ORIGINAL ARTICLE

Outcomes of Cardiac Transplantation in Highly Sensitized Pediatric Patients

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Abstract Despite aggressive immunosuppressive therapy, pediatric orthotopic heart transplant (OHT) candidates with elevated pre-transplant panel reactive antibody (PRA) carry an increased risk of rejection and early graft failure following transplantation. This study has aimed to more specifically evaluate the outcomes of transplant candidates stratified by PRA values. Records of pediatric patients listed for OHT between April 2004 and July 2008 were reviewed ($n = 101$). Survival analysis was performed comparing patients with PRA < 25 to those with PRA > 25, as well as patients with PRA < 80 and PRA > 80. Patients with PRA > 25 had decreased survival compared with those with PRA < 25 after listing ($P = 0.004$). There was an even greater difference in survival between patients with PRA > 80 and those with PRA < 80 ($P = 0.002$). Similar analyses for the patients who underwent successful transplantation showed no significant difference in post-transplant survival between patients with a pre-transplant PRA > 25 and those with PRA < 25 ($P = 0.23$). A difference approaching significance was noted for patients with PRA > 80 compared with PRA < 80 ($P = 0.066$). Patients with significantly elevated pre-transplant PRAs at the time of listing have a significantly worse outcome compared to those with moderately increased PRA values or non-sensitized patients. Further study is necessary to guide physician and family treatment decisions at the time of listing.

Introduction

Allosensitization in pediatric OHT candidates presents a significant challenge for long-term survival. Sensitization in the pediatric population most often occurs after blood transfusion and homograft placement during surgical repairs of congenital heart defects [7, 12]. Sensitization to HLA antigens is augmented after ventricular assist—device placement and after heart transplantation [8]. Although early survival may be comparable, sensitized pediatric transplant patients have been shown to have decreased long-term survival compared with nonsensitized patients [4]. Furthermore, sensitized transplant recipients are at risk for antibody mediated rejection (AMR) of the transplanted heart [7]. AMR is considered a significant cause of biopsy negative, hemodynamically significant rejection episodes and graft failure [1, 6, 9].

Sensitization is often defined as a panel reactive antibody (PRA) measurement > 10% for either class I or II HLA antigens [3–5, 10, 13]. A study of United Network for Organ Sharing data in adults showed significant decreases in 3 year graft survival with each 20% interval increase in pretransplant PRA value [11]. However, the effect of increased PRA values has yet to be as specifically described for the pediatric population. Our institution has historically been aggressive in attempts to successfully transplant pediatric patients with significantly increased PRA levels. We describe wait list survival and long-term graft outcomes in a pediatric population stratified by specific PRA levels (PRA < 25, >25, <80, and >80).

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Materials and Methods

Patients

After receiving Institutional Review Board approval, records of all pediatric patients listed for orthotopic heart transplantation (OHT) between April 2004 and July 2008 were reviewed. Detailed medical histories, including age at transplant, sex, diagnosis, wait time, and cause of death, were recorded. PRA results nearest to listing and transplantation were recorded as were posttransplant endomyocardial biopsy (EMB) results. Patients removed from the wait list due to recovery were excluded.

Immunosuppression

Sensitized transplant candidates routinely underwent desensitization procedures consisting of 5 day courses of plasmapheresis (size permitting) followed by intravenous immune globulin (Baxter International, Deerfield, IL). Rituximab (Genentech, Basal, Switzerland) was also used in select cases. Since 1995, all recipients were managed on triple-drug immunosuppression consisting of corticosteroids, tacrolimus (Astellas Pharma, Japan), and mycophenolate mofetil (Roche, Basel, Switzerland). Corticosteroids were ideally weaned off by 1 year after transplantation based on favorable EMB results and stable clinical course. Tacrolimus was replaced with Sirolimus (Wyeth Pharmaceuticals, Collegeville, PA) in patients with evidence of cardiac allograft vasculopathy (CAV) either by coronary angiography or echocardiography. Recipients with chronic renal insufficiency were maintained on a renal-sparing protocol using Sirolimus in combination with mycophenolate. Symptomatic patients with evidence of AMR were treated with courses of plasmapheresis and intravenous immune globulin.

Panel Reactive Antibody

During the study period, all transplant candidates were screened for anti-HLA antibodies using flow cytometry and microlymphocytoxicity assays. Antibody reactivity in the cytotoxicity assays was measured on a T-lymphocyte panel consisting of 120 reference cells and a B-lymphocyte panel consisting of 60 reference cells. Sample results reflecting >10% reactivity were retested with dithiothreitol to remove immunoglobulin M antibodies. Repeat assays were performed every 4–6 months while the patient was awaiting transplantation. Repeat assays were also performed 2 weeks after blood transfusion or infection.

Donor-Specific Cross-Matching

Prospective cross-matches were performed in candidates with increased PRA levels (>10%). After 2007, virtual cross-matching largely replaced prospective donor-specific cross-matches. Recipient sera were cross-matched directly with donor T and B lymphocytes using the standard microlymphocytotoxicity technique and the three color-flow cross-match technique. Sera used in the cytotoxicity cross-match were pretreated with dithiothreitol.

EMBs

Surveillance EMBs were performed at weeks 1, 3, 12, 26, and 52 during the first year after transplantation. EMBs were performed every 6 months in the second year and then annually thereafter. Additional biopsy procedures were performed in patients with clinical evidence of rejection or after documented cellular rejection of grade 2R (previously 1B) or greater. Recipients presenting with hemodynamic instability were generally treated empirically for rejection without EMB. EMBs were subsequently performed after bolus immunotherapy. Biopsy procedures performed at 1 week, 6 months, and yearly after OHT were screened by immunofluorescence and immunohistochemistry for AMR. EMB specimens from symptomatic patients with suspected AMR based on clinical presentation were also screened for AMR. If AMR was identified, immunofluorescence was performed on all subsequent EMB specimens until a negative result was obtained. EMB specimens were analyzed as previously described.

Statistical Analysis

Survival analysis was performed using Kaplan–Meier curves, which were compared using Breslow test for equality (SPSS version 8.0 for Windows; Chicago, IL). Chi square test (SPSS version 8.0 for Windows) was used to analyze noncontinuous variables, and two-tailed Student t test (Excel; Microsoft, Redmond, WA) was used to analyze continuous variables, and $P < 0.05$ were considered significant.

Results

Patient Demographics

A total of 101 patients listed for OHT between April 2004 and July 2008 were included in this study. Eighty-three patients received transplants, and 18 died while on the wait. One patient was excluded because a pretransplant PRA could not be located, and another was excluded because

Table 1 PRA stratification of transplanted patients by PRA at listing

PRA (n)	% Female sex (n)	P	% CHD Dx (n)	P	Mean age at OHT (y)	P	Mean waitlist time (d)	P	% Graft failure after 2 years (n = 56)	P
<25 (71)	35 (25)	0.90	25 (18)	0.56	11.1	0.91	77.5	0.95	19 (9/47)	0.83
>25 (12)	33 (4)		33 (4)		11.4		76.0		22 (2/9)	
<80 (77)	36 (28)	0.33	26 (20)	0.69	12.7	0.83	76.21	0.93	18 (9/51)	0.23
>80 (6)	17 (1)		33 (2)		10.0		76.0		40 (2/5)	

CHD congenital heart disease

Table 2 PRA stratification of all patients listed for OHT

PRA (n)	% Female sex (n)	P	% CHD Dx	P	Mean age at listing (y)	P	Mean waitlist time (d)	P
<25 (81)	37 (30)	0.51	32 (26)	0.06	11.0	0.92	93.7	0.40
>25 (20)	45 (9)		55 (11)		11.2		125	
<80 (90)	39 (35)	0.87	33 (30)	0.05	11.1	0.66	99.5	0.82
>80 (11)	36 (4)		63 (7)		9.7		108	

CHD congenital heart disease

more than one transplant was performed during the study period. Patients removed from the wait list due to recovery were also excluded. The mean age at transplantation was 11.3 years (range 0.4–20.8) (Table 1). Mean wait list time for patients who were transplanted was 75.8 days (range 1 day–1.3 years), and mean wait list time was 188.1 days (range 12 days–4 years) for patients who did not receive transplants ($P = 0.002$). There were no significant differences in wait list time, age at transplantation, or sex for patients with PRA > 25 and PRA < 25 or for patients with PRA > 80 and PRA < 80 (Tables 1, 2). As expected, a significantly greater number of patients with congenital heart disease (CHD) had extremely high PRA values compared with patients with cardiomyopathies. Graft failure occurred in 19.3% of patients who received transplants in this cohort. Two year posttransplant follow-up was available for 56 patients.

Outcomes After Listing for OHT

A total of 81 patients (80%) had PRA < 25 at listing. Wait-list survival for patients with PRA < 25 was significantly better than for those with PRA > 25 (Fig. 1; $P = 0.004$). Patients with PRA > 25 did not have significantly longer wait times ($P = 0.40$). Ninety patients (89.1%) had PRA < 80 at listing and showed significantly better survival ($P = 0.002$) than patients with PRA > 80 (Fig. 2). During the study period, the mortality for all patients in this cohort, regardless of whether or not they received a transplant, was 33.7% ($n = 34$). Eighteen of the patients
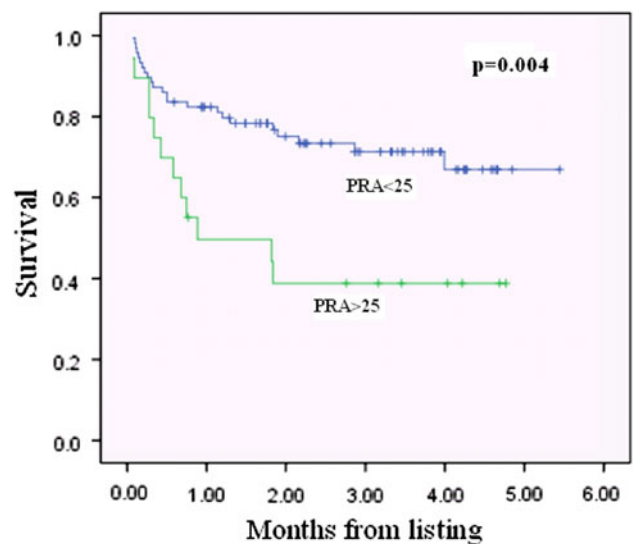

Fig. 1 Survival after listing for patients with PRA > 25 and PRA < 25

who died were still on the wait list at the time of death. Twenty-eight percent of patients who died while on the wait list had PRA > 80 at listing. These patients had a mean survival of 146 days (range 12–261) after listing. Of the patients who died after OHT, 19% had PRA > 80. Mean survival for these patients was 2.1 years (58 days–3.86 years) after OHT. Eleven percent of patients at listing had PRA > 80, and 7% of patients who underwent OHT had PRA > 80.
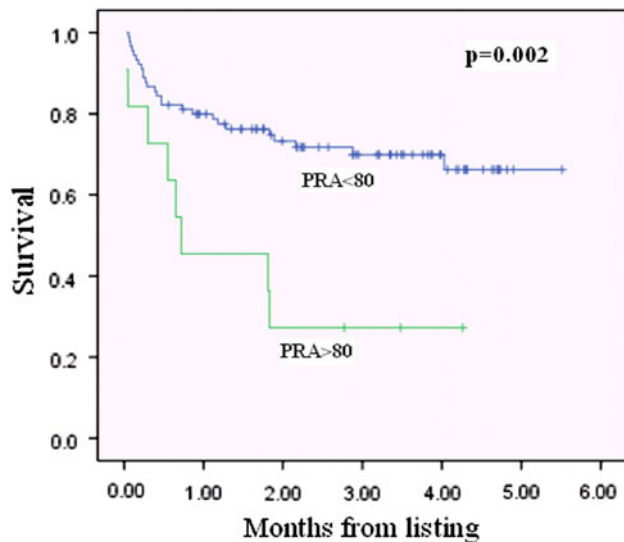
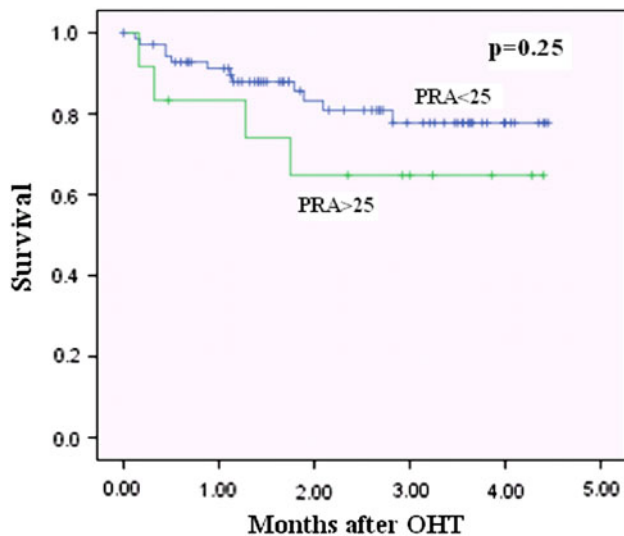**Fig. 2** Survival after listing for patients with PRA > 80 and PRA < 80

Fig. 3 Survival after OHT for patients with PRA < 25 and PRA > 25

Outcomes After OHT

There was not a significant difference in the absolute 2 year survival after OHT for patients with PRA < 25 compared with those with PRA > 25. Although it trended toward significance, the difference in survival for patients with PRA > 80 and PRA < 80 was also not significant. When analyzed by Breslow testing of Kaplan–Meier survival curves (see Fig. 3), posttransplant survival for patients with PRA > 25 was worse than for patients with PRA < 25, although not significantly so ($P = 0.25$). Patients with PRA > 80 also showed decreased survival compared with those with PRA < 80 ($P = 0.066$).

Because of the limited number of patients with PRA levels > 25 and >80, there was not sufficient power to detect a clinical difference if one were to exist. Although the survival plots in Fig. 3 look divergent, we cannot claim that they are definitely different. In our cohort, the presence of class I versus class II alloantibodies did not appear significant. Four of the 16 deaths that occurred after OHT were due to CAV. Two of the three deaths that occurred in the PRA > 80 group were due to CAV, and the third was due to multiorgan system failure. The other two deaths from CAV occurred with patients with PRA < 25. Six of the deaths resulted from acute rejection: Five of these patients had PRA < 25, and one had PRA > 25. Of the five remaining deaths, two were caused by sudden cardiac death, one by sepsis, one by noncompliance and rejection, and one by unknown reasons.

Donor-Specific Cross-Matching

Two patients in this cohort had a positive donor-specific cross-match by flow cytometry. Both patients had pre-transplant PRA > 80 and were transplanted across a weakly positive flow cross-match as it was believed to be the best option given their significantly increased PRA levels and diverse antibody profiles. There were no positive cytotoxic cross-matches. The first patient died 21 months after transplantation from CAV and graft failure. There were no episodes of acute rejection, and antibody-mediated rejection was not detected on endomyocardial biopsy. The second patient is alive and well three years after transplantation at the time of publication. Antibody-mediated rejection was noted on biopsy specimens beginning 1 month after transplantation; however, the patient remained asymptomatic with no evidence of allograft dysfunction by echocardiography or catheterization.

AMR

A total of 584 EMBs were included in this study, with a mean of seven performed for each graft (range 1–19). Twelve patients had documented AMR by at least one EMB. Two of these patients had an EMB specimen positive for HR within 30 days of OHT, although neither patient died during the study period. There was no significant difference in the incidence of AMR in sensitized patients compared with nonsensitized patients. Thirty-three percent of patients with PRA > 80 showed AMR on at least one biopsy specimen compared with 13% of patients with PRA < 80 ($P = 0.28$). Two of the 12 patients with AMR on EMB (16.7%) died during the study period compared with 14 of the 71 patients without AMR (19.7%).

Discussion

Transplantation outcomes of highly sensitized pediatric patients were examined retrospectively. Patients with PRA levels >25 and >80 to either class I or II antigens were specifically considered both at the time of listing for OHT and at the time of transplant. It is our hope that the data provided in this manuscript will help providers to (1) appropriately counsel the families of highly sensitized pediatric OHT candidates and (2) assist in making difficult decisions regarding resource use with regard to highly sensitized children. The study period was fairly long (4 years) and was recent enough to include the more sensitive flow cytometric PRA measurements. However, these data do not fully incorporate the potential benefits of virtual cross-matching for highly sensitized transplant candidates because virtual cross-matching was used sporadically until becoming our standard protocol in 2007, around the time it was established as an effective tool for improving the outcomes after transplantation in highly sensitized patients [14].

Our results indicate that pediatric patients with increased pretransplant PRA values have a significantly worse prognosis at the time of listing compared with nonsensitized patients when a negative donor-specific cross-match is required before transplantation. Thus, the observed difference in survival was likely the result of both sensitization and our management strategy. Although our institution typically required a negative donor-specific cross-match before transplantation, there is evidence that transplanting across a positive cross-match leads to reasonable short-term survival [15]. Because highly sensitized patients may have a wide array of antigen specificities, these patients remain at increased risk, even when virtual cross-matching is used in place of donor-specific cross-matching. Ultimately, transplanting across a positive virtual cross-match is likely the most effective means of decreasing wait list mortality in a highly sensitized patient population. However, the posttransplant implications of such a strategy must be carefully considered.

Although there clearly was a decrease in posttransplant survival for the highly sensitized patients included in our study, our single-institution analysis was not large enough to claim that the effect of high PRA values on posttransplant survival is significant. At listing, patients with a pretransplant PRA < 25 showed significantly better survival than those with PRA > 25 ($P = 0.004$). The difference between survival for patients with PRA < 80 and PRA > 80 was even more pronounced ($P = 0.002$). Of patients who received transplants, those with PRA > 25 showed worse survival than patients with PRA < 25, but this was not a statistically significant difference. A comparison of survival for transplanted pediatric patients with PRA > 80 and those with PRA < 80 showed decreased posttransplant survival for the PRA > 80 patients, which approached statistical significance. Although the presence or absence (and relative abundance) of donor-specific HLA antibodies is likely the most important predictor of posttransplant outcome, we believe that the absolute PRA value may be indicative of overall sensitization status, with high levels of anti-HLA antibodies potentially coinciding with high levels of non-HLA antibodies.

Highly sensitized pediatric patients had much longer wait times (and greater wait list mortality) compared with nonsensitized patients. Early in the study period our standard practice was to require prospective cross-matches for all sensitized patients, which limited the potential donor pool and lengthened wait times for sensitized transplant candidates due to the need for recipient sera to be available near the donor hospital. More recently, we have used virtual cross-match almost exclusively. However, even without the requirement for prospective donor-specific cross-matching, the donor pool for sensitized candidates is necessarily decreased due to unacceptable antigens based on pretransplant PRA. Potential wait times, with the inherent implications for the patient's quality of life, must be considered based on the patient's antibody profile. Every effort should be made to allow transplant candidates with diverse antibody profiles to await transplantation as an outpatient to maximize quality of life. The longevity of the grafts in sensitized patients would also be expected to be inferior given the presence of preformed antibodies.

Certainly, a long-term multi-institutional study of highly sensitized pediatric patients is needed to help transplant teams justify the use of OHT in this patient population. However, the definition of a "successful" outcome is not concrete. In our cohort with PRA levels > 80, one could certainly perceive a 45% graft survival at 3 years after OHT (Fig. 4) as either "acceptable" or "unacceptable," taking into consideration posttransplant quality of life and resource use. In the meantime, desensitization therapy for highly sensitized patients certainly seems warranted given prolonged wait times and waitlist mortality, which is not completely ameliorated with virtual cross-matching. Although an increased incidence of AMR on EMB for patients with increased PRA values was detected, an increased incidence of graft failure and mortality rates for patients with AMR was not documented in this study but was detected in an earlier and longer-term study from our institution [2]. It is likely that if followed-up for a longer period of time, the patients with AMR in this study will also show increased mortality compared with patients without AMR. It is also possible that the difference in outcomes in our newer data represents the beneficial effects of more recent desensitization protocols.
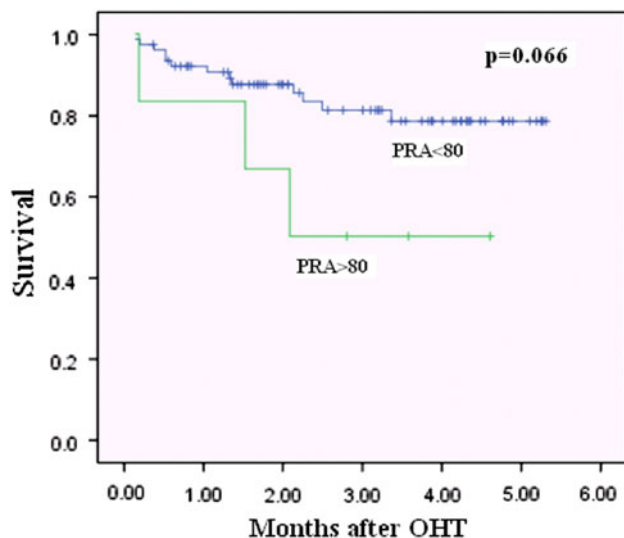
Fig. 4 Survival after OHT for patients with PRA < 80 and PRA > 80

Limitations

In addition to being limited by its retrospective nature, our study is certainly limited by the lack of standardized desensitization protocol during the study period. An additional limitation lies in our use of PRA to assess the level of recipient sensitization as opposed to the more commonly used donor-specific antibody measurement. However, PRA was the appropriate metric given the time the data was collected. Additionally, PRA is still used by many centers.

Conclusion

Increased PRA at the time of listing for pediatric cardiac transplantation is associated with increased mortality. Patients with high PRA values have a greater chance of dying on the wait list or after OHT than do patients with moderately increased PRA values or nonsensitized patients. Further research with large sample sizes and long-term follow-up is needed to generate more conclusive data regarding survival outcomes of patients with high PRA values. We hope that this and future studies will better aid clinicians in risk–benefit analyses when developing treatment plans for highly sensitized pediatric heart transplant candidates.

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