HTx assessed by IVUS and coronary angiography.

The maximal intimal thickness (MIT) is an established prognostic marker in HTx recipients [13,14], and MIT ≥0.5 mm has previously been used as a reliable marker of significant CAV. The primary endpoint of this study was change in MIT between matched segments from BL to 3-year follow-up ($\Delta MIT_{BL\text{-}3Y}$). Secondary endpoints were (i) mean MIT at 3-year follow-up, (ii) incidence of CAV (defined as mean MIT ≥0.5 mm upon IVUS examination) at 3-year follow-up, and (iii) incidence of CAV determined by coronary angiography at 3-year follow-up after HTx.

Allograft rejection

Biopsy-proven acute cellular rejections within the first year after HTx were systematically recorded and graded

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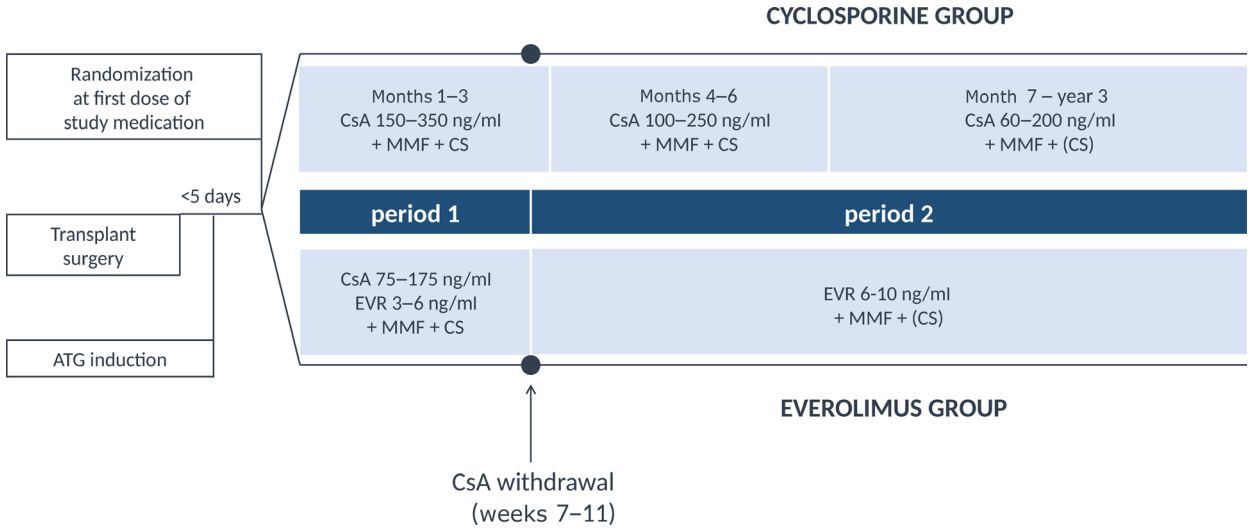

Figure 1 Study design. The study design of the SCHEDULE trial (SCandinavian HEart transplant everolimus De novo stUdy with earLy calcineurin inhibitor avoidancE). ATG, antithymocyte globulin; CS, corticosteroids; CsA, cyclosporine; EVR, everolimus; MMF, mycophenolate mofetil. Modified from Andreassen *et al*. [9].

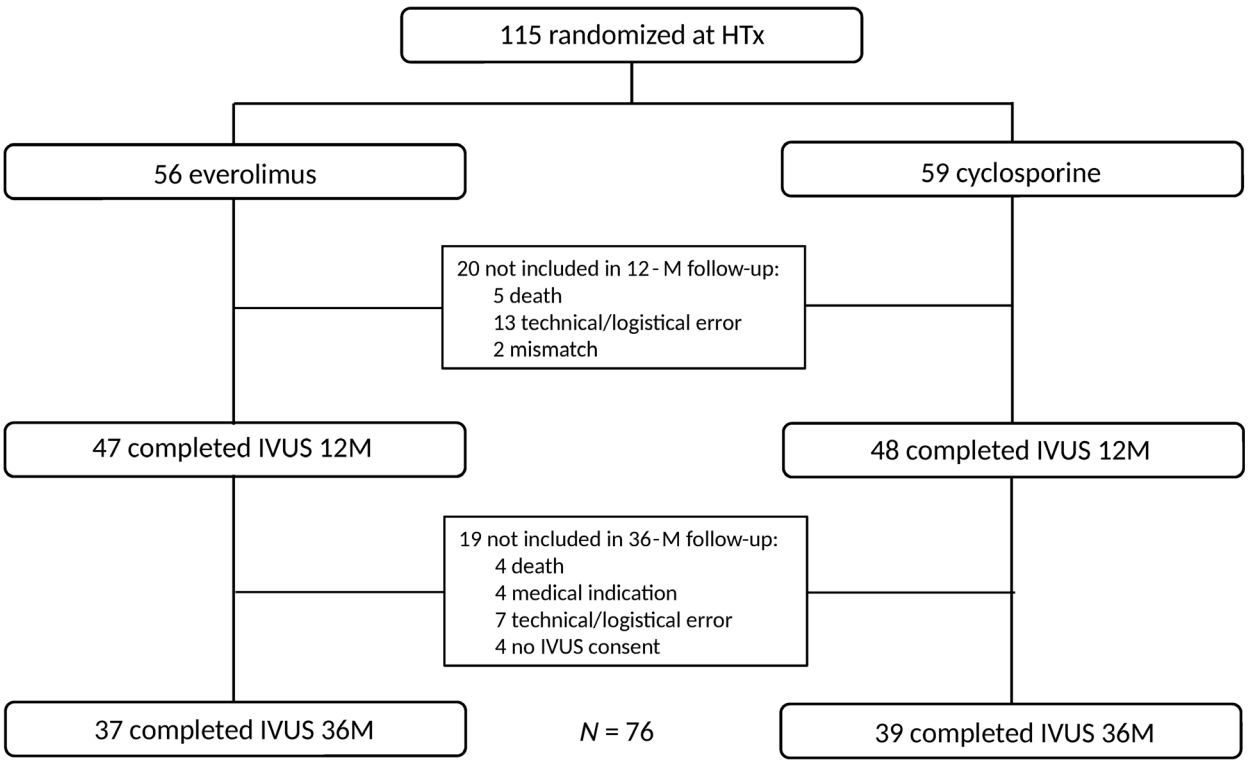

Figure 2 Patient diagram. Substudy patient diagram. Seventy-six patients had matched IVUS examinations at baseline and 3-year follow-up post-HTx. HTx, heart transplantation; IVUS, intravascular ultrasound; M, month. Modified from Arora *et al*. [12].

according to the 2004 ISHLT criteria [15] into mild rejection (1R), moderate rejection (2R), and severe rejection (3R). Patients were grouped by rejection profile into three groups: (i) no ACR within year 1 post-HTx, (ii) only 1R rejection within year 1 post-HTx, and (iii) at least one 2R *or* 3R rejection within year 1 post-HTx.

According to trial protocol, every suspected rejection episode prompted an endomyocardial biopsy within

48 h regardless of anti-rejection therapy or within 24 h of initiation of anti-rejection therapy. Scheduled biopsies were performed at weeks 1, 2, 4, and 8, and months 3, 6, and 12. Additional biopsies were allowed according to local practice and when clinically indicated. Biopsies were read and interpreted by local pathologists at each center, and anti-rejection therapy followed local practice. A rejection episode occurring more than 10 days after the beginning of the preceding one was considered as a new rejection episode. Episodes of antibody-mediated rejection (AMR) and rejection with hemodynamic compromise were also recorded. Exclusion criteria for entering period 2 of the SCHEDULE trial (>weeks 7–11) included ongoing treatment for rejection, biopsy-proven ISHLT grade 3R or ≥2 episodes of ISHLT grade 2R during period 1, and AMR with hemodynamic compromise during period 1.

Intravascular ultrasound

Intravascular ultrasound was performed at BL (weeks 7–11), at 1-year follow-up and at 3-year follow-up after HTx. Examinations were conducted after routine coronary angiography following intracoronary administration of 200 μg nitroglycerin. The same major coronary epicardial artery (preferably the left anterior descending artery, LAD) was imaged using a 20-MHz, 2.9F, monorail electronic Eagle Eye Gold IVUS imaging catheter (Volcano Corporation Inc, Rancho Cordova, CA, USA). The IVUS catheter was placed as distal as possible, and from this point to the ostium, automated mechanical pullback was performed. Images were acquired at a rate of 30 frames/s and a pullback speed of 0.5 mm/s generating 1-mm intervals between every 60 frames. Images were stored for off-line 3D volumetric analysis performed after trial closure and blinded to treatment by a core laboratory (Oslo University Hospital, Rikshospitalet, Oslo, Norway).

Precise matching of recordings was performed, and semi-automated contour detection of the lumen as well as the external elastic membrane (EEM) was conducted at intervals of ~1 mm using dedicated software (QIVUS v.3.0, Medis Medical Imaging Systems, Leiden, the Netherlands). Borders were manually edited according to the guidelines for acquisition and analysis of IVUS images by the American College of Cardiology and European Society of Cardiology [16]. In each patient, the longest possible matching segment between the most distal and proximal side branch visualized in the IVUS pullback was analyzed.

The following parameters were recorded in all patients using the mean result of all analyzed frames: (i) lumen cross-sectional area (CSA), (ii) EEM CSA, and (iii) maximal intimal thickness (MIT). Using CSA measurements, the total atheroma volume (TAV) (by Simpson's method) and percent atheroma volume (PAV) (by equation: $PAV = (\sum (EEM_{area} - Lumen_{area})/\sum EEM_{area}) \times 100$ were calculated. Incidence of CAV was defined as mean MIT ≥0.5 mm over the entire matched segment.

Coronary angiography

Coronary angiography was evaluated by experienced local staff blinded to study treatment. The presence and severity of CAV was graded according to ISHLT criteria [17] into ISHLT CAV0, ISHLT CAV1, ISHLT CAV2, or ISHLT CAV3. Only angiography performed at year 3 post-HTx was evaluated in this substudy.

Immunosuppression and prophylactic treatment

Induction therapy with antithymocyte globulin (ATG) was administered within 12 h of HTx in all included patients and continued for up to 5 days.

In the EVR group, EVR was initiated no later than the fifth postoperative day at a dose of 0.75 mg twice daily. Target trough level of EVR prior to weeks 7–11 was 3–6 ng/ml, and following CNI withdrawal, it was stepped up to 6–10 ng/ml. Target trough level of CsA in the EVR group was 75–175 ng/ml until weeks 7–11. CsA discontinuation took place at week 7, but in case of ongoing rejection, it was allowed to be postponed up to week 11. The target dose of MMF was 1500–2000 mg/day until weeks 7–11 and 1000 mg/day (minimum 750 mg/day) at the time of CNI withdrawal and onwards.

In the CNI group, the target CsA trough level was 150–350 ng/ml during months 1–3, 100–250 ng/ml during months 4–6, and 60–200 ng/ml onwards. The target MMF dose was 2000–3000 mg/day.

All patients received CS at a minimum dose of 0.1 mg/kg during months 1–3 and of 0.05–0.1 mg/kg during months 4–12. Beyond the first year CS could be discontinued at the discretion of the investigator.

Prophylactic treatment for CMV infection was initiated in CMV negative recipients with CMV-positive donors and consisted of valganciclovir for at least 3 months. All patients received lipid-lowering therapy with statin.

Statistics

This was a post hoc sub-analysis of the SCHEDULE trial. Analyses were performed using SAS 9.4 (SAS

Institute Inc., Cary, NC, USA) and GRAPHPAD PRISM 5.01 statistical software (GraphPad Software, Inc., La Jolla, CA, USA). Data were expressed as median (interquartile range) or as count (percentage) as appropriate. Non-parametric statistics were applied. Groups were compared by suitable tests (Kruskal–Wallis test, chi-square test, or Fisher's exact tests). Spearman's correlation analysis and multivariable linear regression analysis were performed to evaluate the association between number of mild rejections and ΔMIT from BL to 3-year follow-up. Variables for the linear regression model were preselected as known or expected risk factors for CAV development. A two-tailed *P*-value of 0.05 was considered statistically significant. All analyses were carried out for the intention-to-treat population ($n = 76$).

Results

The substudy population did not differ significantly from the remaining SCHEDULE population with regard to baseline parameters. Of the 76 patients included, 37 (49%) were randomized to the EVR arm and 39 (51%) to the CNI arm. In terms of rejection, 25 (33%) patients were in the no-ACR group, 32 (42%) patients were in the 1R group, and 19 (25%) patients were in the 2R/3R group. Treatment allocation among rejection groups was as follows: no-ACR group contained 8 (32%) EVR patients vs. 17 (68%) CNI patients, 1R group contained 15 (47%) EVR patients vs. 17 (53%) CNI patients, and 2R/3R group contained 14 (74%) EVR patients vs. 5 (26%) CNI patients ($P = 0.02$).

Baseline characteristics

The baseline characteristics are presented in Table 1. Median recipient age was 53 years (45–61), and 71% were male. Pretransplant hypertension and diabetes were found in 12% and 18%, respectively. One quarter of the patients was supported by an LVAD prior to HTx, and the most common indication for HTx was idiopathic dilated cardiomyopathy (54%). Rejection groups did not differ significantly with regard to baseline parameters, except for systolic blood pressure ($P = 0.01$). The mGFR among rejection groups was not significantly different ($P = 0.08$).

Immunosuppression

Discontinuation of CsA was performed at weeks 7–11 as per protocol in all patients in the EVR group. In the EVR group, a low-dose CNI had been reintroduced in combination with EVR in 6 (16%) patients at 3-year follow-up. In 6 (16%) patients, EVR was discontinued because of adverse events. In 5 (13%) CNI patients, CsA was replaced by EVR because of deteriorating renal function. Thus, 17 (22%) patients in this substudy deviated from the original protocol but were not excluded as analyses were intention-to-treat.

Allograft rejection

The burden of rejection is summarized in Tables 2 and 3. Sixty-seven percent of patients experienced any kind of rejection within the first year post-HTx. Overall, in the current study population ($n = 76$) 1R was recorded in 48 (63%) patients, 2R in 18 (24%) patients, and 3R in 2 (3%) patients. In the 2R/3R group ($n = 19$), 16 (84%) patients also experienced 1R. The number of mild rejections per patient ranged from 0 to 18 episodes with a median of 1 (0–3). Where anti-rejection treatment was indicated, it consisted of corticosteroid in all cases. No cases of humoral rejection or rejection with hemodynamic compromise were observed within the first year post-HTx.

Intravascular ultrasound

The mean length of the analyzed segments at BL, 1-year, and 3-year follow-up was 36.7 ± 7.3, 36.4 ± 8.1, and 36.8 ± 10.5 mm, respectively. Figure 3 depicts the median MIT and median change in MIT at different time points by rejection group, and Table 4 presents IVUS parameters among groups in more detail. After 3 years, MIT had increased by 0.11 (0.04–0.2) mm in the no-ACR group, by 0.09 (0.05–0.14) mm in the 1R group, and by 0.11 (0.05–0.14) mm in the 2R/3R group. Between-group differences were not statistically significant ($P = 0.84$). The MIT at 3 years was numerically higher in the no-ACR group compared with the 1R group and the 2R/3R group but was not significantly different among groups ($P = 0.27$). The incidence of CAV determined by IVUS (mean MIT ≥0.5 mm) at 3-year follow-up was 49% with group distribution as follows: 56% in the no-ACR group, 50% in the 1R group, and 37% in the 2R/3R group ($P = 0.43$).

Figure 4 displays the correlation between number of mild rejections within year 1 post-HTx and $\Delta MIT_{BL\text{-}3Y}$. No correlation was found ($r = -0.025$, $P = 0.83$), and in an adjusted linear regression model, the number of mild rejections within year 1 did not predict $\Delta MIT_{BL\text{-}3Y}$ ($B = 0.003$ [95% CI −0.008 to 0.015], $P = 0.58$). The following variables were included in the model: number

Table 1. Study population baseline characteristics (n = 76).

	All (n = 76)	No ACR (n = 25)	1R (n = 32)	≥2R (n = 19)	P-value
Recipient characteristics					
Recipient age (years)	53 (45–61)	55 (45–59)	53 (47–62)	51 (38–59)	0.41
Female gender (%)	22 (29%)	6 (24%)	10 (31%)	6 (32%)	0.80
BMI (kg/m^2)	24 (22–26)	24 (22–26)	25 (22–27)	23 (21–25)	0.34
Systolic blood pressure (mmHg)	137 (124–150)	129 (118–141)	135 (125–145)	147 (130–159)	0.01
Diastolic blood pressure (mmHg)	80 (76–90)	80 (76–83)	81 (70–92)	83 (80–90)	0.12
Medical history					
Hypertension (%)	9 (12%)	3 (12%)	4 (13%)	2 (11%)	1.00
Diabetes mellitus (%)	14 (18%)	6 (24%)	5 (16%)	3 (16%)	0.75
LVAD (%)	19 (25%)	6 (24%)	8 (25%)	5 (26%)	0.99
History of smoking (%)	40 (53%)	14 (56%)	17 (53%)	9 (47%)	0.85
HTx primary indication					
Idiopathic dilated cardiomyopathy	41 (54%)	12 (48%)	19 (59%)	10 (53%)	0.49
Coronary artery disease	22 (29%)	10 (40%)	6 (19%)	6 (31%)	
Other*	13 (17%)	3 (12%)	7 (22%)	3 (16%)	
Donor characteristics					
Donor age (years)	44 (34–53)	47 (38–53)	44 (35–52)	43 (34–55)	0.99
Donor female gender (%)	28 (37%)	9 (36%)	11 (34%)	8 (42%)	0.85
Cold ischemia time (min)	205 (132–238)	223 (83–247)	184 (138–214)	206 (155–240)	0.32
Renal function†					
mGFR (ml/min/1.73 m^2)	60 (51–74)	67 (59–75)	57 (47–72)	61 (55–78)	0.08
Serum creatinine (mmol/l)	98 (77–120)	93 (76–119)	107 (83–127)	93 (73–100)	0.16
Lipid profile†					
Total cholesterol (mmol/l)	3.8 (3.2–4.7)	3.8 (2.9–4.8)	4.0 (3.4–4.7)	3.6 (3.0–5.1)	0.66
HDL (mmol/l)	0.9 (0.8–1.1)	1.0 (0.8–1.3)	1.0 (0.8–1.1)	0.9 (0.8–1.0)	0.85
LDL (mmol/l)	2.3 (1.9–3.1)	2.2 (1.7–2.9)	2.3 (2.0–3.1)	2.2 (1.9–2.8)	0.55
Triglycerides (mmol/l)	1.3 (0.8–1.9)	1.0 (0.8–1.5)	1.6 (1.0–1.9)	1.4 (0.9–1.9)	0.18

1R, mild rejection; 2R, moderate rejection; 3R, severe rejection; ACR, acute cellular rejection; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVAD, left ventricular assist device; mGFR, measured glomerular filtration rate.

Data are reported as median (IQR) or absolute number (percentage) as appropriate. Groups are compared with qui-square, Fisher's exact, or Kruskal–Wallis test as appropriate.

*Other: HCM, noncompaction, postpartum, myocarditis, congenital, RCM, not specified.

†At time of transplantation.

Table 2. Distribution of biopsy-proven acute cellular rejection within 1 year of HTx (n = 76).

	All (n = 76%)	No ACR (n = 25%)	1R (n = 32%)	≥2R (n = 19%)
Acute cellular rejection episodes* within year 1 post-HTx				
No of patients with no rejection (%)	25 (33)	25 (100)	–	–
No of patients with 1R (%)	48 (63)	–	32 (100)	16 (84)
No of patients with 2R (%)	18 (24)	–	–	18 (95)
No of patients with 3R (%)	2 (3)	–	–	2 (11)
No of patients with any rejection (%)	51 (67)	–	32 (100)	19 (100)

1R, mild rejection; 2R, moderate rejection; 3R, severe rejection; ACR, acute cellular rejection.

Data are reported as absolute number (percentage).

*According to the 2004 ISHLT criteria.

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Table 3. Number of biopsy-proven acute cellular rejections pr. patient within 1 year of HTx (n = 76).

	All (n = 76) Median (IQR)	All (n = 76) Min–Max	All (n = 76) Mean ± SD
Acute cellular rejection episodes* within year 1 post-HTx			
Number of 1R pr. patient	1 (0–3)	0–18	2.17 ± 2.97
Number of 2R pr. patient	0 (0–0)	0–4	0.36 ± 0.76
Number of 3R pr. patient	0 (0–0)	0–1	0.07 ± 0.16
Number of any rejection pr. patient	1 (0–4)	0–18	2.55 ± 3.34

Data are reported as median (IQR), min-max, mean ± SD. Median (IQR) is the most appropriate descriptive measure as the numbers of rejections (1R, 2R, 3R) are not normally distributed.

*According to the 2004 ISHLT criteria.

of 1R, rejection group, recipient age, recipient gender, recipient diabetes, donor age, and treatment arm (EVR versus CNI). We found no significant interaction with treatment arm (P = 0.98). Figure 5 depicts $\Delta MIT_{BL\text{-}3Y}$ by rejection group stratified by treatment arm. We found no significant difference in $\Delta MIT_{BL\text{-}3Y}$ between EVR-treated and CNI-treated patients in neither of the rejection groups (P = 0.50, P = 0.86, P = 0.31, respectively). Tables S1–S7 present CAV-related parameters in further detail.

Coronary angiography

Table 5 summarizes CAV evaluation by coronary angiography at 3-year follow-up. The incidence of any degree of CAV was 26%, and we found that 16% had ISHLT CAV1, 9% had ISHLT CAV2, and 1% had ISHLT CAV3. The proportion of patients with any degree of CAV was numerically higher in the no-ACR group (40%) compared with the 1R group (24%) and the 2R/3R group (7%), but this did not reach statistical significance (P = 0.06). ISHLT CAV1 was found in all three rejection groups, whereas ISHLT CAV2 was only found in the no-ACR group. The only case of ISHLT CAV3 was found in the 1R group.

Discussion

This study found no association between the burden of mild rejection within the first year post-HTx and the development of CAV assessed by IVUS and coronary angiography at 3 years post-HTx. Rejection group was not associated with CAV, and number of mild rejections did not predict $\Delta MIT_{BL\text{-}3Y}$. No interaction with treatment allocation to EVR or CNI was found.

Cardiac allograft vasculopathy is believed to develop secondary to a complex interplay of both immunological and nonimmunological factors, which all contribute to the vascular inflammation and endothelial dysfunction ultimately leading to disease [6,18,19]. The

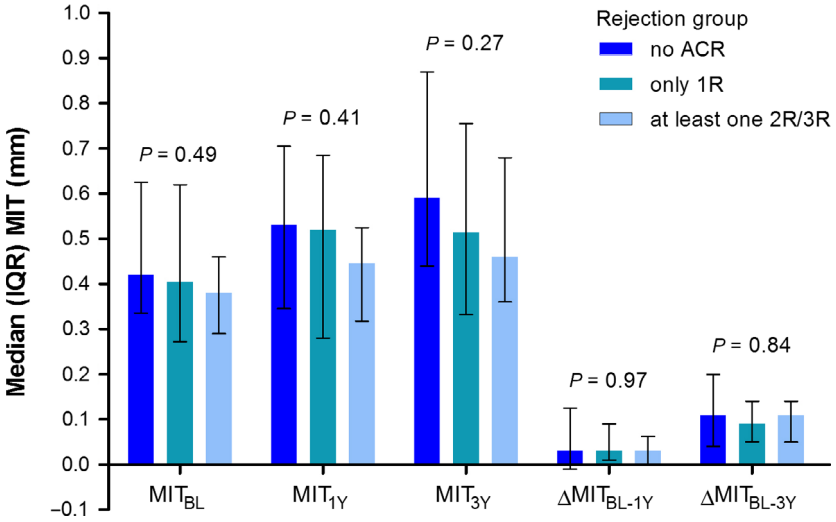

Figure 3 Maximal intimal thickness by rejection group. 1R, mild rejection; 1Y, one year post-HTx; 2R, moderate rejection; 3R, severe rejection; 3Y, three years post-HTx; ACR, acute cellular rejection; BL, baseline; IQR, interquartile range.

Table 4. Cardiac allograft vasculopathy assessed by intravascular ultrasound at 3 years post-HTx (n = 76).

	All (n = 76)	No ACR (n = 25)	1R (n = 32)	≥2R (n = 19)	P-value
CAV assessed by IVUS					
MIT (mm) at 3Y	0.49 (0.38–0.72)	0.59 (0.45–0.86)	0.52 (0.34–0.74)	0.46 (0.36–0.68)	0.27
TAV (mm^3) at 3Y	140 (91–202)	143 (101–201)	132 (82–206)	117 (90–199)	0.80
PAV (%) at 3Y	24 (18–34)	26 (20–36)	24 (18–34)	22 (19–30)	0.64
ΔMIT BL-3Y (mm)	0.09 (0.05–0.15)	0.11 (0.04–0.2)	0.09 (0.05–0.14)	0.11 (0.05–0.14)	0.84
ΔTAV BL-3Y (mm^3)	15 (−1 to 45)	14 (−3 to 44)	13 (−4 to 36)	26 (8–79)	0.35
ΔPAV BL-3Y (%)	6 (4–8)	6 (3–8)	5 (4–7)	6 (3–10)	0.57
MIT ≥0.5 mm at 3Y (incidence CAV)	37 (49%)	14 (56%)	16 (50%)	7 (37%)	0.43
ΔMIT BL-3Y ≥0.5 mm	2 (3%)	1 (4%)	1 (3%)	0 (0%)	1.0

1R, mild rejection; 2R, moderate rejection; ACR, acute cellular rejection; BL, baseline; CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound; MIT, maximal intimal thickness; PAV, percent atheroma value; TAV, total atheroma value; Y, year.

Data are reported as median (IQR) or absolute number (percentage) as appropriate. Groups are compared with qui-square, Fishers exact, or Kruskal–Wallis test as appropriate.

immunological basis of CAV pathogenesis is well established [20] and implicates an interaction between the adaptive and innate immune system, which creates a chronic vascular inflammatory state [18,19]. Activation of T cells and release of pro-inflammatory cytokines cause recruitment of inflammatory cells and upregulation of adhesion molecules by endothelial cells. This pro-inflammatory and pro-fibrotic cascade ultimately leads to smooth muscle cell migration, proliferation, and deposition of extracellular matrix in the intima [21], and CAV lesions are presumably the ultimate result of this cascade of events.

Acute cellular rejection is an inflammatory response predominantly mediated by T-lymphocyte infiltration [22], and emerging evidence supports an association between ACR and CAV [7,8,23,24]. As a manifestation of the immunological contribution to CAV pathogenesis, ACR is believed to progressively increase the inflammatory burden on the allograft and thereby possibly predispose to CAV development [7]. The isolated effect of mild rejection, however, remains largely unknown.

Acute cellular rejection is most frequent within the first 3–6 months after HTx [22]. Mild rejection is generally far

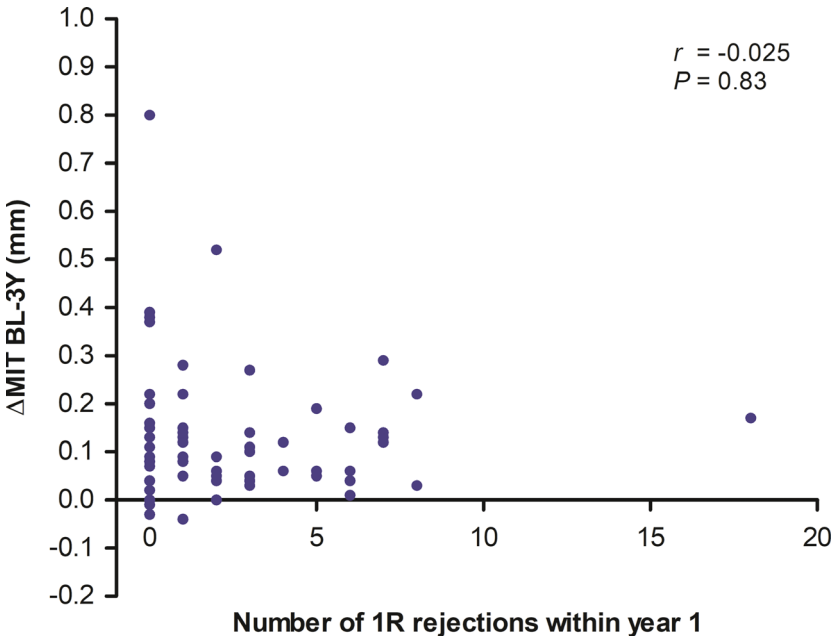

Figure 4 Correlation between number of mild rejections within 1 year post-HTx and the change in maximal intimal thickness from baseline to 3-year follow-up post-HTx (ΔMIT BL-3Y). 1R, mild rejection; 1Y, one year post-HTx; 3Y, three years post-HTx; BL, baseline; MIT, maximal intimal thickness. r = −0.025, P = 0.83.

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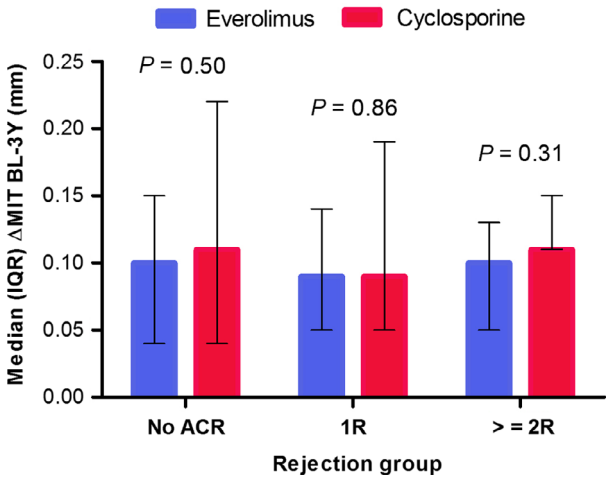

Figure 5 Change in maximal intimal thickness from baseline to 3-year follow-up post-HTx (ΔMIT BL-3Y) by rejection group and stratified by treatment arm (EVR versus CNI). 1R, mild rejection; 1Y, one year post-HTx; 2R, moderate rejection; 3Y, three years post-HTx; ACR, acute cellular rejection; BL, baseline; IQR, interquartile range; MIT, maximal intimal thickness.

more frequent than higher grades of rejection and is less likely treated, as low-grade rejection is believed to resolve spontaneously. In contrast, higher grades of rejection are treated, which presumably results in a suppression of the inflammatory process associated with rejection. The question remains whether more episodes of (untreated) mild rejection have an effect comparable to that of fewer episodes of moderate–severe rejection in terms of increased inflammatory burden and CAV liability.

Stoica *et al.* [7] found that moderate and severe rejection had an independent cumulative effect on the onset of CAV, whereas mild untreated rejection was not associated with CAV. On the contrary, Raichlin *et al.* [8] found that any cellular rejection episode—90% of them low-grade—contributed to CAV development and proposed that moderate and severe rejection had an even more severe cumulative impact. Both studies were predominantly based on angiography and did not apply ISHLT CAV criteria.

Our findings do not align with previous experience as we were not able to demonstrate an association between ACR and CAV. The 1R group did not have more CAV than the no-ACR group determined by IVUS and coronary angiography, and CAV-related IVUS parameters were not more severely affected. Surprisingly, nor did the 2R/3R group suggesting that patients with ≥2R rejection did not experience a higher CAV burden than patients in the 1R group and no-ACR group. In fact, both with regard to IVUS and angiographic evaluation we saw an overall trend toward

Table 5. Cardiac allograft vasculopathy assessed by coronary angiography at 3 years post-HTx (n = 69).

	All (n = 69, %)	No ACR (n = 25, %)	1R (n = 29, %)	≥2R (n = 15, %)	P-value
CAV assessed by coronary angiography*					
No of patients with any degree of CAV (%)	18 (26)	10 (40)	7 (24)	1 (7)	0.06
ISHLT CAV1 (%)	11 (16)	4 (16)	6 (21)	1 (7)	0.02
ISHLT CAV2 (%)	6 (9)	6 (24)	0 (0)	0 (0)	
ISHLT CAV3 (%)	1 (1)	0 (0)	1 (3)	0 (0)	
No of patients with no CAV (%)	51 (74)	15 (60)	22 (76)	14 (93)	

1R, mild rejection; 2R, moderate rejection; ACR, acute cellular rejection; CAV, cardiac allograft vasculopathy; CAV1, mild; CAV2, moderate; CAV3, severe; ISHLT, international society for heart and lung transplantation.
Data are reported as absolute number (percentage). Groups are compared with qui-square or Fishers exact test as appropriate.
*According to the 2010 ISHLT criteria. NB seven patients are missing coronary angiography at 3Y (n = 69).

more CAV in the no-ACR group compared with the 1R group and the 2R/3R group. Prior studies have also examined PAV as a measure of CAV in HTx [25–28]. We did not find excess rejection to result in excess CAV in terms of PAV measures.

The observed tendency toward more CAV in the no-ACR group deserves commentary. Patients in the no-ACR group were numerically older and had older donors and longer cold ischemic time compared with patients in the 1R group and the 2R/3R group. Also, the proportion of patients with diabetes, history of smoking, and coronary artery disease as primary indication for HTx were numerically higher. As these are all among possible risk factors for the development of CAV, this unequal distribution, although not statistically significant, might have been a driver of our findings. Furthermore, the fact that younger recipients generally tend to have more and severe rejections but less CAV might place patients with low a priori risk of CAV in the 2R/3R group. Similarly, the fact that older recipients tend to have less degree of rejection, but more CAV-related risk factors and CAV might place patients with higher a priori risk of CAV in the no-ACR group. This might also in part explain our findings.

Treatment allocation to either EVR or CNI is another possible confounder. Everolimus is a proliferation signal inhibitor (PSI) that inhibits the mammalian target of rapamycin (mTOR) signaling pathway and offers effective immunosuppressive activity combined with antiproliferative properties [29,30]. This is achieved by blocking interleukin (IL)-2- and IL-15-driven proliferation of T and B cells as well as vascular smooth muscle cells, by inhibiting the activation of p70 S6 kinase, and thereby arrest the cell cycle in the G1 phase [31–33]. Combining immunosuppressive and antiproliferative effects, EVR has gained much attention as an alternative to CNI-based immunosuppression in terms of ameliorating the burden of CAV post-HTx. Previous studies have shown that EVR initiation combined with standard or reduced CNI therapy can attenuate intimal thickening after HTx [34,35]. The SCHEDULE trial demonstrated that EVR initiation and early CNI elimination significantly reduced CAV progression at 1 year and that this beneficial effect was sustained at 3-year follow-up post-HTx [11,12]. CNI agents do not seem to possess similar ameliorating effects on CAV development [36]. One could therefore expect EVR patients to demonstrate less CAV than CNI patients in this substudy.

Treatment distribution among rejection groups was unequal as approximately 1/3 of patients in the no-ACR group received treatment with EVR and 2/3 of patients in the 2R/3R group received treatment with EVR. The 1R group had the most equal treatment distribution. This might in part be explained by the fact that EVR patients were more prone to experience ACR compared with CNI patients. As stated in a previous SCHEDULE report, EVR patients had a higher incidence of biopsy-proven acute rejection compared with CNI patients (78.4% in EVR group vs. 56.4% in CNI group) and the proportion of patients with rejection grade ≥2R was significantly higher in the EVR group (41% vs. 13%, $P = 0.01$), seemingly without a proportionately aggravating effect on CAV development [12].

Consequently, the attenuating effect of EVR on CAV development together with the unequal distribution of EVR patients among rejection groups might have blurred our findings and possibly camouflaged an association between rejection profile and CAV. However, we found no interaction with treatment arm in the adjusted model and treatment arm did not seem to affect $\Delta MIT_{BL\text{-}3Y}$ within rejection groups. Furthermore, when evaluating CAV parameters for treatment groups separately, we could not establish an association between mild rejection and CAV in neither of the treatment groups (Supporting Information).

One might argue that the primary endpoint or exposure time was not suited to test the association between mild ACR and CAV development. Change in MIT from BL to 1 year is a more established endpoint among HTx recipients, but on the other hand the 3-year follow-up might be too short to detect a possible effect. Obviously, the exact timespan for a given inflammatory response to translate into evident and detectable CAV is somewhat elusive. By choosing a 3-year follow-up, we believed to increase our chances of detecting an effect. Perhaps even more time is needed. The rationale for selecting ACR within 1 year as exposure was that the highest ACR incidence is observed here. Also, protocolled biopsies in the SCHEDULE trial was only performed within the first year.

Limitations

We acknowledge important limitations to this study, which must be emphasized with respect to interpretation and extrapolation of our findings. The size of the study population and subgroups were small, which could potentially impair statistical power. This was a post hoc analysis of the SCHEDULE trial, which was not designed and powered to test this hypothesis and evaluate the association in question. Only 66% of the original SCHEDULE

population was included because of the availability of matching IVUS examinations, which might constitute a selection bias, and treatment distribution was unequal among rejection groups possibly distorting results. Furthermore, evaluating the isolated effect of mild rejection was challenging as most patients with moderate–severe rejection also experienced mild rejection.

Conclusion

In conclusion, our findings did not support an association between mild rejections within 1 year post-HTx and the development of CAV at 3 years post-HTx. This suggests that the burden of mild rejection should not necessarily lead to alterations in anti-rejection approach and management of HTx recipients with regard to the prevention of CAV development and progression.

Authorship

All authors have contributed substantially to the submitted work through participation in study design, performance, analysis, or reporting. AKA, SA, BA, EG, HE, GR, GD, LG, and FG: have contributed to study design, study conduct, data collection, and revision of the manuscript. LMN and FG: have performed data analysis and drafting of the manuscript. All authors have approved the final submitted version of the manuscript.

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Conflicts of interest

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Cardiac allograft vasculopathy assessed by intravascular ultrasound at 1 year post-HTx (n = 72).

Table S2. Cardiac allograft vasculopathy assessed by intravascular ultrasound at baseline (n = 76).

Table S3. Cardiac allograft vasculopathy assessed by intravascular ultrasound and coronary angiography at 3 years post-HTx, EVR-arm (n = 37).

Table S4. Cardiac allograft vasculopathy assessed by intravascular ultrasound and coronary angiography at 3 years post-HTx, CNI-arm (n = 39).

Table S5. Primary endpoint ΔMIT BL-3Y in rejection group no-ACR stratified by treatment (n = 25).

Table S6. Primary endpoint ΔMIT BL-3Y in rejection group 1R stratified by treatment (n = 32).

Table S7. Primary endpoint ΔMIT BL-3Y in rejection group ≥2R stratified by treatment (n = 19).

REFERENCES

1. Taylor DO, Stehlik J, Edwards LB, *et al.* Registry of the International Society for Heart and Lung Transplantation: twenty-sixth official adult heart transplant report-2009. *J Heart Lung Transplant* 2009; **28**: 1007.
2. Billingham ME. Graft coronary disease: the lesions and the patients. *Transplant Proc* 1989; **21**: 3665.
3. Hiemann NE, Wellnhofer E, Knosalla C, *et al.* Prognostic impact of microvasculopathy on survival after heart transplantation: evidence from 9713 endomyocardial biopsies. *Circulation* 2007; **116**: 1274.
4. Lund LH, Edwards LB, Kucheryavaya AY, *et al.* The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report–2014; focus theme: retransplantation. *J Heart Lung Transplant* 2014; **33**: 996.
5. Pollack A, Nazif T, Mancini D, Weisz G. Detection and imaging of cardiac allograft vasculopathy. *JACC Cardiovasc Imaging* 2013; **6**: 613.
6. Chang DH, Kobashigawa JA. Current diagnostic and treatment strategies for cardiac allograft vasculopathy. *Expert Rev Cardiovasc Ther* 2015; **13**: 1147.
7. Stoica SC, Cafferty F, Pauriah M, *et al.* The cumulative effect of acute rejection on development of cardiac allograft vasculopathy. *J Heart Lung Transplant* 2006; **25**: 420.
8. Raichlin E, Edwards BS, Kremers WK, *et al.* Acute cellular rejection and the subsequent development of allograft vasculopathy after cardiac transplantation. *J Heart Lung Transplant* 2009; **28**: 320.
9. Andreassen AK, Andersson B, Gustafsson F, *et al.* Everolimus initiation and early calcineurin inhibitor withdrawal in heart

transplant recipients: a randomized trial. *Am J Transplant* 2014; **14**: 1828.
10. Andreassen AK, Andersson B, Gustafsson F, *et al*. Everolimus initiation with early calcineurin inhibitor withdrawal in de novo heart transplant recipients: three-year results from the randomized SCHEDULE study. *Am J Transplant* 2016; **16**: 1238.
11. Arora S, Andreassen AK, Andersson B, *et al*. The effect of everolimus initiation and calcineurin inhibitor elimination on cardiac allograft vasculopathy in de novo recipients: one-year results of a scandinavian randomized trial. *Am J Transplant* 2015; **15**: 1967.
12. Arora S, Andreassen AK, Karason K, *et al*. Effect of everolimus initiation and calcineurin inhibitor elimination on cardiac allograft vasculopathy in de novo heart transplant recipients. *Circ Heart Fail* 2018; **11**: e004050.
13. Mehra MR, Ventura HO, Stapleton DD, Smart FW, Collins TC, Ramee SR. Presence of severe intimal thickening by intravascular ultrasonography predicts cardiac events in cardiac allograft vasculopathy. *J Heart Lung Transplant* 1995; **14**: 632.
14. Kobashigawa JA, Tobis JM, Starling RC, *et al*. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005; **45**: 1532.
15. Stewart S, Winters GL, Fishbein MC, *et al*. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005; **24**: 1710.
16. Mintz GS, Nissen SE, Anderson, *et al*. American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 2001; **37**: 1478.
17. Mehra MR, Crespo-Leiro MG, Dipchand A, *et al*. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010; **29**: 717.
18. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008; **117**: 2131.
19. Chih S, Chong AY, Mielniczuk LM, Bhatt DL, Beanlands RSB. Allograft vasculopathy: the Achilles' heel of heart transplantation. *J Am Coll Cardiol* 2016; **68**: 80.
20. Nikolova AP, Kobashigawa JA. Cardiac allograft vasculopathy-the enduring enemy of cardiac transplantation. *Transplantation* 2019; **103**: 1338.
21. Jansen MAA, Otten HG, de Weger RA, Huibers MMH. Immunological and fibrotic mechanisms in cardiac allograft vasculopathy. *Transplantation* 2015; **99**: 2467.
22. Patel JK, Kittleson M, Kobashigawa JA. Cardiac allograft rejection. *Surgeon* 2011; **9**: 160.
23. Yamani MH, Yousufuddin M, Starling RC, *et al*. Does acute cellular rejection correlate with cardiac allograft vasculopathy? *J Heart Lung Transplant* 2004; **23**: 272.
24. Caforio ALP, Tona F, Fortina AB, *et al*. Immune and nonimmune predictors of cardiac allograft vasculopathy onset and severity: multivariate risk factor analysis and role of immunosuppression. *Am J Transplant* 2004; **4**: 962.
25. Starling RC, Armstrong B, Bridges ND, *et al*. Accelerated allograft vasculopathy with rituximab after cardiac transplantation. *J Am Coll Cardiol* 2019; **74**: 36.
26. Stehlik J, Armstrong B, Baran DA, *et al*. Early immune biomarkers and intermediate-term outcomes after heart transplantation: results of clinical trials in organ transplantation-18. *Am J Transplant* 2019; **19**: 1518.
27. Starling RC, Stehlik J, Baran DA, *et al*. Multicenter analysis of immune biomarkers and heart transplant outcomes: results of the clinical trials in organ transplantation-05 study. *Am J Transplant* 2016; **16**: 121.
28. Baran DA, Zucker MJ, Arroyo LH, *et al*. A prospective, randomized trial of single-drug versus dual-drug immunosuppression in heart transplantation: the tacrolimus in combination, tacrolimus alone compared (TICTAC) trial. *Circ Heart Fail* 2011; **4**: 129.
29. Chapman JR, Valantine H, Albanell J, *et al*. Proliferation signal inhibitors in transplantation: questions at the cutting edge of everolimus therapy. *Transplant Proc* 2007; **39**: 2937.
30. Gustafsson F, Ross HJ. Proliferation signal inhibitors in cardiac transplantation. *Curr Opin Cardiol* 2007; **22**: 111.
31. Schuler W, Sedrani R, Cottens S, *et al*. SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. *Transplantation* 1997; **64**: 36.
32. Nashan B. Early clinical experience with a novel rapamycin derivative. *Ther Drug Monit* 2002; **24**: 53.
33. Chapman TM, Perry CM. Everolimus. *Drugs* 2004; **64**: 861.
34. Eisen HJ, Tuzcu EM, Dorent R, *et al*. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med* 2003; **349**: 847.
35. Eisen HJ, Kobashigawa J, Starling RC, *et al*. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. *Am J Transplant* 2013; **13**: 1203.
36. Segovia J, Gómez-Bueno M, Alonso-Pulpón L. Treatment of allograft vasculopathy in heart transplantation. *Expert Opin Pharmacother* 2006; **7**: 2369.