ORIGINAL ARTICLE

De novo HLA Class II antibodies are associated with the development of chronic but not acute antibody-mediated rejection after liver transplantation – a retrospective study

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SUMMARY

Donor-specific antibodies (DSA) cause antibody-mediated rejection (AMR); however, their pathogenic role has not yet been adequately investigated after liver transplantation. The aim of our study was to analyse the clinical significance of DSA and complement-binding DSA for the prediction of AMR after liver transplantation. Our cohort included 120 liver recipients with assessed protocol biopsies one year post-transplant. All patients had defined HLA-specific and complement-binding (C1q + and C3d+) antibodies before and in regular intervals after transplantation. The incidence of DSA was evaluated in relation with clinical and histopathological data in the liver allografts. A higher occurrence of acute AMR was observed in recipients with preformed complement-binding DSA to HLA Class I antigens. Patients who developed chronic AMR had more frequently *de novo*-produced antibodies against HLA Class II antigens ($P = 0.0002$). A correlation was also found between *de novo*-formed C1q + and C3d+-binding antibodies to HLA Class II antigens and the development of chronic AMR ($P = 0.043$). Our study implies that preformed complement-binding DSA to HLA Class I antigens are related to increased risk of acute antibody-mediated rejection, while chronic AMR is more frequent in patients with *de novo*-produced antibodies to HLA Class II antigens after liver transplantation.

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Introduction

Antibody-mediated rejection (AMR) of the transplanted liver is difficult to diagnose; however, if not timely recognized, it may lead to graft failure and a serious life-threatening condition [1–3]. The pathological criteria of liver AMR, which have been recently updated, may resemble non-immunological conditions, like biliary complications, ischemic injury, thrombosis and others [4]. Due to the tolerogenic effects and relative resistance of the liver allograft to the detrimental effects of HLA-specific antibodies, the presence of preformed donor-specific antibodies (DSA) may not be considered as a contraindication to transplantation, even in the presence

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of complement-binding antibodies and a positive crossmatch test with the potential organ donor [5–8]. An earlier study by the group of M. Muro et al. showed a deleterious effect of the positive lymphocytotoxic crossmatch test on liver graft survival in the first post-transplant year [9]. In line with these results, a number of published reports showed increased incidence of rejection, graft dysfunction and worse graft survival in liver recipients with DSA [10–17]; however, the relevance of complement-binding DSA for the risk for the development of liver AMR is still unclear [18–20]. In a pilot study of our centre, all liver recipients with pre-transplant complement-binding DSA developed severe AMR after transplantation; nevertheless, further investigation would be needed to clarify the role of persistent and *de novo*-produced antibodies [21]. The goal of our study was therefore to evaluate the clinical significance of preformed, persistent and *de novo*-produced complement-binding DSA (as defined by solid-phase single-antigen (SA) bead techniques) for prediction of antibody-mediated rejection after liver transplantation.

Materials and methods

Patients

A cohort of 120 liver allograft recipients, who were transplanted in our centre between the years 2015 and 2017, was studied. Our study group did not include paediatric patients and recipients who received split grafts. Three liver allografts failed shortly after transplantation due to immunological reasons, and these patients were retransplanted and again included into the cohort. The patient demographic characteristics are shown in Table 1. All recipients had assessed protocol liver graft biopsies one year after transplantation and were followed regularly for incidence of rejection, graft function and survival for up to 1 year after transplantation. The diagnoses leading to indication for transplantation are shown in Fig. 1. The most frequent indications were alcoholic liver disease (26%), biliary cirrhosis (20%) and HCV hepatitis (10%). Hepatocellular carcinoma was found in 26 patients (22%). The study was approved by the ethics board of the Institute (conformed to the ethical guidelines of the 1975 Declaration of Helsinki), and written informed consent was obtained from all patients. Four patients died during the first year after transplantation. Two of them were diagnosed with chronic AMR, but this was not the direct cause of death. One patient with chronic AMR died due to generalization of HCV, while the reason of death of the other patient with chronic rejection could not be determined.

HLA typing and detection of HLA-specific antibodies

Liver recipients were HLA-typed by the PCR SSOP technique (intermediate resolution) for HLA-A, -B and -DR loci (OneLambda Inc., Canoga Park, USA). Deceased organ donors were HLA-typed by PCR SSP low-resolution kits (Olerup SSP, Stockholm, Sweden and Histo Type SSP, BAG, Lich, Germany) for HLA-A, -B, -DR and -DQ loci. Detection of antibodies specific to HLA–A, –B, –DR and –DQ antigens was performed before, 3, 6 and 12 months after transplantation. For elimination of the prozone effect, all sera were treated with ethylenediaminetetraacetic acid (EDTA) before testing. Pre- and post-transplant serum samples were analysed using the LABScreen Mixed technique and in case of positivity, by the LabScreen Single Antigen (SA) Luminex technique (OneLambda Inc.). A cut-off for positivity of 1000 MFI and 2000 MFI for Class I and Class II SA beads, respectively, was applied. HLA antibodies were tested for complement-binding activity by the C1q Screen (OneLambda) and Lifecodes C3d Detection kits (Immucor, Stamford, USA). Ten liver recipients (seven of whom developed AMR) had a positive complement-dependent cytotoxicity (CDC) crossmatch test before transplantation, and all remaining patients were CDC-crossmatch negative (Table 1).

Evaluation of liver biopsies and immunosuppressive regimens

As indicated above, all patients included into the study had protocol allograft biopsies performed one year after transplantation. T-cell-mediated (TCMR) and antibody-mediated rejection (AMR) were diagnosed according to the criteria of the updated Banff classification published in 2016 (4). The diagnosis of AMR was supported by immunofluorescent staining of diffuse C4d deposits in the sinusoids (>50%) on unfixed frozen tissue sections and if detectable, the simultaneous presence of DSA. Acute AMR cases underwent biopsies due to liver graft dysfunction with laboratory features of severe graft injury with hyperbilirubinemia and thrombocytopenia. Morphology showed severe tissue injury with microvascular pathology involving endothelial enlargement, capillary dilatation with leukocyte margination and also individual 'lytic' hepatocyte necrosis. At the time of histological diagnosis, the presence of DSA was not always known. Patients were treated by plasmapheresis with

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Table 1. Patient and donor demographics.

	AMR	AMR + TCMR	TCMR	No rejection
Number of patients	22 (11 male/11 female)	27 (14 male/13 female)	16 (8 male/8 female)	55 (30 male/25 female)
Recipient age (median)	64.5 (26 – 71)	50 (18 – 73)	56.5 (38 – 66)	58 (34 – 71)
Donor age (median)	50.5 (6 – 85)	47 (11 – 88)	60 (19 – 79)	52 (16 – 75)
Donor gender	14 male/8 female	18 male/9 female	9 male/7 female	36 male/ 19 female
AB0 incompatible transplantation (%)	1 (4.5%)	3 (11.1%)	1 (6.3%)	8 (14.5%)
Positive CDC test before transplantation (%)	4 (17.4%)	3 (11.1%)	0 (0%)	3 (5.5%)
Death during the 1st year after transplantation (%)	2 (9%)	0 (0%)	0 (0%)	2 (3.6%)
Basiliximab before transplantation (%)	3 (13.6%)	3 (11.1%)	0 (0%)	5 (9.1%)
HLA mm (mean)	4.2	4.9	4.9	4.6
PRA last (mean)	9%	2.7%	13.0%	6.5%
Preformed DSA Class I (%)	6 (27.3%)	4 (14.8%)	3 (18.8%)	5 (9%)
Preformed DSA Class II (%)	2 (9.1%)	3 (11.1%)	1 (6.3%)	4 (7.3%)
Preformed non-DSA Class I (%)	12 (54.5%)	7 (25.9%)	6 (37.5%)	15 (27.3%)
Preformed non-DSA Class II (%)	4 (18.2%)	3 (11.1%)	3 (18.8%)	7 (12.7%)
De novo DSA Class I (%)	0 (0%)	1 (3.7%)	2 (12.5)	0 (0%)
De novo DSA Class II (%)	1 (4.5%)	4 (14.8%)	2 (12.5)	1 (1.8%)
De novo non-DSA Class I (%)	9 (40.9%)	5 (18.5%)	7 (43.8%)	10 (18.2%)
De novo non-DSA Class II (%)	6 (27.3%)	9 (33.3%)	2 (12.5)	4 (7.3%)

AMR, antibody-mediated rejection; TCMR, T-cell-mediated rejection; CDC, complement-dependent cytotoxicity; PRA, panel reactive antibodies; DSA, Donor-specific antibodies.

very good response, usually with rapid decrease of bilirubinemia and normalization of the liver tests. The standard immunosuppressive protocol after transplantation included calcineurin inhibitors, mycophenolate mofetil (MMF) and corticosteroids.

Statistics

All data were evaluated according to the type of rejection (acute/chronic AMR/TCMR) and HLA-specific antibodies (DSA/non-DSA; preformed/persisting/*de novo* formed; complement-binding/non-complement binding) and calculated using the chi-square test, respectively, Fisher's exact test in frequency tables. Data were considered statistically significant for P-values < 0.05.

Results

Incidence of rejection and graft loss

Twenty-two patients were diagnosed with AMR, 16 patients had TCMR only, while 27 patients experienced AMR and TCMR simultaneously. AMR was subdivided into acute and chronic. Out of the 49 patients with AMR, 20 had acute AMR, 6 developed acute AMR and later chronic AMR, while 23 had chronic AMR. TCMR was evaluated as acute and late-onset. Twenty-six liver recipients had acute TCMR, 16 had late-onset TCMR, and 1 patient developed acute and then late-onset TCMR. Fifty-five patients remained free of rejection during first year after transplantation (Table 1). No higher incidence of AMR during the early post-transplant period was observed. No substantial differences in gender, HLA matching, panel-reactive antibodies and several other demographic parameters were found between liver recipients who experienced rejection and those without rejection (Table 1). Intriguingly, patients with TCMR (including TCMR with late onset) were significantly younger than patients free of rejection ($P = 0.0307$ and $P = 0.0151$, respectively). During the 1-year follow-up, 15 liver grafts failed, 12 due to early thrombosis (these 12 recipients did not have pre-transplant HLA antibodies and were excluded from further analysis) and 3, as indicated in Materials and Methods, due to immunologic complications. Two of those patients had preformed DSA to both HLA Class I and

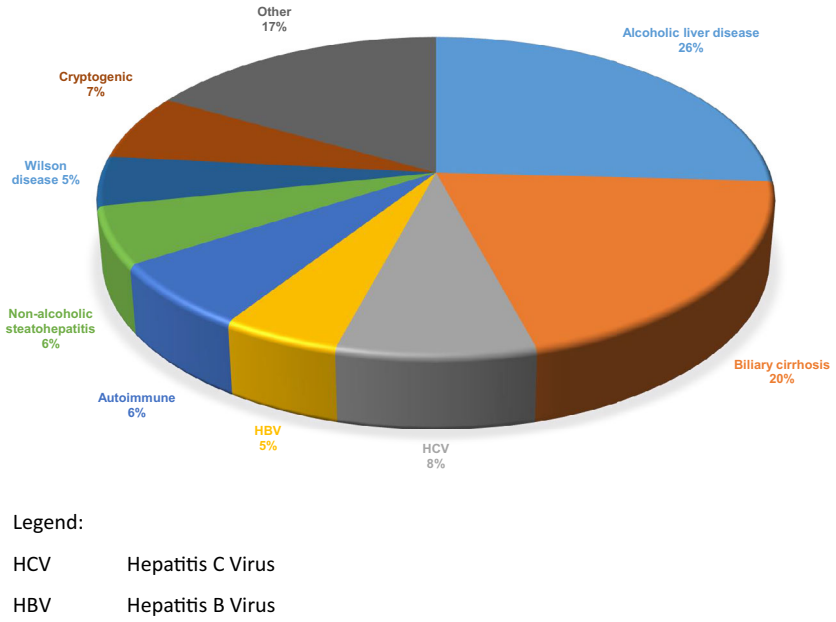

Figure 1 Diagnoses leading to indication for liver transplantation.

Class II antigens with high MFI levels (>5000), while the third patient had DSA to HLA Class I antigens only. Because of the low number of failed grafts due to immunological reasons, the effect of DSA on graft failure could not be assessed.

Pretransplant HLA-specific antibodies in relation to the incidence of rejection

Totally, there were 27 patients with anti-HLA Class I and 4 with anti-HLA Class II antibodies only. Thirteen patients had anti-HLA Class I and anti-HLA Class II antibodies simultaneously (Table 2). Twenty-two liver recipients had preformed DSA before transplantation (12 recipients to HLA Class I antigens only, 6 – concurrently to HLA Class I, and II and 4 – to HLA Class II antigens only). Forty-four patients had non-DSA (27 to HLA Class I, 4 – to HLA Class II and 13 simultaneously to HLA Class I and Class II antigens). In general, antibodies against HLA Class I antigens (DSA and non-DSA) occurred more frequently. Eight patients who were diagnosed with acute AMR did not have detectable DSA in the periphery.

We could not find an effect of preformed HLA-specific antibodies (both DSA and non-DSA) on the incidence of acute AMR after transplantation (Table 2). In contrast, liver recipients with preformed complement-binding HLA Class I antibodies (C1q + and C3d+), both DSA and non-DSA, developed more frequently AMR than patients without HLA Class I complement-binding antibodies (Table 3). However, pre-transplant complement-binding HLA Class II DSA and non-DSA were not associated with increased occurrence of AMR (Table 3). All complement-binding antibodies had high MFI values (>5000) in the SA Luminex tests, both for HLA Class I and Class II.

Post-transplant HLA antibodies and relation to the incidence of rejection

During the first year after transplantation, 18 (86%) out of 22 recipients lost their preformed DSA (pretransplant DSA-positive); four recipients had persisting DSA. The detection of *de novo* DSA and non-DSA was prevailingly during the first six months after transplantation. Ten recipients formed *de novo* DSA (2 to HLA Class I, 7 to HLA Class II and 1 to both Class I and Class II antigens). Two of these ten patients had pretransplant non-DSA antibodies. Interestingly, persistent DSA (pre- and post-transplant) were not related to a higher risk for development of acute AMR. There were no detectable persisting complement-binding DSA in our cohort. Furthermore, no correlation was found between *de novo* HLA-specific antibodies and the incidence of acute AMR. This was valid for DSA, non-DSA and complement-binding non-DSA (results not shown). Anti-HLA-B and anti-HLA-DQ were the most frequent *de novo*-produced antibodies. Eleven patients developed

Table 2. Pretransplant antibodies to HLA Class I, Class II antigens, DSA and non-DSA and incidence of acute AMR.

		Acute AMR		
n = 120		–	+	*P*
HLA Class I (%)	–	64 (53%)	16 (13%)	0.5308
	+	30 (25%)	10 (8%)	
HLA Class II (%)	–	81 (68%)	22 (18%)	0.8405
	+	13 (11%)	4 (3%)	
DSA Class I (%)	–	81 (68%)	21 (18%)	0.4948
	+	13 (11%)	5 (3%)	
DSA Class II (%)	–	88 (73%)	22 (18%)	0.2207
	+	6 (5%)	4 (3%)	
Non-DSA Class I (%)	–	64 (53%)	16 (13%)	0.5308
	+	30 (25%)	10 (8%)	
Non-DSA Class II (%)	–	81 (68%)	22 (18%)	0.8405
	+	13 (11%)	4 (3%)	

AMR, Antibody-mediated rejection; DSA, Donor-specific antibodies.

Table 3. Preformed complement-binding DSA and non-DSA specific to HLA Class I and Class II antigens and incidence of acute AMR.

		Acute AMR		
n = 120		–	+	*P*
DSA Class I C1q+	–	94 (78%)	23 (19%)	0.0093
	+	0 (0%)	3 (3%)	
DSA Class I C3d+	–	93 (78%)	23 (19%)	0.0316
	+	1 (1%)	3 (3%)	
Non-DSA Class I C1q+	–	87 (73%)	20 (17%)	0.0341
	+	7 (6%)	6 (5%)	
Non-DSA Class I C3d+	–	90 (75%)	21 (18%)	0.0224
	+	4 (3%)	5 (4%)	
DSA Class II C1q+	–	93 (78%)	25 (21%)	0.3878
	+	1 (1%)	1 (1%)	
DSA Class II C3d+	–	93 (78%)	26 (22%)	1.0000
	+	1 (1%)	0 (0%)	
Non-DSA Class II C1q+	–	91 (76%)	25 (21%)	1.0000
	+	3 (3%)	1 (1%)	
Non-DSA Class II C3d+	–	91 (76%)	25 (21%)	1.0000
	+	3 (3%)	1 (1%)	

AMR, Antibody-mediated rejection; DSA, Donor-specific antibodies.

simultaneously *de novo* anti-HLA-B and DQ antibodies, and three of them developed chronic AMR. An intriguing finding was that, when analysing the influence of *de novo*-produced antibodies on the occurrence of chronic liver AMR, we observed that *de novo* anti-HLA Class II antibodies strongly correlated with the development of chronic AMR (Table 4). There was a specifically strong dependency between the incidence of chronic AMR and the presence of *de novo* non-DSA to HLA Class II antigens ($P = 0.0001$). For prediction of chronic AMR by assessment of the levels of HLA Class II non-DSA, the cut-off value was defined as MFI >2000; that is, any detectable antibodies were clinically significant (sensitivity = 41%, specificity = 90%, $P = 0.0015$); and an ROC curve is shown in Fig. 2. On the contrary, the cut-off value of DSA to HLA Class II antigens was not statistically relevant ($P = 0.1663$) (ROC not shown).

A correlation between *de novo*-formed complement-binding non-DSA against HLA Class II antigens and the occurrence of chronic AMR was also observed ($P = 0.0434$) (Table 4). Nineteen patients (66%) out of 29 who were diagnosed with chronic AMR did not have detectable DSA in the periphery.

Discussion

Our study was aimed to assess the clinical relevance of the detection of complement-binding and non-

Table 4. *De novo*-produced antibodies to HLA antigens and incidence of chronic AMR.

		Chronic AMR		
n = 120		–	+	*P*
HLA Class I	–	68 (57%)	21 (18%)	0.8044
	+	23 (19%)	8 (7%)	
HLA Class II	–	81 (68%)	17 (14%)	0.0002
	+	10 (8%)	12 (10%)	
DSA Class I	–	89 (74%)	28 (23%)	0.5674
	+	2 (2%)	1 (1%)	
DSA Class II	–	87 (73%)	25 (21%)	0.0952
	+	4 (3%)	4 (3%)	
C1q + DSA Class II*	–	90 (75%)	27 (23%)	0.1446
	+	1 (1%)	2 (2%)	
C3d + DSA Class II	–	90 (75%)	27 (23%)	0.1446
	+	1 (1%)	2 (2%)	
Non-DSA Class I	–	68 (57%)	21 (18%)	0.8044
	+	23 (19%)	8 (7%)	
Non-DSA Class II	–	82 (68%)	17 (14%)	0.0001
	+	9 (8%)	12 (10%)	
C1q + non-DSA Class I	–	89 (74%)	27 (23%)	0.2458
	+	2 (2%)	~~3~~ 2 (2%)	
C3d + non-DSA Class I	–	89 (74%)	27 (23%)	0.2458
	+	2 (2%)	2 (2%)	
C1q + non-DSA Class II	–	90 (75%)	26 (22%)	0.0434
	+	1 (1%)	3 (3%)	
C3d + non-DSA Class II	–	90 (75%)	26 (22%)	0.0434
	+	1 (1%)	3 (3%)	

AMR, Antibody-mediated rejection; DSA, Donor-specific antibodies.

*No *de novo* complement-binding DSA against HLA Class I antigens were detected.

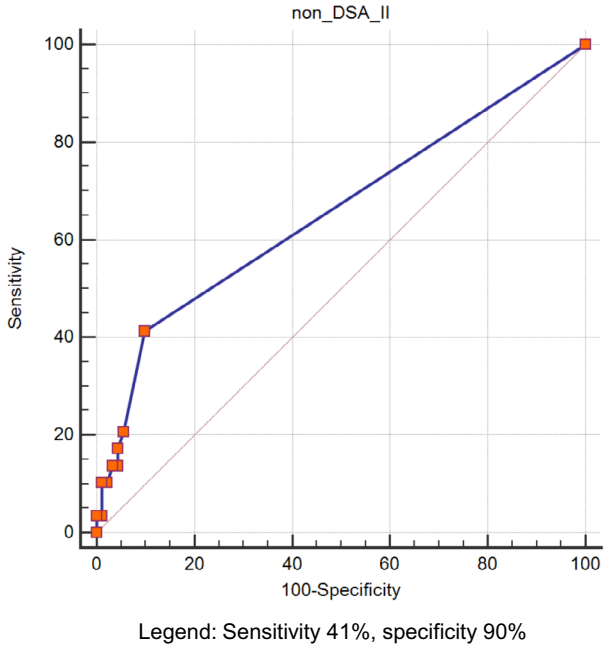

Figure 2 ROC curve representing the significance of HLA Class II non-DSA detection for prediction of chronic liver allograft rejection.

complement-binding HLA-specific antibodies for the prediction of the risk of acute and chronic rejection after liver transplantation. The deleterious effects of DSA on transplanted organs are now clearly recognized in kidney, heart and pancreas transplantation [4]. Besides the increasing number of studies supporting data about the harmful consequences of DSA on liver transplant survival, as indicated above, the role played by DSA as defined by solid-phase techniques detecting complement-binding antibodies needs further analysis.

Our demographic data concerning the incidence of TCMR are supporting a previous report of a large multicentre study, showing that younger recipient age is associated with higher risk for development of TCMR [22]. As far as acute AMR, in contrast with a recent publication of Del Bello [23], we found no correlation between AMR and pretransplant HLA-specific complement-non-binding DSA. However, Legaz et al. also, like in our report, did not find a higher frequency of acute rejection in recipients with preformed DSA [24]. A probable explanation of these contradictory results might be differences in patient demographics and immunosuppressive regimens, and/or other unknown factors. On the other hand, in our cohort, preformed complement-binding DSA directed to HLA Class I antigens, as defined by the C1q and C3d techniques, came up as a clear predictor for the development of acute AMR. This finding is in concordance with our preliminary results [21] and also with the report of Kozlowski et al., showing higher risk of AMR in recipients with pretransplant C1q-binding DSA [25]. This indicates that liver recipients with preformed complement-binding HLA Class I DSA may be considered as a high-risk group and should be carefully monitored after transplantation.

With respect to the histological diagnosis of AMR, to our best knowledge, detection of C4d deposits in liver allografts is performed in the majority of centres by the immunoperoxidase technique on formalin-fixed, paraffin-embedded tissue samples. The significantly lower sensitivity of immunoperoxidase staining in comparison with the immunofluorescence (IF) C4d detection in frozen tissue samples is well documented for kidney, heart and also liver grafts [26,27]. For this reason, the more sensitive IF method was applied in our cohort. Just a few reports have correlated the presence of C4d on liver sinusoidal endothelial cells with the presence of circulating DSA [28,29]. Due to the fact that protocol biopsies and C4d detection are frequently missing, and besides, chronic AMR may be associated with normal or nearly normal liver function [30], the incidence of both acute and chronic AMR is according to our assessment, underestimated and might at least in part explain the higher incidence of AMR in our study. In addition, as indicated by L. Wozniak and R. Venick [30], AMR might be occasionally miss-recognized, especially in the setting with concurrent TCMR, as has been also reported by O'Leary *et al.* [31].

As far as the relevance of detection of post-transplant antibodies, the finding of persistent HLA-specific antibodies after transplantation (DSA and non-DSA), in our experience, did not contribute for predicting the incidence of acute AMR. Conversely, an important result was that *de novo* HLA antibodies to Class II antigens (including non-DSA) in our cohort were strongly associated with the incidence of chronic AMR. Considering the calculated ROC curves and cut-off values, there was no statistically significant correlation between the development of chronic AMR and the formation of *de novo* DSA to Class II antigens. Surprisingly, we found that *de novo*-formed non-DSA to Class II antigens were a clear predictor for the risk of chronic AMR. The cut-off value of antibody levels was defined as> 2000 MFI, so the presence of any non-DSA to Class II antigens formed after transplantation could be considered as a risk factor for the development chronic AMR. The above-mentioned finding is in contradiction with the reports of Cousin *et al.* [32] and Tokodai *et al.* [33], who reported that inflammation and fibrosis in

paediatric liver transplant biopsies, which are considered as morphological features of chronic rejection, were related to the presence of circulating *de novo* DSA, especially DSA to HLA Class II antigens. It can be speculated about the reason why non-DSA to HLA Class II antigens in our study predicted an increased risk for the development of chronic AMR. A probable explanation could be that DSA (both HLA Class I and Class II antibodies) might be bound to HLA antigens in the graft and extracted from the recipient blood circulation; furthermore, both studies mentioned above were performed in paediatric recipients who have reduced graft capacity for antibody binding. In kidney transplantation, Everly *et al.* [34] suggested that the production of *de novo* DSA occurs more frequently during the first year after transplantation and that these antibodies are concomitant with higher incidence of graft failure, an observation that will have to be verified in liver transplant recipients.

Our study has a few limitations. Firstly, the influence of non-HLA antibodies on the incidence of liver allograft rejection could not be assessed. Secondly, even though peripheral DSA have to be detected for the unambiguous diagnosis of AMR [4], we were not able to identify DSA in all patients who had clear histopathological signs of acute/or chronic AMR. We understand the current strict criteria for chronic AMR which help avoid over diagnosis until the entire spectrum of morphological features is perceived.

In conclusion, detection of preformed complement-binding DSA to HLA Class I antigens appears to be a significant risk factor for the development of acute AMR after liver transplantation. *De novo*-produced antibodies to HLA Class II molecules including non-DSA may predict increased risk for chronic AMR, which suggests that regular monitoring of patients for antibody production after liver transplantation would be appropriate. Detection of antibody formation after liver transplantation will be perhaps important also in the near future when new types of treatment currently tested in kidney transplant recipients will become available.

Authorship

BK: performed experiments, analysed data and wrote the article. AS: contributed to the study design, analysed data and wrote the article. EH: analysed liver allograft biopsies and wrote the article. DE: analysed clinical data and revised the article. JS: statistically evaluated clinical data and revised the article. OV: contributed to the study design and revised the article. PT: contributed to the study design, analysed clinical data and revised the article.

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Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

1. Kim PT, Demetris AJ, O'Leary JG. Prevention and treatment of liver allograft antibody-mediated rejection and the role of the 'two-hit hypothesis'. *Curr Opin Organ Transplant* 2016; **21**: 209.
2. Demetris AJ, Zeevi A, O'Leary JG. ABO-compatible liver allograft antibody-mediated rejection: an update. *Curr Opin Organ Transplant* 2015; **20**: 314.
3. Hübscher SG. Antibody-mediated rejection in the liver allograft. *Curr Opin Organ Transplant* 2012; **17**: 280.
4. Demetris AJ, Bellamy C, Hübscher SG, *et al.* 2016 Comprehensive update of the Banff Working Group on liver allograft pathology: introduction of antibody-mediated rejection. *Am J Transplant* 2016; **16**: 2816.
5. Demetris AJ, Bellamy CO, Gandhi CR, Prost S, Nakanuma Y, Stolz DB. Functional immune anatomy of the liver-as an allograft. *Am J Transplant* 2016; **16**: 1653.
6. Shin M, Moon HH, Kim JM, *et al.* Significance of true-positive and false-positive pretransplantation lymphocytotoxic crossmatch in primary liver allograft outcomes. *Transplantation* 2013; **95**: 1410.
7. Wesson RN, Etchill EW, Garonzik-Wang J. Application and interpretation of histocompatibility data in liver transplantation. *Curr Opin Organ Transplant.* 2017; **22**: 499.
8. Muro M, Moya-Quiles MR, Mrowiec A. Humoral alloresponse in liver transplantation: role of Human Leucocyte Antigens (HLA) antibodies. *Curr Prot Pept Sci* 2016; **17**: 776.
9. Muro M, Marina L, Miras M, *et al.* Liver recipients harbouring anti-donor preformed lymphocytotoxic antibodies exhibit a poor allograft survival at the first year after transplantation: experience of one centre. *Transpl Immunol* 2005; **14**: 91.
10. Musat AI, Agni RM, Wai PY, *et al.* The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. *Am J Transplant* 2011; **11**: 500.
11. Del Bello A, Congy-Jolivet N, Muscari F, *et al.* Prevalence, incidence and risk factors for donor-specific anti-HLA antibodies in maintenance liver

transplant patients. *Am J Transplant* 2014; **14**: 867.
12. O'Leary JG, Kaneku H, Jennings LW, *et al*. Preformed class II donor-specific antibodies are associated with an increased risk of early rejection after liver transplantation. *Liver Transpl* 2013; **19**: 973.
13. Kaneku H, O'Leary JG, Banuelos N, *et al*. De novo donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. *Am J Transplant* 2013; **13**: 1541.
14. O'Leary JG, Demetris AJ, Friedman LS, *et al*. The role of donor-specific HLA alloantibodies in liver transplantation. *Am J Transplant* 2014; **14**: 779.
15. Del Bello A, Congy-Jolivet N, Danjoux M, Muscari F, Kamar N. Donor-specific antibodies and liver transplantation. *Hum Immunol* 2016; **77**: 1063.
16. Vandevoorde K, Ducreux S, Bosch A, *et al*. Prevalence, risk factors, and impact of donor-specific alloantibodies after adult liver transplantation. *Liver Transpl* 2018; **24**: 1091.
17. Kaneku H, O'Leary JG, Taniguchi M, Susskind BM, Terasaki PI, Klintmalm GB. Donor-specific human leukocyte antigen antibodies of the immunoglobulin G3 subclass are associated with chronic rejection and graft loss after liver transplantation. *Liver Transpl* 2012; **18**: 984.
18. Wozniak LJ, Hickey MJ, Venick RS, *et al*. Donor-specific HLA antibodies are associated with late allograft dysfunction after pediatric liver transplantation. *Transplantation* 2015; **99**: 1416.
19. O'Leary JG, Kaneku H, Banuelos N, Jennings LW, Klintmalm GB, Terasaki PI. Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. *Am J Transplant* 2015; **15**: 1003.
20. Ducreux S, Guillaud O, Bosch A, *et al*. Monitoring efficiency of humoral rejection episode therapy in liver transplantation: any role for complement binding Luminex Single Antigen assays? *Transpl Immunol* 2016; **35**: 23.
21. Kovandova B, Slavcev A, Sekerkova Z, Honsova E, Trunecka P. Antibody-mediated rejection after liver transplantation-relevance of C1q and C3d-binding antibodies. *HLA* 2018; **92** (Suppl 2): 34.
22. Wiesner RH, Demetris AJ, Belle SH, *et al*. Acute hepatic allograft rejection: incidence, risk factors, and impact on outcome. *Hepatology* 1998; **28**: 638.
23. Del Bello A, Neau-Cransac M, Lavayssiere L, *et al*. Outcome of liver transplant patients with preformed donor-specific anti-human leukocyte antigen antibodies. *Liver Transpl* 2020; **26**: 256.
24. Legaz I, Boix F, López M, *et al*. Influence of preformed antibodies in liver transplantation. *J Clin Med* 2020; **9**: 708.
25. Kozlowski T, Weimer ET, Andreoni K. C1q Test for identification of sensitized liver recipients at risk of early acute antibody-mediated rejection. *Ann Transplant* 2017; **22**: 518.
26. Sapir-Pichhadze R, Curran SP, John R, *et al*. A systematic review of the role of C4d in the diagnosis of acute antibody-mediated rejection. *Kidney Int* 2015; **87**: 194.
27. Seemayer CA, Gaspert A, Nickeleit V, Mihatsch MJ. C4d staining of renal allograft biopsies: a comparative analysis of different staining techniques. *Nephrol Dial Transplant* 2007; **22**: 568.
28. Kozlowski T, Rubinas T, Nickeleit V, *et al*. Liver allograft antibody-mediated rejection with demonstration of sinusoidal C4d staining and circulating donor-specific antibodies. *Liver Transpl* 2011; **17**: 357.
29. Watson R, Kozlowski T, Nickeleit V, *et al*. Isolated donor specific alloantibody-mediated rejection after ABO compatible liver transplantation. *Am J Transplant* 2006; **6**: 3022.
30. Wozniak L, Venick R. Donor-specific antibodies following liver and intestinal transplantation: clinical significance, pathogenesis and recommendations. *Int Rev Immunol* 2019; **38**: 106.
31. O'Leary JG, Kaneku H, Demetris AJ, *et al*. Antibody mediated rejection as a contributor to previously unexplained early liver allograft loss. *Liver Transpl* 2014; **20**: 218.
32. Cousin VL, Rougemont AL, Rubbia-Brandt L, *et al*. Peripheral donor specific antibodies are associated with histology and cellular subtypes in protocol liver biopsies of pediatric recipients. *Transplantation* 2020; **104**: 1633. https://doi.org/10.1097/TP.0000000000003099
33. Tokodai K, Miyagi S, Nakanishi C, *et al*. Association of post-transplant donor-specific HLA antibody with liver graft fibrosis during long-term follow-up after pediatric liver transplantation. *Pediatr Transplant* 2018; **22**: e13169.
34. Everly MJ, Rebellato LM, Haisch CE, *et al*. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation* 2013; **95**: 410.