# Viral Endomyocardial Infection Is an Independent Predictor and Potentially Treatable Risk Factor for Graft Loss and Coronary Vasculopathy in Pediatric Cardiac Transplant Recipients

Mousumi Moulik, MD,* John P. Breinholt, MD,† William J. Dreyer, MD,‡ Debra L. Kearney, MD,§
Jack F. Price, MD,‡ Sarah K. Clunie, RN,‡ Brady S. Moffett, PHARMD,‖ Jeffrey J. Kim, MD,‡
Joseph W. Rossano, MD,‡ John Lynn Jefferies, MD, MPH,‡ Karla R. Bowles, PHD,#
E. O'Brian Smith, PHD,¶ Neil E. Bowles, PHD,** Susan W. Denfield, MD,‡ Jeffrey A. Towbin, MD††

*Houston, Texas; Indianapolis, Indiana; Salt Lake City, Utah; and Cincinnati, Ohio*

**Objectives**     This study sought to evaluate the outcome and prevalence of viral endomyocardial infection after cardiac transplantation.

**Background**     Viral myocardial infection causes heart failure, but its role after cardiac transplantation is unclear. We hypothesized that viral infection of the cardiac allograft reduces graft survival.

**Methods**     Between June 1999 and November 2004, 94 pediatric cardiac transplant patients were screened for the presence of viral genome in serial endomyocardial biopsies (EMBs) using polymerase chain reaction (PCR) assays. Graft loss, advanced transplant coronary artery disease (TCAD), and acute rejection (AR) were compared in the PCR-positive (n = 37) and PCR-negative (n = 57) groups, using time-dependent Kaplan-Meier and Cox regression analyses. From November 2002 to November 2004, intravenous immunoglobulin therapy (IVIG) was administered to patients with PCR-positive EMBs. The outcomes of the IVIG-treated, PCR-positive patients (n = 20) were compared with IVIG-untreated, PCR-positive patients (n = 17).

**Results**     Viral genomes were detected in EMBs from 37 (39%) patients; parvovirus B19, adenovirus, and Epstein-Barr virus (EBV) were the most common. The PCR-positive group (n = 37, 25% graft loss at 2.4 years) had decreased graft survival (p < 0.001) compared with the PCR-negative group (n = 57, 25% graft loss at 8.7 years) and developed advanced TCAD prematurely (p = 0.001). The number of AR episodes was similar in both groups. On multivariate analysis, presence of viral genome was an independent risk factor for graft loss (relative risk: 4.2, p = 0.015). The time to advanced TCAD after becoming PCR-positive was longer in the IVIG-treated patients (p = 0.03) with a trend toward improved graft survival (p = 0.06).

**Conclusions**     Viral endomyocardial infection is an independent predictor of graft loss in pediatric cardiac transplant recipients. This effect appears to be mediated through premature development of advanced TCAD. IVIG therapy in this subgroup may improve survival and merits further investigation.     (J Am Coll Cardiol 2010;56:582–92) © 2010 by the American College of Cardiology Foundation

Over the last 2 decades, the prevalence of heart failure has significantly increased in the developed world (1). Simultaneously, cardiac allograft transplantation has become the definitive therapy for end-stage heart disease. However, the long-term survival after cardiac transplantation is limited (2,3). Several donor- and recipient-specific risk factors for

cardiac graft loss have been identified (2,3), the majority of which are not amenable to modification. Viral allograft infection is a potential risk factor amenable to therapy and deserves further evaluation.

Viral myocarditis of the native heart is an established etiology for dilated cardiomyopathy (4,5). We have hypothesized that viral infection of the post-transplant heart is also detrimental. The histologic diagnostic criteria for myocarditis, the Dallas criteria, rely heavily on the combination of inflammatory infiltrate, myocyte necrosis, edema, and fibrosis (6). Cardiac transplant rejection, as defined by the International Society of Heart and Lung Transplantation (7), appears similar to the criteria for myocarditis. It is possible, therefore, to speculate that the 2 disorders are related, both triggered by viral infection. Viral genome has been detected in the cardiac allograft after transplantation (8–11) and is associated with an increased risk for rejection and graft loss (8,11). A similar association has also been shown in lung and renal transplant recipients (12,13).

Viral infections, especially cytomegalovirus (CMV), have been implicated in the pathogenesis of coronary atherosclerosis in the general population and transplant coronary artery disease in cardiac transplant patients (14–16). Treatment with ganciclovir and anti-CMV immunoglobulin decreases the risk of transplant coronary artery disease (TCAD) in cardiac transplant recipients with systemic CMV infection (17,18). Intravenous immunoglobulin (IVIG) therapy for acute viral myocarditis is common in many centers, based on studies suggesting a beneficial role of IVIG in these patients (19,20). In addition, IVIG has been utilized for its immunomodulatory effects in transplant recipients with viral infection as well as other conditions with possible immune-mediated, infectious agent–triggered etiologies (21,22).

In this study, we compared the outcomes of cardiac transplant patients with viral polymerase chain reaction (PCR)-positive versus -negative endomyocardial biopsies (EMBs), as well as the outcomes of IVIG-treated PCR-positive patients with that of PCR-positive, IVIG-untreated counterparts.

## Methods

**Patient cohort.** All consecutive cardiac transplant patients followed in Texas Children's Hospital between June 1, 1999, and November 30, 2004, were eligible for selection. Five patients who had undergone cardiac transplantation in another institution and 2 patients who did not undergo any EMBs due to lack of vascular access or clinical instability were excluded. The final study cohort consisted of 94 patients. Seven patients transferred care to another institution prior to completion of the study and were censored after the last day of patient encounter.

Baseline recipient and donor characteristics, immunosuppressive regimen, and post-transplant patient course data were retrospectively collected from hospital records. The study cohort was divided into 2 exposure groups, based on the presence or absence of viral genome in their EMBs. An overview of the study design and analysis is given in Figure 1.

**EMB, histopathology, and PCR.** Serial surveillance right ventricular EMBs were performed as per an established schedule (Online Table 1). Patients with concern for acute graft dysfunction also underwent nonscheduled EMBs. Histological grading of biopsy specimens was performed according to Texas Heart Institute and International Society of Heart and Lung Transplantation criteria (7,23). PCR analysis of all EMBs for the presence of adenovirus, parvovirus B19, Epstein-Barr virus (EBV), CMV, and enteroviral genomes was performed by individuals who were blinded to the clinical course, as previously described (11). Detection of viral genome in EMBs was considered diagnostic of viral endomyocardial infection (current or past) for purposes of this study.

**Outcomes.** The patient cohort was followed for the outcomes of all-cause graft loss, advanced TCAD, and acute rejection (AR) until June 30, 2005. All patients underwent baseline coronary angiograms 3 months after transplantation followed by annual screening coronary angiograms, starting from 1 year after transplantation. TCAD was diagnosed on the basis of either the coronary angiograms or the histological evaluation of the cardiac allograft in patients who suffered graft loss. Severity of TCAD was graded as defined by Cardiac Transplant Research Database (CTRD) criteria (Online Table 2) (24) and classified as advanced TCAD if criteria for moderate or severe TCAD were met. AR was diagnosed by the treating physicians in conjunction with the cardiac pathologist on the basis of the clinical picture and histopathology results of EMBs. All rejection episodes were treated by pulse steroid therapy plus a change in short- and/or long-term immunosuppressive therapy.

**IVIG treatment.** From November 2002 to November 2004, the management of viral endomyocardial infection in cardiac transplant patients at our institution included administration of a single dose of 1 g/kg IVIG, given after each PCR-positive biopsy, either during the same hospital visit or on a follow-up visit. All patients with any virus-positive EMBs were eligible to receive IVIG. This treatment protocol was discontinued after November 2004 due to an institutional restriction on off-label use of IVIG, triggered in part by a shortage of IVIG supplies nationally. As a subgroup analysis, outcome data from the viral PCR-positive IVIG-treated patients were compared with their IVIG-untreated viral PCR-positive counterparts (historic or concurrent) as controls.

| | |
|---|---|
| **Figure 1** | **Overview of Study Design With Patient Selection, Follow-Up, and Analysis** |

IVIG = intravenous immunoglobulin; PCR = polymerase chain reaction; TCAD = transplant coronary artery disease.
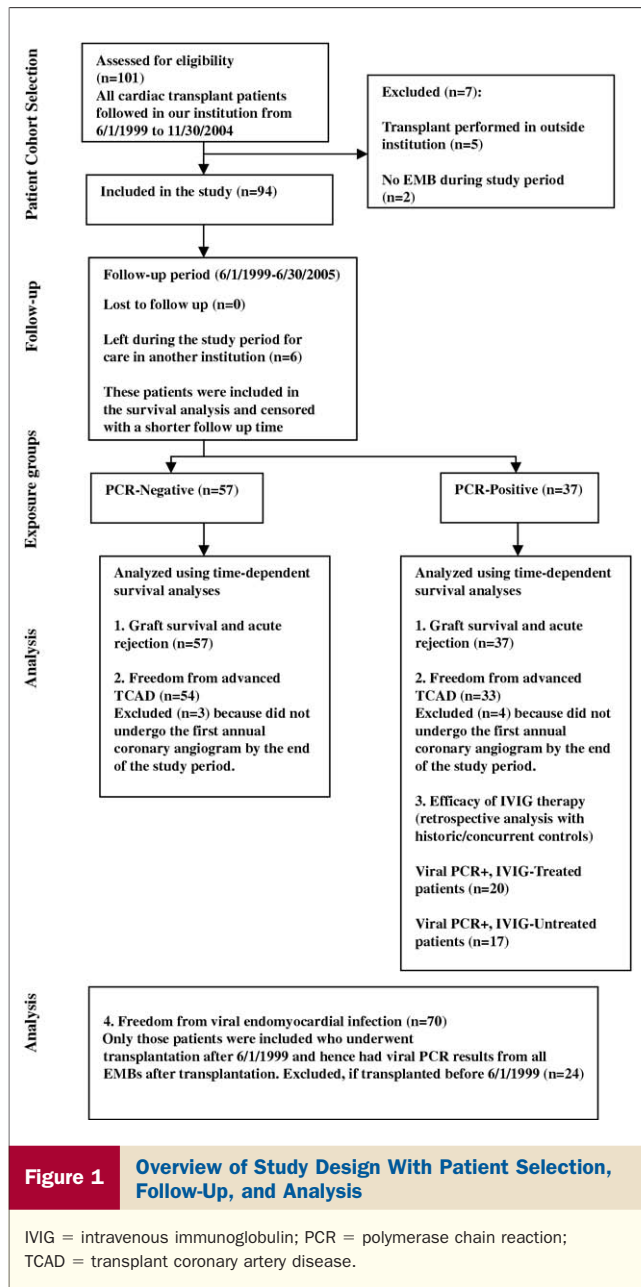
**Statistical analysis.** Univariate analysis for freedom from an event was performed using Kaplan-Meier analysis and log-rank test, and multivariate analysis using Cox proportional hazards regression. Viral endomyocardial infection was treated as a time-dependent binary covariate for survival analyses. AR episodes were compared using Mann-Whitney test and Poisson regression. Covariates between the 2 groups were compared using *t* test, chi-square, or Fisher exact tests. A p value of <0.05 was considered significant. Patients who had transferred care to another institution prior to completion of the study were considered lost to follow-up and were censored as "alive" on the last day of their follow-up in the survival analysis. Analysis was done

with Stata version 8.2 software (Stata Corp., College Station, Texas).

## Results

**Patient cohort and baseline characteristics.** The study cohort (n = 94) had 39 females (41.5%) and 55 (58.5%) males, with a mean age of 6.5 ± 5.5 years (range 0.06 to 18.3 years) at the time of transplantation. The mean follow-up period from the time of transplantation to the completion of the study was 4.5 years (range 0.16 to 16.22 years) for a total of 420 patient-years. Congenital heart disease (34 of 94, 36%) was the most common indication for cardiac transplantation, followed by dilated cardiomyopathy (33 of 94, 35%), restrictive cardiomyopathy (13 of 94, 14%), and cardiac retransplantation (12 of 94, 13%). All children were initially treated with a standard triple-drug immunosuppressive regimen consisting of cyclosporine or tacrolimus, prednisone, and azathioprine or mycophenolate mofetil. No induction therapy was used. The antiproliferative agent (azathioprine or mycophenolate) was discontinued in 41% of the patients at an average of 1.5 years after transplantation, due to persistent leukopenia. The patients were then maintained on dual therapy with steroids and calcineurin inhibitors. The baseline characteristics of the viral PCR-positive and -negative groups are shown in Table 1. Differences between the 2 groups included; the PCR-positive group had a higher mean recipient age (p = 0.007) and weight (p = 0.002) at transplant, higher mean donor age (p = 0.004), higher number of retransplants (p = 0.02), more patients with viral genome detected in the explanted heart (p = 0.03), and more patients on left ventricular assist device/extracorporeal membrane oxygenation at transplant (p = 0.05). There was no difference in the pattern of immunosuppression between the PCR-positive and -negative groups. The PCR-positive and -negative groups were similar with respect to the initial triple-drug therapy combination. There was no statistically significant difference in the 2 groups with respect to the percentage of patients in whom azathioprine or mycophenolate was discontinued and immunosuppression transitioned to a combination of prednisone and a calcineurin inhibitor alone. No adjustments in immunosuppression were made if virus was detected in the myocardium.

Of the 94 patients in the study cohort, 24 underwent transplantation before the onset of the study in June 1999. Of these, 19 remained PCR-negative and 5 became PCR-positive during the course of the study. At their entry into the study, the mean time from transplantation was 5.19 years in the 19 PCR-negative patients and 3.84 years in the 5 PCR-positive patients. The average follow-up period after transplantation was 4.99 years in the PCR-negative patients and 3.64 years in the PCR-positive patients.

**Viral PCR results of EMBs.** PCR was performed on 928 serial EMBs from the 94 study patients. Viral genome was

amplified from the myocardium of 39% (37 of 94) patients and 8.9% (83 of 928) of the biopsies. Parvovirus B19 genome was amplified most commonly and was found in 71.1% of all positive biopsies, from 24.5% (23 of 94) patients (62.1% of PCR-positive patients). Adenoviral genome was detected in 9 (9.6%) patients (24.3% of PCR-positive patients), EBV in 8 (8.5%) patients (21.6% of PCR-positive patients), CMV in 4 (4.3%) patients, and enterovirus in 1 (1%) patient (Fig. 2A). Eight patients (8.5%) had more than 1 virus type amplified, either in the same (3 cases) or follow-up EMB (5 cases). Viral genome persisted for >6 months in 40% (15 of 37) of the virus-positive patients; 14 of these patients were parvovirus B19–positive and 1 was EBV-positive. Majority of patients with parvovirus-positive EMBs showed evidence of chronic persistence of viral genome, with 61% (14 of 23) positive for parvovirus >6 months after the initial detection, and in the subgroup where a follow-up EMB was available more than a year after the initial parvovirus-positive EMB, 88% (14 of 16) showed persistence or recurrence of parvoviral genome >1 year after the initial detection. A change in the infecting virus was noted over the study period (Fig. 2B) with increasing incidence of parvovirus B19 from 0% of the biopsies in 1999 to 18% in 2004 (p < 0.001, Fisher exact) and decreasing incidence of adenovirus from 7.5% of the biopsies in 1999 to 0% in 2004 (p < 0.001, Fisher exact). Most of the viral endomyocardial infections were clinically silent and detected on routine surveillance biopsies. Only 1 patient with parvovirus-positive biopsy developed aplastic anemia in our patient cohort.

**Risk for viral endomyocardial infection.** Patients were most prone to develop viral endomyocardial infection in the first year after cardiac transplantation; 59% (22 of 37) of the viral PCR-positive patients had their first viral PCR-positive EMB within the first year after transplantation. To get a more accurate estimation of how the time period from transplantation affected the risk for viral endomyocardial infection, we performed a Kaplan-Meier analysis of freedom from viral endomyocardial infection after transplantation on the 70 patients (transplanted after June 1, 1999) on whom viral PCR status of all the EMBs performed after transplantation was known. The cumulative probability of developing viral endomyocardial infection was 35% (95% confidence interval [CI]: 26% to 48%) by the end of the first year after transplantation and 63% (95% CI: 48% to 78%) by the end of the fifth year after transplantation (Fig. 2C).

**Long-term graft survival.** Graft survival was decreased (p < 0.001, log-rank) in the PCR-positive group compared with the PCR-negative group. The risk for graft loss in the PCR-positive group was 4.2 (p = 0.015, 95% CI: 1.33 to 13.29) times that of the PCR-negative group, after adjusting for recipient age, recipient weight, recipient sex, donor age, retransplantation, and AR episodes, using Cox regression analysis. The median graft survival was 4.8 years in the PCR-positive group (Fig. 2D) compared with 12.4 years in

the whole cohort. The median graft survival time in the PCR-negative patients could not be estimated due to lack of enough graft failures during the study period. Eleven (35.5%) of the PCR-positive patients and 11 (19.3%) of the PCR-negative patients lost their grafts (death or retransplantation) during the study period. The causes of graft loss are listed in Online Table 3. After excluding patients with nonallograft-specific causes of mortality (malignancy, aplastic anemia, and pulmonary vein stenosis), the PCR-positive group still had a higher risk for premature graft loss (p = 0.01, log-rank). Persistence of viral genome in EMBs for longer than 6 months did not further increase the risk of graft loss. Risk factors evaluated for and predictive of graft loss are listed in Online Tables 4 and 5, respectively.

**Advanced TCAD.** Data on coronary arteries were not available in 7 of the 94 study patients, as they were within the first year after transplantation and had not undergone the first annual screening coronary angiography by the end of the current study. Of the remaining 87 patients on whom coronary angiography or coronary histopathology results were available, 33 (38%) patients were PCR-positive and 54 (62%) patients were PCR-negative. One patient developed advanced TCAD before detection of viral endomyocardial infection and was considered as PCR-negative for purposes of this analysis. Average follow-up time after transplantation was 5.15 years in the PCR-negative patients compared with 3.87 years in the PCR-positive patients. Fourteen patients (16%) developed advanced TCAD: 7 (21.2%) of the PCR-positive and 7 (12.9%) of the PCR-negative patients. PCR-positive patients developed advanced TCAD prematurely compared with the PCR-negative group (p = 0.001, log-rank) and had an 8.3 times higher risk for developing advanced TCAD (p = 0.001, 95% CI: 2.48 to 28.02) (Fig. 2E). After adjusting for time from transplantation at entry into the study, being PCR-positive remained a risk factor for premature development of advanced TCAD (p = 0.002, log-rank). The risk for developing premature advanced TCAD in the PCR-positive group was 6.8 (p = 0.01, 95% CI: 1.47 to 32.06) times that of the PCR-negative group, after adjusting for recipient weight, age, and sex, donor age, retransplantation, time from transplantation, and AR episodes, using Cox regression analysis. Persistence of viral genome in EMBs for longer than 6 months did not further add to the risk of developing advanced TCAD. The median time to developing advanced TCAD was 4.8 years in the PCR-positive group compared with 12.2 years in the whole cohort. The median time to developing advanced TCAD in the PCR-negative group could not be calculated due to insufficient number of events during the study period.

**Acute graft rejection.** A total of 101 rejection episodes occurred in 62 of the 94 patients during the study period. No statistically significant association was found between viral endomyocardial infection and AR. Concomitant rejection was present in 8.3% of the PCR-positive biopsies and 8.6% of the PCR-negative biopsies.

| Table 1 | Baseline Demographics and Covariates in the PCR-Positive and -Negative Groups | | |
|---|---|---|---|
| **Characteristic** | **PCR-Positive Group** | **PCR-Negative Group** | **p Value** |
| **Patients** | 37 | 57 | |
| Recipient sex | | | NS |
| Male | 20 (54%) | 35 (61.4%) | |
| Female | 17 (46%) | 22 (38.6%) | |
| Recipient ethnic group | 34/37 | 52/57 | NS |
| White | 13 (38.2%) | 23 (44.2%) | |
| Black | 5 (14.7%) | 10 (19.2%) | |
| Hispanic | 15 (44.1%) | 18 (34.6%) | |
| Asian | 1 (2.9%) | 1 (1.9%) | |
| Others | 0 (0%) | 0 (0%) | |
| Recipient age | 37/37 | 57/57 | 0.01 |
| Mean ± SD | 7.9 ± 5.64 | 5.1 ± 5.03 | |
| Median | 7.5 | 3.0 | |
| Range | 0.1–18.3 | 0.06–17.6 | |
| Recipient age category, yrs | | | NS |
| <1 | 4 (10.8%) | 10 (17.5%) | |
| >1 | 33 (89.2%) | 30 (82.5%) | |
| Recipient weight, kg | 28.3 ± 3.28 | 17.1 ± 1.74 | 0.002 |
| Indication for transplantation | | | NS |
| CHD | 16 (35.6%) | 18 (36.7%) | |
| DCM | 14 (31.1%) | 20 (40.8%) | |
| RCM | 4 (8.9%) | 9 (18.4%) | |
| Retransplant | 9 (20.0%) | 2 (4.1%) | |
| Other | 2 (4.4%) | 0 (0%) | |
| UNOS waiting-list status | | | NS |
| 1 | 30 (68.2%) | 31 (63.3%) | |
| 2 | 14 (31.8%) | 18 (36.7%) | |
| Wait time, days (mean) | 132 | 153 | NS |
| Ventilator dependence pre-transplantation | 12 (29.3%) | 11 (26.8%) | NS |
| LVAD/ECMO dependence pre-transplantation | 6 (13.9%) | 1 (2.4%) | 0.05 |
| Ionotrope dependence pre-transplantation | 27 (65.9%) | 21 (51.2%) | NS |
| Hospitalization pre-transplantation | | | NS |
| ICU | 18 (41.7%) | 14 (33.3%) | |
| Non-ICU | 10 (23.3%) | 8 (19.1%) | |
| Catheterization | | | |
| PSP, mm Hg (mean) | 29.0 | 30.0 | NS |
| PCWP, mm Hg (mean) | 13.6 | 15.2 | NS |
| PVR, Woods Units (mean) | 3.2 | 2.7 | NS |
| CMV status seropositivity | | | |
| Recipient | 17 (42.5%) | 17 (39.5%) | NS |
| Explant myocarditis | 10 (29.4%) | 10 (21.3%) | NS |
| Viral PCR-positive explant | 8 (26.7%) | 2 (6.1%) | 0.03 |
| **Donors** | | | |
| Donor cold ischemia time, min (mean) | 246 | 230 | NS |
| Donor sex | | | NS |
| Male | 22 (57.9%) | 23 (59%) | |
| Female | 16 (42.1%) | 16 (41%) | |
| Donor ethnicity | | | NS |
| White | 22 (61.1%) | 18 (50.0%) | |
| Black | 5 (13.9%) | 10 (27.8%) | |
| Hispanic | 8 (22.2%) | 8 (22.2%) | |
| Asian | 1 (2.8%) | 0 (0.0%) | |
| Other | 0 (0.0%) | 0 (0.0%) | |
| Donor age, yrs | | | 0.004 |
| Mean ± SD | 9.0 ± 6.60 | 5.2 ± 5.13 | |
| Median | 8.5 | 3.0 | |
| Range | 0.33–23 | 0.06–19 | |

| Table 1 | Continued | | | |
|---|---|---|---|---|
| **Characteristic** | **PCR-Positive Group** | **PCR-Negative Group** | **p Value** | |
| Donor age category, yrs | | | NS | |
| 0–1 | 4 (12.5%) | 9 (19.6%) | | |
| 10–20 | 16 (50.0%) | 27 (58.7%) | | |
| 20–30 | 11 (34.4%) | 10 (21.7%) | | |
| 30–40 | 1 (3.1%) | 0 (0.0%) | | |
| Donor CMV seropositivity | 25 (58.1%) | 27 (56.3%) | NS | |
| Recipient–donor mismatch variables | | | | |
| Sex mismatch | 17 (44.7%) | 17 (43.6%) | NS | |
| Ethnicity mismatch | 21 (60.0%) | 22 (64.7%) | NS | |
| Recipient–donor age ratio | 1.0 ± 0.49 | 1.3 ± 1.18 | NS | |
| Recipient–donor weight ratio | 0.8 ± 0.23 | 0.84 ± 0.18 | NS | |
| CMV mismatch: negative recipient, positive donor | 14 (36.8%) | 15 (35.7%) | NS | |
| HLA crossmatch positive | 4 (13.8%) | 1 (3.1%) | NS | |
| Post-transplantation course | | | | |
| Initial post-transplant hospital stay, days | 38.1 ± 30.44 | 27.2 ± 66.93 | NS | |
| Baseline immunosuppression | | | | |
| Cyclosporin | 94.6% | 90.9% | NS | |
| Tacrolimus | 5.4% | 9.1% | NS | |
| Azathioprine | 31.4% | 41.2% | NS | |
| Mycophenolate | 68.6% | 55.8% | NS | |
| Prednisone | 100% | 100% | NS | |
| Percentage of patients transitioned to 2-drug regimen due to persistent leukopenia | 32.4% | 46.4% | NS | |
| Acute rejection episodes per patient-year | 0.52 ± 0.83 | 0.58 ± 1.09 | NS | |
| Number of rejection episodes in the first year after transplantation | 1.0 ± 1.19 | 0.8 ± 0.89 | NS | |
| CMV viremia | 54.1% | 42.6% | NS | |
| EBV viremia | 62.6% | 66.7% | NS | |

Values are n, n (%), or mean ± SD unless otherwise indicated.

CHD = congenital heart disease; CMV = cytomegalovirus; DCM = dilated cardiomyopathy; EBV = Epstein-Barr virus; ECMO = extracorporeal membrane oxygenation; HLA = human leukocyte antigen; ICU = intensive care unit; LVAD = left ventricular assist device; NS = not significant; PCR = polymerase chain reaction; PCWP = pulmonary capillary wedge pressure; PSP = pulmonary systolic pressure; PVR = pulmonary vascular resistance; RCM = restrictive cardiomyopathy; UNOS = United Network for Organ Sharing.

**Virus subtype and outcome.** After adjusting for viral subtypes using Cox regression, adenoviral (p = 0.03) and EBV (p = 0.002) endomyocardial infections were associated with decreased graft survival (Fig. 2F). The risk for graft loss was 4.1 (95% CI: 1.13 to 14.73) times higher in the adenovirus-positive patients and 8.5 (95% CI: 2.18 to 33.56) times higher in the EBV-positive patients compared with the PCR-negative patients. However, the predicted graft survival for parvovirus endomyocardial infection was similar to that of PCR-negative patients. This was confounded by the fact that the majority (17 of 23, 74%) of the parvovirus-positive patients received IVIG therapy after a PCR-positive biopsy, which could have had a beneficial effect on the outcome. After adjusting for viral subtypes using Cox regression, EBV (p = 0.03) and parvovirus (p = 0.005) endomyocardial infections were associated with premature development of advanced TCAD. The risk for advanced TCAD was 7.5 (p = 0.03, 95% CI: 1.76 to 45.07, Cox regression) times higher in the EBV-positive patients and 7.1 (p = 0.005, 95% CI: 1.78 to 28.02, Cox regression) times higher in the parvovirus-positive patients compared with the PCR-negative patients. No statistically significant

association was detected between AR and individual virus subtypes in our study cohort.

**Effect of IVIG therapy on the outcome of patients with PCR-positive biopsies.** Of the 37 patients with viral PCR-positive EMBs, 20 (54%) were treated with IVIG following a PCR-positive biopsy. The remaining 17 (46%) who did not receive IVIG therapy served as treatment-naive controls. The mean time to IVIG treatment was 16.2 days from the PCR-positive biopsy (range 0 to 136 days). The number of IVIG doses that a patient received ranged from 1 to 3 doses (mean 1.6 doses, median 1 dose), with 7 patients receiving more than 1 IVIG dose due to multiple PCR-positive biopsies. The baseline characteristics of the IVIG-treated patients and IVIG-untreated controls are given in Table 2. Due to the surge in parvovirus-B19– positive EMBs in 2003 and 2004, the years during which IVIG therapy was administered for PCR-positive viral endomyocardial infection, the IVIG-treated PCR-positive group had a disproportionately higher number of parvovirus-positive patients compared with the IVIG-untreated PCR-positive group. The other viral subtypes were not significantly different in the 2 groups. Three (15%)
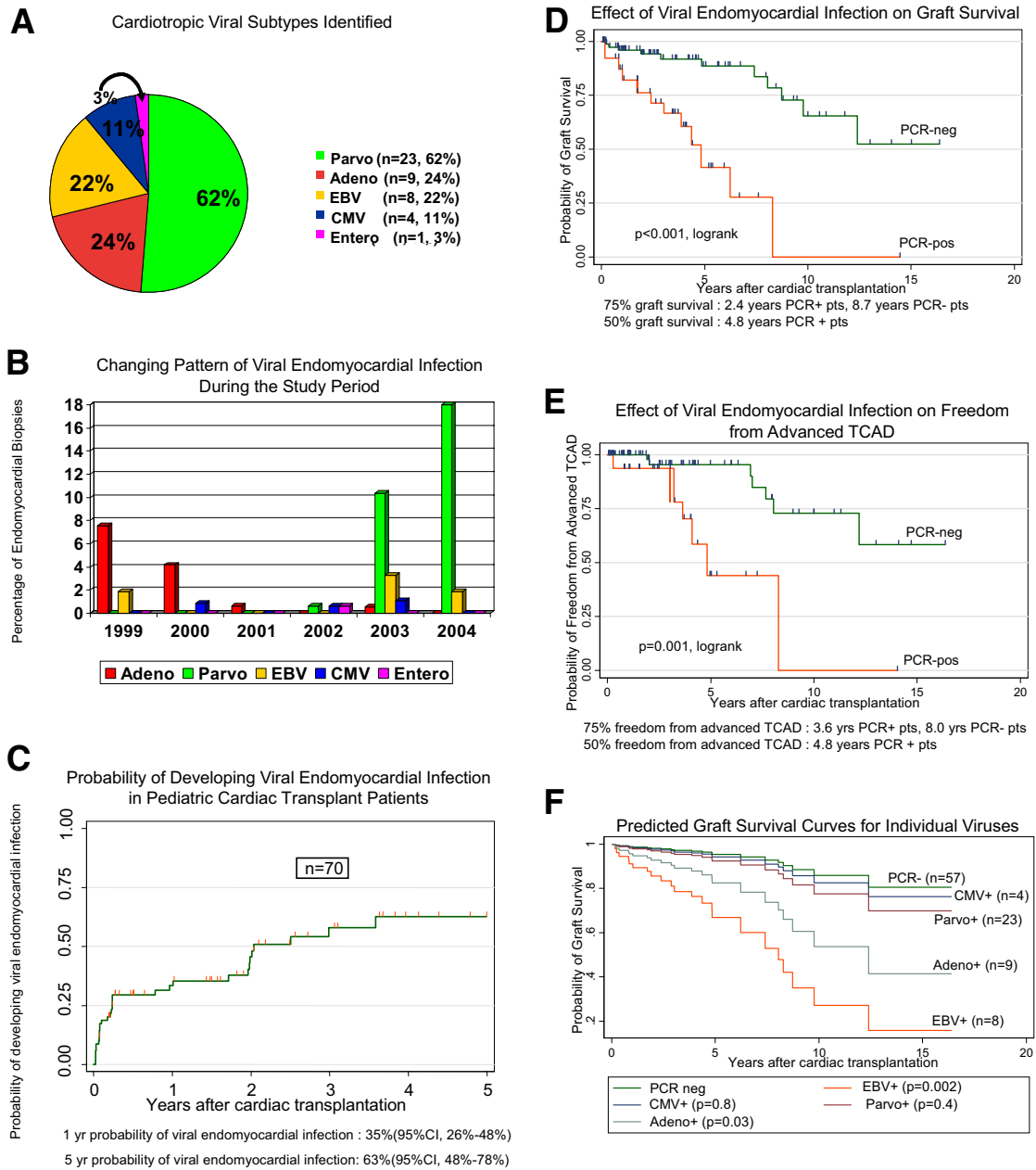
**Figure 2** | **Prevalence and Outcome of Viral Endomyocardial Infection in a Pediatric Cardiac Transplant Cohort**

**(A)** Pie chart showing the viral subtypes of the polymerase chain reaction (PCR)-positive viral endomyocardial infections identified; parvovirus B19, adenovirus, and Epstein-Barr virus (EBV) were most commonly identified. **(B)** Bar graph showing a changing trend in the viral composition of PCR-positive viral endomyocardial biopsy during the study period from 1999 to 2004 with a surge in parvovirus B19 in 2003 and 2004, and a concomitant decrease in adenovirus-positive biopsies. **(C)** Kaplan-Meier curve for freedom from viral endomyocardial infection after cardiac transplantation shows that the first year post-transplantation is the highest risk period. **(D)** Kaplan-Meier curves for graft survival show that graft survival is worse in the viral PCR-positive patients compared with the viral PCR-negative patients (p < 0.001, log-rank). **(E)** Kaplan-Meier curves for freedom from advanced transplant coronary artery disease (TCAD) show that the viral PCR-positive patients develop advanced TCAD prematurely compared with the PCR-negative group (p = 0.001, log-rank). **(F)** Predicted survival curves for individual viruses after Cox regression show that patients with adenoviral and EBV endomyocardial infection have a worse outcome compared with PCR-negative or cytomegalovirus (CMV)-positive or parvo-positive patients.

of the 20 IVIG-treated patients and 8 (47%) of the 17 IVIG-untreated patients suffered graft loss. Graft survival from the date of transplant (p = 0.09, log-rank) (Fig. 3A), and graft survival after becoming PCR-positive (p = 0.06, log-rank) (Fig. 3B) trended to be better in the IVIG-treated

group compared with the IVIG-untreated group. The 3-year survival after becoming PCR-positive was 86% (95% CI: 54% to 96%) in the IVIG-treated patients, compared with 33% (95% CI: 8% to 62%) in the IVIG-untreated patients (p = 0.03, log-rank).

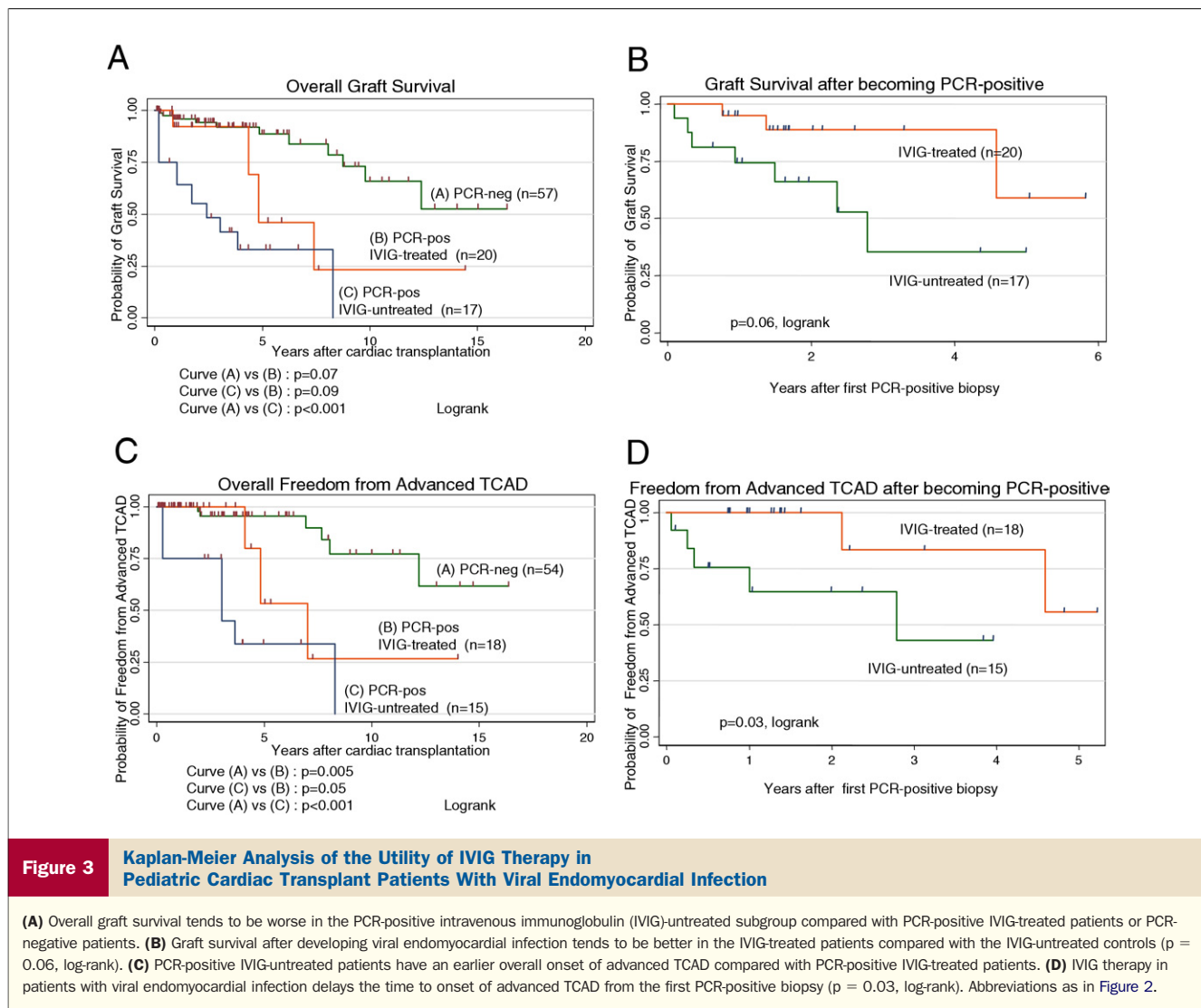| Table 2 | Baseline Characteristics of the IVIG-Treated and -Untreated Viral PCR-Positive Patients | | |
|---|---|---|---|
| | **PCR-Positive** | | |
| **Characteristic** | **IVIG-Treated Group** | **IVIG-Untreated Group** | **p Value** |
| **Patients** | 20 | 17 | |
| Recipient sex | | | NS |
| Male | 9 (45%) | 11 (65%) | |
| Female | 11(55%) | 6 (35%) | |
| Recipient ethnic group | | | NS |
| White | 7 (39%) | 6 (38%) | |
| Black | 2 (11%) | 3 (19%) | |
| Hispanic | 8 (44%) | 7 (43%) | |
| Asian | 1 (6%) | 0 (0%) | |
| Recipient age, yrs | 7.1 ± 6.01 | 9.8 ± 5.19 | NS |
| Recipient weight, kg | 24.4 ± 20.63 | 35.0 ± 22.05 | NS |
| Indication for transplantation | | | NS |
| CHD | 8 (40%) | 3 (18%) | |
| CM | 9 (45%) | 7 (41%) | |
| Retransplantation | 3 (15%) | 5 (29%) | |
| Other | 0 (0%) | 2 (12%) | |
| UNOS waiting-list status | | | NS |
| 1 | 13 (76%) | 11 (65%) | |
| 2 | 4 (24%) | 6 (35%) | |
| Wait time, days (mean) | 157 | 153 | NS |
| **Donors** | | | |
| Donor cold ischemia time, min | 259 | 231 | NS |
| Donor sex | | | NS |
| Male | 11 (73%) | 9 (56%) | |
| Female | 4 (27%) | 7 (44%) | |
| Donor ethnicity | | | NS |
| White | 8 (57%) | 11 (73%) | |
| Black | 2 (14%) | 0 (0%) | |
| Hispanic | 4 (29%) | 3 (20%) | |
| Asian | 0 (0%) | 1 (0%) | |
| Donor age, yrs | 7.5 ± 6.79 | 11.3 ± 6.93 | NS |
| CMV mismatch: negative recipient, positive donor | 4 (21%) | 7 (50%) | NS |
| **Post-transplant viral endomyocardial infection** | | | |
| Viral subtypes | | | |
| Adenovirus | 4 (20%) | 5 (29%) | NS |
| EBV | 5 (25%) | 3 (18%) | NS |
| Parvovirus B19 | 17 (85%) | 6 (35%) | 0.03 |
| CMV | 1 (5%) | 3 (18%) | NS |
| Enterovirus | 1 (5%) | 0 (0%) | NS |
| Endomyocardial infection with >1 virus | 6 (30%) | 2 (12%) | NS |
| Mean time to first PCR+ EMB after transplantation, yrs | 1.5 | 2.0 | NS |

Values are n, n (%), or mean ± SD unless otherwise indicated.
CM = cardiomyopathy; IVIG = intravenous immunoglobulin; other abbreviations as in Table 1.

Freedom from advanced TCAD, measured from the time of transplant, was better in the IVIG-treated patients (p = 0.05, log-rank) (Fig. 3C). The onset of advanced TCAD after becoming PCR positive was delayed in the IVIG-treated patients compared with the untreated control group (p = 0.03, log-rank) (Fig. 3D). The 3-year freedom from advanced TCAD after the first PCR-positive biopsy was 82% (95% CI: 24% to 97%) in the IVIG-treated patients compared with 45% (95% CI: 11% to 75%) in the IVIG-untreated patients (p = 0.03, log-rank). IVIG therapy did not affect the number of AR episodes after becoming PCR positive. In addition, it did not seem to help in the clearance of viral genome. Viral genome was amplified from the follow-up biopsy in 48% of the IVIG-treated biopsies and 37% of the IVIG-untreated biopsies (p = NS, chi-square). A valid comparison of virus-free time after a PCR-positive biopsy could not be performed as the follow-up biopsies were obtained at varying time intervals.

**Figure 3**    **Kaplan-Meier Analysis of the Utility of IVIG Therapy in Pediatric Cardiac Transplant Patients With Viral Endomyocardial Infection**

**(A)** Overall graft survival tends to be worse in the PCR-positive intravenous immunoglobulin (IVIG)-untreated subgroup compared with PCR-positive IVIG-treated patients or PCR-negative patients. **(B)** Graft survival after developing viral endomyocardial infection tends to be better in the IVIG-treated patients compared with the IVIG-untreated controls (p = 0.06, log-rank). **(C)** PCR-positive IVIG-untreated patients have an earlier overall onset of advanced TCAD compared with PCR-positive IVIG-treated patients. **(D)** IVIG therapy in patients with viral endomyocardial infection delays the time to onset of advanced TCAD from the first PCR-positive biopsy (p = 0.03, log-rank). Abbreviations as in Figure 2.

## Discussion

In this retrospective single-institution study, we show that occult viral endomyocardial infection (diagnosed by viral PCR-positive EMBs) after transplantation is common in pediatric cardiac transplant patients and is associated with premature graft loss. We also show that viral endomyocardial infection is an independent risk factor for premature loss of the cardiac allograft and appears to mediate this effect through premature development of advanced TCAD. The greatest risk for developing viral endomyocardial infection is in the first year after transplantation (which is also the time of maximal immunosuppression), parvovirus B19 infection being the most common, followed by adenovirus and EBV. Parvoviral B19 infection has a tendency for chronicity. IVIG therapy may improve graft survival and delay onset of advanced TCAD in the viral PCR-positive patients and merits further evaluation.

**Study limitations.** Our study is limited by its retrospective nature and low number of events, raising concerns for

inability to compensate for all baseline differences and overfitting. The possibility that some of the patients classified as viral PCR-negative could have had undetected transient viral endomyocarditis may have resulted in an inadvertent selection bias and inflated or deflated the true strength of association between viral endomyocardial infection and an adverse outcome. However, these results replicate our findings from a prior prospective trial performed in a completely different cohort of pediatric cardiac transplant patients followed in a completely different institution and, hence, are less likely to represent an alpha error (11). The IVIG therapy subgroup analysis is limited by an uncontrolled, retrospective analysis, small sample size, and unequal distribution of viral subtypes in the IVIG-treated and -untreated groups.

The prevalence of advanced TCAD was much higher in our patient cohort compared with recently published prevalence rates in pediatric heart transplant recipients in a multi-institutional study, with a 17% probability of devel-

oping advanced TCAD within 5 years in our cohort, compared with a 6% 5-year probability in the Pahl et al. study (25). This could be secondary to a difference in patient demographics, immunosuppressive regimens, acute rejection episodes, incidence of viral endomyocarditis, or differences in case ascertainment, as coronary angiography interpretation is limited by subjectivity.

As indicated in Figure 2F, the presence of parvovirus does not appear to be associated with decreased graft survival (even though it was associated with higher risk for developing advanced TCAD). It is possible that since the majority of parvovirus infections were detected in the later half of the cohort, there may not have been enough time to demonstrate an adverse effect on graft survival. In addition, since many of the parvovirus-positive patients were treated with IVIG, it is also possible that the graft survival curve has been normalized due to the treatment. The number of patients was too small to provide a significant comparison between the IVIG-treated and -untreated parvovirus groups.

An association between the presence of adenovirus, enterovirus, or CMV genome in the myocardium and AR has been previously described (8,10,11), but the lack of such an association in our study (which had a parvovirus predominance) leads us to speculate that unlike adenovirus, enterovirus, and CMV, parvovirus does not result in AR, but may still cause premature TCAD by chronic host-mediated immunologic response. The isolation of predominantly parvovirus genome from cardiac allograft biopsies compared with adenovirus genome as previously reported by our group (11) leads us to speculate that similar to recent reports in myocarditis patients (26), there has been an epidemiologic shift in the predominant virus responsible for endomyocardial infection after cardiac transplantation, from adenoviral to a parvoviral predominance.

Therapeutic measures such as interferon beta therapy, immunoglobulins, or cellular immune therapy have been found to be beneficial in persistent viral myocarditis (27,28) and need to be explored in cardiac transplant patients who develop viral endomyocardial infection.

## Conclusions

We show that viral infection of the endomyocardium is common in pediatric cardiac transplant recipients and is an independent risk factor for graft loss. This effect on graft loss seems to be mediated through premature development of advanced TCAD. Our data suggest that an adverse outcome may be delayed by using IVIG therapy. Hence, serial PCR screening of surveillance endomyocardial biopsies for the presence of cardiotropic viruses is indicated in pediatric cardiac transplant recipients. This will be crucial to the development and evaluation of antiviral or other novel therapeutic measures and potentially could improve long-term survival outcomes in these at-risk children.

**Reprint requests and correspondence:** Dr. Jeffrey A. Towbin, The Heart Institute, Division of Pediatric Cardiology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, Ohio 45229. E-mail: jeffrey.towbin@cchmc.org.

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▶ **APPENDIX**

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**For supplemental tables, please see the online version of this article.**