ORIGINAL ARTICLE

Diminished impact of cytomegalovirus infection on graft vasculopathy development in the antiviral prophylaxis era – a retrospective study

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SUMMARY

Evidence concerning an association between cytomegalovirus (CMV) infection and accelerated cardiac allograft vasculopathy (CAV) is inconclusive. Data were analyzed retrospectively from 297 consecutive heart transplants between 1.1.2002 and 31.12.2012. Patients ≤18 years of age, survival, and follow-up ≤1-year post-transplant and patients with early CAV were excluded. CMV-infection was diagnosed and monitored closely in the first year. CAV was diagnosed by coronary angiography via left heart catheterization, and results were categorized according to the International Society of Heart and Lung Transplantation (ISHLT) scoring system. Risk factors for CAV were tested in a multivariable model. Median follow-up was 7.5 years (IQR: 5.6–10.3). CMV infection in the first year after transplantation occurred in 26% of patients (n = 78), CMV disease in 5% (n = 15). CAV ≥1 ISHLT was detected in 36% (n = 108). Incidence of CAV >1 ISHLT and severity of CAV increased over time. No statistically significant association between CMV infection and disease within the first year and risk of CAV after 1-year post-HTx was detected in the univariate (P = 0.16) and multivariable [hazard ratio (HR), 1.36; confidence interval (CI), 0.89–2.07; P = 0.16] Cox regression. In the multivariable Cox regression, donor age (HR, 1.04; 95% CI, 1.02–1.06; P < 0.01) and acute cellular rejection (ACR) ≥2R in the first year after HTx (HR, 1.77; 95% CI, 1.06–2.95; P = 0.03) were independent risk factors for CAV development. In our cohort, CMV infection and disease in the first year after transplantation did not significantly influence the risk of CAV in the long-term follow-up.

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Introduction

Cytomegalovirus (CMV) infection remains an important challenge in heart transplantation (HTx) recipients. Recent studies have reported an overall incidence of 16–30% in high-risk CMV-sero-negative recipients of a CMV-seropositive graft receiving conventional triple-drug immunosuppression, even when antiviral

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prophylaxis is used [1,2]. The direct effects of CMV infection include CMV syndrome, characterized by flu-like symptoms and neutropenia, and tissue-invasive CMV disease, with manifestations such as myelosuppression, pneumonitis, hepatitis, and gastric ulceration [3]. Indirect effects are also clinically significant. CMV infection is associated with an increased risk of acute rejection [4–6] and secondary infections [7]. Of particular concern is evidence that CMV infection may promote the development of cardiac allograft vasculopathy (CAV), a leading cause of death after the first post-transplant year [8]. Early studies from the era before CMV prophylaxis showed that CAV, as detected by coronary angiography, was accelerated in the presence of CMV infection [9–12]. More recently, a systematic review of retrospective and prospective studies found that the majority of analyses found no association between CMV infection and risk of CAV [13]. Contradictory evidence, however, comes from nonrandomized trials which have reported that universal CMV prophylaxis [12], or aggressive prophylaxis comprising CMV immunoglobulin (CMVIg) with antiviral therapy in high-risk patients [4], reduces intimal thickening when assessed by intravascular ultrasound. Clearly, the question of whether CMV infection affects progression of CAV has not yet been conclusively answered.

Prophylactic therapy for CMV infection is now frequently prescribed in HTx patients. It is generally considered mandatory after high-risk transplantation in which a CMV-seronegative patient receives a graft from a seropositive donor (R−/D+) [14–16] . In this subgroup, combined prophylactic therapy with ganciclovir and CMVIg has been shown to achieve a low rate of CMV disease and may reduce the rate of CAV progression [4,17,18] At our center, we routinely use induction with rabbit antithymocyte globulin (rATG; Thymoglobulin®), prompting universal CMVIg prophylaxis, as the high rates of CMV infection observed with early high-dose rATG regimens no longer apply, rATG induction still appears to increase the risk for CMV [19].

At the Medical University of Vienna, CMVIg is given prophylactically to all HTx recipients since 2002 (low-risk group since 2009), with the addition of valganciclovir in R−/D+ patients. A retrospective analysis was undertaken to evaluate the long-term effects of CMV infection or CMV disease on the incidence of CAV, in this setting.

Methods

This retrospective analysis was based on data obtained from the Medical University of Vienna Heart Transplant Database for consecutive patients receiving a heart transplant between 1 January 2002 and 31 December 2012. Patients were excluded if they were aged 18 years or less at time of transplant, if they survived less than 1-year post-transplant or had less than 1 year of follow-up data. First routine angiography is performed at 1-year post-transplant unless there exist clinical findings that suggest CAV. Unless the donor heart had an undetected coronary artery disease at time of transplant, CAV appears by 1 year at the earliest. Some patients had their first routine angiography not exactly 1 year after HTx but due to logistic reasons in month 11 or 12. Therefore, only patients with early CAV in the first 10 months after HTx were excluded. If a patient received more than one transplant, only the first transplant was evaluated. Approval for the study was obtained from the institutional review board.

During this period, all patients received induction with rATG. Maintenance immunosuppression comprised a calcineurin inhibitor (cyclosporine or tacrolimus) with either mycophenolate mofetil (MMF) or everolimus/sirolimus and steroids. Calcineurin inhibitor therapy was started after rATG induction, once serum creatinine was <1.5 mg/dl. Where used, MMF was administered at a dose of 2 g/day adjusted according to leukocyte and neutrophil counts. In patients given everolimus, the starting dose was 1.5 mg/day, adjusted to target levels of 3–8 ng/ml. For sirolimus, the starting dose was 3 mg/day with target levels of 5–10 ng/ml.

All patients received statins as prophylaxis against development of CAV [16].

In all cases where either the donor or the recipient were seropositive for CMV, 100 ml of CMVIg (Cytotect®; Biotest Pharmaceuticals Corporation, Dreieich, Germany) was administered intravenously on post-transplant days 1, 7, 14, 21, and 28. The center protocol was amended in late 2008, after which CMVIg was also given to CMV-seronegative recipients of a graft from a seronegative donor. Patients at high risk for CMV infection (R−/D+) received antiviral therapy comprising oral valganciclovir (450 mg b.i.d.) for 3 months. CMV infection was monitored by CMV deoxyribonucleic acid (DNA) on real-time polymerase chain reaction (PCR) weekly during month 1, then monthly until month 12, and subsequently when clinically indicated. CMV infection was defined as >1000 copies/ml. CMV disease was diagnosed if clinical signs of CMV infection were accompanied by a positive CMV DNA test. Clinical signs included new-onset leukopenia, flu-like clinical symptoms, or organ manifestations such as nonspecific gastrointestinal symptoms, CMV-related colitis with

diarrhea, CMV-related pneumonitis, fever of unknown origin, or subfebrile raised temperature.

All patients with CMV DNA >1000 copies/ml on any PCR test were treated preemptively with valganciclovir adjusted according to renal function.

Cardiac allograft vasculopathy was monitored by coronary angiography via left heart catheterization at 1, 3, 5, 7 years post-transplant and when clinically indicated by clinical signs and symptoms of CAV or any signs of CAV in coronary computed tomography angiography (CTA). If any intervention was carried out during the angiographic procedure, angiography was repeated 6 months later. Results were categorized according to the International Society of Heart and Lung Transplantation (ISHLT) scoring system [20]. In addition to angiography, the noninvasive CTA for surveillance of CAV has been used increasingly. Whenever there are signs of CAV detected in CTA (Agatston Score elevated/increasing or stenosis), coronary angiography is performed.

Acute cellular rejection was defined according to the ISHLT nomenclature [21]. All patients with cellular rejection ≥2R were treated and counted as acute cellular rejection in our analysis.

Patients were stratified according to CMV infection risk: low risk (R−/D−), intermediate risk (R+/D+ or R+/D−), or high risk (R−/D+).

Categorical variables are described by absolute and relative frequencies, continuous variables by the median (interquartile range, IQR). Associations between categorical variables were tested using the Chi-square test. The inverse Kaplan–Meier method was used to calculate the median follow-up time [22]. To evaluate the potential effect of the time-dependent event of a CMV infection on the incidence of CAV, a landmark analysis [23] was performed. Thereby, the time point 1-year post-HTx was defined as a landmark, and patients were grouped with respect to the occurrence or nonoccurrence of a CMV infection in the first year after HTx. Then, starting at the landmark time point, the time to first positive CAV (≥1) evaluation after 1-year post-HTx was considered as primary outcome variable. As a consequence of this landmark approach, patients with follow-up less than 1 year or with an event (death or CAV) before 1-year post-HTx, were not included in the analyses [23]. The probability of developing CAV was estimated by the cumulative incidence function (CIF), accounting for death as a competing event, and differences in the CIFs were compared between groups of patients calculating the Gray's test. The associations between risk factors for developing CAV and CAV post-transplant were analyzed using univariate and multivariable Cox regression analyses, including the following variables: CMV risk status, CMV infection, recipient and donor sex, recipient and donor age, acute cellular rejection ≥2R in the first year. *P*-values below 0.05 were considered statistically significant. All statistical computations were performed using SAS® version 9.2 software (SAS Institute, Cary, NC, USA).

Results

Study population

In total, 398 HTx procedures were performed during the study period. Twenty-nine patients were under 18 years old, and 59 patients died within the first year. After excluding 13 patients with early CAV in the first 10 months, 297 patients were available for analysis with a median follow-up of 7.5 years (IQR 5.6–10.3). 76% (n = 226) of the recipients and 71% (n = 210) of the donors were men. The median recipient age was 55 (IQR 45–61), and the median donor age was 39 (IQR 26–48) years (Table 1a and b). Indication for heart transplantation was most frequently dilative (62%; n = 185) and ischemic cardiomyopathy (28%; n = 82), with the remaining 10% accounted for hypertrophic cardiomyopathy, restrictive cardiomyopathy, congenital disease, and other rare conditions. Ten patients had their re-transplantation during the study period. Immunosuppression consisted of steroids in all patients, 64% (n = 189) were treated with cyclosporine, and the other 36% (n = 108) with tacrolimus. MMF was administered in 90% (n = 266) of cases, everolimus in 8%, and sirolimus in 2%. Cause of death was CAV associated with 4% (n = 13). Other causes of death were malignancy in 5% (n = 15), infection in 2% (n = 7), and in 7% (n = 20), other reasons like multi-organ failure (MOF), rejection, or unknown reasons.

Cellular rejection ≥ISHLT Grade 2 was detected in 15% (n = 46) in at least one biopsy, 38 of them in the first year after HTx.

CMV events according to CMV risk status

Asymptomatic CMV infection occurred in 26% (n = 78) and CMV disease in 5% (n = 15) of the patients in the first year after HTx. Only eight patients developed CMV infection and three patients CMV disease after the first year.

Dividing all patients into the CMV risk groups, 24% (n = 72) were in the high (R−/D+), 57% in the intermediate (R+/D−, n = 60; R+/D+, n = 108), and 19% (n = 57) in the low-risk group (R−/D−). Across the

Table 1. (a) Recipient and (b) donor demographics and baseline characteristics.

(a)	
n =	297
Follow-up, years, median (IQR)	7.5 (5.6–10.3)
Age, median (IQR)	55 (45–61)
Sex, male, % (no.)	76 (226)
CAV, % (no.)	36 (108)
CAV 1, % (no.)	31 (91)
CAV 2, % (no.)	9 (26)
CAV3, % (no.)	3 (10)
CMV infection, % (no.)	26 (78)
CMV disease, % (no.)	5 (15)
CMV risk group	
High risk (R−/D+), % (no.)	24 (72)
Intermediate risk (R+/D and R+/D+), % (no.)	57 (168)
Low risk (R−/D−), % (no.)	19 (57)
Immunosuppression, % (no.)	
Cyclosporine, % (no.)	64 (189)
Tacrolimus, % (no.)	36 (108)
Mycophenolate Mofetil, % (no.)	90 (266)
Everolimus, % (no.)	8 (26)
Sirolimus, % (no.)	2 (5)
Ischemic time, median (IQR)	180 (149–215)
Primary graft dysfunction, % (no.)	12 (35)
ACR >2 in the first year, % (no.)	13 (38)
(b)	
Age, median (IQR)	39 (26–48)
Sex, male, % (no.)	71 (210)
Hypertension, % (no.)	30 (82)
NIDDM, % (no.)	9 (25)
IDDM, % (no.)	8 (24)
BMI, median (IQR)	25 (23–28)

study population, CMV disease occurred only in the high- (n = 11) and the intermediate-risk group (n = 4).

Of the 93 cases with CMV infection or disease, all but two were treated successfully with valganciclovir, with CMV DNA levels decreasing to the normal range of <200 copies/ml after 2 weeks of treatment. The remaining two cases, both in R−/D+ patients, showed laboratory-confirmed ganciclovir resistance. One patient was successfully treated with cidofovir but developed renal failure, dying 6 months later due to septic complications. The other patient was treated in a compassionate use program with maribavir and experienced no complications.

Cardiac allograft vasculopathy

After excluding 13 patients with CAV in the first 10 months, 108 of the remaining 297 patients developed CAV ≥1 with a probability (CIF) of 16% (CI, 12–21%) at 2 years, 26% (CI, 22–32%) at 5 years and 40% (CI, 33–46%) at 10 years after HTx. The incidence and severity of CAV increased over time (Table 1a). The probability (CIF) of developing CAV ≥2 was 1% (CI, 0.3–3%), 5% (CI, 3–8%), and 12% (CI, 8–17%) and for CAV 3: 0%, 1% (CI, 0.3–3%) and 4% (CI, 2–7%) 2, 5, and 10 years after HTx, respectively. The cumulative incidence of CAV ≥1, CAV ≥2, and CAV 3 overtime is shown in Fig. 1.

Donor comorbidities (Table 1b) as hypertension (P = 0.81), IDDM (P = 0.23) and NIDDM (P = 0.93) were not associated with CAV in the univariate analysis (Cumulative Incidence Function), but older donor age (HR, 1.03; 95% CI 1.02–1.05; P < 0.01) and higher BMI (HR, 1.06; 95% CI 1.0–1.12; P = 0.04) were statistically significant associated with CAV in the univariate Cox regression model. In the multivariable Cox regression model, donor age (HR, 1.04; 95% CI, 1.02–1.06; P < 0.01) was associated with development of CAV (Table 2).

Neither primary graft dysfunction (PGD) (HR, 1.078; 95% CI 0.62–1.87; P = 0.79) nor longer ischemic time (HR 1.0; 95% CI 0.99–1.00; P = 0.58) was associated with CAV.

There is a borderline statistical significant difference with respect to the onset of CAV between patients with and without acute cellular rejection ≥2 in the first year after HTx (Fig. 2; Gray's test: P = 0.065). In the univariate (HR, 1.69; 95% CI 1.04–2.75; P = 0.03) and multivariable Cox regression model (HR, 1.77; 95% CI, 1.06–2.95; P = 0.03), acute cellular rejection

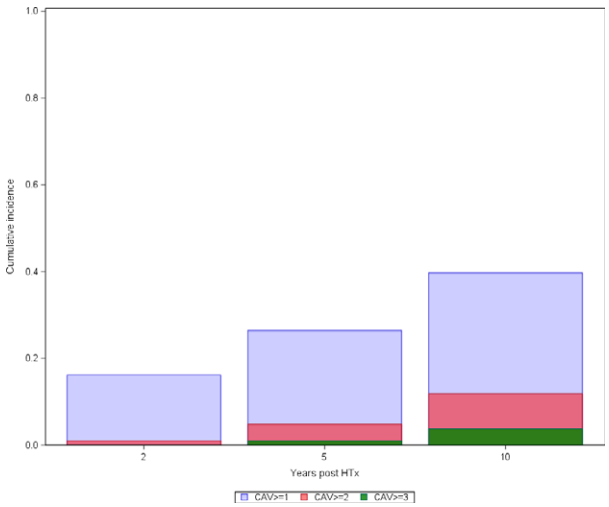

Figure 1 Cumulative incidence for cardiac allograft vasculopathy (CAV) ≥1, CAV ≥2, and CAV 3 angiographically detected 2, 5, and 10 years after heart transplantation.

Table 2. Multivariable Cox regression model for risk of cardiac allograft vasculopathy among patients surviving the first year post-transplant.

Prognostic factor	Hazard ratio	95% Confidence limit	*P*-value
Recipient age (per year)	0.99	0.98–1.01	0.455
Donor age (per year)	1.04	1.02–1.06	**<0.0001**
CMV group*			
(negative/positive)	0.89	0.47–1.72	0.986
(positive/negative)	0.96	0.51–1.82	
(positive/positive)	0.98	0.55–1.74	
CMV infection within 12 months post-HTx	1.36	0.89–2.07	0.157
Recipient sex (male versus female)	1.14	0.66–1.98	0.639
Donor sex (male versus female)	1.56	0.94–2.58	0.087
Rejection ≥2R within 12 months post-HTx	1.77	1.06–2.95	**0.028**

Significant *P*-values are shown in bold.

*CMV group (negative/negative) performs as reference category.

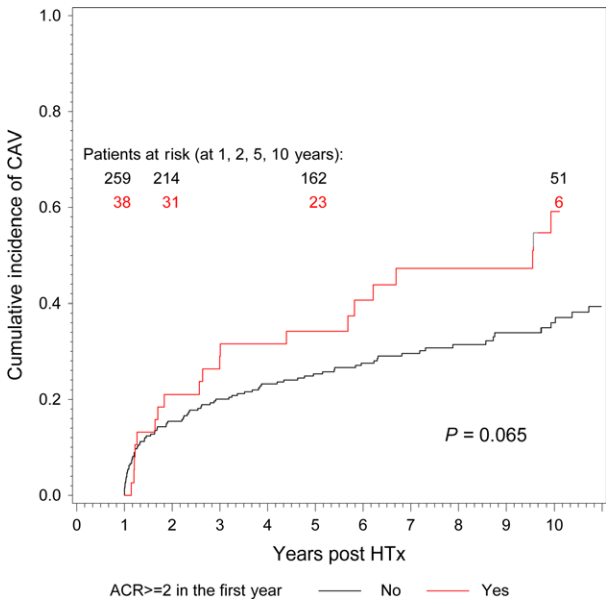

Figure 2 Cumulative Incidence of angiographically detected cardiac allograft vasculopathy according to acute cellular rejection ≥2 in the first 12 months.

(ISHLT ≥ Grade 2), in the first year after HTx was independently associated with CAV (Table 2).

CAV according to CMV infection

When onset of CAV was analyzed according to whether patients had developed CMV infection in the first year post-transplant, no statistically significant association between CMV infection and late risk of CAV was observed (Gray's test: $P = 0.16$) (Fig. 3). The estimated probability (CIF) of CAV ≥1 after 2, 5, and 10 years post-transplant for patients with CMV infection during the first year was 15% (CI, 9–23%), 29% (CI, 20–39%), and 46% (CI, 33–57%) and for patients without infection 17% (CI, 12–22%), 25% (CI, 19–31%), and 37% (CI, 29–45%).

In a subanalysis, we defined CAV either as ≥1 ISHLT by coronary angiography or as an Agatston Score >10 or stenosis in CTA or both. Twenty-three patients had an Agatston Score >10 or stenosis in CTA but no signs of CAV in coronary angiography. No statistically significant association between this definition of CAV and CMV infection was observed (Chi-square test: $P = 0.45$).

Discussion

In the analysis of this large cohort of heart transplant patients managed in a consistent manner with modern Immunosuppression protocols at a single center, neither CMV infection nor CMV disease in the first year was associated with significantly increased risk for late CAV. With aggressive systemic CMV, prophylaxis consisting of valganciclovir and CMVIg therapy, consequent monitoring and treatment of CMV infection and disease and the modern immunosuppression protocols the incidence of CAV 5 and 10 years after HTx is very low in our cohort. However, we cannot rule out if some or all of the above-mentioned factors attributed to these outcomes.

Our center is using CMVIg for all heart transplant recipients regardless of risk status which is not recommended in the ISHLT Guidelines for the Care of HTx recipients [16]. CMVIg is an expensive therapy which

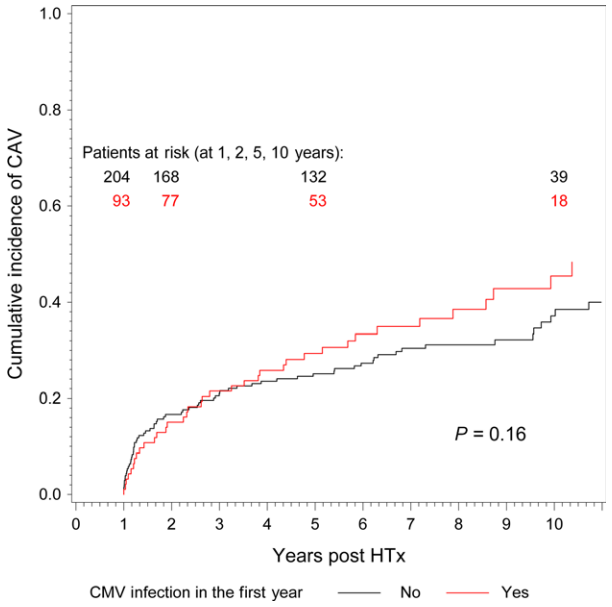

Figure 3 Cumulative Incidence of angiographically detected cardiac allograft vasculopathy according to cytomegalovirus infection in the first 12 months.

needs to be given intravenously and there is a lack of prospective, randomized trials about CMVIg prophylaxis but retrospective data suggest that addition of CMVIg to antiviral prophylaxis may lead to reduction in CMV-related complications [24]. We amended the protocol in late 2008 to extend CMVIg prophylaxis to low-risk CMV-seronegative recipients of a seronegative graft, based on evidence suggesting that CMVIg therapy is associated with a reduced risk of lymphoma in the first year after kidney transplantation [25].

Our findings are based on a large recent cohort of patients in whom CMV diagnosis was based on rigorous PCR monitoring during the first year post-transplant, with a median follow-up time of 7.5 years. Early detection and successful treatment of all patients with diagnosed CMV infection might be associated with our extremely low number of CMV disease and may have an impact on the low rate of late CAV as well. After the first year post-transplant, protocol-stipulated CMV monitoring stopped, and PCR was only performed in the event of clinically suspected CMV infection. Asymptomatic or misdiagnosed CMV infection therefore remained undetected, although such late-onset cases are rare, this is unlikely to have affected the results significantly. Detection of CMV infection varies between the HTx centers [26]. We implemented CMV-PCR routinely for detection of CMV infection in our center in 2002. As CMV-PCR could not detect <1000 copies/ml at that time, CMV infection was defined as >1000 copies/ml.

We used valganciclovir for CMV prophylaxis and treatment, CMVIg, a modern immunosuppressive regimen and early diagnosis of asymptomatic CMV infection with PCR. These factors may account for the difference between our findings and studies that showed a significant association of CMV and CAV. Therefore, our results might even underscore the importance of aggressive CMV prophylaxis and therapy. In a recent analysis reporting that CMV infection is associated with development of CMV, 166 patients undergoing heart transplant during 1995–2002 received only a 14-day course of intravenous ganciclovir with maintenance immunosuppression based on cyclosporine (and azathioprine up to 2002), without PCR-based monitoring of CMV [26]. In our series, the CMV management strategy remained consistent over time, other than introduction of CMVIg in the low-risk subgroup. Protocol coronary angiographic examinations were undertaken, the current standard for diagnosis of CAV [20]. While imperfect, angiography provides an effective screening tool for detection of CAV and is universally available and clinically acceptable. We are aware that intravascular ultrasound has the advantage of detecting angiographically silent CAV and is a more sensitive tool than angiography [27,28], but it is not established in our center for routine, longitudinal surveillance, mainly due to high costs [20]. It should be noted that the incidence of angiographically detected CAV in our cohort (40% probability at 10 years) was relatively low compared to published data [29–31].

Several other studies have documented that CMV infection does not influence occurrence of CAV, based on various prophylactic regimens and different diagnostic criteria [13]. Many studies examining this issue have not stated how CAV was defined or what diagnostic tools were used, while others were published more than 20 years ago, prior to modern antiviral treatments for CMV infection and when CMV infection was undetectable before the onset of CMV disease. These discrepancies may at least partly account for the variability of findings in the literature.

Other authors have previously described a benefit for intensive CMV prophylaxis regimens in limiting the progression of CAV [4,17,18,32]. In a prospective study of 66 heart transplant recipients reported by Potena *et al.* [4] in 2006, R−/D+ patients received CMVIg with antiviral prophylaxis (intravenous ganciclovir for 1 week, then valganciclovir for 2 months). CMV-seropositive recipients were given no CMVIg and only intravenous ganciclovir for 4 weeks. Intravascular ultrasound revealed slower progression of CMV in the aggressively

managed high-risk patients. Earlier, a retrospective study by Valantine *et al.* [18] demonstrated a lower prevalence and severity of intimal thickening following R−/D+ heart transplantation when CMVIg was added to ganciclovir. Using angiographic diagnosis, Bonaros and colleagues observed in a retrospective analysis that intensive prophylaxis with CMVIg and antiviral therapy significantly reduced the risk of CAV in high-risk recipients [17]. Antiviral prophylaxis alone, without CMVIg, also appears to reduce intimal thickening, as detected by intravascular ultrasound, compared to preemptive management [33]. These findings are compatible with evidence from heart transplant populations that recipient CMV-seropositivity [32] or CMV infection requiring treatment [34] are significantly associated with intimal thickness and lumen loss. Accordingly, aggressive anti-CMV therapy could be expected to suppress intimal thickening.

In our cohort, donor age and acute cellular rejection (≥2R) in the first year after HTx are independent risk factors associated with CAV, which is in line with several other studies [13,30,35]. As one could hypothesize that older donors have more cardiovascular comorbidities compared to younger donors and therefore higher incidence of CAV, we analyzed donor comorbidities as hypertension, IDDM, NIDDM, and BMI. Only donor BMI was associated with CAV in the univariate analysis.

There are some limitations beside the retrospective character of our study. A profound limitation is that not all of our patients (109 angiographic results after 5 years of follow-up, 21 results after 10 years) were followed consistently with angiography but with coronary (coronary CTA). If the patient was asymptomatic, without any clinical signs of CAV and a normal angiographic result after the first year, follow-up with coronary CTA was performed. Whenever there was a stenosis or Agatston Score >10 in coronary CTA, coronary angiography was performed. Based on our and others experience, coronary CTA seems to be a reliable and noninvasive imaging alternative for the detection of CAV [36]. Another limitation of the analysis is the relatively short median follow-up of 7.5 years compared to other studies and the ISHLT registry data [16,26]. Furthermore, all patients received CMVIg and therefore the lack of a control group is a major limitation.

In conclusion, the salient points from this large retrospective analysis are as follows. (i) In our cohort, CMV infection and disease in the first year after transplantation did not significantly influence the risk of CAV after the first year. (ii) Donor age and acute cellular rejection (≥2R) in the first year after HTx are independent risk factors for the development of late CAV.

Prospective, randomized trials are needed to determine the effect of CMV on the development of CAV under prophylactic CMV therapy, CMVIg prophylaxis, and modern Immunosuppression protocols.

Authorship

JG, AZ, PA, EO-J, RM, KU-U, F-MK, MM, GL and AZA-Z: collected and analyzed the data. AK: performed all statistical procedures. JG, AZA-Z and AZ: developed the manuscript. All authors critically reviewed the manuscript before publication.

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Conflicts of interest

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