Article 1

Acute Cellular Rejection in Paediatric Liver Transplants: Does a Living Donor Ameliorate the Risk of Rejection in Our Patients? A Retrospective Review at Wits Donald Gordon Medical Centre, South Africa

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ABSTRACT

Background: Despite the enlarging pool of paediatric liver transplants (LT), there is a paucity of data-detailing risk factors for acute cellular rejection (ACR).

Objective: To identify risk factors associated with ACR.

Method: We reviewed the data of 98 paediatric patients at Wits Donald Gordon Medical Centre who underwent LT between 2015 and 2018, and subsequent histologically determined ACR.

Results: Of the 98 patients who received a LT, 52% of donors were deceased donors and 48% were living donors. Twenty-two per cent of the patients were diagnosed with ACR during the first 90 days post LT. Sixty-eight per cent of living donor liver transplants were in the shortest (less than 2.5 h) cold ischaemic time (CIT) tertile, while 0% of deceased donor organs were transplanted prior to 2.5 h. We identified decreased CIT and living donor status as factors, both closely related to each other and associated with a decreased risk of ACR.

Conclusion: CIT is associated with a decreased risk of ACR. Living donor LT is associated with a decreased CIT and as a result a less inflammatory milieu in the early post LT period. Further research should be conducted, with particular reference to a decreased risk of ACR in living donor paediatric LT, in order to better inform immunosuppressive therapeutic regimens.

Keywords: Paediatric liver transplant, Acute cellular rejection, Living donor liver transplants, Cold ischaemic time

INTRODUCTION

Following Starzl's pioneering work in paediatric liver transplantation (LT) in 1967, LT has been established as the standard of care for paediatric liver failure and liver-based metabolic disease.(1) Advances in surgical techniques, especially those associated with related-living donor, organ preservation and immunosuppression have improved clinical outcomes over the last few decades.(2) However, graft rejection, which can affect up to 45% of recipients within 5 years of their initial liver transplant,(3,4) often requires hospitalisation and contributes to significant morbidity. The clinical significance of early graft rejection is uncertain, and the side-effects of immunosuppression are sometimes severe.(5) Despite the enlarging pool of paediatric LT recipients, there is a paucity of extant literature identifying factors that would allow clinicians to predict graft rejection allowing earlier detection and the more careful modulation of immunotherapy. In the present study, we sought to describe the paediatric LT recipients in our transplant programme and investigate risk factors for acute allograft liver rejection after LT.

PATIENTS AND METHODS

Study population

We collected clinical, haematological and histological data from all paediatric patients aged between 1 day and 18 years who received a liver transplant at the Wits Donald Gordon Medical Centre (WDGMC) in Johannesburg, South Africa, between 1 January 2015 and 30 August 2018, and who were followed up for at least 90 days after the transplant. These patients were referred to WDGMC from referral centres within South Africa as well as neighbouring countries. Data were sourced from the research database at the transplant unit and from the anatomical pathology department run by the National Health Laboratory Service (NHLS). The study was approved by the University of the Witwatersrand Human Research Ethics Committee.

Acute cellular rejection

Acute cellular rejection (ACR) was detected by a rise in transaminases or gamma glutamyl-transferase pre-symptomatically but diagnosed histologically at the NHLS by an expert pathologist and reported by use of the Banff score to classify the severity of rejection. Histological diagnosis was made by identifying a pattern of eosinophilia, ductal proliferation and endothelialitis.(6) Treatment for ACR was initiated with high dose (10 mg/kg) intravenous corticosteroids for three days. Rejection which did not respond to two pulses of steroid was categorised as 'steroid resistant rejection' and required therapy with a polyclonal antilymphocyte preparation or a newer monoclonal drug against lymphocytes.(3) Steroid resistant ACR was treated with antithymocyte thymoglobulin.

Variables

Recipient data collected were on gender, age at time of LT, race (self-reported), aetiology of liver failure, paediatric end-stage liver disease (PELD) score at time of transplant, mid-upper arm circumference (MUAC) *z*-score pre-LT and blood group. Donor data collected were on type (deceased or living), blood group and living donor body mass index (BMI). Post-transplant data collected included: cold ischaemic time (CIT), surgical re-exploration post-operative complications, first recorded tacrolimus level and time to therapeutic tacrolimus level. Data pertaining to the ACR, including the time to rejection, tacrolimus level at the time of rejection, Banff score at the time of rejection and the therapy of the ACR were recorded.

Sample size

Sample size estimation was based on the estimation of the relative risks of study variables for ACR. Assuming equal risk factor group size, and a 20% prevalence of the outcome, a sample size of 98 allowed the detection of relative risk (RRs) of 1.7 and greater, with a power of 80% and a 5% significance level.(7)

Data analysis

Continuous risk factors were categorised into tertiles in preparation for analysis. Binomial regression analysis was used to determine the relative risk of the study factors for the development of ACR. The hazard ratio of patient and graft survival for ACR vs non-ACR groups was determined using Cox Proportional Hazards regression. Analysis was not done where missing data exceeded 30% or for small group sizes ($n < 10$). Data analysis was carried out using SAS version 9.4 for Windows. The 5% significance level was used.

RESULTS

Ninety-eight patients received liver transplants during the study period and followed up for 90 days. Their baseline clinical and demographic information is summarised in Table 1. Patients are described in terms of gender (60% female, 40% male), age tertiles (30% between 0 and 18 months, 36% between 18 and 48 months, and 35% between 48 and 96 months). The aetiology of the underlying liver disease was classified as chronic (this included biliary atresia, Alagille's syndrome, Wilson's disease, hepatocellular carcinoma, Budd–Chiari veno-occlusive disease, autoimmune hepatitis, Gaucher's disease, cryptogenic cirrhosis and rejection of previous transplant graft), acute fulminant (hepatitis A, Epstein Barr virus hepatitis, adenovirus hepatitis, hepatic artery thrombosis, primary graft non-function, portal vein thrombosis, mushroom poisoning, drug-induced hepatitis) or acute on chronic when there was a known diagnosis of chronic liver disease, but presentation was an acute fulminant clinical picture (e.g. known with Wilson's disease, but acute decompensating due to viral hepatitis). Fifty-two per cent of donors were deceased donors, and 48% were living donors.

The proportion of living donor liver donations in the shortest CIT tertile (less than 2.5 h) was 68%, while 0% of deceased donor organs were transplanted prior to 2.5 h (Table 2). There was a significant association between CIT and donor type (chi-squared test: $p < 0.0001$; Cramer's $V = 0.77$).

Twenty-two per cent of the patients were diagnosed with ACR during the first 90 days post LT. The ACR occurred at a median of 9 days (interquartile range 6–19 days). The clinical characteristics of the patients with ACR are presented in Table 3.

There was no significant risk of ACR in terms of recipient gender, race, age, underlying diagnosis, PELD-score, MUAC *z*-score, living donor BMI, occurrence of biliary complication, first recorded tacrolimus level or hours required to achieve a therapeutic tacrolimus level (Table 4). There was, however, a significant risk of ACR with an increased CIT, although this finding was only marginally significant in the longest CIT subgroup.

The median patient and graft follow-up time was 1.2 years. Neither patient survival (Figure 1; $p = 0.28$) nor

Table 1: Demographic and clinical characteristics of study group ($n = 98$).

Characteristic	Category	*n*	%
Recipient gender	Female	59	60
	Male	39	40
Recipient age (months)	0–18	29	30
	18–48	35	36
	48–96	34	35
Recipient race	Black	65	66
	White	20	20
	Mixed racial origin	10	10
	Indian	3	3
Aetiology of liver disease	Chronic	74	76
	Acute	18	18
	Acute on chronic	2	2
	Metabolic	4	4
Recipient PELD at transplant	<15	27	29
	15–29	48	52
	>29	17	18
	Unknown	6	
MUAC z-score at transplant, age under 5 years (n = 69)	<−2.1	11	22
	(−2; −1.01)	15	31
	>−1	23	47
	Unknown	20	
Donor type	Deceased donor	51	52
	Living donor	47	48
ABO compatibility	Yes	95	97
	No	3	3
Congruent blood group	Yes	82	84
	No	16	16
Living donor BMI kg/m^2 (n = 47)	18–24	23	50
	25 or more	23	50
	Unknown	1	
Post-operative biliary complications	Yes	32	33
	No	65	67
	Unknown	1	
Cold ischaemic time (h)	0–2.5	32	33
	2.6–7	33	34
	>7	33	34
First recorded tacrolimus level (ng/ml)	0–2.5	25	30
	2.6–7	30	36
	>7	29	35
	Unknown	14	
Time to therapeutic tacrolimus level (h)	12–48	26	31
	49–96	31	36
	>96	28	33
	Unknown	13	

MUAC = mid-upper arm circumference, PELD = paediatric end-stage liver disease.

Table 2: Proportion of patients per CIT tertile by donor type.

	Donor type	
CIT	**Deceased donor (%)**	**Living donor (%)**
0–2.5 h	0	68.1
2.6–7 h	39.2	27.7
>7 h	60.8	4.3
Total	100	100

graft survival (not shown; $p = 0.36$) differed significantly between the ACR and non-ACR groups.

DISCUSSION

ACR represents a complicated but important end-point that must be avoided in the medical management of paediatric LT patients in the post-transplant period. With advances in immunosuppression and the advent of calcineurin inhibitors, ACR risk has been greatly reduced. Immunosuppression is prescribed as per local protocol in the transplant unit, but not individualised, except in the case of Blood group ABO incompatible LT. Even though, as our data show, well-managed ACR does not impact graft survival,(3,8) untreated ACR will lead to graft failure, and more immunosuppression leads to infection, malignancy and exposure to the considerable side-effect profile of immunosuppressive medication.(3,9) Therefore, finding the right balance to allow for sufficient but not excessive immunosuppression is complicated but essential for post LT survival.

The risk of ACR is thought to be highest in the early post-transplant period, and this risk declines over time with development of tolerance for the liver allograft.(10) In published studies, the incidence of ACR is reported between 7% (11) and 20%,(8,12) and even up to half of all recipients (3,4,13) although the time period over which 'acute rejection' can be diagnosed varies between studies, ranging between 3 (3,12,14) and 12 months.(11) The incidence of ACR of 22% in our cohort at 3 months is in keeping with

Table 3: Clinical characteristics of patients with acute cellular rejection ($n = 22$).

Characteristic	Measure/category		
Time to first ACR (days)	Median (interquartile range)	9 (6–19)	
Banff score at rejection	Mean (SD)	6.3 (1.6)	
Acute rejection therapy		*n*	%
	Methylprednisone	18	85
	Methylprednisone and MMF	1	5
	Steroid resistant, treated with ATG	1	5
	Plasmapheresis	1	5
	Unknown	1	

ATG = antithymocyte globulin, MMF = mycophenolate mofetil, SD = standard deviation

Table 4: Relative risk for identified factors for ACR.

		ACR at 90 days			
		No	Yes	RR for ACR	
		n (%)	*n* (%)	RR	95% CLs
Recipient gender	Female	46 (61)	13 (59)	1.00	
	Male	30 (39)	9 (41)	0.95	(0.45; 2.02)
Recipient race	Black	46 (63)	19 (86)	1.00	
	White	17 (23)	3 (14)	0.51	(0.17; 1.56)
	Mixed racial origin	10 (14)	0 (0)	No. of ACR cases	
	Indian	3 (3)	0 (0)	No. of ACR cases	
Recipient age (months)	0–18	20 (26)	9 (41)	1.00	
	18–48	27 (36)	8 (36)	0.74	(0.33; 1.66)
	48–96	29 (38)	5 (23)	0.47	(0.18; 1,.6)
Aetiology	Chronic	53 (76)	21 (95)	1.00	
	Acute	17 (24)	1 (5)	0.20	(0.03; 1.36)
PELD	<15	22 (31)	5 (23)	1.00	
	15–29	32 (46)	16 (73)	1.80	(0.74; 4.37)
	>30	16 (23)	1 (5)	0.2	(0.04; 2.49)
MUAC z-score	<−2.1	6 (19)	5 (29)	1.00	
	(−2; −1.01)	13 (41)	2 (12)	0.29	(0.07; 1.24)
	>−1	13 (41)	10 (59)	0.96	(0.43; 2.12)
Donor type	Deceased	36 (47)	15 (68)	1.97	(0.88; 4.42)
	Living	40 (53)	7 (32)	1.00	
Blood group congruency	No	14 (18)	2 (9)	0.51	(0.13; 1.98)
	Yes	62 (82)	20 (91)	1.00	
Donor BMI (kg/m^2)	18–24	22 (56)	2 (25)	1.00	
	>25	17 (44)	6 (75)	3.13	(0.70; 13.95)

Biliary complications	Yes	23 (31)	9 (41)	1.41	(0.67; 2.94)
	No	52 (69)	13 (59)	1.00	
Cold ischaemic time (h)	0–2.5	30 (39)	2 (9)	1.00	
	2.6–7	21 (28)	12 (55)	**5.82**	**(1.41; 23.97)***
	>7	25 (33)	8 (36)	3.88	(0.89; 16.89)
First recorded tacrolimus level (ng/ml)	0–2.5	18 (28)	7 (35)	1.00	
	2.6–7	26 (41)	4 (20)	0.48	(0.16; 1.44)
	>7	20 (31)	9 (45)	1.11	(0.48; 2.54)
Time to therapeutic tacrolimus level (h)	12–48			1.00	
	49–96			0.70	(0.24; 2.03)
	>96			1.39	(0.58; 3.37)

Bold values indicate $P = 0.05$.
MUAC = mid-upper arm circumference, PELD = paediatric end-stage liver disease
*Significantly increased risk.

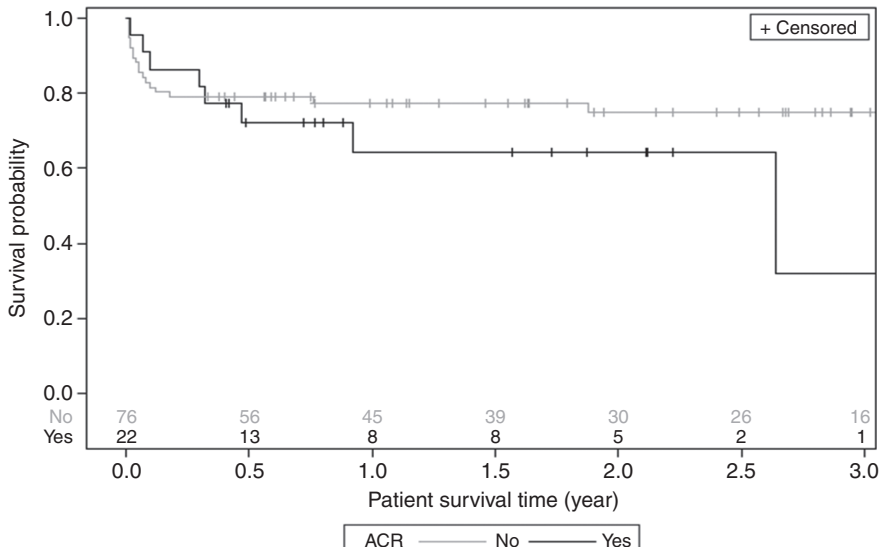

Fig 1: Patient survival over time in ACR and non-ACR groups

extant literature, and on par with international standards. Patient survival drops after an episode of ACR, and this is likely related to graft function, complications surrounding altered and more aggressive immunosuppressive regimens, and due to the inflammatory milieu established during an acute rejection which may predispose to chronic rejection. This needs to be examined more fully and was beyond the scope of this study.

Intuitively it would make sense for there to be a lower risk of ACR in those recipients who receive a liver from a living donor. CIT is closely associated with donor type, in that living donors have a shorter CIT, a finding confirmed by our analysis. Thus, our finding of a significantly lower risk of ACR with shorter CIT possibly explains why the risk of ACR may be reduced with living donors. Shaked et al. demonstrated no immunological benefit in related living donor transplant recipients and, in fact, showed an increased risk of rejection (46% in living donor vs 38% in deceased donor).(15) It is difficult to reconcile the latter group's findings to our data, but one possible explanation is that the differences may in part be related to varying immunological responses in different population groups.

The development of ACR is initiated by the stimulation of the antigen-mediated adaptive immune response. However, it is now understood that the immune environment in the hyperacute (hour to days) post LT period probably plays an important role in priming the adaptive immune response. A pro-inflammatory milieu occurring as

a result of ischaemia-reperfusion injury, sepsis or chemical-related inflammation will trigger the development of damage and pathogen-associated molecular patterns, which are recognised by pattern recognition receptors. This results in the activation of an innate immune response: an up-regulation of co-stimulator molecules and secretion of pro-inflammatory cytokines. Antigen-presenting cells, natural killer cells and complement create an environment in which a cellular immune response is activated and promoted. It is further thought that T-memory cells that are generated in an inflammatory environment act as a barrier to tolerance induction.(10) This explanation gives credence to the observation of increasing ACR related to longer CITs (8,15–18) and biliary complications,(9) which are known to cause inflammation. Our data illustrates the increased risk of ACR with longer CIT; however, the expected increased risk of ACR was not shown in those patients with biliary complications.

In this study, CIT was identified as the only significant factor in increasing the risk of ACR. The greatest risk for ACR occurred when CIT exceeded 2 .5 h, and the risk was significantly increased, but only marginally when CIT exceeded 7 h. A prolonged CIT likely predisposes to ACR by causing inflammation. The mechanism of the inflammatory reaction is likely related to depletion of ATP, increase of free radical and cytokines, progressive cellular dysfunction and apoptosis resulting from progressive ischaemia. (16) CIT is potentially a modifiable factor in LT, and although it is clear that the risk of 3 am surgery may outweigh the risk of a longer CIT,(17) it is noteworthy that we were able to transplant with significantly lower CITs when the donors were living and present at our centre.

A younger recipient age at LT has previously been shown to increase the rate of rejection.(8,18) This is thought to be related first to relatively higher CD8 counts in younger children, which decrease to near adult levels with increasing age and second to altered Th1 vs Th2 responses known to occur in children. This pattern was not elicited from our database but could be better explored in further prospective studies, as age may be important in developing more individualised immunosuppression protocols.

Gender, and in particular female donors, donor–recipient gender mismatch has been shown to predispose to rejection.(9) PELD and MELD are thought not to affect ACR.(8) To our knowledge, there is no literature examining the relationship between race and race mismatch and ACR. Our data identified no association between PELD, recipient race or recipient gender and ACR.

Interestingly, sarcopenia has been shown to be associated with lower rates of rejection (10.6% vs 30.2% in those with normal muscle mass).(19) In the paediatric population in our setting, LT is only performed once MUAC has reached at least the -2 z-score. This analysis did not identify any increased risk of ACR with increasing MUAC.

Tacrolimus tortuosity is thought to be one of the most important predictors of rejection post LT.(12,20,21) Intra-patient tacrolimus variability occurs because of drug interactions, gastrointestinal events and circadian rhythm changes. Inter-patient tacrolimus variability is expected due to differing cytochrome genetics, drug transporter heterogeneity and, of course, varying compliance. Even though we believe that this is likely to have had an impact on ACR in our patients, it was difficult to demonstrate this effect due to limited tacrolimus monitoring data. It is certainly an area where future research should be directed.

This study has some obvious limitations, particularly in terms of its retrospective nature, small sample size and some missing data, as it was unable to fully assess many of these risk factors. It has illustrated that more information about pre-transplant transfusion, donor information and tacrolimus drug monitoring should be collected in our database.

In conclusion, our data suggests that living donor LT shows a trend towards decreased risk of ACR. This we believe is related to the fact that living donor LT is associated with decreased CIT and as a result a less inflammatory milieu in the early post LT period. We believe that these data will add to the increasing pool of evidence that should enable transplant clinicians to individualise immunosuppressive regimens and dosing in a scientifically guided manner in the future. Further research should be conducted, with particular reference to a decreased risk of ACR in living donor paediatric LT, in order to better inform immunosuppressive therapeutic regimens.

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Authorship

JKS: Participated in research design, data collection and writing of the article. PG: Participated in data collection and statistical analysis. HM: Participated in research design and data collection. JB: Participated in research design and writing of the article.

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Conflicts of interest

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