Use of an allograft patch in repair of hypoplastic left heart syndrome may complicate future transplantation


Objective: Cryopreserved allograft tissue used in the Norwood procedure for infants with hypoplastic left heart syndrome (HLHS) has the potential to cause marked immunologic sensitization which may complicate potential future heart transplantation, if required. The purpose of this study was to assess the anti-HLA antibody response to allograft patches used in the initial repair of HLHS. Methods: A prospective cohort study was conducted comparing the panel-reactive antibody levels (PRA) in 12 infants undergoing repair of HLHS with cryopreserved allograft patch to 10 infants undergoing arterial switch for transposition of the great arteries (no allograft tissue used). PRA for Class I (HLA-A, B, C) and Class II (HLA-DR, DQ) antibodies were assessed preoperatively and postoperatively using flow cytometry. Results: The two groups were well matched at the time of surgery (age, weight, gender). Infants in both groups received blood from multiple donors; however, the allograft group received significantly more (12 ± 10 vs. 5 ± 1 units; \( P < 0.001 \)). By 4 months, most infants receiving allograft tissue had become highly sensitized for both Class I PRA (62 ± 40 vs. 0; \( P = 0.002 \)) and Class II PRA (49 ± 42 vs. 2 ± 3; \( P = 0.022 \)). This response continued to increase at 12 months: Class I PRA (79 ± 21 vs. 0; \( P = 0.008 \)) and Class II PRA (66 ± 27 vs. 5 ± 6; \( P = 0.008 \)). Specificity analysis confirmed antibodies were specific for the donor allograft HLA type. In addition, infants who were coincidently HLA-matched with their allograft did not develop an elevated PRA. Conclusions: Allograft tissue used in the repair of HLHS is associated with profound donor specific immunologic sensitization in the majority of recipients and may complicate or jeopardize future transplantation. Methods to reduce the immunogenicity of cryopreserved allograft tissue used for arch reconstruction require further investigation.

Keywords: Allograft tissue; Panel reactive antibody; Transplantation; Hypoplastic left heart syndrome

1. Introduction

The Norwood operation followed by a staged Fontan procedure has become the accepted standard of care for infants born with hypoplastic left heart syndrome (HLHS). While the results of the Norwood operation are steadily improving, the children are left with a single ventricle Fontan circulation which is known to have a reduced life expectancy [1]. It is probable that cardiac transplantation will eventually be required in some, if not most, of these children.

Evidence is accumulating from both clinical [2,3] and laboratory studies [4,5] that cryopreserved allograft tissue used in congenital cardiac surgery appears to be immunogenic in the majority of patients and may elicit an early and intense cellular immune response. Less, however, is known about the humoral response to the cryopreserved allograft tissue. It is possible that the allograft tissue used in the infant’s initial repair may sensitize them and jeopardize the success of potential future transplantation. This is particularly concerning given the evidence that the presence of anti-HLA antibodies prior to transplantation have deleterious consequences for the transplant recipient.

It has been demonstrated that pretransplant antibodies are associated with early development of high grade cellular rejection and increased annual rejection frequency [6,7], increased graft vasculopathy [8], and decreased survival [9,10]. Notably, Jacobs et al. recently reported that in pediatric transplantation (median age 130 days), a PRA > 10% was associated with increased 30 day (25%) and longterm (50%) mortality compared with those with
a PRA <10% (8 and 15%, respectively). Moreover, elevated pretransplant panel reactive antibodies (PRA) increase the time on the wait list and complicate perioperative management at the time of transplantation. Thus, the purpose of this study was to assess the anti-HLA antibody response to allograft patches used in the initial repair of HLHS.

2. Materials and methods

2.1. Study design

A prospective cohort study was conducted to compare the effect of exposure to cryopreserved allograft tissue (patch of allograft adult pulmonary artery) on panel reactive antibody. PRA levels were assessed preoperatively and at 1, 4, and 12 months postoperatively in two groups of infants undergoing standard congenital cardiac procedures either with or without allograft tissue. The study was approved by the local ethics committee for human research and written consent was obtained from patients (parents).

2.2. Study cohort

Twelve infants undergoing aortic arch reconstruction with cryopreserved pulmonary artery patch. There were 10 infants with hypoplastic left heart syndrome undergoing first-stage palliation (Norwood procedure), one infant with transposition of the great arteries with arch hypoplasia/coarctation (arterial switch and repair of arch with allograft patch), and one infant with tricuspid atresia with arch hypoplasia (repair of interrupted arch with allograft patch). Allograft tissue was provided by comprehensive tissue centres at two Canadian University Hospitals (University of Alberta Hospital, Edmonton, Alberta; Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia).

2.3. Control cohort

Ten infants undergoing an arterial switch operation for transposition of the great arteries. No allograft tissue was used for this procedure.

2.4. Variables

Preoperative variables to ensure similarity between the two groups included age, gender, preoperative length of hospitalisation, and blood product exposure (amount and type). Perioperative factors included duration of cross-clamping and cardiopulmonary bypass, use of hypothermic circulatory arrest, and blood product exposure (amount and type). Postoperative variables included length of stay in ICU, length of stay in hospital, and blood product exposure. All infants received CMV negative, leukocyte depleted blood.

2.5. Donor and recipient HLA typing

Donor and recipient Class I and II HLA typing was tested by molecular methodology. Recipient DNA was purified from whole blood using QIAamp® DNA Blood Mini Kit (Quiagen, Valencia, CA). Donor DNA was purified from bone marrow tissue. HLA A, B, and DR antigen typing was performed using the low resolution Micro SSP™ DNA typing kit (One Lambda, Inc., Canoga Park, CA). DNA fragments were separated by agarose gel electrophoresis. HLA antigens were determined through a combination of One Lambda DNA/LMT software analysis and manual interpretation of the electrophoresis results.

2.6. HLA antibody analysis

Screening for anti-HLA antibodies was performed using the Flow PRA® Screening Test (One Lambda, Inc.). Serum samples were analysed according to the manufacturer’s recommendations. Test control sera included a negative control from One Lambda, Inc. (Catalogue number FL-NC) as well as a positive control which was a 1/32 dilution of a local positive pool made from many high PRA patient sera. A 10 µL mixture of class I and class II beads as well as control beads were added to every tube. Patient sera (20 µL) were added and the tubes were incubated for 30 min. The tubes were washed twice and 100 µL of diluted FITC conjugate (anti human F(ab')2) was added. A final wash step was performed and the beads were analysed using a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA). Samples that tested positive for the presence of either Class I and/or Class II antibodies were then further tested for specificities using the FlowPRA® Specific Antibody Detection Test kit (One Lambda, Inc.). Specificity analysis was also performed by the use of single antigen beads if the PRA >50% (catalogue numbers FL2HD and FL1HD, One Lambda, Inc.). In a few cases antibody specificity for class II was also done by ELISA methodology. The ELISA kit used was Class II ID (GTI, Waukesha, WI).

2.7. Data analysis/statistics

All outcomes were expressed as means and standard deviation. Comparisons between continuous data were made with Mann-Whitney U-test and comparisons between nominal data were made with Chi-square or Fisher’s exact test where appropriate. Differences were considered significant for a value of P<0.05. Simple linear regression analysis was used to assess the relationship between transfusions and PRA. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 11.5).

3. Results

Patient demographics are summarized in Table 1. Except for the transposition group being somewhat longer (50.5 ± 2.4 vs. 52.6 ± 3.0 cm; P=0.041), the two groups were well matched preoperatively. Cardiopulmonary bypass time was similar for the two groups (127.1 ± 59.7 vs. 122.6 ± 19.1 min; P=0.314) but the arterial switch procedure for the transposition group required a significantly longer cross clamp time (42.6 ± 27.7 vs. 58.9 ± 11.5 min; P=0.004). The increased use of circulatory arrest for the Norwood procedure is reflected in the significantly increased total circulatory arrest time for the allograft group.
were coincidently HLA matched with their donor allograft. For Class I (79.2 months confirmed the persistence of the humoral response type of the donor allograft (Table 2). Equally important was many of the antibodies generated were specific for the HLA antibody specificity was analyzed. This confirmed that the 27.4 vs. 5.2 HLA mismatch in individuals (e.g. patient 7; Table 2) who the failure to generate anti-HLA antibodies in the absence of (49.3 PRA for Class I (61.9 PRA for both Class I (19.6 PRA for Class II (17.1 PRA for Class I (16.6 vs. 3.8 units; P<0.001) and cryoprecipitate (3.8 vs. 0.9 units; P=0.011).

Both groups had minor elevations in PRA preoperatively, most likely reflecting maternally transmitted antibodies (Figs. 1 and 2). By 1 month postoperatively, there was evidence for a humoral response with modest elevations in PRA for both Class I (19.6 ± 30.1 vs. 3.8 ± 10.6; P=0.270) and Class II (17.1 ± 27.5 vs. 4.0 ± 5.7; P=0.792) antibodies. However, at 4 months there was a significant elevation in PRA for Class I (61.9 ± 39.9 vs. 0; P=0.002) and Class II (49.3 ± 41.9 vs. 1.8 ± 3.3; P=0.022) antibodies associated with the use of cryopreserved allograft patches. PRA at 12 months confirmed the persistence of the humoral response for Class I (79.2 ± 21.1 vs. 0; P=0.008) and Class II (65.6 ± 27.4 vs. 5.2 ± 6.6; P=0.008) antibodies.

In an attempt to identify the source of sensitization, antibody specificity was analyzed. This confirmed that the many of the antibodies generated were specific for the HLA type of the donor allograft (Table 2). Equally important was the failure to generate anti-HLA antibodies in the absence of HLA mismatch in individuals (e.g. patient 7; Table 2) who were coincidently HLA matched with their donor allograft.

(28.0 ± 10.6 vs. 2.7 ± 5.2 min; P<0.001). The allograft group also required more blood products perioperatively, especially packed red blood cells (12.3 ± 9.6 vs. 5.3 ± 1.2 units; P<0.001) and cryoprecipitate (3.8 ± 4.3 vs. 0.9 ± 1.4 units; P=0.011).

In addition, simple linear regression analysis was used to assess the relationship between transfusions and PRA. This identified a negative relationship between the number of units of packed red blood cells transfused and Class I PRA (β = −2.229; R² = 0.286; P=0.073) and Class II PRA (β = −0.833; R² = 0.036; P=0.554) at 4 months (Fig. 3A). A similar relationship existed between the number of units of platelets transfused and Class I PRA (β = −2.607; R² = 0.238; P=0.108) and Class II PRA (β = −1.658; R² = 0.087; P=0.352) at 4 months (Fig. 3B).

**4. Discussion**

Despite previous beliefs that allograft tissue is immuno-privileged, recent investigations have provided evidence that allograft tissues activate alloreactive immune responses. Clinical studies by Baskett et al. correlated enhanced cellular viability of aortic valve allografts with increased antigenicity and increased rate of valve failure [2]. Studies have also correlated HLA mismatch with increased rate of allograft valve failure [11,12]. Other studies in humans and animals have demonstrated an increase in donor-specific cytotoxic [13] and helper T-lymphocyte precursors [3]. Using a rat model our group [4] and others [5] have also demonstrated an early and intense cytotoxic T-lymphocyte response along with complete destruction of valve leaflets in allogeneic rats.

Despite the aforementioned cell-mediated response, there is a relative paucity of definitive evidence for a humoral immune response to cryopreserved allograft tissue in infants. This is somewhat concerning for infants with HLHS because, despite improved results of the Norwood operation for HLHS [1], it is probable that many of these children will eventually require cardiac transplantation. The unfavorable impact of elevated PRAs on the outcome of cardiac transplantation is well documented. Elevated pretransplant PRAs significantly increase the risk of early allograft failure and reduced patient survival [6–10]. In a review of 14,535 heart transplants performed between 1987 and 1996 from the United Network of Organ Sharing Registry, it was found that elevated PRA at transplantation significantly increased...
the relative risk of graft failure \( (P=0.0001) \) [9]. Moreover, a PRA > 60% was found to be associated with a 2.242 relative risk of graft failure. Such PRA levels were not uncommon in our HLHS population after receiving allograft tissue in the Norwood operation. Jacobs et al. recently reported that in pediatric transplantation (median age 130 days) a PRA > 10% was associated with increased 30-day mortality (25 vs. 8%; \( P=0.178 \)) and overall mortality (50 vs. 15%; \( P=0.0434 \)) when compared to children with PRA < 10% [10]. These findings occurred despite aggressive preoperative/perioperative efforts to reduce the PRA including (in various combinations) intravenous immunoglobulin G (IVIG), cyclophosphamide, mycophenolate mofetil, exchange transfusions, and/or plasmapheresis. Leech et al. reported evidence of acute or hyperacute rejection on endomyocardial biopsy at postoperative day 7 in three of four patients who received orthotopic heart transplants despite being highly sensitized [14]. Similarly, Itescu et al. reported that pretransplantation anti Class II antibodies were associated with early development of high grade cellular rejection \( (P<0.0001) \) and increased annual rejection frequency \( (P<0.001) \) [6].

As more sensitive methods for detecting antibodies have become available, the impact of allosensitization has also become even more apparent. Using the more sensitive method of PRA-STAT, Kerman et al. reported that recipients with pretransplant PRA-STAT sera >10% were at increased risk for graft rejection \( (P<0.05) \), more rejection episodes/recipient \( (P<0.02) \), and graft rejection within 30 days \( (P<0.001) \) [8]. The increased sensitivity of the FlowPRA technique was recently reported by Tambur et al. [7]. When compared to the CDC method, FlowPRA detected a pretransplant PRA >10% in 34.8% of the patients who initially tested negative by CDC methodology. Moreover, pretransplant antibodies detected by Flow PRA were highly associated with rejection episodes \( (P<0.001) \) and 1-year graft survival \( (P<0.004) \). Despite the aforementioned negative consequences of an elevated PRA on transplant outcomes, this factor is not an absolute contraindication to surgery. Leech et al. recently reported that in four individuals with elevated PRA and positive prospective lymphocyte crossmatch, good medium-term success (follow up: 17–57 months) can be expected with the use of aggressive perioperative immunosuppression despite evidence for acute or hyperacute rejection in three of four patients [14].

Our study clearly demonstrates an intense humoral response to allograft tissues as evidenced by PRA levels approaching 100% in many of those exposed to such tissue. Moreover, we provided evidence that the PRA level continues to increase with time between 4 and 12 months. Our findings are consistent with those of other institutions [15–18]. Hoekstra et al. found that panel reactive antibodies developed in 78% of 32 recipients of cardiac valve allografts [16]. Smith et al. similarly noted a strong donor HLA-specific antibody response with HLA antibodies detected in 56% of recipients of antibiotic-preserved allografts and 100% of homovital (fresh) allograft recipients [17]. Hawkins et al. identified a significant increase in alloreactive antibodies in 24 children receiving cryopreserved allografts: at 3.3 months after operation panel reactive antibodies had risen to 92% (1.9% preoperative) and Class II antibodies had risen to 70% [18].

In support of the hypothesis that the allograft tissue was responsible for the elevations in PRA, specificity analysis confirmed that a substantial number of antibodies generated were specific for the HLA type of the donor allograft. Specificity analysis did, however, identify antibodies, which were not specific for the HLA type of the allograft. One possible explanation for this finding are cross-reactive groups (CREGs) [19]. When an individual is mismatched for a HLA antigen they may make antibodies to all

Table 2

Donor-recipient HLA antigen mismatch and antibody specificities at 4 months

<table>
<thead>
<tr>
<th>Pt</th>
<th>Antigen mismatches a,b</th>
<th>PRA 4 months</th>
<th>Antibody specificities</th>
<th>Donor specific antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class I</td>
<td>Class II</td>
<td>Class I (%)</td>
<td>Class II (%)</td>
</tr>
<tr>
<td>1</td>
<td>A2 A11 B51</td>
<td>DR11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>A2 B7 B14 (64 or 65)</td>
<td>DR7 DR15</td>
<td>100</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>A1 A29 B8 B44</td>
<td>DR3 (DR17 or 18)</td>
<td>99</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>A11 A28 B14 B27</td>
<td>DR3 (DR17 or 18)</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>A24 B50</td>
<td>DR13</td>
<td>60</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>DR1 DR15</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>A2 B14 (64 or 65)</td>
<td>DR13</td>
<td>65</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>A3 B35 B44</td>
<td>DR14</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
<td>98</td>
<td>88</td>
</tr>
<tr>
<td>11</td>
<td>A11 B27 B61</td>
<td>DR1</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

a Antigen mismatches: donor antigens at which recipient was not matched.
b N/A: donor or recipient typing was not performed.
the mismatched epitopes on this antigen. These epitopes are shared with other HLA antigens. Most of the antibody reactivity we observed can be explained by cross-reactivity with public epitopes shared by the donor antigen and very few antibody specificities cannot be explained on the basis of CREGs. Moreover, in the case of infant number seven who received an allograft matched for HLA class I antigens there was an absence of a PRA response. This finding, to the best of our knowledge, has not been previously reported in the literature for this population. Others have also reported a failure to develop an elevated PRA in response to allograft tissue; however, in these studies the HLA mismatch was not reported and thus the reason for the failure to develop antibodies is unclear [12,16].

Admittedly, there is potential for the results to be confounded by the increased use of blood products in recipients of allograft tissue. FFP may contain soluble HLA antigens; however, the exposure to this product did not differ significantly between the two groups. Cryoprecipitate use did differ significantly between the two groups but the exposure to soluble antigens is usually considered to be negligible with this product. The increased use of packed red blood cells by the HLHS group, however, is concerning. Despite being leukodepleted, there is the chance that some of the children may have developed antibodies in response to these transfusions. In addition, the expression of Class I HLA antigens by platelets could also be a source for sensitization. Despite these concerns, simple linear regression failed to identify a significant relationship between red blood cell or platelet transfusion and PRA levels at 4 months. In fact, a negative relationship between transfusions and PRA was noted which may suggest an immunomodulatory effect of transfusions [20]. This observation is only speculative and limited by the small sample size in our study. In addition, we have clearly demonstrated that the majority of the antibodies are specific for the HLA type of the allograft, and thus can, cautiously, assume that the impact of blood transfusions is minimal. Furthermore, the difference in PRA levels between the two groups is between 10-fold and infinite, whereas, there are much smaller differences in the number of blood products used. Lastly, in those instances where the infant undergoing the Norwood procedure coincidentally received an HLA-matched allograft there was no PRA response, again suggesting that it is the antigenicity of the allograft, not blood product usage, that caused the antibody response.

These findings provide impetus to find methods to reduce the immune response to allograft tissue, to use alternative tissue, or to not use any tissue at all. In a pilot study Shaddy et al. recently demonstrated that a 3-month postoperative course of mycophenolate mofetil significantly, but not completely, abrogated the humoral immune response to valved allografts in children undergoing cardiac surgery. The long term effect of this therapy on the development of anti-HLA antibodies, however, is still not known and the use of this agent is not without consequences due to its toxicity [21]. Decellularization has the potential to remove immunogenic cellular elements from the allograft tissue. Hawkins et al. reported that decellularized grafts significantly reduce Class I and Class II antibody levels after implantation in children (mean age 8.5 ± 7.9 years) [22]. None of 14 patients maintained PRA levels <10% in the first year of follow up. These findings are consistent with those in our laboratory investigating the humoral immune response to decellularized allograft tissue in a rat model (unpublished results). HLA mismatch and ABO mismatch has been significantly associated with allograft failure [11]. These findings along with our observation that lack of HLA matching is associated with failure to develop antibodies (patient #7) suggests that greater efforts to avoid HLA and ABO mismatch would be beneficial. However, this would require tissue banks much larger than most institutions maintain, a problem that could potentially be overcome by an organ-sharing network [23]. Others have used an autologous [24] or bovine pericardial patch with acceptable results. Lastly, primary repair has been advocated by some groups. Ishino et al. reported that they were able to perform a direct pulmonary artery-to-aorta anastomosis in 85% cases with survival very comparable to that reported by other groups [1] during the same time period, albeit with a 23% recoarctation rate in the survivors [25].

In conclusion, in our experience the use of cryopreserved allograft tissue in the Norwood procedure is associated with
a significant humoral response in the majority of patients, especially in those who were mismatched for HLA type. Although the exact number of hypoplastic left syndrome children treated with surgical palliation who will require subsequent transplantation is unknown, we do know that prior sensitization portends a significant risk for early graft failure and poorer patient survival. Taken together, these findings suggest that the use of allograft tissue in the Norwood procedure may complicate future transplantation. Norwood stage I should preferably be performed without an allograft patch. Alternatives such as an autologous pericardial patch, direct anastomosis, or methods such as decellularization to make the allograft tissue less immunogenic should be considered.

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References


Appendix A. Conference discussion

Dr A. Boening (Kiel, Germany): We do around 20 Norwood I procedures per year. 5 years ago we stopped using homografts totally and started using bovine pericardium for every patient. Could that be a solution for you?

Dr Meyer: There are a number of alternatives including bovine pericardium. We haven’t looked at that. It’s a great suggestion. I guess the one thing that you would obviously have to look at is the potential for...
a neo-aortic obstruction afterwards. I don’t know if you’ve had any problem with tissue shrinkage.

Dr Boening: It occurs in the same percentage as in homografts.

Dr M. Hazekamp (Leiden, The Netherlands): Do you know if there’s any evidence or did you have any personal experience with transplant complications after Norwood operation by more than normal rejection? Because that would be interesting to know.

Dr Meyer: That’s actually what’s prompted this study as we’ve had a few children who have had Norwood procedures, have come back, and have required transplantation subsequently. We’ve had 2 children, one who had a panel-reactive antibody that was 100%. We were eventually, with intense therapy with rituximab, to get his PRA down, but he died before we transplanted him. A second child we did transplant before we were able to get his panel-reactive antibodies down. He died in hospital within about 6 weeks of what appeared to be acute rejection.

Mr V. Tsang (London, UK): I may have missed part of your talk. Can you explain the possibility of blood sensitization here.

Dr Meyer: If I may, if the chairman will allow me to show one more slide, we did actually look at this, because this is very concerning to us. What we looked at here was the correlation between percent PRA, which is on the Y-axis, and the units of packed red blood cells that were transfused. On the right-hand side, we looked at the units of platelets transfused. What we actually found with simple linear regression was a negative relationship between the units of packed cells as well as units of platelets transfused and the PRA at 4 months, both class I and class II PRA. It’s a little surprising, but it’s consistent with some evidence in the literature for an immunomodulatory effect of transfusions.

Dr F. Lacour-Gayet (Denver, CO, USA): I would like to ask you, if you would extend this word of caution, that you’re giving around the use of homograft, to other pathology? And when dealing with complex patients that may require at some point a transplantation; when doing complex biventricular repair, will you suggest that we should not use allograft but heterograft material?

Dr Meyer: When we designed the study, we wanted to look at a very narrow group of infants; hence, looking at the hypoplats compared to the transpositions. But I would tend to assume that you’re going to see a similar response in other children undergoing congenital surgery with cryopreserved tissue.