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ORIGINAL ARTICLE

Acute rejection after paediatric heart transplantation: far less common and less severe

Astrid E. Lammers,[1] Philip Roberts,[1] Katherine L. Brown,[2] Matthew Fenton,[1] Philipp Rees,[1] Neil J. Sebire[3] and Michael Burch[1]

1 Department of Paediatric Cardiology, Great Ormond Street Hospital for Children, London, UK
2 Department of Paediatric Cardiac Critical Care, Great Ormond Street Hospital for Children, London, UK
3 Department of Pathology, Great Ormond Street Hospital for Children, London, UK

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Correspondence
Dr Michael Burch, Consultant Paediatric Cardiology/Paediatric Cardiothoracic Transplantation, Great Ormond Street Hospital for Children, Great Ormond Street, London WC1N 3JH, UK. Tel.: +44 207 4059200; fax: +44 207 9052321; e-mail: burchm@gosh.nhs.uk

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Summary

Despite improved immunosuppression, rejection accounts for significant morbidity and mortality in children after heart transplantation. We report the incidence and outcome of rejection of 105 children (male = 50; mean age of 8.3 ± 5.8 years) following heart transplantation between January 2002 and August 2007. A multi-variant model was constructed for risk factors associated with significant rejection. In 271.9 patient-years of follow-up, there were 23 episodes of significant rejection (≥3A) in 21 patients (20%). Five presented in haemodynamic collapse requiring extracorporeal membrane oxygenation support 1.6–35.9 months after transplantation; four of five survived the rejection episode. Overall rejection episodes were more common in older children, boys and those treated with sirolimus. Whereas the risk for rejection in patients on an immunosuppression regime containing tacrolimus was significantly lower. The latter finding persisted on multivariate analysis ($P < 0.002$). Interestingly, none of the patients who presented with haemodynamic collapse was on mycophenolate mofetil. While our experience is of a far lower incidence of rejection than registry data, rejection remains a serious problem after paediatric heart transplantation. Sirolimus without a calcineurin inhibitor was associated with more rejection episodes, whereas tacrolimus and mycophenolate appeared to provide the best protective profile.

Introduction

Despite improved immunosuppressive regimes, rejection remains a major concern in patients after heart transplantation and is associated with significant morbidity and mortality [1–4]. The recent ISHLT registry data have shown that over 40% of children required treatment for rejection in the first year post-transplantation and survival was worse in those that suffered rejection [5,6].

Asymptomatic cellular rejection can be diagnosed on endomyocardial biopsies – routinely performed in our centre before discharge, 3 and 6 months following transplantation [7]. Other patients present with clinical symptoms of congestive heart failure, e.g. increasing lethargy, shortness of breath or peripheral oedema. Rejection with haemodynamic compromise is a great concern after heart transplantation in adults and children. Defined as a clinical event more than 1 week postoperatively that leads to augmentation of immunosuppression and inotropic therapy, the incidence has been shown to be 11% with a 60% mortality in the paediatric heart transplant data collection [8]. It has also been shown to have a high mortality when associated with late rejection in children [9].

Humoral rejection is typically early and associated with haemodynamic compromise, although recent work has shown a high prevalence of late antibody-mediated rejection [10]. The complement split product C4d has been used to assess humoral rejection in renal transplantation

[11] and appears important in adult heart transplantation [12], however, the value in paediatrics is less clear. High levels of donor-specific circulating allo-antibodies and positive immuno-stains for C4d complement are widely used to diagnose humoral rejection [13,14]. Human leucocyte antigen (HLA) matching, particularly the DR group appears important in limiting rejection [15], although again there are limited paediatric data.

Although the registry data are clearly important, large single-centre experience can allow detailed analysis of outcome, risk and rescue strategies. In our experience, the incidence of acute rejection is low and while rejection with haemodynamic compromise remains a great concern, rescue with extracorporeal membrane oxygenation (ECMO) support has proven useful. To illustrate this, we report our experience with acute rejection from a large paediatric, single-centre recent cohort and have also investigated risk factors in a multivariate model including age, ethnicity, HLA mismatches and C4d staining.

Patients and methods

We retrospectively reviewed 105 consecutive patients who underwent orthotopic heart transplantation in our centre between February 2002 and August 2007, as transplantation protocols underwent major revisions in our centre and Basiliximab (monoclonal antibody to the IL-2Rα receptor of T cells) was introduced as standard induction therapy [16].

We report patient demographics and incidence of severe rejection [Grade 3A and above (before 2005)/>2R according to the revised ISHLT classification from 2005], including any episodes of haemodynamic collapse [17]. We tried to identify predisposing factors, including population risk factors (gender, ethnicity, number of HLA mismatches and ABO mismatch) and immuno suppression regime (induction and maintenance therapy) as well as other risk factors such as cytomegalovirus (CMV) status (and mismatch). Univariate analysis was performed separately for overall rejection, symptomatic rejection as well as rejection episodes with haemodynamic collapse.

Immunosuppression practice

Immunosuppression therapy in the reported cohort varied. When possible children were given tacrolimus, unless renal dysfunction was severe and sirolimus was used instead.

A cell cycle inhibitor was used, where tolerated and this was azathioprine in children transplanted in the earlier era (before April 2005), and more recently this was replaced by mycophenolate mofetil (MMF). Steroid treatment was withdrawn in the majority of patients following a negative second endomyocardial biopsy after 3 months of therapy. Only in those children who presented with rejection was steroid treatment continued.

For 6 months after transplantation, we aim for tacrolimus levels between 10 and 14 ng/ml. In patients with renal impairment postoperatively, we introduce the tacrolimus slowly and accept levels <10 ng/ml during the first week after transplantation. From 6 months to 1 year after transplantation, target levels are 8–12 ng/ml, after a year 5–8 ng/ml. The same levels apply for sirolimus.

Assessment for acute cellular and humoral rejection

Surveillance biopsies are still routinely performed in our centre. Three are undertaken within the first 6 months after transplantation (one before hospital discharge, the second 3 months following transplant, and the third 6 months following transplant). Further biopsies were taken only when there had been previous rejection or clinical symptoms occurred.

Significant rejection was defined as either a histopathological diagnosis from an endomyocardial biopsy ≥3A/2 R rejection [17–19] or a patient presenting with symptoms of severe heart failure or haemodynamic collapse.

To assess for any underlying humoral rejection, the endomyocardial biopsies of all patients with a rejection episode were investigated and C4d staining was performed. Endothelial complement deposition resulting in a positive staining was suggestive of humoral rejection. We used 20 routine biopsies of patients following heart transplantation without any clinical or histopathological evidence for cellular rejection episode as normal controls for C4d staining.

Statistical analysis

All values are presented as mean ± standard deviation. Comparisons between groups were made using the Mann–Whitney *U*-test or chi-square test as appropriate.

The relationship between co-variables and event-free survival was studied by univariate Cox proportional hazard analysis initially. The hazard ratio with 95% confidence interval (CI) and *P*-values are presented. Hazard ratios for continuous variables apply per unit of the analysed variable. Parameters significant on univariate analysis were entered into a multivariate Cox proportional hazard analysis model.

Kaplan–Meier cumulative survival plots were constructed to illustrate the results. In patients who suffered more than one rejection episode, only the first rejection was used for statistical analysis. For all analyses, a *P*-value <0.05 was considered significant. All tests were performed two-tailed. MedCalc 9.3 (MedCalc Software, Mariakerke, Belgium) was used for statistical analysis.

Results

One hundred and five consecutive children [male $n = 50$; mean age of 8.3 ± 5.8 years (0.1–17.9 years)] underwent an orthotopic heart transplantation in our centre between February 2002 and August 2007. Mean waiting time for a suitable donor organ was 1.8 ± 2.9 months (range 0–19.4 months).

Patients' demographics, HLA tissue typing and morbidity are shown in Table 1.

Outcome

Mean follow-up after transplantation was 2.6 ± 1.6 years (range 0–5.5 years), accumulative time of follow-up was 271.9 patient-years following transplantation. During follow-up, seven patients died (6.7%). Five died early after transplantation (during the same admission of their transplantation) from acute graft failure/pulmonary hypertension (4.8%). These patients were censored from further analysis.

Occurrence of clinically or histologically proven rejection, clinical presentation and treatment

We observed 23 episodes (21.9%) of significant rejection in 271.9 patient-years in 21 patients (20%) (Fig. 1).

Thirteen (12.4%) of the children were asymptomatic, in whom the routine surveillance endomyocardial biopsy revealed significant rejection on histopathological examination according to the ISHLT classification (≥3A before 2005/≥2R). On five (4.8%) occasions, children presented with symptoms of cardiac dysfunction (lethargy, tachypnoea and ankle oedema) suggestive of a rejection episode with cardiac compromise. Five children (4.8%) presented in haemodynamic collapse 1.6–35.9 months after transplantation requiring ECMO support for recovery, of whom four survived the rejection episode. Though all patients survived the course of ECMO (5–15 days) and ventricular function improved after the rejection was successfully treated, one patient died from fulminant sepsis 20 days after presentation. All other children with confirmed rejection recovered from their rejection episode and survived following medical antirejection treatment. All patients received high dose pulsed steroids as initial rejection treatment. The five patients who presented with haemodynamic collapse received antithymocyte globulin (ATG), and one of those patients was treated with additional OKT3.

None of the patients who had ECMO support or a Berlin Heart preoperatively, presented within our follow-up period with a rejection episode. One patient died from acute graft failure.

Table 1. Patients' demographics, HLA-tissue typing and morbidity.

Patients (*n*)	*n* = 105	Percentage
Male/female	50/55	
Age at transplantation (years)	8.3 ± 5.8	
Waiting time on list (months)	1.75 ± 2.9	
Weight at transplantation (kg)	30.1 ± 21.6	
Height at transplantation (cm)	121.0 ± 40.0	
Ethnical background		
Caucasian	89	84.8
Asian	12	11.4
African/Afrocaribean	4	3.8
Diagnosis		
Dilated cardiomyopathy	66	62.9
Restrictive cardiomyopathy	12	11.4
Congenital heart disease	27	25.7
Blood group		
O	54	51.5
A	42	40
B	9	8.5
AB	0	0
ABO mismatch	13	12.4
HLA-A (*n* = 95)		
0 Mismatches	5	5.5
1 Mismatch	48	50.5
2 Mismatches	42	44.2
HLA-B (*n* = 95)		
0 Mismatches	6	6.3
1 Mismatch	29	30.5
2 Mismatches	60	63.2
HLA-DR (*n* = 95)		
0 Mismatches	6	6.3
1 Mismatch	37	38.9
2 Mismatches	52	54.7
HLA total mismatches (*n* = 95)		
1 Mismatch	2	2.1
2 Mismatches	4	4.2
3 Mismatches	15	15.8
4 Mismatches	17	17.9
5 Mismatches	40	42.1
6 Mismatches	17	17.9
CMV positive (IgG)	20	19.4
CMV mismatch	26	24.8
Bridged to transplantation		
On ECMO	21	20
Median days on ECMO pre-Tx	9	
On assist device (Berlin Heart)	4	3.8
Median days on Berlin Heart	28	
Morbidity post-transplantation		
ECMO	7	6.7
RVAD	1	0.9
Temporary renal replacement therapy	15	14.3
CHB – pacemaker insertion	2	1.8
Tracheostomy/slow weaning	4	3.8
Diaphragmatic palsy	2	1.8
Cerebral insult (1 prior/1 post-Tx)	2	1.8
Mean duration of stay on ICU (days)	16 ± 28	
Mean hospitalization (days)	30 ± 28	

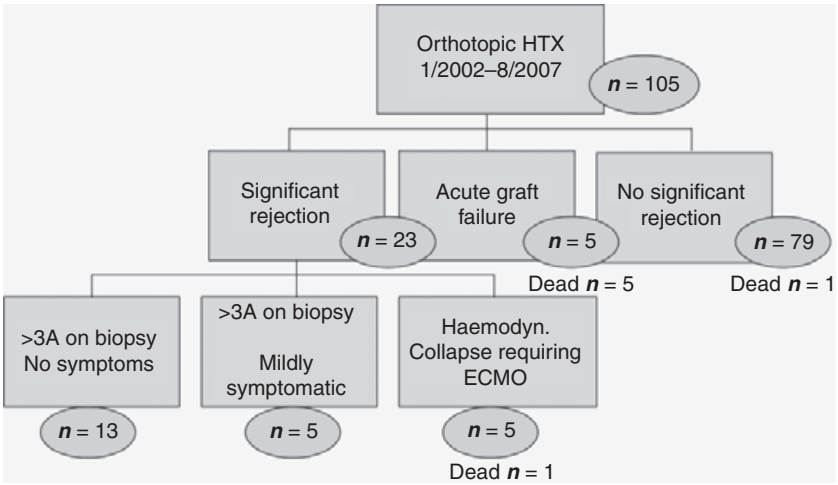

Figure 1 Flowchart showing total number of orthotopic heart transplantations between January 2002 and August 2007, occurrence of rejection/graft failure and outcome, including biopsy findings.

Seven patients received ECMO post-transplantation. Indication for post-transplant ECMO support was acute graft failure in six patients (failure to come off bypass, pulmonary hypertension) and in one child it was observed early rejection. Four of the seven patients, who received ECMO support post transplantation, had ECMO before their transplantation.

Of the seven patients who received ECMO post-transplantation, one died from acute graft failure. Two patients presented with a rejection episode, 4 and 20 months following transplantation, subsequently. Six of the seven patients who required ECMO support post-transplantation were alive at the end of the follow-up period.

Endomyocardial biopsy findings of patients presenting with haemodynamic compromise

In two patients, the initial biopsies were not performed because of the critical condition at presentation. All other patients with haemodynamic compromise or congestive heart failure had grade 3/>2R rejection. Only two children who had cellular rejection also had evidence for humoral rejection on their endomyocardial biopsy (positive C4d stains). Neither of these patients presented with haemodynamic compromise.

Immunosuppression regime

Of the 100 children who survived and were discharged from hospital after their transplantation, 50 patients had an immunosuppression regime including tacrolimus and MMF. Six patients were on sirolimus and mycophenolate (five with additional prednisolone). Thirty-one patients were managed on tacrolimus and azathioprine, 13 were on tacrolimus without a cell cycle inhibitor (six were on additional predinsolone).

Figure 2 demonstrates that the tacrolimus levels at various time points following transplantation were in the desired range. We could show that the levels did not differ between patients with rejection or without rejection episode. The same applies to sirolimus levels; the last documented levels before the rejection episode were within the recommended range. In children who had sustained severe diarrhoea or sustained lymphopenia, MMF was discontinued.

Survival/late mortality

During follow-up, one patient died following an acute rejection episode requiring ECMO. Fulminant sepsis occurred, after successfully coming off ECMO. One

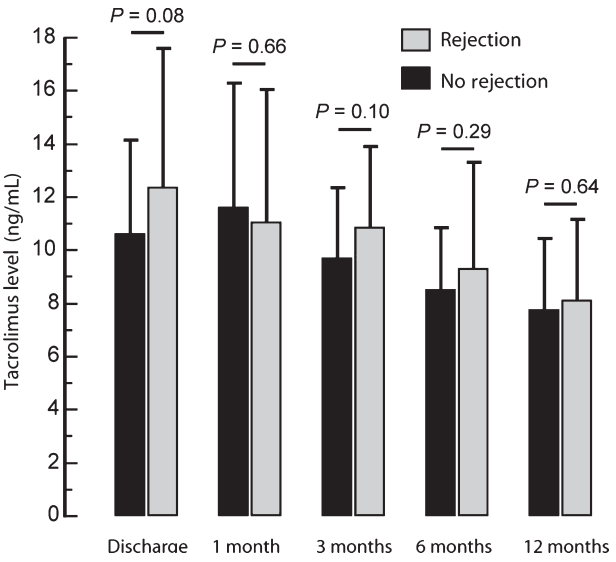

Figure 2 Tacrolimus levels of children with and without rejection at discharge, 1, 3, 6 and 12 months following transplantation.

further patient died during follow-up, of a pulmonary haemorrhage, which was unrelated to a rejection episode. All other children are still alive and well. Post-transplantation lymphoproliferative disease (PTLD) was seen in one patient. One other required long-term renal replacement therapy.

Analysis of predictors of outcome

We performed a univariate Cox proportional hazard analysis stratified for overall rejection, symptomatic rejection and rejection leading to haemodynamic collapse requiring ECMO support. The aim was to delineate whether different factors or immunosuppression regimes are associated with a higher risk for rejection.

Rejection with haemodynamic collapse

Sirolimus therapy without a calcineurin inhibitor, CMV (IgG) positive status before transplantation as well as non-Caucasian ethnicity was associated with haemodynamically compromising rejection on univariate analysis. None of the patients presenting with haemodynamic collapse was on MMF. Of note among the patients whose immunosuppression regime contained mycophenolate, none presented with a rejection resulting in haemodynamic collapse (Fig. 3).

Symptomatic rejection

Patients with a lower weight and height (representing a younger age in a paediatric cohort) had a lower incidence for rejection ($P < 0.05$). Sirolimus was associated with a higher incidence of rejection, whereas an immunosuppression regime with tacrolimus showed a lower risk for rejection (Table 2).

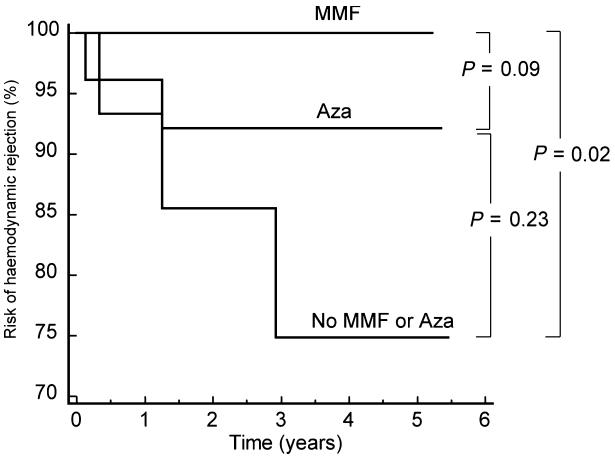

Figure 3 Kaplan–Meier plots showing freedom from rejection leading to haemodynamic collapse requiring ECMO of patients stratified by their antiproliferative agent as part of the immunosuppression regime (AZA, azathioprine; MMF, mycophenolate mofetil or no antiproliferative agent), showing that no patient who had been on MMF had a rejection episode leading to haemodynamic collapse.

Overall rejection

Analysing for overall rejection we had similar findings for sirolimus, which again was associated with a higher incidence of rejection, whereas patients on tacrolimus were at lower risk for rejection ($p < 0.0001$). Other

Table 2. Univariate Cox proportional hazard analysis.

Parameter	Hazard ratio (95% CI)	*P*-value
Overall rejection		
Female gender	0.383 (0.155–0.946)	0.04
Age > 5 years	4.640 (1.375–15.550)	0.01
Weight (kg)	1.024 (1.005–1.043)	0.01
Height (cm)	1.020 (1.006–1.034)	0.004
Sirolimus monotherapy	7.817 (2.247–27.192)	0.001
Sirolimus + MMF	4.146 (1.396–12.311)	0.01
Tacrolimus in combination	0.159 (0.064–0.398)	<0.0001
Rejection with haemodynamic collapse		
Ethnicity (non-White)	8.600 (1.440–51.376)	0.02
Sirolimus monotherapy	45.747 (6.310–331.668)	<0.0001
Tacrolimus in combination	0.092 (0.015–0.578)	0.02
Symptomatic rejection		
Weight (kg)	1.032 (1.001–1.063)	0.04
Height (cm)	1.028 (1.003–1.053)	0.03
Sirolimus monotherapy	35.738 (7.800–163.700)	<0.0001
Sirolimus + MMF	6.888 (1.384–34.286)	0.02
Tacrolimus in combination	0.390 (0.009–0.163)	<0.0001

Parameters significantly associated with rejection on univariate Cox proportional hazard analysis. MMF, mycophenolate mofetil.

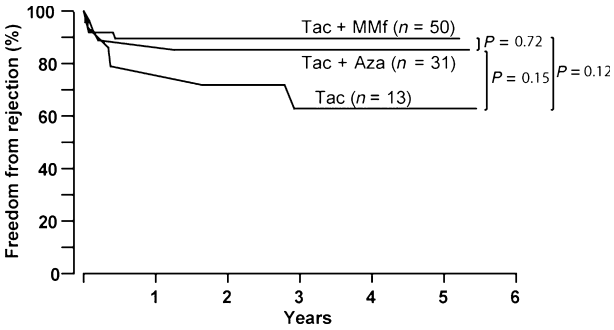

Figure 4 Kaplan–Meier analysis, showing freedom from rejection stratified by immunosuppression therapy. Tac, tacrolimus; MMF, mycophenolate mofetil; Aza, azathioprine.

factors associated with a higher risk for rejection were an older age (>5 years, $P = 0.014$, higher weight and height, respectively) as well as male gender ($P = 0.004$) (Table 2).

Other factors such as body mass index, diagnosis (dilated cardiomyopathy, restrictive cardiomyopathy and congenital heart disease), CMV status of the recipient/mismatch, ABO mismatch, HLA-A, -B and -DR mismatches, morbidity as expressed by duration of stay on the intensive care unit as well as hospitalization time post-transplantation did not seem to play a role predicting an individual patients' risk for the occurrence of a rejection episode. Figure 4 shows Kaplan–Meier curves illustrating the freedom from rejection on different immunosuppression therapies.

A multivariate analysis was performed for overall rejection, which showed that tacrolimus and a lesser height (representative for a younger age) independently were associated with a lower incidence for rejection.

Discussion

Our single-centre experience offers a differing perspective on acute rejection after paediatric heart transplantation. Although we concur with the registry data on the importance of older age on rejection, we report an incidence of rejection approaching half of the recent registry data [5,6]. Clearly, the incidence of asymptomatic rejection depends on the frequency of surveillance and our biopsy schedule of 3 in the first 6 months may miss some episodes. However, rejection with haemodynamic compromise is less open to debate. By using the same criteria as the Pediatric Heart Transplant Study [8], we report a reduction of over 50% from that data with an incidence of severe rejection with haemodynamic impairment of <5%.

We found the lowest incidence of rejection with immunosuppressive regimes including tacrolimus and MMF (Fig. 4). However, the difference between tacrolimus and MMF compared to the combination of tacrolimus and azathioprine was not statistically different. The combination with myocophenolate may even be protective against rejection resulting in haemodynamic collapse, as none of the patients in our series who required ECMO as rescue therapy to overcome their rejection episode were on maintenance mycophenolate, and no cases of severe haemodynamic compromise were seen in the tacrolimus-/mycophenolate-treated patients (Fig. 3).

In our cohort, tacrolimus and mycophenolate appeared to be the maintenance combination immunosuppressive therapy of choice to prevent (haemodynamically relevant) rejection following orthotopic heart transplantation in children. The observed trend of a lower incidence of rejection with tacrolimus and MMF than with tacrolimus and azathioprine or tacrolimus alone is in keeping with emerging adult data on mycophenolate therapy and preliminary experience in children as reported by other centres [20]. A multicentre study in adult patients following cardiac transplantation also revealed a lower incidence in the patient group treated with tacrolimus and mycophenolate compared to cyclosporine and mycophenolate at 1 year [21]. Similar observations were made by Teebken *et al.* [22], who stressed the superior properties preventing allograft rejection in patients on a combination of tacrolimus and mycophenolate compared to a cohort, who received cyclosporine and azathioprine. Fuchs *et al.* [23] also reported lesser rejection episodes requiring treatment in the group treated with tacrolimus and mycophenolate compared to a group, who received a combination of tacrolimus and cortisone only.

In contrast Kim *et al.* [24] suggested that paediatric patients' outcome in patients with a low white cell count (WBC) who discontinued their antiproliferative agent (MMF/azathioprine) was superior to those treated with an antiproliferative agent and a normal WBC.

Our hesitation to withdraw the antiproliferative agent permanently from a child's standard immunosuppression regime is based on our observation that all five patients who presented with haemodynamic collapse, who required temporary ECMO support for recovery, were not on MMF at the time. We aim to restart the patients on a smaller dose of MMF, after a short temporary omission of the drug, to allow the WBC to recover or symptoms to abolish. The WBC should be kept under close follow-up to reassess the drug tolerance.

It is perhaps not surprising that sirolimus patients had more frequent rejection (both haemodynamically significant, symptomatic and asymptomatic) than those treated with tacrolimus. Several papers including a meta-analysis have shown this in solid organ transplantation [25,26]. Our data do underscore the need for caution in patients

with renal dysfunction who were treated with a TOR inhibitor early after transplantation.

Interestingly, C4d staining did not appear to provide significant additional clinically important information in those with symptomatic rejection. Furthermore, the number of HLA mismatches was not significantly associated with severe rejection. While the importance of C4d staining in paediatric heart transplantation remains somewhat controversial [13] and there was a trend to significance with over four mismatches on Kaplan–Meier analysis (data not shown), it would appear that antibody-mediated rejection is not a major component of rejection in our series.

The availability and an early recourse to mechanical support were crucial to the survival of 4/5 children. Despite this, there was one death and this child had improved graft function but died of overwhelming sepsis. That particular case underlines the need for antiviral and antifungal prophylaxis in heavily immunosuppressed children on ECMO support. However, it also shows that there is time to wait for graft function to improve and it is perhaps cautionary to note that this child was not only given a course of ATG but also OKT3 as graft function did not improve initially. It may well be that supportive therapy on ECMO can allow time to wait for graft recovery without compromising the immune system to such a degree that overwhelming sepsis occurs. Morales *et al.* [27] reported the use of mechanical circulatory support for acute graft rejection in seven children with an acceptable weaning rate (71%), which is comparable to ours (80%). There are no generally accepted criteria for starting ECMO support. In our centre, the decision to transfer a patient onto ECMO support is the result of a discussion of a multidisciplinary team as well as the parents/and patient – if appropriate. All risks and potential benefits are evaluated. Points particularly considered in this discussion are poor ventricular function with sustained symptoms refractory to inotropic support, gravity of presentation, prevention of secondary organ damage and preservation of endorgan function, and expected/anticipated waiting time for a suitable organ.

Humoral sensitization with raised panel-reactive antibodies is described in patients with ventricular assist devices. Despite this, finding analysis of registry data did not show an impact on rejection rates [28]. Interestingly, in our series none of the patients who had ECMO support or a Berlin Heart preoperatively, presented within our follow-up period with a rejection episode.

The short-term follow-up (32 ± 14 months) for the patients with treated rejection is encouraging in that all survivors of the episodes remain well.

It is also interesting that only one case of lymphoproliferative disease was seen in this cohort of 100 transplant survivors, which offers encouragement that the immunosuppression is not too intense.

Limitations

The drug selection process itself may confound the risk of rejection. For example, we use sirolimus as immunosuppressant in patients with renal impairment. Therefore, if renal impairment would augment the risk of rejection, this would be wrongly attributed to sirolimus in the statistical analysis. This is a general limitation of retrospective analyses such as this one.

In comparison with other studies [6,24], consideration of the Ethnic distribution of the cohort of children need to be taken into account, as it appears that different Ethnic groups have a differing response to immunosuppressant drugs. It has been suggested that gene polymorphisms may play a role explaining the differing results between Ethnic groups [29,30]. It may well be that 'one combination fits all' is a simplification of a much more complex problem.

Ongoing registries such as the ISHLT database with rising numbers and expertise in the field of paediatric transplantation may shed more light into the optimal immunosuppressant strategy for individual patients from different Ethnicities.

Considering that survival following heart transplantation in children exceeds 15 years according to the latest ISHLT data, the overall follow-up of our reported patient group is relatively short. Problems like Epstein–Barr virus infection and PTLD may occur later.

Conclusion

Although our experience is of a far lower incidence of both rejection and rejection with haemodynamic compromise than registry data, rejection remains a serious problem after paediatric heart transplantation. ECMO support offers hope in the most severe cases. In our experience, antibody-mediated rejection was uncommon and not associated with haemodynamic collapse.

In our study, sirolimus without a calcineurin inhibitor appeared to be associated with more rejection episodes, whereas the combination of tacrolimus and MMF appeared to have the best protective properties against rejection, although these findings need to be confirmed by larger studies.

Authorship

MB: designed the study. AEL and KLB: collected and analysed the data. AEL and MB: wrote the manuscript. NJS: performed the C4d staining and contributed to data

analysis. PR, KB, PR, MF, NJS and MB: contributed to the critical review of the manuscript.

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