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# The Association Between Cytomegalovirus Infection and Cardiac Allograft Vasculopathy in the Era of Antiviral Valganciclovir Prophylaxis

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**Background.** Previous studies on the association between cytomegalovirus (CMV) infection and cardiac allograft vasculopathy (CAV) were conducted on patients transplanted in the prevalganciclovir prophylaxis era. The aim of our study is to evaluate this relation in heart transplantation (HTx) recipients treated according to current prophylactic and immunosuppressive regimens. **Methods.** This single-center retrospective study included all consecutive adult patients that underwent HTx between January 1, 2000, and May 31, 2018. Clinically relevant CMV infection was defined as either plasma CMV DNAemia  $\geq 1000$  IU/mL with/without clinical symptoms or  $<1000$  IU/mL with symptoms. The primary endpoint was first manifestation of CAV diagnosed by coronary angiography. For statistical analysis, the cause-specific hazard regression model was applied, with clinically relevant CMV infection and any CMV infection as time-dependent variables. **Results.** In total, 260 patients were included in the analysis. The median (interquartile range) follow-up was 7.88 (4.21–12.04) years. During the follow-up, clinically relevant CMV infection was diagnosed in 96 (37%) patients and CAV in 149 (57%) patients. In the multivariate regression analysis, independent predictors of CAV were: number of rejection episodes (cause-specific hazard ratio [95% confidence interval]: 1.18 [1.04–1.34],  $P = 0.01$ ), hypertension (1.61 [1.11–2.34],  $P = 0.01$ ), treatment with mycophenolate mofetil (0.68 [0.47–0.97],  $P = 0.03$ ). No significant association was observed between CMV infection and CAV, except for patients who experienced a breakthrough CMV infection ( $n = 24$ ) during prophylaxis (1.94 [1.11–3.40],  $P = 0.02$ ). **Conclusions.** In the era of contemporary immunosuppression and valganciclovir prophylaxis, a significant effect of CMV infection on the risk of CAV was seen only among HTx recipients with CMV breakthrough infection.

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## INTRODUCTION

Cardiac allograft vasculopathy (CAV) is a form of coronary atherosclerosis in heart transplant (HTx) patients characterized by progression of intimal hyperplasia, which leads to diffuse narrowing of coronary arteries and is usually clinically silent because of allograft denervation.<sup>1,2</sup> Although the dynamics of its development are usually slow, in some patients, a rapid progression to occlusive disease within months is observed.<sup>3</sup> With no effective

etiological treatment strategy established to date, CAV is an independent predictor of major cardiovascular events in this population and one of the main factors that compromise long-term patient survival after HTx.<sup>4–6</sup>

Available evidence indicates that CAV results from an interplay between various immunologic and metabolic risk factors that include acute cellular and antibody-mediated rejection, anti-HLA and antiendothelial antibodies, older age and male sex of the donor (D), hyperlipidemia, insulin

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resistance, and endothelial dysfunction. Some researchers report on the involvement of cytomegalovirus (CMV) disease or even subclinical CMV replication in the development of CAV.<sup>5</sup> Potential mechanisms of damage caused by CMV are direct endothelial assault and impairment of proper vascular remodeling.<sup>7</sup> However, until now, clinical studies on the association between CMV and CAV yielded conflicting results.<sup>8–10</sup> Moreover, over the recent decades, we observed major changes in both D and recipient (R) characteristics (a 33% prevalence of Ds aged >50 y, more stroke-related Ds with a higher burden of atherosclerotic cardiovascular disease and higher proportion of Rs aged >60 y) but also modifications in post-HTx treatment (early statin treatment) and surveillance.<sup>11</sup> In 2000, treatment with mycophenolate mofetil (MMF) was introduced, and international consensus guidelines on anti-CMV prophylaxis have been issued in the following years.<sup>12</sup> There is a gap in evidence on to what extent these changes influenced the prevalence of CAV in HTx Rs because previous studies on adult Rs included only patients transplanted before 2002.<sup>8,9,13,14</sup> Therefore, the aim of this study was to evaluate the impact of CMV infection on prevalence and outcome of CAV in a cohort of HTx Rs treated according to current immunosuppressive and antiviral prophylactic regimens.

## PATIENTS AND METHODS

### Patient Cohort

All the adult patients who underwent HTx at our institution between January 2000 and May 2018 were screened for eligibility for the study. Second heart transplant was regarded as exclusion criterion unless it was performed because of primary graft failure early in the perioperative period. Those patients who did not survive until successful discharge after the surgery were excluded from the study. All patients received pravastatin 10 mg and aspirin 80 mg daily starting within 2–4 weeks posttransplant. Pravastatin dose was uptitrated gradually to 40 mg at 1-year post-HTx.

This study was approved by the local Ethical Committee of Erasmus MC (MEC-2017-421). The data were derived from the patients' medical records and local registries according to the standards set by the Declaration of Helsinki.

### Rejection Surveillance and Immunosuppressive Therapy

Rejection surveillance was performed by routine endomyocardial biopsy (EMB) of median (interquartile range [IQR]) 16 (14–18) times (during the first year after transplantation and at 4 y post-HTx). After that period, EMBs were only taken when rejection was suspected. All patients included presented for routine EMB. Grading of the histological findings was done according to International Society for Heart and Lung Transplantation (ISHLT) standards.

Several regimens of induction therapy (intravenous anti-T-cell antibodies immediately after transplantation) have been used over time (Table S1, SDC, <http://links.lww.com/TP/B827>). From 2000, the maintenance immunosuppression therapy changed from cyclosporine-based to a tacrolimus-based scheme combined with prednisone and MMF.

The standard dose of MMF used at our institution is from 2 × 750 mg/d (if combined with tacrolimus) up to 2 × 1500 mg/d (if combined with cyclosporin), modified based on trough level (optimum 1–3 mg/L). Either MMF or prednisone was stopped at 1-year posttransplant depending on the amount of rejections and on the side effects of these drugs (eg, infections, diabetes mellitus, obesity). Acute cellular rejection episodes were treated with pulsed high-dose methylprednisolone; in the case of steroid-resistant rejection, rabbit antithymocyte globulin was used. Antibody-mediated rejection was treated only in case of signs of graft failure in combination with histological and immunopathologic findings.

### Coronary Allograft Vasculopathy Definition and Diagnosis

The diagnosis of CAV was based on coronary angiography results and grading was applied according to ISHLT criteria published in 2010.<sup>15</sup> Patients underwent routine CAV screening according to the protocol established at our institution: coronary angiography at 1 and 4 years after HTx unless contraindicated or patient refused. After 4 years, coronary angiography was performed when clinically indicated or significant abnormalities on annual surveillance myocardial perfusion imaging.<sup>11</sup> No patients missed the annual myocardial perfusion imaging.

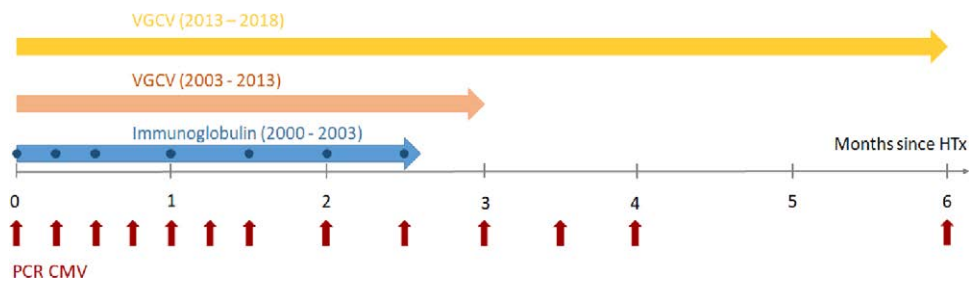
### CMV Prophylaxis and Screening Protocol

CMV prophylaxis and screening protocol are presented in Figure 1. Only CMV seronegative patients that received an organ from a seropositive D (D+/R-) received prophylaxis against CMV infection. Since 2000, there were 3 types of anti-CMV prophylactic regimes. Until October 2003, patients received human CMV immunoglobulin (CMVIG) (Megalotect CP, Biotest) on day 0, 7, 14, 28, 42, 56, and 70 posttransplant. Between November 2003 and October 2013, a standard of 3 months valganciclovir (VGCV) prophylaxis was given. After October 2013, 6 months VGCV prophylaxis was applied routinely. Prophylaxis with VGCV started at a standard dose of 450 mg/d from day 5 to 7 postoperatively (adjusted for renal function when necessary).

Screening for plasma CMV DNAemia was performed with quantitative real-time polymerase chain reaction (PCR) weekly for the first 6 weeks after HTx, then every 2 weeks until the end of third-month post-HTx, then every 2 months until the end of first-year post-HTx. With CMV seropositive Rs, there was no routine follow-up, that is, CMV PCR was performed only if CMV disease was suspected. Besides pre-HTx CMV serostatus evaluation, CMV serology was not applied for the diagnosis of CMV. CMV prophylaxis together with immunosuppressive regimens is summarized in Table S1 (SDC, <http://links.lww.com/TP/B827>).

### CMV Definitions

Clinically relevant CMV infection was defined as either plasma CMV DNAemia ≥1000 IU/mL independently of the presence of symptoms and signs or plasma CMV DNAemia <1000 IU/mL if accompanied with symptoms or signs that were typical for CMV disease (eg, fever, gastrointestinal disease, leukopenia, abnormal liver function



**FIGURE 1.** Cytomegalovirus (CMV) prophylactic regimens and screening protocol used throughout the study period. HTx, heart transplantation; PCR, polymerase chain reaction; VGCV, valganciclovir.

tests).<sup>16</sup> The reason for choosing this definition was that we aimed to investigate only clinically relevant infection instead of asymptomatic CMV replication, which may be temporarily observed during infections caused by other pathogens or in any severe disease. The VGCV preemptive treatment of asymptomatic CMV infection was started if plasma CMV DNAemia exceeded 1000 IU/mL. Breakthrough CMV infection was defined as the detection of CMV DNA in plasma by PCR while the patient was still receiving VGCV prophylaxis (regardless of CMV DNAemia level and symptoms). Additionally, we have evaluated any CMV infection defined as the detection of CMV DNA in plasma independent of the presence of symptoms and plasma CMV DNAemia level, which will be referred to as “CMV infection.”

### CMV Monitoring

Plasma CMV DNAemia was monitored using a dual-target, laboratory developed, quantitative real-time PCR (see Text S1 and Table S2, SDC, <http://links.lww.com/TP/B827>). Results are reported in international unit per milliliter. All data generated before December 2012, were obtained with a different protocol, described previously<sup>17,18</sup> and reported in copies/mL. These test results were converted to IU/mL using a conversion factor of 2, as was established during validation of the dual-target assay. The limit of quantification was 100 IU/mL before and 50 IU/mL after December 2012. All virological tests were performed in an International Organization for Standardization 15189:2012 accredited (or equivalent) clinical diagnostic setting.

### CMV Treatment

Asymptomatic CMV infection with plasma CMV DNAemia  $\geq 1000$  IU/mL was treated with VGCV for at least 14 days until negative CMV PCR results in 2 consecutive plasma samples. Symptomatic CMV infection was treated for at least 14 days until disappearance of symptoms, and negative CMV PCR results in 2 consecutive plasma samples. Preferably, the MMF dose was halved or temporarily halted during preemptive treatment of CMV and during treatment of CMV disease. If there was a confirmed CMV resistance against VGCV, treatment with intravenous foscarnet was commenced.

### Statistical Analysis

Distributions of continuous variables were tested for normality using the Shapiro-Wilk test. Normally distributed continuous variables are presented as mean and SD, nonnormally distributed continuous variables as median

and IQR. Categorical data are displayed as count and percentage. In case of skewed distributions, continuous variables were logarithmically transformed (log base 2) for further analyses.

The primary endpoint analyzed was the combined endpoint, which comprised first manifestation of CAV grade 1 or higher according to ISHLT 2010 nomenclature,<sup>15</sup> elective percutaneous coronary intervention or acute coronary syndrome, or CAV-associated death. Death caused by other causes was regarded as competing risk. We evaluated the associations between clinical characteristics and the primary endpoint by calculating cause-specific hazard ratios within Cox proportional hazards regression models. Clinically relevant CMV infection, CMV infection, and CMV breakthrough infection were entered as time-dependent variables into these models. First, we performed univariable analyses. After that we performed a multivariable analysis with CMV breakthrough infection as time-dependent covariate and all the covariates that were significant by univariable analysis at a significance level of  $P < 0.05$ . We did not include the effect of recurrent CMV infection in the analysis. For the variables associated with rejection, we chose one (episodes of acute cellular rejection  $\geq 2$  R/AMR) to avoid collinearity.

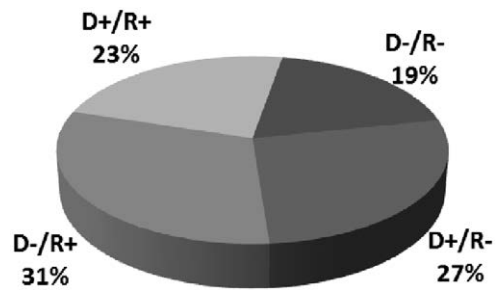
## RESULTS

In total, 260 patients were included in the analysis. Among them, 3 patients received a second heart transplant. No patient was lost to follow-up. Median follow-up was 7.9 (4.2–12.0) years, with 218 (84%) patients having at least 4 years of follow-up completed. Characteristics of the study population are presented in Table 1. CMV mismatch (D+/R–) was present in 71 (27%) patients. CMV serological status at transplantation is presented in Figure 2. All the D+/R– patients received prophylaxis with CMVIG ( $n = 14$ ; 20%) before 2003 and with VGCV ( $n = 57$ ; 80%) after 2003. Over the follow-up, clinically relevant CMV infection was diagnosed in 96 (37%) patients. In CMV mismatch patients, clinically relevant CMV infection was more frequent than in the rest of the Rs (44 of 71 patients, 62% versus 52 of 189 patients, 27%,  $P < 0.001$ ), but it occurred at a later time point from HTx (138 [57–226] versus 30 [18–61] days,  $P < 0.001$ ). The same was observed for any CMV infection (46 of 71, 65% and 76 of 189, 40%,  $P < 0.001$ ; 138 [56–239] versus 32 [19–66] days,  $P < 0.001$ ). The follow-up of mismatch patients was not longer than the rest of the study population (2917 [1512–5121] versus 3613 [2161–5228] days),  $P = 0.14$ . Although the rates of clinically relevant CMV infection were not significantly different in VGCV-treated versus immunoglobulin-treated

**TABLE 1.**  
**Characteristics of the study population (N = 260)**

Variable	
<b>Donor characteristics</b>	
Age, y	45 (34–53)
Males	107 (41%)
BMI, kg/m <sup>2</sup>	24 (22–26)
<b>Recipient characteristics</b>	
Age at HTx, y	50 (43–58)
Males	172 (66%)
BMI, kg/m <sup>2</sup>	24 (22–26)
<b>Primary disease</b>	
Cardiomyopathy	163 (63%)
Ischemic heart disease	81 (31%)
Valvular disease	5 (2%)
Congenital	8 (3%)
Retransplantation	3 (1%)
PRA >10%	9 (3.5%)
<b>Perioperative</b>	
Urgent transplantation	98 (38%)
Mechanical support pre-HTx	57 (22%)
Ischemic time, min	188 (159–219)
CVWH	40 (15%)
<b>Immunosuppressive regimen</b>	
<b>Induction therapy</b>	
ATG	237 (92%)
Anti-CD25	6 (2%)
OKT3	2 (1%)
None	13 (5%)
<b>Tac-based therapy</b>	
CsA-based therapy	37 (14%)
MMF	131 (50%)
Steroids	125 (48%)
<b>CMV-related</b>	
Clinically relevant CMV infection	96 (37%)
Time from transplantation, d	57 (27–146)
CMV primary infection	49 (19%)
CMV infection	122 (47%)
Time from transplantation, d	52 (26–143)
CMV breakthrough infection	24 (9%)
Time from transplantation, d	62 (42–92)
<b>Rejection episodes</b>	
ACR ≥ 2R/AMR (first y)	114 (44%)
<b>Episodes of ACR ≥ 2R/AMR</b>	
0	88 (34%)
1	82 (32%)
2	56 (22%)
≥3	30 (12%)
<b>Comorbidities and treatment</b>	
Hypertension	151 (58%)
DM pre-HTx	30 (11%)
DM post-HTx	101 (39%)
eGFR (CKD-EPI), pre-HTx, mL/min/1.73 m <sup>2</sup>	59 (43–73)
eGFR (CKD-EPI), 1 y post-HTx, mL/min/1.73 m <sup>2</sup>	54 (42–71)
Kidney transplantation post-HTx	7 (3%)
Total cholesterol at 1 y post-HTx, mmol/L	5.1 (4.3–6.0)
LDL at 1 y post-HTx, mmol/L	2.64 (2.23–3.60)
Statin treatment	246 (95%)

Data are presented as count (percentage) for categorical variables, and mean (SD) for continuous normally distributed variables, and median (25th–75th percentile) for continuous nonnormally distributed variables. Time from transplantation for clinically relevant CMV infection defined as time till first CMV PCR ≥1000 IU/mL independent of symptoms or <1000 if accompanied by symptoms. Primary infection was defined as CMV infection (CMV PCR ≥1000 IU/mL independent of symptoms or <1000 IU/mL and accompanied by symptoms) in seronegative recipients. ACR, acute cellular rejection; AMR, antibody-mediated rejection; ATG, anti-thymocyte immunoglobulin; BMI, body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration; CMV, cytomegalovirus; CsA, ciclosporin A; CVWH, continuous venovenous hemofiltration; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate based on CKD-EPI equation; HTx, heart transplantation; LDL, low-density lipoprotein; MMF, mycophenolate mofetil; OKT3, monoclonal anti-human T lymphocyte antibody; PCR, polymerase chain reaction; PRA, panel reactive antibodies; Tac, tacrolimus.

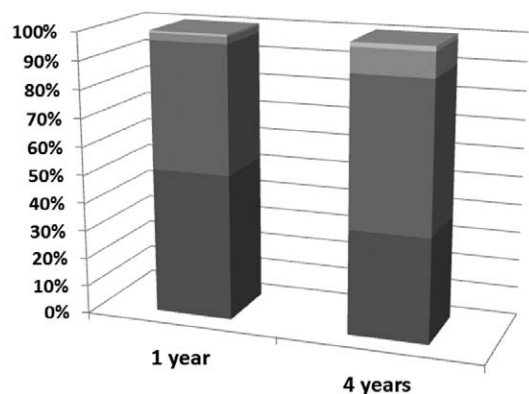


**FIGURE 2.** CMV serological status at heart transplantation. CMV, cytomegalovirus; D, donor; R, recipient.

subgroup (33 of 57 patients, 58% versus 11 of 14 patients 79%,  $P = 0.13$ ), it occurred significantly later if VGCV was given (143 [111–239] versus 42 [39–44],  $P < 0.001$ ). Out of the total of study population, 14 (5.4%) patients experienced 1 recurrence of any CMV infection, and 5 (1.9%) patients experienced 2 recurrences. Foscarnet was applied in 2 patients infected with VGCV-resistant strains.

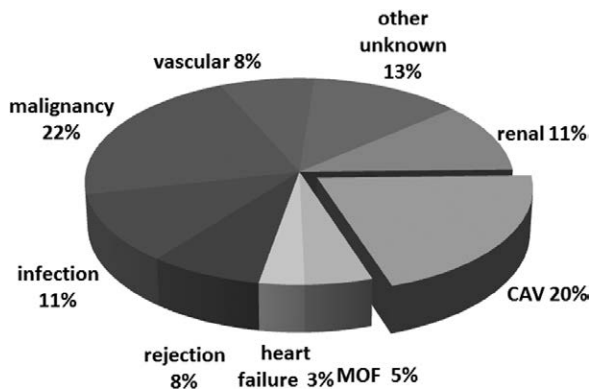
In patients with clinically relevant CMV infection, 72 (75.0%) were treated with MMF before infection occurred with 1500 (1000–2000) mg total daily MMF preinfection dose (defined as dose used on the last visit before infection). In 11 (15.3%) patients, the dose was reduced, and in 44 (61.1%), MMF was stopped during infection so that median dose was 0 (0–1000) mg. The postinfection dose (defined as after 2× negative PCR CMV or the last MMF dosage before the end point or censoring date in patients who did not turn negative) was 0 (0–1000) mg. At the day of the first positive PCR, patients with clinically relevant CMV infection presented the following blood count values: 6.90 (4.20–0.52) × 10<sup>9</sup>/L leukocytes with 9.70 (4.00–18.80) % lymphocytes (0.56 [0.28–1.10] × 10<sup>9</sup>/L).

By the end of follow-up, CAV was diagnosed in 149 (57%) patients. Most patients presented mild CAV (CAV1, according to ISHLT). The severity of CAV classified according to ISHLT at the 2 screening moments is presented in Figure 3. Twenty-two (8%) patients experienced



	1 year	4 years
CAV3	0,8%	1,6%
CAV2	2,4%	8,7%
CAV1	45,1%	52,7%
CAV0	51,6%	37,0%

**FIGURE 3.** Cardiac allograft vasculopathy (CAV) prevalence at 1-y and 4-y posttransplant. Grading, according to International Society for Heart and Lung Transplantation (ISHLT). Figure presents only patients who completed 4-y follow-up (n = 218).



**FIGURE 4.** Causes of death (n = 64). CAV, cardiac allograft vasculopathy; MOF, multiorgan failure.

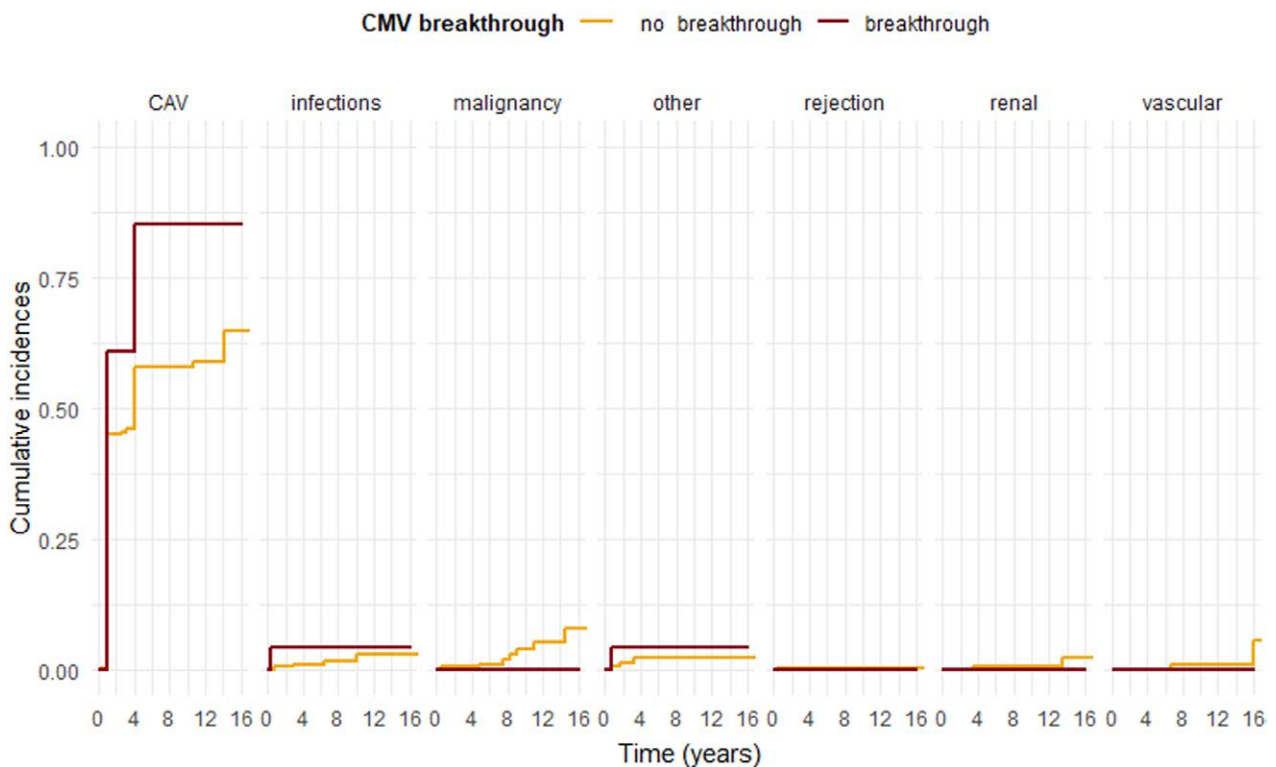
acute coronary syndrome. Among the study population, 64 (25%) patients died during the follow-up time mostly due to malignancy (n = 14, 22%), followed by CAV-related deaths (n = 13 [20%]; see Figure 4). The temporal trends for CAV and various causes of death are presented in Figure 5 (stratified according to the presence of breakthrough infection), with CAV being the major early complication and malignancies contributing to mortality in the late post-HTx period.

Univariable regression analysis showed that neither CMV serological status of D and R at transplantation (D+/R-, D+/R+, D-/R+, D-/R-) nor the occurrence of clinically relevant CMV infection nor CMV infection were associated with CAV (Table 2). However, we observed that out of 71 CMV mismatched patients (D+/R-), 24 experienced a

breakthrough CMV infection (34%). These breakthroughs occurred more frequently in patients from immunoglobulin prophylaxis group (n = 9, 64%) than in VGCV prophylaxis group (n = 15, 26%), P = 0.01 (Table S3, SDC, <http://links.lww.com/TP/B827>). CMV breakthroughs increased the risk of CAV when analyzed both univariably and multivariably (cause-specific hazard ratio, cause-specific hazard ratio, 95% [confidence interval]: 1.94 [1.11-3.40], P = 0.02), as demonstrated in Tables 2 and 3. After excluding immunoglobulin-treated patients from the analysis (n = 246), there was still a trend towards higher risk of CAV in patients with CMV breakthrough infection among patients on VGCV prophylaxis by univariable analysis (1.85 [0.96-3.53], P = 0.06), although the association did not persist in multivariable analysis (Table S4, SDC, <http://links.lww.com/TP/B827>). Other factors, that were independent predictors of CAV in multivariable analysis were the number of acute rejection (AR) episodes (1.15 [1.01-1.30], P = 0.03) and hypertension (1.75 [1.21-2.53], P = 0.003), whereas the use of MMF as maintenance immunosuppression was associated with lower risk of CAV (0.68 [0.48-0.98], P = 0.04). CAV-free survival stratified by CMV-related factors: clinically relevant CMV infection, CMV infection, and CMV breakthrough infection is presented in Figure 6. CAV-free survival according to the other 3 significant predictors (rejection episodes, hypertension, and MMF) is presented in Figure 7.

Rejection episodes (of grade 2R or more) occurred with similar frequency in patients in whom clinically relevant CMV infection was diagnosed (1 [0-2] versus 1 [0-2], P = 0.93) compared with the rest of the study group. Neither were they more frequent in patients with any

### Cumulative incidence functions



**FIGURE 5.** Cumulative incidence curves for major complications: combined endpoint cardiac allograft vasculopathy (CAV) grade 1 or higher and CAV-related death in comparison with death from other causes. CMV, cytomegalovirus.

**TABLE 2.**

**Univariable regression analysis of combined composite endpoint consisting of first manifestation of CAV grade 1 or higher according to ISHLT 2010 nomenclature, elective PCI or ACS or CAV-associated death**

Covariate	CSHR (95% CI)	P
Donor characteristics		
Age (per y)	1.01 (1.00-1.03)	0.02
Sex (female)	0.73 (0.52-1.00)	0.05
BMI, kg/m <sup>2</sup>	2.85 (1.32-6.14)	0.008
Recipient characteristics		
Age at HTx (per y)	1.00 (0.99-1.02)	0.27
Sex (female)	0.79 (0.56-1.12)	0.19
BMI (kg/m <sup>2</sup> )	1.05 (1.00-1.09)	0.02
PRA >10%	0.73 (0.27-1.99)	0.73
Perioperative/procedural		
Mechanical support pre-HTx	0.89 (0.60-1.32)	0.59
Ischemic time, min	1.24 (0.78-1.98)	0.36
Rethoracotomy (count)	1.16 (0.90-1.49)	0.23
ICU hospitalization/stay, d	0.98 (0.97-1.00)	0.12
CVWH post-operatively	0.87 (0.53-1.42)	0.57
Immunosuppressive regimen		
Induction: ATG	0.82 (0.42-1.63)	0.59
Induction: anti-CD25	0.99 (0.30-3.23)	0.99
Induction: OKT3	0.66 (0.08-5.25)	0.69
Tac-based therapy	0.80 (0.52-1.21)	0.27
CsA-based therapy	1.26 (0.83-1.94)	0.27
MMF	0.63 (0.46-0.88)	0.007
CMV-related		
CMV recipient status (R+)	1.00 (0.72-1.38)	0.99
Clinically relevant CMV infection	1.01 (0.73-1.40)	0.96
Primary infection	1.05 (0.70-1.59)	0.80
CMV infection	0.97 (0.70-1.35)	0.88
Time until infection from HTx, d	1.00 (1.00-1.003)	0.05
CMV breakthrough	1.94 (1.18-3.18)	0.009
Duration of clinically relevant CMV infection	1.00 (0.99-1.003)	0.14
Prophylaxis scheme (VGCV)	0.66 (0.35-1.23)	0.19
Number of recurrences	0.98 (0.61-1.58)	0.93
Rejection episodes		
ACR≥2R/AMR in the first-y post-HTx	1.43 (1.03-2.00)	0.03
Episodes of ACR ≥2R/AMR (first y)	1.18 (1.01-1.39)	0.04
Time till first ACR≥2R/AMR episode, d	1 (0.99-1.00)	0.42
Episodes of ACR ≥2R/AMR (total)	1.13 (1.00-1.27)	0.04
Comorbidities and treatment		
Hypertension	1.66 (1.18-2.34)	0.003
DM pre-HTx	1.21 (0.74-1.98)	0.45
DM post-HTx	0.86 (0.62-1.20)	0.38
eGFR (CKD-EPI), pre-HTx, mL/min/1.73 m <sup>2</sup>	1.00 (0.99-1.01)	0.61
Kidney transplantation	1.63 (0.67-3.99)	0.28
Total cholesterol at 1 y post-HTx (mmol/L)	1.16 (1.03-1.30)	0.02
Statin treatment	1.10 (0.52-2.40)	0.79

CSHR for Ischemic time and time till reactivation are presented per log, changes. - CSHR for other variables is presented per 1 unit of increase. The duration of clinically relevant infection was defined as number of days between the first positive and first negative CMV PCR. Clinically relevant CMV infection, CMV infection and CMV breakthrough infection were analyzed as time-dependent variables. ACR, acute cellular rejection; ACS, acute coronary syndrome; AMR, antibody-mediated rejection; ATG, antithymocyte immunoglobulin; BMI, body mass index; CAV, cardiac allograft vasculopathy; CI, confidence interval; CKD-EPI, chronic kidney disease epidemiology collaboration; CMV, cytomegalovirus; csA, ciclosporin A; CSHR, cause-specific hazard ratio; CVWH, continuous venovenous hemofiltration; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate based on CKD-EPI equation; HTx, heart transplantation; ICU, intensive care unit; ISHLT, International Society for Heart and Lung Transplantation; MMF, mycophenolate mofetil; OKT3, monoclonal antihuman T lymphocyte antibody; PCI, percutaneous coronary intervention; PCR, polymerase chain reaction; PRA, panel reactive antibodies; R, recipient; Tac, tacrolimus; VGCV, valganciclovir.

CMV infection (1 [0–2] versus 1 [0–2],  $P = .76$ ) nor CMV breakthrough (1 [0–2] versus 1 [0–2],  $P = 0.75$ ). In 19 (19.8%) patients with clinically relevant CMV, infection followed the diagnosis and treatment of AR. In 8 patients,

the rejection continued during the treatment of infection, and in 13 (13.5%) patients, AR was newly diagnosed during the treatment of infection. Among CMV mismatch patients, 27 (38.0%) experienced rejection during VGCV

**TABLE 3.**  
**Multivariable analysis of CAV any grade**

Covariate	CSHR (95% CI)	P
Donor BMI, kg/m <sup>2</sup>	1.01 (0.98-1.08)	0.18
Donor age, y	1.01 (1.00-1.02)	0.10
Recipient BMI, kg/m <sup>2</sup>	1.03 (0.98-1.08)	0.19
CMV breakthrough	1.94 (1.11-3.40)	0.02
Number of ACR≥2R/AMR episodes	1.18 (1.04-1.34)	0.01
Hypertension	1.61 (1.11-2.34)	0.01
MMF	0.68 (0.47-0.97)	0.03
Total cholesterol at 1 y (mmol/L)	1.08 (0.95-1.23)	0.21

ACR, acute cellular rejection, AMR, antibody-mediated rejection, BMI, body mass index, CAV, cardiac allograft vasculopathy, CI, confidence interval; CMV, cytomegalovirus; CSHR, cause-specific hazard ratio; MMF, mycophenolate mofetil.

prophylaxis. Rejection rate did not differ significantly between patients on prophylaxis with VGCV (7, 50.0%) versus immunoglobulin (20, 35.1%),  $P = 0.36$ . The temporal trend in rejection related-death and other posttransplant complications with regard to the type of prophylaxis are presented in **Figure S1** (SDC, <http://links.lww.com/TP/B827>). Rejection episodes occurred with similar frequency in patients with CMV infection independently of change in MMF dosage during infection (median [IQR] rejection episodes: 1 [0–2] if MMF dosage maintained, 2 [1–3] if halved, 1 [0–2] if stopped,  $P = 0.22$ ).

## DISCUSSION

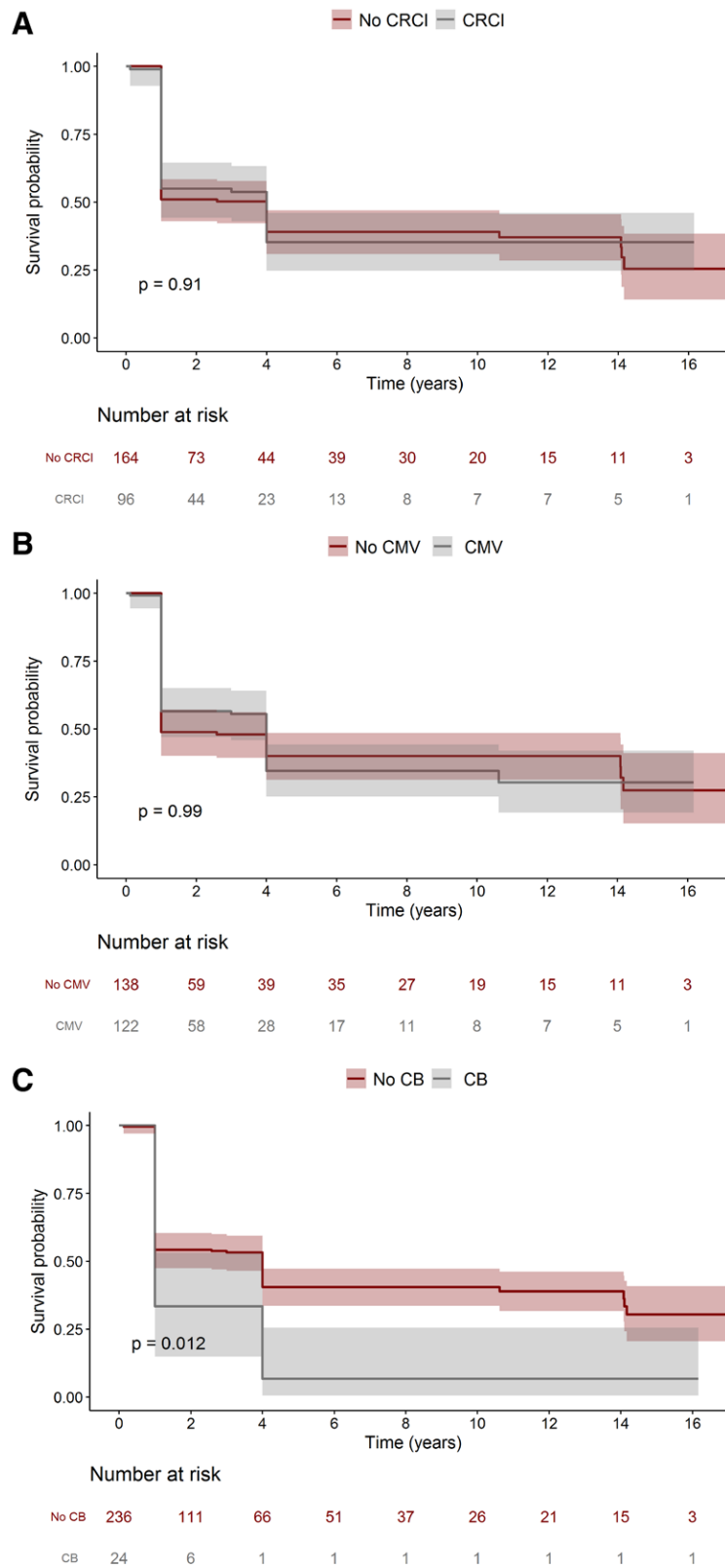
To the best of our best knowledge, this is the first study in HTx patients in the contemporary immunosuppressive and antiviral prophylactic era of the new millennium, showing that there is no association between CMV infection and development of CAV in HTx patients. The only exception is the patients in whom infection occurred during prophylactic treatment, that is, CMV breakthrough infection, who present significantly higher risk of CAV. AR and hypertension are associated significantly with CAV, whereas treatment with MMF reduces that risk.

The results of our study are only partially in concordance with previous retrospective studies with long-term follow-up by Delgado et al<sup>9</sup> or Johansson et al,<sup>8</sup> on this topic with the largest sample sizes to date ( $N = 166$  and  $N = 226$  respectively). They reported a significant association between CMV disease, infection and asymptomatic infection, and CAV over long-term follow-up. These studies presented different approaches towards CMV screening and prophylaxis than is maintained in our institution and is the current standard of care for HTx Rs. Johansson et al<sup>8</sup> described a cohort of patients, the majority of whom were not subjected to CMV prophylaxis, which was introduced only in the last 2 years of the 12-years long study period and which comprised oral ganciclovir for 14 weeks. Delgado et al<sup>9</sup> included patients with universal prophylaxis, but only within the first 14 days, whereas current guidelines recommend 3 to 6 months ganciclovir or VGCV prophylaxis in high-risk patients (D+/R-) so this approach could not prevent CMV infection and disease in the first months post-HTx. Such a strategy was probably the cause of higher rate of CMV infection (defined by the authors as a positive phosphoprotein 65 antigenemia) compared with our study (75%–89% versus 47%). On the other hand, the

rate of symptomatic CMV infection and disease described Johansson et al<sup>8</sup> was similar to the prevalence of clinically relevant CMV infection in our cohort (36% versus 42%, respectively). However, it should be taken into account that our patients were subjected to CMV screening with a more sensitive method (quantitative real-time PCR) compared to the above mentioned reports (phosphoprotein 65 antigenemia<sup>9</sup> and posttransplant seroconversion, virus culture, and qualitative CMV DNA PCR<sup>8</sup>), which could contribute to prompt detection, earlier treatment initiation, and continuation until there is no more active replication, which could decrease overall CMV burden in our study patients.<sup>19</sup> It should be noticed that more potent maintenance immunosuppression was applied in our center, with the use of MMF in half of our study population, a higher proportion than in previous studies (0%–22%).<sup>8,9</sup>

However, one should be aware that there are patients who experience CMV infection during prophylaxis, which increased the risk of CAV in multivariable analysis by 94%. These patients comprise 34% of the CMV mismatch (D+/R-) patients within our study population, and the rate of CMV breakthrough on VGCV is high (26%). The rate of unsuccessful prophylaxis is higher than in renal transplant Rs treated with both standard (900 mg/d for 6 months) and reduced (450 mg/d for 6 months) dose of VGCV—2% and 13%, respectively<sup>20</sup> as well as lung transplant Rs (900 mg/d, for longer than 6 months in 86% of patients)—9%.<sup>21</sup> This could be influenced by higher immunosuppression applied for HTx compared with renal transplantation and shorter period of VGCV prophylaxis compared with both groups. Unfortunately, CMV breakthroughs among HTx Rs were not investigated before. We cannot exclude that these patients might have been predisposed to CMV infection by other concomitant infection, increased immunosuppression for treatment of rejection, drug malabsorption, nonadherence to prophylaxis, or a combination of the above. A larger cohort of patients with CMV mismatch is required to verify this association as well as to evaluate the efficacy of VGCV and other CMV antivirals and to determine the optimum timespan, dosage, and drug level monitoring for VGCV prophylaxis. Further investigation is warranted to identify patients who could benefit from CMV-specific immunity evaluation.

Based on the results of multivariable analysis, the number of AR episodes (both cellular and antibody-mediated taken together) was associated with higher risk of CAV. This association was mentioned in previous clinical studies,<sup>22,23</sup> also those with the application of near-infrared spectroscopy, intravascular ultrasound (IVUS) imaging, or optical coherence tomography.<sup>24-26</sup> The involvement of the immune system in the development of coronary lesions in HTx Rs is also supported by the data on increased immune system activity in CAV positive patients<sup>27</sup> and histopathological evidence.<sup>28-30</sup> The negative impact of rejections stays in accordance with a positive influence of MMF exerted on the risk of CAV observed in our study population. However, the design of our study does not allow to draw the definitive conclusion on role of MMF in reducing the risk of CAV. The efficacy of MMF in CAV prevention was analyzed in only one prospective study to date, in which de novo treatment with low-dose tacrolimus/siroliimus ( $n = 61$ ) was compared to full-dose tacrolimus/MMF ( $n = 64$ ) in HTx Rs. No significant benefit was observed

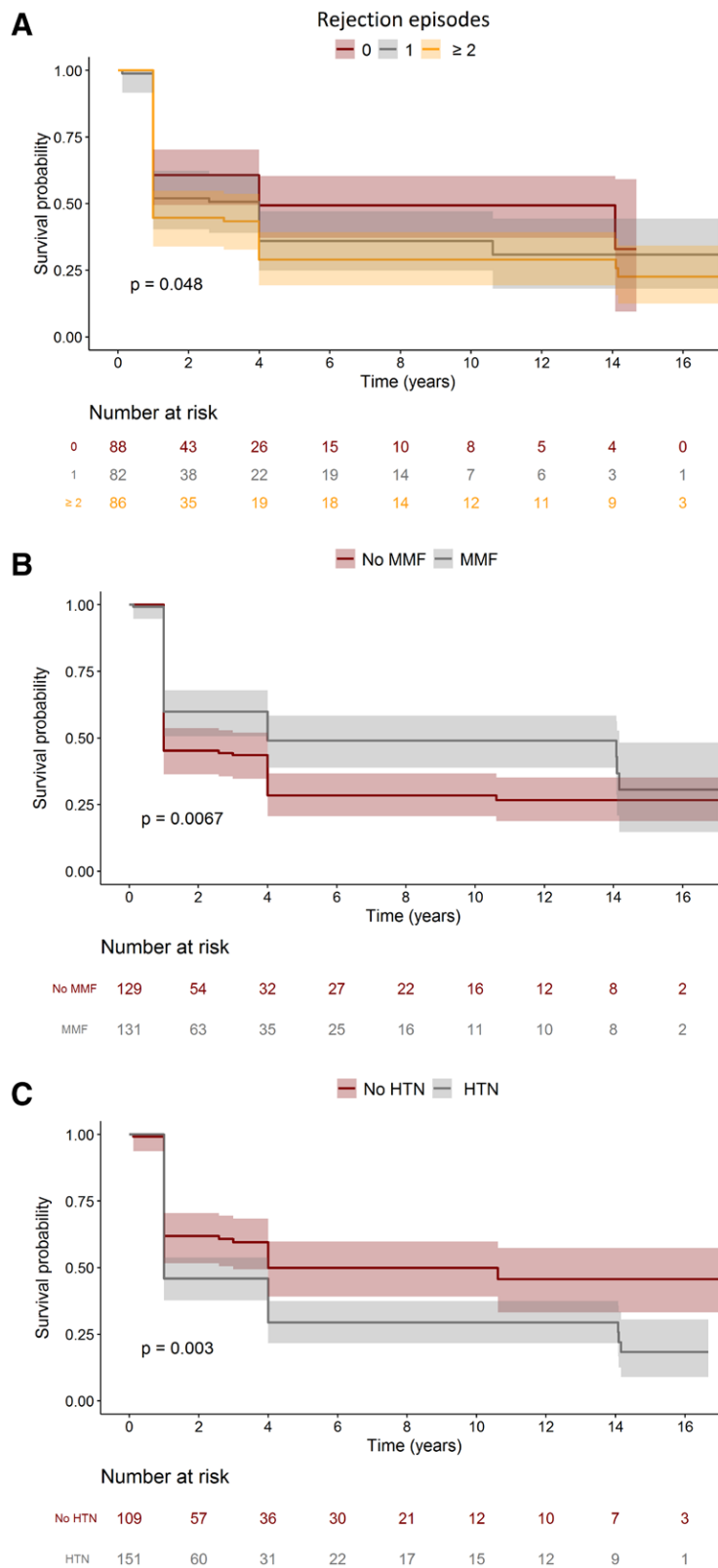


**FIGURE 6.** Cardiac allograft vasculopathy (CAV)-free survival curves—cytomegalovirus (CMV)-related factors: (A) clinically relevant CMV infection (CRCI), (B) CMV infection, (C) CMV breakthrough infection (CB). CMV denotes any CMV infection.

for any of the 2 treatment regimens.<sup>31</sup> Taking into account relatively small sample size of the study by Guethoff et al,<sup>31</sup> more prospective randomized trials are necessary to evaluate the potential benefit of immunosuppression in preventing the development of CAV.

Among nonimmunologic risk factors, we have observed a positive association between hypertension and CAV. Hypertension contributes to vascular endothelial inflammation and was mentioned as a risk factors by other studies.<sup>32</sup> No impact of diabetes or total cholesterol level was





**FIGURE 7.** Cardiac allograft vasculopathy (CAV)-free survival curves for statistically significant factors: (A) number of rejection episodes, (B) mycophenolate mofetil (MMF) treatment, (C) hypertension (HTN).

observed in multivariable model, although both D and R BMI as well as total cholesterol level were significant at univariable analysis. The results might be influenced by protective effect exerted by very high rate of treatment with pravastatin (95%) in our study cohort, which

is the standard approach, based on our knowledge that statins diminish the rate of CAV progression and patient survival.<sup>33,34</sup>

The main limitation of the study is heterogeneity with regard to type and duration of prophylaxis. Although most

of the patients were treated with VGCV (80%), still longer follow-up with uniformly treated group and larger numbers of CMV mismatch patients are required.

Based on the results of our study, we could identify clinically relevant concepts worth further investigation. First, it is necessary to maintain a balance between overimmunosuppression and underimmunosuppression, with CMV infection and ARs being the consequences of these states and, at the same time, factors predisposing for CAV. There is a need for a tool to evaluate the level of immunosuppression, which has not been fulfilled to date. Second, there is a group of patients at increased risk of CMV, for instance, patients who experience a breakthrough infection, who may benefit from more aggressive prophylaxis. Based on our results, immunoglobulins proved less effective than VGCV in preventing such breakthrough infection episodes. However, a combined therapy with VGCV and CMVIG could improve the efficacy of anti-CMV prophylaxis in selected patients (although selection of these patients may still pose a challenge). To date, no randomized direct comparison of CMVIG versus CMVIG plus VGCV has been performed. In a retrospective analysis, Snyderman et al<sup>35</sup> did not observe significant difference in mortality compared with antiviral monotherapy. However, there is interesting data from an IVUS retrospective analysis by Valantine et al<sup>36</sup> which show benefits for the addition of CMVIG, as well as encouraging data that show lower CMV DNA titers in D+/R- patients after a combined CMVIG + ganciclovir prophylaxis followed by 6 weeks VGCV compared with R+ patients, together with lesser extent of CAV measured by IVUS in this subgroup.<sup>37,38</sup> CMVIG could also be an additional option for those subjected to potent immunosuppression during rejection episodes.<sup>39</sup> Especially that >20% patients with clinically relevant CMV infection required concomitant treatment against AR and CMVIG was reported to provide additional benefit in the form of reduced rejection rates, an effect probably related to CMVIG immunomodulatory effects.<sup>38</sup>

In conclusion, in the era of contemporary immunosuppression and VGCV prophylaxis, a significant effect of CMV infection on the risk of CAV was observed only among HTx Rs with breakthrough CMV infection during anti-CMV prophylaxis. Further studies are warranted to evaluate the efficacy, dosage, and optimal timespan of currently applied anti-CMV VGCV prophylaxis.

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