**TRANSPLANTATION FOR CONGENITAL HEART DISEASE IS ASSOCIATED WITH AN
INCREASED RISK OF EPSTEIN-BARR VIRUS-RELATED POST-TRANSPLANT
LYMPHOPROLIFERATIVE DISORDER IN CHILDREN.**

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38 **Abbreviations**

- 39 ACM – Acquired Cardiomyopathy
- 40 ATG – Anti-Thymocyte Globulin
- 41 CHD – Congenital Heart Disease
- 42 CI – Confidence Intervals
- 43 CNI – Calcineurin Inhibitor
- 44 DLBCL – Diffuse Large B-cell Lymphoma
- 45 EBV – Epstein-Barr Virus
- 46 EBER – Epstein-Barr Virus Encoded RNA
- 47 HR – Hazard Ratio
- 48 IHC – Immunohistochemistry
- 49 ISH – In situ Hybridisation
- 50 IQR – Inter-Quartile Range
- 51 OHT – Orthotopic Heart Transplant
- 52 PTLT – Post-transplant Lymphoproliferative Disorder
- 53 PHTS – Pediatric Heart Transplant Study
- 54 SOT – Solid Organ Transplant
- 55 TCR – T cell receptor
- 56 TRECs – T-lymphocyte Receptor Excision Circles

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Abstract

Background

Children undergoing heart transplant are at higher risk of developing post-transplant lymphoproliferative disorder (PTLD) compared to other solid organ recipients. The specific factors driving that risk are unclear. This study investigated risk factors for PTLD in children transplanted at one of two UK pediatric cardiac transplantation centres.

Methods

Clinical characteristics of all children (<18 years) transplanted at our institution over a 16-year period were analysed. Freedom from PTLD was assessed using the Kaplan-Meier method and Cox-proportional regression.

Results

Cumulative PTLD incidence for the study cohort was 21.1% at 5-years post-transplant. PTLD occurred in 17/71 transplanted for congenital heart disease (CHD) and 18/129 transplanted for acquired cardiomyopathy (ACM). Median time from transplant to PTLD was 2.9 years (IQR 0.9–4.6). Negative EBV serostatus pre-transplant (Adjusted HR=2.7; 95%CI=1.3–5.6, p=0.01) and underlying CHD (Adjusted HR=3.2; 95%CI=1.4–7.4, p=0.007) were independently associated with higher risk of PTLD. Age at thymectomy was significantly different between children with CHD and ACM (0.4 vs 5.5 years, p<0.01). Median CD4⁺ and CD8⁺ T-lymphocyte counts at 2-years post-transplant were significantly lower in children transplanted for CHD vs ACM (CD4⁺ 391/μl vs 644/μl, p=0.01 and CD8⁺ 382/μl vs 500/μl, p=0.01). At 5-years post-transplant, those differences persisted amongst patients who developed PTLD (CD4⁺, 430/μl vs 963/μl, p<0.01 and CD8⁺, 367/μl vs 765/μl, p<0.01).

Conclusion

Underlying CHD is an independent risk factor for PTLD and is associated with a younger age at thymectomy. A persistent association with altered T-lymphocyte subsets may contribute to the impaired response to primary EBV infection and increase the risk of PTLD.

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a rare but conspicuous complication of solid organ transplantation (SOT), especially in children. It constitutes a heterogeneous group of conditions that are predominantly characterised by pathological B-lymphocyte proliferation driven by Epstein-Barr virus (EBV).^[1] The spectrum of PTLD includes indolent, polyclonal, non-destructive infiltrates that often involve the adenoids and tonsils and leave residual lymphoid architecture intact, and destructive lesions which efface normal tissue structures. The latter comprise polymorphic PTLDs which are typically monoclonal but contain B-lymphocytes at all stages of differentiation, and monomorphic PTLDs which are monoclonal lymphomas, histologically indistinguishable from their sporadic counterparts.^[1, 2]

While there are few data on the incidence of non-destructive PTLD in pediatric recipients of an orthotopic heart transplant (OHT),^[3] the rate of destructive polymorphic and monomorphic PTLD in OHT recipients is estimated at up to 9.2 % at 10-years post-transplant, which is notably higher than in adults after OHT and children with abdominal SOTs.^[4-6] This has been attributed to a greater proportion of childhood recipients being EBV naïve at transplantation and a more aggressive approach to post-transplant immunosuppression compared to other transplanted organs, with the use of higher intensity induction and maintenance therapies to prevent graft rejection.^[7] This degree of sustained immunocompromise likely impairs cytotoxic T-lymphocyte function in controlling abnormal proliferation of EBV--infected transformed B-lymphocytes, leading to an increased risk of PTLD.

In recent years, significant improvements in transplant-related outcomes and survival have led to growing emphasis on identifying modifiable risk factors for PTLD in pediatric heart transplant patients.^[8] Previous observational studies have consistently identified primary EBV infection as a significant risk factor for PTLD in this cohort.^[9-11] However, the evidence for other factors (e.g. choice of induction/maintenance immunosuppression) remains inconclusive^[4, 12], and few studies have considered risk factors that may be unique to childhood cardiac transplantation. As one of two UK centres commissioned to perform pediatric cardiac transplantation, we sought to address this within an examination of clinical and laboratory risk factors in a large single centre analysis of children undergoing cardiac transplant.

Methods

Study participants and variables

All patients who received a primary OHT aged 18 years or less at our institution between January 2000 and December 2015 were reviewed. Those who died within 30 days of transplantation were excluded from analysis. Clinical and laboratory data from January 2000 to December 2017 were collected. Demographic and clinical variables were obtained from our centre's pediatric heart transplant database and cross referenced with information from electronic patient records. Data for univariate analysis were collected on gender, ethnicity, age at transplantation, induction and maintenance immunosuppression therapy, donor and recipient EBV serostatus at transplantation, pre-transplant cardiac diagnosis and age at thymectomy. As thymectomy is standard at median sternotomy in children, ^[12] age at first median sternotomy was used as a proxy measure for age at thymectomy. Information on CD4⁺ and CD8⁺ T-lymphocyte counts at clinically relevant time points after transplantation were also collected. Pre-transplant cardiac diagnosis was classified as either congenital heart disease (CHD) or acquired cardiomyopathy (ACM).

PTLD case ascertainment

Cases of PTLD were identified from the heart transplant database and by review of all local histopathology reports from pediatric cardiac transplant patients. Pathology slides for PTLD and potential PTLD cases were subjected to expert hematopathology review (CMB, KW). PTLDs were characterised using routine immunohistochemistry (IHC) and *in-situ* hybridization for EBV-encoded early RNA (EBER ISH) and were classified according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue (Revised 4th Edition). ^[2] A diagnosis of florid follicular hyperplasia-type PTLD required an increased number of EBER-positive cells above the level typically seen incidentally in the lymphoid tissue of EBV carriers.

Immunosuppression regimen

All patients underwent induction immunosuppression with either Anti-Thymocyte Globulin (ATG) or Basiliximab. Maintenance immunosuppression involved the combination of a calcineurin inhibitor

(CNI), corticosteroid and/or antimetabolite. Induction and maintenance therapies were based on our institution's standard immunosuppression protocol [Table S1].

Data analysis

Continuous variables were analysed using the Wilcoxon rank sum test while chi-square and Fisher's exact tests were used to investigate associations between categorical covariates. Continuous variables were summarised as median values and interquartile ranges (IQR). Estimates of freedom from PTLD and the cumulative incidence of a PTLD event occurring were obtained using the Kaplan-Meier method and p-values for stratified comparisons were determined using the log rank test. Death before PTLD was treated as a competing event. Cox proportional regression was used to calculate Hazard Ratios (HR) with 95% confidence intervals (CI) and multivariate analysis was performed to adjust for potential confounding from relevant covariates using a stepwise technique. All prognosticators with a p-value below 0.10 were considered and successively removed if the p-value in the Cox multivariate model was above 0.05.

Schoenfeld residuals were used to investigate the validity of the proportional hazards assumption and the global score test of proportional hazards based on the scaled Schoenfeld residual was used for significant covariates. All study variables were investigated as potential predictors of PTLD except for T-lymphocyte counts which were excluded from Cox modelling as 50% of the study cohort who developed PTLD only had available data from T-lymphocyte assays performed after the PTLD diagnosis. Data analysis was performed using STATA version 15.0.

Ethical Considerations

The study has been conducted in full accordance with the principles of the "Declaration of Helsinki", Good Clinical Practice (GCP) and within the laws and regulations of the United Kingdom.

Results

Patient Characteristics

Patient demographics and clinical characteristics are illustrated in Table 1. A total of 200/208 children survived more than 30 days after undergoing a primary orthotopic heart transplant during the study period. The median age at transplantation was 5.8 years (IQR 1.6 – 12.5 years) with a median post-transplant follow up time of 6.5 years (IQR 3.6 – 10.3 years). ACM was the primary indication for transplantation in 129 children (65%) while the remaining 71 (35%) had various CHD diagnoses [Table 1]. Post-transplant immunosuppression was induced using ATG in 62% of patients and Basiliximab in the remaining 38%. Maintenance immunosuppression at 1-year post-transplantation was with tacrolimus in 60% and ciclosporin in 40% of children. Two-thirds of children were also on at least one adjunct immunosuppressant at 1-year post-transplant [Table 1].

There was no association between cardiac diagnosis and EBV serostatus at transplantation, with 19/59 CHD patients and 38/102 ACM patients being seronegative (n=161 patients with data available, p=0.52) and no significant difference in age at transplantation between children with CHD and ACM (5.5 years vs 6.2 years; p=0.33). Overall freedom from PTLT for the study cohort was 93% (95% CI; 88% – 96%) at 1-year, 79% (95% CI; 71% – 85%) at 5-years, and 73% (95% CI; 62% – 81%) at 10-years post-transplantation, while freedom from destructive PTLT was 95% (95% CI; 91% - 97%) at 1-year, 89% (95% CI; 82% - 93%) at 5-years and 86% (95% CI; 78% - 92%) at 10-years.

Development of PTLT

The cumulative incidence of PTLT in the study cohort at 5-years post-transplantation was 21.1% (95% CI; 15.3% – 28.9%). PTLT occurred in 35/200 patients (17.5%), with destructive PTLT diagnosed in 19/35 children (54%) and non-destructive PTLT in 16/35 (46%) [Table S2]. One patient (PT12) also developed an intracranial EBV-associated smooth muscle tumour as a separate event to her PTLT. No patient developed both destructive and non-destructive PTLT during the study period. Histological features are summarized in Table 1. Monomorphic disease made up 12/19 destructive PTLT cases and comprised 8 diffuse large B-cell lymphomas (DLBCL) and 4 Burkitt lymphomas. There was 1 case of classical Hodgkin lymphoma and the remaining 6 destructive PTLTs were polymorphic. The median

overall time from transplantation to PTLD diagnosis was 2.9 years (IQR 0.9 – 4.6 years) and there was no significant difference in time to PTLD by cardiac diagnosis or type of PTLD ($p > 0.05$). All PTLDs were of B-lymphocyte lineage and were EBV-positive. Pre-transplant EBV serostatus was established for 30/35 children who developed PTLD, of whom 17 (57%) were EBV negative. Twenty-eight children (80%) were on tacrolimus at the time of diagnosis while the remaining 7 (20%) were receiving ciclosporin. Eighteen children (51%) were also on adjunctive therapy with a combination of prednisolone and azathioprine/mycophenolate mofetil. The median post-PTLD follow up time was 3.1 years (IQR 1.8 – 8.5 years). Overall survival (OS) following development of destructive PTLD was 94% (95% CI; 63% – 99%) at 3-years and 87% (95% CI; 57% – 97%) at 5-years, while one death unrelated to PTLD occurred within 18 months of PTLD diagnosis in the non-destructive group.

Risk factors for PTLD

i. Univariate analysis

Consistent with published literature,^[8-9] we observed a significant association between recipient EBV seronegativity at transplantation and an increased risk of PTLD [Figure 1A]. Within our cohort, pre-transplant cardiac diagnosis and age at thymectomy were identified as additional risk factors for PTLD. Children who had been transplanted for an underlying CHD had a significantly higher risk of developing PTLD compared to those transplanted for ACM (crude HR= 2.8; 95% CI: 1.4–5.4) [Figure 1B]. This was a consistent finding for both destructive and non-destructive PTLD [Figure S1]. Similarly, children with age of thymectomy at ≤ 1 year had a higher risk of developing PTLD compared to those undergoing a similar procedure at > 1 year (crude HR= 2.7; 95% CI: 1.3–5.2) [Figure 1C]. In the setting of all patients receiving induction immunosuppression, neither the choice of that induction therapy (basiliximab v ATG, HR=1.5; 95% CI: 0.8 – 2.9, $p=0.27$) nor maintenance immunosuppressive therapy (tacrolimus v ciclosporin, HR=1.3; 95% CI: 0.7 – 2.7, $p=0.72$) was associated with risk of PTLD [Table 1]. Finally, age at transplantation was also found not to be a significant risk factor for developing PTLD ($p = 0.09$) [Table 1].

Age at Thymectomy and T-Lymphocyte Counts

The median age at thymectomy for all children with CHD was 0.4 years (IQR 0.02 – 3.4 years) and for those with ACM was 5.5 years (IQR 1.4 – 13.2 years), $p < 0.001$. This age difference was independent of PTLTD status [Figure 2]. It is notable that amongst children with CHD who developed PTLTD, the median age at thymectomy was particularly early (0.06 years; IQR 0.01 – 1.6 years). $CD4^+$ and $CD8^+$ T-lymphocyte counts measured at 2 years post-transplant were significantly lower in the CHD group compared to the ACM group ($CD4^+$ 391/ μ l vs 644/ μ l, $p=0.01$ and $CD8^+$ 382/ μ l vs 500/ μ l, $p=0.01$) [Figures 3A and 3B], with the difference in T-lymphocyte counts still persisting at 5 years post-transplant ($CD4^+$, 530/ μ l vs 620/ μ l, $p=0.02$ and $CD8^+$ 342/ μ l vs 475/ μ l, $p=0.02$). None of the study participants with underlying CHD were transplanted for DiGeorge syndrome or were known to have a pre-existing thymic/immunodeficiency disorder.

To determine if the disparity in $CD4^+$ and $CD8^+$ T-lymphocyte counts was influenced by PTLTD status, the study cohort was stratified into 2 groups, those developing PTLTD and those not developing PTLTD, and T-lymphocyte counts were analyzed according to pre-transplant cardiac diagnosis [Figure 4]. $CD4^+$ T-lymphocyte counts at 2 years post-transplant were significantly lower in children transplanted for CHD versus ACM in both the PTLTD (263/ μ l vs 797/ μ l, $p= 0.01$) and non-PTLTD groups (310/ μ l vs 592/ μ l, $p= 0.04$). However, at 5 years post-transplant the difference in $CD4^+$ T-lymphocyte count between the two groups was found only to be significant in children who had developed PTLTD (430/ μ l vs 663/ μ l, $p < 0.01$). Similarly, $CD8^+$ T-lymphocyte counts at 2 years post-transplant were significantly lower in children with CHD for both the PTLTD (404/ μ l vs 1055/ μ l, $p < 0.01$) and non-PTLTD groups (360/ μ l vs 486/ μ l, $p= 0.03$), while counts at 5 years post-transplant were again found only to be significant in children who developed PTLTD (367/ μ l vs 765/ μ l, $p < 0.01$).

ii. Multivariate analysis

Multivariate Cox analysis identified two independent risk factors for developing PTLTD after cardiac transplantation [Table 2]. EBV seronegative status at transplant was, as expected, associated with a significantly greater risk of developing PTLTD (HR= 2.7; 95% CI: 1.3 – 5.6, $p=0.010$). Importantly, pre-transplant cardiac diagnosis of CHD also remained significantly associated with risk of PTLTD,

conferring a three-fold higher risk of developing PTLD compared to ACM (HR= 3.2; 95% CI: 1.4 – 7.4, p = 0.007).

Discussion

Our study provides a detailed analysis of PTLD characteristics in the largest UK cohort of pediatric heart transplant patients reported to date. The cumulative incidence of PTLD was 21.1% at 5-years post-transplant, with 9.5% developing destructive PTLD, in keeping with previous reports.^[1, 9, 13-14] Equally, we were able to demonstrate a clear risk association between primary EBV infection after transplantation and PTLD development^[1, 9-12, 15]. Two distinctive findings were the significantly higher risk of PTLD in children transplanted for CHD compared to ACM and in children undergoing a first sternotomy at ≤ 1 -year-old. Only two other studies have reported CHD to be a risk factor for PTLD following OHT,^[12, 14] with earlier age (< 1 year) at sternotomy, hypoplastic left ventricle and number of surgical events identified as additional risk-factors by one group.^[12] As expected, EBV seronegativity at transplant was also a risk-factor, but these somewhat smaller studies were unable to perform multivariate analysis to investigate the independent effect of these potentially interacting variables. In contrast, recent findings by the Pediatric Heart Transplant Study (PHTS) did not show CHD to be a significant risk factor for late-onset destructive PTLD (i.e. PTLD occurring > 3 years post-transplant).^[16] It is important to recognise, however, that only a third of PTLD tumours in this PHTS study were shown to be EBV-positive, indicating that the relationship between CHD and the risk of PTLD is likely to be dependent on the timing of post-transplant viral infection and alternative mechanisms driving lymphoproliferation.^[15, 16] In our cohort of EBV-positive PTLD we have been able to demonstrate that a pre-transplant diagnosis of congenital heart disease remains a risk factor for development of PTLD, independent of EBV serostatus. Furthermore, our study expands on the previously published data by comparing serial post-transplant CD4⁺/CD8⁺ T-lymphocyte counts from children with and without PTLD. We demonstrate significantly lower CD4⁺ and CD8⁺ T-lymphocyte counts in our CHD cohort compared to those with ACM, a finding which persisted at 5 years post-transplant specifically within the subgroup that developed PTLD. We believe this likely represents the effect of very early thymectomy within the CHD patients who developed PTLD.

In our study we included cases with a histologically proven non-destructive PTLD, predominantly of adeno-tonsillar tissue. The clinical relevance of non-destructive PTLD in children remains uncertain but the occasional demonstration of clonal cytogenetic abnormalities in non-destructive PTLD and reports of destructive PTLD occurring concurrent with or subsequent to non-destructive PTLD provide some evidence to support the potential for transformation into more aggressive, destructive disease.^[17] It is important to note that our registry-based data may not include all episodes of adeno-tonsillectomy performed outside our centre and we are therefore unable to provide an accurate figure on the incidence of non-destructive PTLD following OHT. Nevertheless, our central finding of the increased risk of PTLD in patients with CHD is consistent within both destructive and non-destructive PTLD sub-groups and we recommend that future studies of the role of EBV-specific T-lymphocyte immunity should include patients with non-destructive PTLD.

Cardiac surgery via median sternotomy in early childhood is necessarily associated with a total or partial thymectomy in order to facilitate access to the heart and great vessels. The thymus plays a crucial role in the development of robust cell-mediated immunity by providing a microenvironment for precursor T-lymphocytes to proliferate and differentiate into mature (naïve) T-lymphocytes.^[18] The long-term clinical implications of early thymectomy remain contentious although some data identify the possibility of precipitating premature immunosenescence.^[19, 20] Numerous studies have demonstrated a significant alteration in the composition of adaptive immune cells in thymectomised CHD patients. Overall reduction in the total CD4⁺ and CD8⁺ T-lymphocyte counts alongside progressive lymphopenia and long-term decline in the number of T-cell receptor excision circles (TRECs) have been noted, indicating a pervasive restriction in the diversity of the T-cell receptor (TCR) repertoire.^[19, 21–24]

In spite of the demonstrated role of impaired T-lymphocyte surveillance as a risk predictor for EBV-positive PTLD in adult and pediatric recipients of hematopoietic transplants and other SOTs,^[25–27] its relationship with pediatric cardiac transplantation and age at thymectomy is yet to be fully explored.^[12] CHD patients undergoing thymectomy at less than two weeks old have been shown to exhibit a loss of TCR repertoire diversity and an altered immune response to CMV infection.^[21] It is plausible that an overall restriction of the cell mediated immune compartment of transplanted CHD patients could similarly impair the development of EBV-specific immunity and lead to uncontrolled EBV proliferation

in B-lymphocytes with an ensuing increase in the risk of PTLD. Interestingly, however, the Single Ventricle Survivorship Program did not identify abnormally severe EBV infection and reported only one case of EBV-positive lymphoid malignancy amongst 178 survivors of the Fontan procedure.^[28] The implication is that any restriction of the TCR repertoire post-thymectomy is insufficient to allow EBV-driven lymphoproliferation and that additional factors are instrumental in causing EBV-positive PTLD. This highlights the need for detailed, functional immunological studies to demonstrate a causal link between early thymectomy and risk of PTLD.

In addition to the importance of pre-transplant immunological repertoire, the impact of induction therapy on risk of PTLD remains unclear^[13, 29-31] and is complicated by varied choice and intensity of induction agent and subsequent maintenance immunosuppression. All patients transplanted at our centre received induction of immunosuppression with either lymphocyte depleting ATG or non-depleting Basiliximab. We did not identify a difference in PTLD risk between these two patient groups, neither did we identify a difference in risk based on maintenance immunosuppression. This may result from a limited cohort size or from the challenge of accurately quantifying single time-point or cumulative immunosuppression. Interestingly, the PHTS identified that patients receiving induction were more likely to have underlying CHD^[31], highlighting the importance of careful multivariate modelling in future studies.

In summary, our study provides compelling evidence to support a role for early thymectomy in the development of PTLD through T-lymphocyte dysregulation and impaired response to primary EBV infection. However, the overall risk of PTLD is likely to reflect a complex interplay of immunological development, timing of EBV infection and overall immunosuppressive exposure. Additional research focussed on elucidating the interaction(s) between the host immune microenvironment and EBV is needed in order to better understand which patients are at greatest risk of developing PTLD and how that risk might be modified. Explicit profiling of the T-lymphocyte repertoire and immune response to EBV infection in thymectomised pediatric heart transplant patients is warranted to further investigate this theory and provide data to inform future changes to clinical practice.

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Figure 1: Kaplan-Meier curves of cumulative incidence of PTLD with unadjusted hazard ratios, 95% confidence intervals and log rank P values in **A)** Overall study cohort, **B)** EBV positive and negative children, **C)** Children having thymectomy before or after 1 year of age, **D)** Children with CHD and ACM

Figure 2: Age at thymectomy for all transplanted children stratified by cardiac diagnosis and PTLD status (CHD: Congenital Heart Disease; ACM: Acquired Cardiomyopathy)

Figure 3: Box plots showing A) CD4 T-lymphocyte counts at 2- and 5 years post-transplant in children with CHD and ACM, B) CD8 T-lymphocyte counts at 2- and 5 years post-transplant in children with CHD and ACM.

Figure 4: Stratification by PTLT status of CD4 T-lymphocyte counts at **A)** 2 years post-transplant and **B)** 5 years post-transplant, and CD8 T-lymphocyte counts at **C)** 2 years post-transplant and **D)** 5 years post-transplant for children with CHD and ACM

Number at risk					
CHD	71	23	4	0	0
ACM	129	73	25	3	0

Number at risk					
CHD	71	23	4	0	0
ACM	129	73	25	3	0

Supplementary Figure 1: Kaplan-Meier curves of the cumulative incidence of PTLD according to cardiac diagnosis with unadjusted hazard ratios, 95% confidence intervals and log rank P values for **A)** Non-destructive PTLD lesions and **B)** Destructive PTLD lesions

Table 1: Study cohort characteristics and *p* values for univariate Cox regression analysis

Patient Demographics	n of 200 (%)	PTLD (n = 35)	No PTLD (n = 165)	<i>p</i>
Age group at Transplant (years)				
<1	36 (18)	10	26	0.09
1 – 10	103 (52)	14	89	
≥ 10	61 (30)	11	50	
Median age at PTLD (IQR)		9 (4.8 – 19.2)	6.7 (6 – 11)	0.47
Gender				
Male	98 (49)	18	80	0.86
Female	102 (51)	17	85	
Ethnicity				
Caucasian	167 (84)	27	140	0.08
Black	7 (3)	4	3	
Asian	26 (13)	4	22	
Cardiac Diagnosis				
Congenital Heart Disease	71 (36)	18	53	0.003
• Single Ventricle defect	34 (17)	10	24	
• Biventricular – Cyanotic	21 (11)	2	19	
• Biventricular – Acyanotic	16 (8)	6	10	
Acquired Cardiomyopathy	129 (64)	17	112	
EBV Status Pre-transplant				
Positive	104 (52)	13	91	0.02
Negative	57 (29)	17	40	
Unknown	39 (19)	5	34	
Age at Thymectomy				
≤ 1 year	88 (44)	21	67	0.005
> 1 year	112 (56)	14	98	
Induction Therapy				
Anti-Thymocyte Globulin	124 (62)	20	104	0.27
Basiliximab	76 (38)	15	61	
CNI at 1-year post-transplant				
Ciclosporin	80 (40)	7	73	0.72
Tacrolimus	120 (60)	28	92	
Adjunct used at 1-year post-transplant†				
Prednisolone only	24 (12)	6	18	0.22
Aza/MMF only	70 (35)	3	67	
Prednisolone + Aza/MMF	40 (20)	3	37	
None	43 (22)	23	20	
PTLD Histology				
Overall	35 (18)			
Non-destructive PTLD	16 (46)†			
Destructive PTLD	19 (54)†			
• Monomorphic PTLD	12 (63)*			
• Polymorphic PTLD	6 (32)*			
• Hodgkin lymphoma-type PTLD	1 (5)*			

CNI: Calcineurin Inhibitor; Aza: Azathioprine; MMF: Mycophenolate Mofetil; IQR: Interquartile Range † n of 35 (%); * n of 19 (%); † excluding 23 patients who died or were lost to follow up at < 1-year post-transplant

Table 2: Multivariate Cox regression analysis with adjusted hazard ratios and *p* values

Study variable	Adjusted HR (95% CI)	<i>p</i>
Pre-transplant Cardiac diagnosis		
ACM	1.0	0.007
CHD	3.2 (1.4 – 7.4)	
EBV serostatus at Transplantation		
Positive	1.0	0.010
Negative	2.7 (1.3 – 5.6)	
Age at Thymectomy		
> 1 year	1.0	0.89
≤ 1 year	0.9 (0.4 – 2.2)	

HR: Hazard Ratio; ACM: Acquired Cardiomyopathy; CHD: Congenital Heart Disease; EBV: Epstein-Barr Virus

Supplementary Table 1: Peri-operative and long-term immunosuppression regimen for children < 18 years undergoing an orthotopic heart transplant at Newcastle upon Tyne Hospitals

Pre-operative	Intra-operative	Post-operative
Ciclosporin <ul style="list-style-type: none">4mg/kg orally if eGFR >90 ml/min/1.73 m22mg/kg orally if eGFR 60-90 ml/min/1.73 m2Omit if eGFR <60ml/min/1.73 m2 Azathioprine <ul style="list-style-type: none">4 mg/kg orally (no max dose)Titrate dose depending on severity of induced thrombocytopenia and/or leucopenia Basiliximab (Simulect) <ul style="list-style-type: none">IV 10mg if body weight < 35kgIV 20mg if body weight > 35kgAdminister within 2 hours pre-transplant	Methyl prednisolone <ul style="list-style-type: none">IV 15mg/kg (max dose 500mg) when the cross-clamp is released	Anti-thymocyte globulin (rATG) <ul style="list-style-type: none">IV 2.5mg/kgRepeat daily doses for up to 7 days post-op if CD3 count > 0.05x10⁹/l <p><u>OR</u></p> Basiliximab (Simulect)* <ul style="list-style-type: none">IV 10mg if body weight < 35kgIV 20mg if body weight > 35kgAdminister on day 4 post-op Methylprednisolone <ul style="list-style-type: none">3 mg/kg (max dose 125 mg). THREE doses only are given Calcineurin inhibitors <ul style="list-style-type: none">Ciclosporin IV 1mg/kg twice daily initiated day 1 post-op. Switched to enteral route when absorption is reliable (3x IV dose). Peri-transplant trough level of 250mcg/l is gradually reduced to 100-150mcg/l over the first year.Tacrolimus Used if significant side effects with Ciclosporin (typically switched at 3 months post-transplant). Initial trough levels target of 10-15 mcg/l are reduced to 5-10 mcg/l over time. Metabolic inhibitors <ul style="list-style-type: none">Azathioprine 2 mg/kg daily (no max dose). Initiated a few days after transplant when the platelets and LFTs have normalised. Stopped if the WCC < 4.0 - 6.0 x 10⁹/l.Mycophenolate mofetil Substituted for Azathioprine if needed. Target dose is 600 mg/m2/dose bd. Prednisolone <ul style="list-style-type: none">Given to older patients> 5 years 0.2mg/kg/day from day 2 – 6 weeks. Gradually wean off if no rejection> 10 years 1mg/kg/day from day 2 and then tailor to a maintenance dose of 0.2mg/kg/day. Gradually wean to 0.1mg/kg/day by 1 year of transplant if no rejection then may be discontinued or maintained for life depending on rejection history.

* Used as **an alternative to ATG** in patients at high risk of sepsis or in those with severe renal dysfunction as it selectively blocks the CD25 Interleukin-2 receptor, preventing T-lymphocyte activation. It is used in patients bridged with ECMO but not necessarily a VAD.

Supplementary Table 2. PTLD cohort details. **ASO** - arterial switch operation, transposition of the great arteries, **CoA** - coarctation of the aorta, **CHL** - congenital hypoplastic left ventricle, **IM** - infectious mononucleosis, **LVAD** - left ventricular assist device

Patient	Gender	Cardiac Diagnosis	Age at Transplant (years)
PT1	M	DCM	15.9
PT2	M	TGA	13.1
PT3	M	cAVSD + right atrial isomerism	7.4
PT4	F	HLHS	3
PT5	F	HLHS	5.1
PT6	F	HLV	4.3
PT7	M	pAVSD	13.2
PT8	M	HLHS	8.8
PT9	F	DCM	8.5
PT10	M	DCM	11.4
PT11	M	DCM	14.2
PT12	F	cAVSD + left atrial isomerism	11.1
PT13	F	DCM	16
PT14	F	DCM	10.1
PT15	F	PA/IVS	1.9
PT16	F	DCM	5.7
PT17	F	DCM	0.9
PT18	F	DCM	0.5
PT19	M	DORV	16
PT20	M	HLHS	2.1

PT21	F	ccTGA	11.4
PT22	M	DCM	5.4
PT23	M	Severe AVR	2.3
PT24	M	HLHS	0.5
PT25	M	HLV	3.9
PT26	F	RCM	14.8
PT27	M	CoA	0.1
PT28	M	DCM	0.7
PT29	M	DCM	4
PT30	F	TGA	0.9
PT31	M	HCM	0.5
PT32	F	RCM	14.1
PT33	F	DCM	0.6
PT34	M	DCM	0.3
PT35	F	HLHS	0.4

in, **ATG** - AntiThymocyte Immunoglobulin, **AVR** - aortic valve regurgitation, **AZA** - Azathioprine, **BL** - B-cell lymphoma, **BMT** - bone marrow transplant, **CNS** - central nervous system, **DLBCL** - diffuse large B-cell lymphoma, **CNI** - calcineurin inhibitor, **DCM** - dilated cardiomyopathy, **DLBCL** - diffuse large B-cell lymphoma, **ECLS** - extracorporeal life support, **ECMO** - extracorporeal membrane oxygenation, **FIM** - Functional Independence Measure, **Graft** - organ or tissue transplanted from one individual to another, **HIV** - Human Immunodeficiency Virus, **IABP** - intra-aortic balloon pump, **ICU** - intensive care unit, **LDL** - low-density lipoprotein cholesterol, **LVEF** - left ventricular ejection fraction, **MACE** - major adverse cardiac events, **MMF** - Mycophenolate Mofetil, **n.a.** - not available, **PA/IVS** - pulmonary atresia with intact ventricular septum, **PAP** - pulmonary artery pressure, **PCPD** - percutaneous catheter placement device, **PE** - pulmonary embolism, **PPV** - percutaneous transluminal tracheostomy, **RBC** - red blood cells, **SVC** - superior vena cava, **TBI** - traumatic brain injury, **TJ** - thoracic junction, **VAD** - ventricular assist device, **WBC** - white blood cells, **XRT** - external beam radiation therapy.

Recipient EBV Status at Transplant	Donor EBV Status at Transplant	Induction Immunosuppression	CNI
Positive	Positive	ATG	Tacrolimus
Negative	Positive	ATG	Ciclosporin
Negative	Positive	ATG	Tacrolimus
Negative	Negative	Basiliximab	Tacrolimus
Positive	Positive	ATG	Tacrolimus
Positive	Negative	Basiliximab	Ciclosporin
Negative	Negative	ATG	Tacrolimus
Positive	Negative	Basiliximab	Ciclosporin
Negative	Negative	Basiliximab	Tacrolimus
Positive	Negative	ATG	Tacrolimus
Negative	Positive	ATG	Tacrolimus
Positive	Positive	Basiliximab	Tacrolimus
Negative	Positive	ATG	Tacrolimus
n.a	n.a	Basiliximab	Tacrolimus
Negative	Positive	Basiliximab	Tacrolimus
Negative	Positive	Basiliximab	Tacrolimus
Negative	Positive	ATG	Tacrolimus
Negative	Negative	ATG	Ciclosporin
Negative	Negative	ATG	Tacrolimus
Positive	Positive	ATG	Ciclosporin

Positive	Positive	ATG	Ciclosporin
Positive	n.a	ATG	Tacrolimus
n.a	n.a	Basiliximab	Tacrolimus
Negative	Negative	Basiliximab	Tacrolimus
Negative	n.a	ATG	Tacrolimus
Positive	n.a	ATG	Tacrolimus
n.a	n.a	ATG	Tacrolimus
n.a	n.a	ATG	Tacrolimus
Positive	Positive	ATG	Tacrolimus
Negative	Negative	ATG	Tacrolimus
n.a	n.a	Basiliximab	Tacrolimus
Positive	Positive	Basiliximab	Ciclosporin
Positive	n.a	Basiliximab	Tacrolimus
Negative	Positive	Basiliximab	Tacrolimus
Negative	Positive	Basiliximab	Tacrolimus

Burkitt Lymphoma, **BiVAD** - Biventricular Assist Device, **cAVSD** - complete atrioventricular large B-cell lymphoma, **DORV** - double outlet right ventricle, **HCM** - hypertrophic cardiomyopathy, **IVS** - intact ventricular septum, **RCM** - restrictive cardiomyopathy, **TGA** - transposition of the great vessels

Adjunctive Immunosuppression	PTLD Histology	Age at PTLD diagnosis (years)
None	DLBCL	16.8
Aza	DLBCL	13.9
None	DLBCL	9.4
Pred	BL	11.4
Pred	DLBCL	6.6
Pred	DLBCL	4.9
None	BL	17.5
Pred/Aza	DLBCL	9
Pred	BL	11.5
None	BL	13.5
Pred/Aza	CHL	18.8
Pred	DLBCL	14
None	DLBCL	16.3
None	Polymorphic	15.3
None	Polymorphic	2.3
Pred	Polymorphic	6.5
None	Polymorphic	2.8
None	Polymorphic	4.8
Pred/MMF	Polymorphic	19.1
None	Florid follicular hyperplasia	5.3

Aza	Florid follicular hyperplasia	14.4
MMF	Florid follicular hyperplasia	10.3
None	IM-like	7.6
None	Florid follicular hyperplasia/IM-like	5.8
None	Florid follicular hyperplasia	14.1
None	Florid follicular hyperplasia	17
MMF	Florid follicular hyperplasia	3.5
None	Florid follicular hyperplasia/IM-like	1.1
MMF	Florid follicular hyperplasia	5.5
None	Florid follicular hyperplasia/IM-like	1.7
Aza	Florid follicular hyperplasia/IM-like	1.7
None	Florid follicular hyperplasia/IM-like	20.6
MMF	IM-like	2.6
MMF	Florid follicular hyperplasia/IM-like	4.5
MMF	Florid follicular hyperplasia	5.5

septal defect, **ccTGA** - congenitally corrected
 athy, **HLHS** - hypoplastic left heart syndrome, **HLV** -
 eat arteries

Indication for first median sternotomy	Age at Thymectomy (years)	CD4 count at 2-years post-transplant (cell/uL)
Transplant	15.9	1735
Senning procedure	1.7	n.a
Glenn procedure	1.6	n.a
Norwood I	0.01	n.a
Norwood I	0.01	n.a
Glenn procedure	0.01	102
AVSD repair	0.9	n.a
Norwood I	0.06	44
LVAD insertion	8.5	905
Transplant	11.4	797
Transplant	14.2	1019
Glenn procedure	2.1	n.a
Transplant	16	n.a
Transplant	10.1	644
BiVAD insertion	1.4	263
BiVAD insertion	5.6	530
Transplant	0.9	997
Transplant	0.5	20
DORV repair	0.3	54
Norwood I	0.01	1500

Transplant	11.4	420
LVAD insertion	5.1	586
BiVAD insertion	2	954
Norwood I	0.02	291
Glenn procedure	0.02	107
Transplant	14.8	390
CoA repair	0.06	202
Transplant	0.7	712
LVAD insertion	3.8	566
ASO	0.02	288
BiVAD insertion	0.3	1133
Transplant	14.1	871
LVAD insertion	0.3	928
LVAD insertion	0.2	895
Norwood I	0.01	167

CD8 count at 2-years post-transplant (cell/uL)	CD4 count at 5-years post-transplant(cell/uL)	CD8 count at 5-years post-transplant (cell/uL)
n.a	n.a	460
n.a	n.a	n.a
n.a	798	720
230	709	204
n.a	191	178
202	175	290
n.a	n.a	n.a
51	33	32
1210	560	785
1972	240	1918
1917	1028	400
n.a	234	83
n.a	n.a	n.a
633	1180	529
141	115	n.a
1720	765	455
269	1257	718
143	984	961
61	360	n.a
480	516	306

561	589	368
963	451	469
455	n.a	n.a
404	198	426
296	530	490
269	1016	765
320	768	561
1843	470	812
1458	975	1024
234	n.a	n.a
498	1002	432
1148	963	1163
549	712	1820
330	526	376
233	499	367