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Safety and Early Outcomes Using a Corticosteroid-Avoidance Immunosuppression Protocol in Pediatric Heart Transplant Recipients

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Abstract

Background—Long-term oral corticosteroids have been a mainstay of maintenance immunosuppression in pediatric heart transplantation. In this study, we report early clinical outcomes in a cohort of pediatric heart transplant recipients managed using a steroid-avoidance protocol.

Methods—Of the 70 patients who underwent heart transplantation during the study period, 55 eligible recipients, including 49 non-sensitized and 6 sensitized (all 55 with negative cross-match) patients, entered a steroid-avoidance immunosuppression protocol consisting of thymoglobulin induction followed by a 2-drug tacrolimus-based, corticosteroid-free regimen. The primary outcome variable was freedom from moderate rejection (International Society for Heart and Lung Transplantation [SHLT] grade 2R/3A or antibody-mediated rejection).

Results—The median age at transplant was 7.1 years (range 2 weeks–22 years) and median follow-up 19 months (range 2–46 months). Fifty patients survived to discharge after transplantation. Of these patients, 2 (4%) were discharged on steroids and 8 (16%) started on maintenance steroids at follow-up. Rejection was diagnosed in 8 patients (Grade 2R cellular rejection in 3 and antibody-mediated rejection in 5). Freedom from rejection was 92% at 6 months (95% confidence interval [CI], 80% to 97%) and 87% at 1 year (CI 73% to 94%). Post-transplant survival was 91% at 6 months (CI 79% to 96%) and 88% at 12 and 24 months (CI 75% to 95%). There was 1 death due to rejection (antibody-mediated) 8 months after transplantation

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Disclosures

There are no conflicts of interest to disclose.

Conclusions—An immunosuppression protocol consisting of induction followed by corticosteroid avoidance appears to achieve acceptable rejection rates during the first year post transplant in pediatric heart transplant recipients.

Keywords

Corticosteroids; children (pediatric); immunosuppression; rejection; heart transplantation

Long-term oral corticosteroids remain a mainstay of standard maintenance immunosuppression in adult and pediatric heart transplant recipients. A common clinical practice consists of a gradual weaning of oral steroids with a goal of discontinuation within the first year post-transplant.^{1, 2} However, 60% of all children reported to the registry of the International Society of Heart and Lung Transplant (ISHLT) Registry, who underwent heart transplantation between 2001 and 2005, remained on maintenance prednisone therapy at 1 year after transplant and 40% remained on maintenance prednisone therapy at 5-years post-transplant.³ As the survival after heart transplantation continues to improve in the pediatric population, there is a concern that morbidity complications associated with long-term corticosteroid therapy such as stunted growth, hypertension, glucose intolerance/diabetes, hyperlipidemia, weight gain and osteoporosis may affect long-term outcomes and quality of life in these children. These metabolic side effects may be minimized by a “steroid-avoidance” approach to immunosuppression in pediatric heart transplant recipients.

The term “steroid-avoidance” usually suggests complete withdrawal of corticosteroids from immunosuppression regimen after the induction period (first week) but has been used to describe immunosuppression with steroid-use up to the first 6 weeks post-transplant in pediatric heart transplantation.^{4–9} Steroid-avoidance immuno suppression has indeed been reported previously in pediatric heart transplantation but almost exclusively from institutions that used echocardiography-based rejection surveillance and/or had a large neonatal heart transplant population.^{4–9} In this report, we describe outcomes in a cohort of pediatric heart transplant recipients who entered a steroid-avoidance maintenance immunosuppression protocol at the time of their transplant with rejection surveillance consisting of frequent endomyocardial biopsies.

METHODS

Subjects

This is a retrospective analysis of 55 consecutive children who underwent heart transplantation at one of two heart transplant centers, Children’s Hospital Boston or Cleveland Clinic Children’s Hospital, during the study period (2005–2009) and entered a steroid-avoidance immunosuppression protocol at the time of transplant. All patients who were non-sensitized against human leukocyte antigen (defined as those with panel reactive antibodies (PRA) <10%) and had a negative donor-specific flow cytometry cross-match at the time of transplant were managed using the protocol (n=49). In addition, 6 patients who were sensitized but had a negative cross-match at the time of transplant were also managed using the protocol. Fifteen patients who underwent heart transplant during the study period were not considered eligible for this protocol at the time of their transplant due to their

higher risk for antibody-mediated rejection. The study was approved by the Institutional Review Boards of the two centers with a waiver of informed consent.

Immunosuppression

All patients received induction therapy using rabbit antithymocyte globulin (Thymoglobulin; Genzyme Corp.) at 1.5 mg/kg/dose, and pre-treated with an intravenous steroid dose (methylprednisolone) for a median duration of 5 days (range 3–6 days). Patients were started on oral tacrolimus 48 to 72 hours after transplant (divided 2 or 3 times a day based on age) as their renal function improved, and on mycophenolate mofetil (1,200 mg/m² per day, divided 2 or 3 times per day) after completing induction therapy. The target trough levels for tacrolimus were 8 to 12 ng/ml during the first three months, 6 to 10 ng/ml 3 to 12 months post-transplant, and 6 to 8 ng/ml after the first year. Mycophenolate mofetil dose was adjusted to achieve a target trough level of 2 to 4 mg/dl.

Rejection Surveillance

All patients underwent serial, routine cardiac catheterization studies with endomyocardial biopsy after transplant to assess right heart hemodynamics and evaluate for allograft rejection. Biopsies were performed approximately 2, 4, 6, 9, 12, 16, and 24 weeks, and at 9 and 12 months after heart transplantation. The biopsy schedule was modified for infants <1 year of age after the first two biopsies (if there was no rejection) to limit the number of first-year biopsies to 4 to 6. All patients also underwent a routine transthoracic echocardiographic assessment of graft function on the day of each endomyocardial biopsy. Patients were monitored non-invasively between biopsies and underwent at least one echocardiography study during the time period between two consecutive biopsies.

Infection Prophylaxis

Patients who were seropositive for cytomegalovirus (CMV) infection pre-transplant received CMV prophylaxis post-transplant using intravenous ganciclovir followed by oral valganciclovir. Patients who were CMV-negative but received transplant from a seropositive donor (primary mismatch) also received prophylaxis as just described. When both the transplant recipient and the donor were seronegative, no prophylaxis was used beyond the induction therapy. The duration of prophylaxis was different for patients at the two centers (3 months in Boston and one month in Cleveland). Periodic screening for CMV activation using a quantitative CMV antigen assay (blood) was performed after completing CMV prophylaxis at both centers. Periodic screening for Epstein-Barr Virus (EBV) viremia using a quantitative polymerase chain reaction (PCR) was also performed.

All patients received trimethoprim-sulfamethoxazole for prophylaxis against *Pneumocystis jirovecii* infection, and oral nystatin (or clotrimazole) for prophylaxis against candidal infection.

Study Outcomes

The primary outcome variable was time to first rejection episode, defined as meeting one of the following criteria: (1) an endomyocardial biopsy showing ISHLT grade 2R (old grade 3A) cellular rejection, or (2) antibody-mediated rejection.¹⁰ Antibody-mediated rejection

was defined when three of the following four criteria were met: (1) graft dysfunction, (2) presence of a circulating donor-specific antibody, (3) positive immunofluorescence for C4d in endomyocardial biopsy, and (4) endothelial swelling with interstitial edema on light microscopy. The secondary outcomes analyzed were infections (CMV, EBV, others) and patient survival.

Statistical Analysis

The study cohort was described using median (range or interquartile range as indicated) and counts (percent). The Kaplan-Meier method was used to estimate event-free rates with their 95% confidence intervals at 6, 12, and 24 months post-transplant. Patients were censored at the time of death or on June 22nd, 2009, the last day of data collection. Analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC).

RESULTS

Study Population

The baseline characteristics of the study cohort are outlined in Table 1. There were only 3 neonates < 30 days old in the cohort at transplant. Seven of the 17 patients with congenital heart disease had previously undergone Fontan single-ventricle palliation; 4 of these had protein-losing enteropathy and 1 had plastic bronchitis. The median stay in the intensive care unit after heart transplantation was 6 days (Inter-quartile range, [IQR] 5 to 10 days) and median hospital stay was 19 days (IQR 14 to 32 days). Median post-transplant follow-up was 19 months (range 2 to 46 months, IQR 8 to 27 months).

Patient Survival

Overall post-transplant survival at the two participating centers (including 15 patients not in the study cohort) was 93% at 6 months, 89% at 12 months, and 87% at 24 months post-transplant respectively. Post-transplant survival in the study cohort (n=55) was 91% at 6 months (95% confidence interval [CI] 79% to 96%), and 88% at both 12 and 24 months [CI] 75% to 95%, Figure 1). There were 6 deaths, 5 of which were early hospital deaths in the post-transplant period due to multiple organ failure. None of the hospital deaths involved rejection. Three deaths involved infants who received a heart transplant while supported on extracorporeal membrane oxygenation support (two with post-operative Norwood palliation for hypoplastic left heart syndrome and one with hypertrophic cardiomyopathy with systolic dysfunction). The fourth death involved a 6-month old infant with scimitar syndrome, hypoplastic right lung and total anomalous pulmonary venous return to the left innominate vein who developed pulmonary hemorrhage, right heart failure and renal failure post-operatively. The fifth death involved a 10-year old child who underwent transplantation due to debilitating plastic bronchitis several years after a Fontan operation. He developed anuric renal failure in the immediate post-operative period, was managed using hemodialysis but died of pulmonary hemorrhage 2 weeks post-transplant.

Fifty patients (89%) survived transplant hospitalization to discharge. Of these, one patient died due to antibody-mediated rejection 10 months after transplant. This patient was

sensitized pre-transplant, had a negative cross-match at the time of transplant but developed donor-specific antibodies on follow-up. There were no deaths due to infection.

Steroid-Free Immunosuppression

Maintenance immunosuppression after completing induction was modified in all 5 patients who died during their transplant hospitalization. To avoid/delay calcineurin-inhibitor administration in the presence of acute renal dysfunction, steroids were continued beyond the first 5 post-operative days in these patients. Among the 50 hospital survivors, 2 patients were discharged on maintenance steroids - an infant with ABO-incompatible heart transplant who was weaned off steroids over 4 months and a teenager with post-operative Fontan and pre-transplant renal dysfunction whose renal function worsened on tacrolimus-based immunosuppression which was then switched to sirolimus plus prednisone.

Eight patients (16%) were started on maintenance steroids after a variable duration of steroid-free regimen. Seven of these were started on maintenance steroids to augment immunosuppression following a rejection episode or after they developed a donor specific antibody. One patient with protein losing enteropathy pre-transplant was started on maintenance steroids 6 months post-transplant due to persistent protein-losing enteropathy.

Graft Rejection

Rejection episodes occurred in 8 of 55 (14.5%) patients (cellular rejection in 3 and antibody-mediated rejection in 5 patients). Six of these 8 patients were > 10 yrs old at the time of their transplant. Freedom from first rejection was 92% at 6 months (CI 80% to 97%), 87% at 1 year (CI 73% to 94%) and 81% at 2 years (CI 64% to 90%) after heart transplant (Figure 2). Freedom from first *cellular* rejection was 97% at 6 months (CI 88% to 99%), 95% at 1 year (CI 86% to 98%) and 95% at 2 years (CI 86% to 98%). Of the 5 patients who developed antibody-mediated rejection, only one patient had PRA >10% prior to transplant. Rejection episodes were treated using intravenous pulse steroids in 4 patients, plasmapheresis in 2 patients, oral steroid pulse in one patient, and intra-venous immune globulin in one patient.

Infections and Other Complications

Eleven patients (20%) in the study cohort were detected to have CMV antigenemia following transplant, all within 6 months of transplant. Ten of these 11 patients had a primary CMV mismatch. Nine of the 11 patients were asymptomatic and were diagnosed on routine monitoring. One patient presented with CMV syndrome (fever, weight loss) and one developed asymptomatic CMV antigenemia during augmented immunosuppression for graft rejection. EBV viremia was detected using a quantitative viral PCR in 8 patients. Two of these patients were sero-positive pre-transplant, 3 had a primary mismatch with their donor and 3 developed a primary EBV infection. In 7 of these 8 patients, EBV viremia was asymptomatic and diagnosed on routine surveillance. These patients were managed with serial monitoring and/or adjustment in immunosuppression. One patient developed primary infectious mononucleosis that progressed to post-transplant lymphoproliferative disease.

One teenage transplant recipient developed insulin-dependent diabetes mellitus 2 years post-transplant. Another teenage patient developed transient glucose intolerance one-week post-

transplant and was treated with insulin for 2 weeks. Both patients had a family history of diabetes. Thirty-six (72%) of the 50 hospital survivors were treated with an anti-hypertensive medication at the time of discharge; antihypertensive treatment was continued beyond 3 months post-transplant in 31 patients and beyond one year in 17 patients.

DISCUSSION

This is the first report of a tacrolimus-based steroid-avoidance immunosuppression protocol in pediatric heart transplant recipients and demonstrates its clinical safety in this patient population. None of the early post-transplant mortality in the cohort was related to immunosuppression. Although the percentage of patients who achieved a steroid-free state is similar to that described using a steroid-weaning approach, the steroid-avoidance approach achieved this with very little overall corticosteroid exposure (limited to the first week post-transplant).¹ The incidence of rejection after heart transplantation appears to have declined in the recent era recipients due to efficacious immunosuppressant regimens.¹¹ In the current study cohort, the risk of rejection during the first 6 months after transplant was less than 10% and appeared to be lower than that reported in pediatric heart transplant recipients in ISHLT 2009 report.¹² The incidence of antibody-mediated rejection in the cohort (5 patients) may be related to steroid avoidance, may be an aberration (due to small sample size) or may reflect its improved recognition in practice due to frequent monitoring for donor specific antibodies and immune fluorescence staining of biopsies. Although 16% of transplant recipients in follow-up were started on maintenance steroids to augment their immuno suppression, the protocol was not associated with increased risk of heart allograft loss or patient death. The early survival appears similar to that reported in pediatric recipients in ISHLT 2009 report.¹²

Steroid-avoidance immunosuppression in pediatric heart transplantation has been previously reported from programs in Loma Linda, California and Denver, Colorado in the United States, and Harefield and Newcastle-upon-Tyne in the United Kingdom.^{4-6, 8, 9, 13, 14} In all these reports, the immunosuppression was cyclosporine-based and the rejection-surveillance was performed non-invasively using echocardiography imaging. Because moderate cellular rejection (ISHLT grade 3A/2R) by endomyocardial biopsy is not necessarily associated with accompanying echocardiographic changes in clinical practice¹⁵, a widespread acceptance of these protocols (or that of echocardiography-based rejection surveillance) has not occurred. Furthermore, the majority of heart transplant recipients in the Loma Linda program have been neonates or young infants who may need less immunosuppression than older children to prevent graft rejection.^{6, 13, 14} Our study describes a tacrolimus-based immunosuppression protocol using biopsy-based rejection surveillance in transplant recipients distributed across the pediatric age-group.

Induction therapy with T-cell lymphocyte depletion therapy (anti-thymocyte globulin) was given as a part of steroid avoidance protocol. We used intermittent rather than continuous dosing of anti-thymocyte globulin.¹⁶ Induction therapy using either thymoglobulin or an interleukin-2 (IL-2) receptor antagonist (dacluzimab or basiliximab) has facilitated steroid-free immunosuppression in renal transplant recipients with low rejection rates and excellent clinical outcomes.¹⁷⁻²⁰ A study in adult heart transplant recipients recently reported results

of a steroid avoidance protocol in a small cohort of 16 patients following thymoglobulin induction.²¹ It is unknown whether induction using an IL-2 receptor antagonist rather than lymphocyte-depletion at transplant would allow successful steroid avoidance immunosuppression in heart transplant recipients.

This study has the same limitations that apply to all retrospective descriptive studies. There were no concurrent controls with baseline characteristics similar to patients in this series. Fifteen patients who were not initiated on a steroid-avoidance protocol during the study period (such as those who were highly sensitized and/or those with a positive cross match) received a different regimen including induction, 3-drug immunosuppression (and antibody depletion followed by IVIG in presence of a positive cross match) because they were at higher-risk of antibody-mediated rejection. Furthermore, the clinical care protocols for patients in this series were similar in the two participating institutions but not identical (for example, different duration of CMV prophylaxis). These may have affected the morbidity rates in the two institutions that we chose not to compare due to small sample size.

In conclusion, an immunosuppression protocol consisting of thymoglobulin induction followed by steroid-avoidance was associated with low incidence of rejection during the first year after transplant in pediatric heart transplant recipients. A minority of patients were initiated on maintenance steroids to augment immunosuppression. A larger cohort with longer follow-up is required to assess long-term safety and efficacy of this strategy of immunosuppression in pediatric heart transplant recipients.

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Figure 1.
Kaplan-Meier survival curve with 95% confidence intervals illustrating post-transplant patient survival for the study cohort

Figure 2.

Kaplan-Meier survival curve with 95% confidence intervals depicting freedom from rejection (ISHLT 2R *or* antibody-mediated) for the study cohort

Table 1

Baseline Characteristics

Characteristic	<i>n</i> (total = 55)
Age at transplant	7.1 y (2 wk to 22 y)
Age <1 y	15 (27.3%)
Age 1–10 y	17 (30.9%)
Age ≥10 y	23 (41.8%)
Female gender	28 (50.9%)
Diagnosis	
Congenital heart disease	17 (30.9%)
Dilated cardiomyopathy	26 (47.3%)
Hypertrophic cardiomyopathy	4 (7.3%)
Restrictive cardiomyopathy	4 (7.3%)
Other ^a	4 (7.3%)
Mechanical support	
Ventricular assist device	7 (12.7%)
ECMO	4 (7.3%)
CMV-negative	41 (74.5%)
PRA >10%	5 (9.1%)

Data are presented as number (percent) for categorical and median (range) for continuous characteristics. CMV, cytomegalovirus; ECMO, extracorporeal membrane oxygenation; PRA, panel-reactive antibody; wk, weeks; y, years.

^aTwo patients were retransplants: 1 had ischemic cardiomyopathy and 1 fulminant myocarditis.