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Donor specific HLA-Antibodies as a Trigger for Ischemic Type Biliary Lesions (ITBL) after Orthotopic Liver Transplantation-A Case Controlled Study

Katharina Schulte[1], Gero Puhl[2*], Safak Gül[3], Constanze Schönemann[3], Nils Lachmann[3] and Johann Pratschke[3]

Abstract

Despite recent surgical developments, biliary complications following orthotopic liver transplantation (OLT) are an important cause of morbidity and mortality. Ischemic Type Biliary Lesions (ITBL) is particularly challenging accounting for a high proportion of bile-duct associated complications following OLT. We aimed to better predict and reduce the incidence of ITBL through detection of donor specific human leucocyte antibodies (DSA). These could be involved in the pathogenesis of this disease. This approach allows to standardized perioperative DSA-screening in OLT-patients. Prospective database of ITBL-patients (n=15) that had undergone OLT from February 2008 to October 2011 was analysed. Clinical parameters, biochemical data including preoperative HLA-status and postoperative DSA-status, complications, morbidity and mortality were studied. The ITBL-patients were matched in a 1:1 ratio accounting for demographic and clinical variables to a control cohort of OLT-patients. Propensity modelling with matched cohort analysis was employed. There was a significance between the detection of de novo DSA and the development of an ITBL (p=0,003) and an asymptomatic elevation of alkaline phosphatase (AP). This suggested a strong relationship between de novo DSA and future development of ITBL. This implies a need for a protocolisation of care with standardized postoperative DSA-status allowing earlier diagnosis and therapeutic intervention.

Keywords: ITBL; Orthotopic liver transplantation; DSA; Donor specific HLA-Antibodies; Complications; Outcomes

Introduction

Liver transplantations are the only curable treatment for a wide spectrum of liver diseases when other therapeutic options have failed. Despite recent surgical developments biliary complications following orthotopic liver transplantation (OLT) are an important cause of morbidity and mortality.

Ischemic Type Biliary Lesion (ITBL) is particularly challenging and responsible for a high proportion of mortality and morbidity of bile-duct associated complications following OLT.

Donor specific human leucocyte antibodies (DSA) and recipient specific chemokine receptor polymorphisms have been associated with the development of biliary strictures and may contribute to enhanced fibrotic tissue remodelling and biliary stricture formation. In pediatric patients a higher incidence of fibrosis [1] and unexplained biliary complications were associated with DSA [2]. DSA were not linked to a reduced survival rate after OLT or an increased rate of re-transplantation [3,4].

We aimed to better predict and reduce the incidence of ITBL through detection DSA that could be involved in the pathogenesis of this disease.

Finding a link between the development of ITBL and the presence of DSA would allow the implementation of standardized pre- and post-operative DSA-screenings. This already plays an important role in kidney [5,6] heart [7] and lung [8] transplantations.

Screening of DSA antibodies might be useful for early identification of patients at risk who could benefit from closer surveillance and tailored immunosuppressive regimen [9]. Our findings have relevance for prediction and management of post-OLT biliary complications such as ITBL.

Data regarding the prevalence, incidence and impact of DSA after OLT are still controversial. To stimulate future work, we summarize the considerable advances of perioperative DSA-testing in order to highlight the open and controversial issues remaining. We add our data to a growing body of evidence advocating DSA testing to predict outcomes in transplantation.

Patients and Methods

Patients

Primary liver allograft recipients (n=394) at the Charité University of Berlin, between February 1, 2008 and October 30, 2011 (Table 1), were reviewed. All patients underwent pre- and postoperative DSA testing. 15 patients of the total cohort developed postoperatively ITBL. The ITBL-patients were matched in a 1:1 ratio accounting for demographic and clinical variables to a control cohort of OLT patients (Table 2). Clinical parameters, biochemical data including pre-operative HLA-status and their postoperative DSA-status, complications, morbidity and mortality were analysed. Propensity modelling with matched cohort analysis was employed. Clinical, histopathological and laboratory data were obtained from the Electronic Data Base.

Luminex test and DSA detection

Samples of recipients and donors were routinely and prospectively screened for anti-HLA antibodies using the Luminex (Luminex SA;

Table 1: Propensity matching criteria for the control group.

Age of the donor (+/- 6 years)
Same donor gender
Recipients age (+/- 7 years)
Same recipient gender

***Corresponding author:** Gero Puhl, Asklepios Hospital Hamburg-Altona Paul-Ehrlich-Straße 1, 22763 Hamburg, Germany, Tel: (040) 18 18 81 1600; E -mail: g.puhl@asklepios.com

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One Lambda, Canoga Park, CA) single-antigen assay the test was carried out as per manufacture guidelines [10]. The results of the DSA levels normalized for background are reported as the mean fluorescence index (MFI). MFI ≥ 2000 was considered positive for a single DSA.

Statistical analysis

The values reported are shown as the mean (SD) or median (inter quartile range). Proportions were compared using Fisher's exact test or Chi square test. Quantitative variables were compared using the Mann-Whitney nonparametric test or T-tests. A p-value of <0.05 was considered statistically significant. Transplant and patient survival was determined using the Kaplan Meier survival curve.

Results

Prevalence of HLA preoperatively

One ITBL-patient was preoperatively positive for HLA. In our control group there were three patients positive for HLA prior to OLT. This was statistically not significant p=0.224 (Table 3).

De novo DSA postoperatively

The ITBL-group was screened for a significantly high incidence of de novo DSA post OLT (Table 4) p=0.003. In total 46.7% of the ITBL-patients were postoperatively developed de novo DSA. No DSA were detected postoperatively in the control group.

In 33.3% of the ITBL patients DSA class I were detected (p=0.021). 20% of the ITBL patients showed DSA class II (p=112). Statistically only the presence of DSA class I was significant.

De novo NDSA

The control group had 46.7% increased incidence of de novo NDSA, whereas only 6.7% of all patients in the ITBL showed de novo NDSA (Table 4).

DSA and outcomes

The postoperative course showed significant differences concerning the postoperative interventions. ERC and stent implantations were needed in a significantly higher proportion in the ITBL-group than in the control group p=0.001 and p=0.021 (Table 5).

No statistical differences were found papillotomies (p=0,390), antibody mediated rejections (AMR) (p=0,466), the need for plasmapheresis (p=0,483) or steroid therapies (p=0,146).

Perioperative laboratory results

Patients were tested for GGT, ALAT, ASAT, and AP on day 1-7, 14, 21, and 28 postoperatively. Perioperative results showed a significant increase in AP in the ITBL group at multiple time points (Table 6). No statistical differences were found between the groups for ALAT, ASAT and GGT.

Table 2: Biographical-sociological factors between ITBL- and control group.

	ITBL-Group (n=15)	Control group (n=15)	p-value
Age recipient M (SD)	58,8 (± 10,92)	57,1 (± 10,43)	ns
Gender recipient: male/female	11/4	11/4	ns
CMV-positive recipients	5	8	ns
Age donor M (SD)	57,8 (± 11,01)	56,07 (± 10,93)	ns
Gender donor: male/female	8/7	8/7	ns
CMV-positive donor	8	10	ns
Preoperative MELD-Score	23,46 (± 12,37)	20,5 (± 10,11)	ns
Perfusion solution (HTK/UW)	15/0	13/2	ns
Cold ischemia time (min)	635,2 (± 225,05)	506,36 (± 128,87)	ns
Warm ischemia time (min)	40,8 (± 14,07)	41,27 (± 10,13)	ns
Operative time (min)	298,73 (± 57,09)	320,27 (± 117,78)	ns
ITBL diagnosis postoperatively (days)	235,5 (± 103,4)		

Table 3: Preformed HLA pre OLT.

		ITBL group (n=15)	Control group (n=15)	p-value
preformed HLA		0	3	ns
preformed HLA class I		0	3	ns
preformed HLA	*class II*	*0*	*1*	*ns*

Transplant and patients' survival

The transplants showed a cumulative 3-years-survival rate of 72.7% in the ITBL group *vs.* 73.3% in the control group. No significant difference was shown between both groups (Appendix). The cumulative patient survival rate 3-years post OLT was 72.7% in the ITBL group *vs.* 73.3% in the control group (Tables 7 and 8).

Discussion

Biliary complications are present with a constant incidence 10-15% in patients after OLT [11]. ITBL is one of the major postoperative complications accounting for up to 38% of morbidity and mortality rates of all biliary complications [12,13].

The impact DSA on the liver allograft remains controversial and patients are not regularly tested for DSA perioperatively. Recently, several groups have provided convincing evidence that DSA could be linked to poor liver allograft survival [14,15], chronic, ductopenic rejection [16], liver fibrosis [17] and biliary complications [18].

HLA class I and II are expressed on all cells in the liver and in a high density on endothelial cells. Hepatocytes and biliary epithelial cells exclusively express MHC class I, antibody binding of class I DSA could therefore directly injure the transplant. HLA class II are exclusively found on dendritic cells, partly on macrophages and there is a low expression on portal microvasculature. They are up-regulated in case of infection, inflammation and ischemic injuries [19] and can

Table 4: De novo DSA and NDSA post OLT.

	ITBL GROUP (N=15)	CONTROL GROUP (N=15)	P-VALUE
DSA	7	0	0,003
DSA CLASS I	5	0	0,021
DSA CLASS II	3	0	0,112
NDSA	1	7	0.035

Table 5: Results of the postoperative variables.

postoperative variables		ITBL group (n=15)	control group (n=15)	p-value
ICU stay recipient	(days)	17,54 (19,69)	8,87 (± 6,63)	ns
ICU stay donor (days)		4,6 (± 3,87)	4,13 (± 4,57)	ns
Hospitalization	recipient (days)	55,6 (± 39,16)	48,00 (± 52,55)	ns
Normal t-drain visualization (5POD)		11	12	ns
ERC (%)		86,67	20,0	0.001
Stent (%)		60,00	13,33	0.021
Papillotomies (%)		33,33	13,33	ns
Acute rejection (%)		40,00	60,00	ns
Steroid therapy	(%)	33,33	66,67	ns
Plasmapheresis (%)		0	13,33	ns

Table 6: AP perioperatively, day 0-28.

Alkaline Phosphatase	ITBL group		Control group	p-value
	M (± SE)		*M (± SE)*	
Day 0, preoperative	*261,08*	*(± 78,40)*	*164,87 (± 37,79)*	*0,259*
Day 1	*120,20*	*(± 23,764)*	*81,47 (± 8,36)*	*0,135*
Day *2*	*117,20*	*(± 20,71)*	*79,80 (± 7,69)*	*0,101*
Day *3*	*131,53*	*(± 22,49)*	*81,40 (± 6,18)*	*0,040*
Day *4*	*148,67*	*(± 29,19)*	*83,60 (± 6,91)*	*0,039*
Day *5*	*154,87*	*(± 29,33)*	*92,53 (± 8,72)*	*0,051*
Day *6*	*143,27*	*(± 24,50)*	*120,46 (± 21,38)*	*0,496*
Day *7*	*143,07*	*(± 21,58)*	*139,93 (± 28,28)*	*0,930*
Day *14*	*253,57*	*(± 56,60)*	*233,46 (± 60,27)*	*0,810*
Day *21*	*341,75*	*(± 58,56)*	*192,14 (± 43,06)*	*0,047*
Day *28*	*402,45*	*(± 88,49)*	*249,90 (± 48,96)*	*0,137*

Table 7: Patient survival post OLT, Kaplan Meyer Survival Curve, Log Rank P=0,999.

Days post OLT	50	150	250	750	100
ITBL-Group	93,3	86,7	80,0	80,0	72,7
Control group	93,3	86,7	86,7	73,3	73,3

Table 8: Transplant survival post OLT, Kaplan Meyer Survival Curve, Log Rank P=0,999.

Days post OLT	50	150	250	750	100
ITBL-Group	93,3	86,7	80,0	80,0	72,7
Control group	93,3	86,7	86,7	73,3	73,3

(Kaplan Meier Survival Curve; Log Rank P=0,999)

activate endothelial, fibroblast and smooth muscle cell proliferation [20]. Circulating immune complexes can then trigger inflammatory and fibrotic processes in small vessels of the biliary tree which lead to ischemic injuries [21,22].

Vascular and hepatocyte DSA could play an important role that favours the development of ITBL and biliary tree fibrosis. According to the timing of onset biliary complications post OLT can be divided into early and late. Two thirds of the patients manifest within the first three months post OLT [11]. Biliary complications that present several months or years post OLT are intra- or extra hepatic fibrotic lesions, stricture mediated cholestasis and biliary insufficiency [23,24].

Studies in adult and pediatric patients post OLT showed an increased risk between DSA and fibrosis/cirrhosis [1,16,25,26]. Biliary strictures are increasingly seen in patients who are positive for DSA [2]. In our study the ITBL group showed a significantly incidence of de novo DSA post OLT when compared to a matched control cohort, which could be detected even before the diagnosis of the ITBL.

Several studies were able to show the presence of DSA before the onset of transplant failure [14,15,27,28]. In pediatric OLT patients, those with DSA had a higher frequency of bridging fibrosis or cirrhosis, a higher frequency of diffuse/local endothelial C4d staining and mild or intermediate acute rejection compared to those with no DSA [1]. Yamada et al. performed an immuno-histochemical study of 28 patients who had received a living-donor OLT suggesting an influence of DSA on the development of pericentral fibrosis [26]. Del Bello et al. reported increased liver fibrosis in patients with DSA compared to those without DSA [25]. Fontana et al. cohort had 4.2% with DSA. All DSA-positive patients developed biliary complications [18]. 46.6% of the ITBL-patients presented with de novo DSA, demonstrating a ten times higher postoperative incidence of DSA than our or other control groups mentioned in other studies. These results show an important predisposition for DSA-positive patients for ITBL.

In the literature only few studies describe the NDSA-status of patients post OLT. A study described an NDSA incidence of 24% in a patient cohort post OLA without an association of biliary complications [18]. In our study 46.7% of the patients in the control group showed NDSA whereas no NDSA were detected in the ITBL-group. Our results show that DSA are associated with biliary complications like ITBL whereas NDSA are not associated with biliary complications.

No differences were shown between the groups for the preoperative HLA-status.

AP is a sensitive marker in liver and biliary disease. According to Pascher et al. and Iacob et al. patients with biliary complications present with an elevated GGT, AP and transaminases [11,29]. Hilscher et al. described an asymptomatic elevation of AP in patients with primary sclerosing cholangitis as a negative prognostic factor for the outcome and long term survival [30]. According to Del Bello at al. DSA testing should be performed in patients with increased liver enzyme levels, alkaline phosphatase and graft dysfunction, especially in cases where immunosuppression is not optimal [25].

In our study an AP elevation in the ITBL group was already present during the initial postoperative hospital stay and before the actual diagnosis of ITBL had been made and could serve as a predictive marker for an early diagnosis in patients with a positive DSA status.

ERCs and stentings were significantly higher necessary in the ITBL group. The imaging of the t-drain 5 days postoperatively showed no differences. There was no significant association shown between DSA or NDSA and rejections.

The postoperative ICU and hospital stay did not differ between the groups. A model of explanation is the mean time to the diagnosis of ITBL of 235, 5 days. This point lies outside the mean duration of hospitalization and shows that the ITBL is not initially symptomatic in this patient cohort. It also shows the importance of tight long term controls, especially in the first year postoperatively even in an uneventful initial postoperative course.

Despite circulating DSA and the presence of ITBL we did not find clinical outcomes to be inferior in our ITBL-group. Survival rates of the transplant and the graft recipient was similar in both groups up to 3-years post OLT. Taner et al. reported similar findings in DSA positive patients in their study.

Nevertheless, given the possible long-term impact of the DSA, continued monitoring of the antibody levels is warranted in these patients. There is an association of DSA and the development of ITBL in OLT patients. De novo DSA in combination with an asymptomatic increase of AP are a potential diagnostic indicator for development of ITBL.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

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Author Affiliations

Top

[1]*St. Hedwig-Hospital, Berlin, Germany*

[2]*Asklepios Hospital Hamburg-Altona, Hamburg, Germany*

[3]*Charité Virchow Hospital, Berlin, Germany*