ANTIBODY-MEDIATED REJECTION AFTER LIVER TRANSPLANTATION - RELEVANCE OF C1q AND C3d-BINDING ANTIBODIES

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RUNNING TITLE: Antibody-mediated rejection of the transplanted liver

Abbreviations

AMR	antibody-mediated rejection
ACR	acute cellular rejection
DSA	donor-specific antibodies
HLA	human leukocyte antigens
SA	single antigen

Author contributions:

Barbora Kovandova: performed experiments, analysed data, wrote the article

Antonij Slavcev: contributed to the study design, analysed data, wrote the article

Zuzana Sekerkova: performed experiments, analysed data

Eva Honsova: analysed liver allograft biopsies, revised the article

Pavel Trunecka: analyzed clinical data, revised the article

Abstract

The aim of our study was to evaluate the relevance of complement-binding donor-specific antibodies (DSA) for prediction of antibody-mediated rejection after liver transplantation. Sera from 123 liver transplant recipients were retrospectively defined for HLA specificity and complement-fixing activity using the single antigen beads, C1q and C3d techniques. Liver-recipients´ sera were tested before transplantation, 3, 6 months and one year after transplantation. Patients were followed up for graft survival and rejection incidence for one year after transplantation. All patients with pre-transplant complement-binding DSA developed severe antibody-mediated rejection after transplantation, while three recipients out of four, who produced de novo complement-fixing DSA, developed AMR. Definition of DSA with respect to complement-fixing activity may provide clinically relevant information about the risk of antibody-mediated rejection after liver transplantation

Keywords: liver transplantation, antibodies, complement, C4d, rejection, HLA, crossmatch

Acute antibody-mediated rejection (AMR) after liver transplantation is rare, however, there is increasing awareness that AMR may cause deterioration of graft function and impaired survival of transplanted organs (1) (2) (3). Due to the complex pathological characteristics which may overlap with other non-immunological complications, like biliary obstruction, ischemic injury and others, the diagnosis of AMR after liver transplantation is difficult (4). The transplanted liver has tolerogenic (immunosuppressive) properties and is relatively resistant to the harmful effects of donor-specific antibodies (DSA), because of the large absorbing surface of the organ, clearance of immune complexes by Kupffer cells, and various other (probably also unknown) factors (5). Quite a few studies have already indicated worse graft and patient survival in recipients with persistent DSA and *de novo*-produced DSA (6) (7) (8) (9) (10). On the other hand, data on the role of complement-binding DSA in comparison with non-complement fixing DSA in the assessment of the risk for liver transplant rejection remain still limited (11). The goal of our retrospective study was therefore to evaluate the clinical relevance of complement-binding donor-specific antibodies (as defined by the single-antigen bead techniques) for prediction of the risk of antibody-mediated rejection after liver transplantation.

One hundred twenty three liver recipients transplanted in our centre between the years 2015 and 2017 were included into the study. All patients received grafts from deceased donors. Recipients were followed for allograft function, incidence of rejection and graft survival for up to 1 year after transplantation. The research was approved by the ethics committee of the institute and written informed consent was obtained from all patients. The demographic characteristics of patients with biopsy-proven rejection in comparison with patients without rejection are shown in Table 1. Liver recipients were HLA typed by the PCR SSOP technique (intermediate resolution) for HLA-A, -B and –DR loci (OneLambda, Canoga Park, USA). Organ donors were HLA typed by PCR SSP low resolution kits (Olerup and BAG) for HLA-A, -B, -DR and -DQ loci. No allele-specific antibodies or antibodies solely to the DQ-alpha chain were detected, so high-resolution typing was not necessary.

Detection and specification of antibodies were performed before, 3, 6 and 12 months after transplantation. For elimination of the prozone effect, all sera were pre-treated with EDTA. One recipient had a positive complement-dependent cytotoxicity (CDC) crossmatch test before transplantation, all remaining patients had negative CDC crossmatch test. No unacceptable HLA antigens were defined in the liver graft allocation process. During the one year follow up 6 grafts failed, 3 livers were lost due to immunologic complications, and three due to other reasons (thrombosis and subsequent graft ischemia). Pre- and post-transplantation sera were analysed retrospectively using the Luminex technique. In total, 355 serum samples were tested. Detection of HLA antibodies was performed using the

LABScreen Mixed technique; positive sera were then screened for HLA specificity using single antigen (SA) beads (One Lambda Inc., Canoga Park, USA) according to the manufacturer's instructions. A cut-off for positivity of 1000 MFI and 2000 MFI which was previously validated by the laboratory was applied for class I and class II SA beads, respectively. Further, positive sera were tested for complement-binding activity by the C1q Screen (OneLambda) and Lifecodes C3d Detection kits (Immucor, Stamford, USA). Donor-specific antibodies were defined against HLA-A, -B and -DR and -DQ antigens.

Cellular (CR) and antibody-mediated rejection (AMR) were diagnosed in graft biopsies according to the criteria of the updated Banff classification published recently (4). The diagnosis of AMR was supported by immunofluorescent detection of diffuse C4d deposits (> 10%) and the simultaneous presence of DSA. The standard immunosuppressive protocol after transplantation included calcineurin inhibitors, mycophenolate mofetil and corticosteroids.

Our analysis had two parts. HLA antibodies were first defined as donor-specific or non-donor specific (DSA or non-DSA), and subsequently as complement-binding /or non-complement-binding (DSA and non-DSA). DSA were present in 27 (21%) and non-DSA in 30 (25%) patients. 19 patients had pretransplant DSA, while 8 recipients produced *de novo* DSA during the first year after transplantation (Table 2). In the second part of our study, as indicated above, we compared two different techniques for detecting complement-binding antibodies, i.e. the C1q Screen and Lifecodes C3d detection tests. With exception of one recipient, who produced *de novo* complement-fixing non-DSA, all C3d-positive patients were also positive in the C1q test (Table 3). C1q-positive DSA were detected in 7 (5.7%) patients and complement-binding non-DSA in 7 (5.7%) recipients (Table 3). Complement-binding DSA (both C1q and C3d positive) had MFI values >9000 in the SA bead test (MFI HLA-B = 17483, HLA-DQ = 16182). Complement-fixing non-DSA had MFI >6000 (HLA A = 11224, HLA B = 9549 and HLA DQ = 7541). As far as clinical outcome, there were totally 28 antibody mediated rejections, 21 cellular rejections (CR), 8 concurrent AMR and CR and in 28 patients the lack of rejection against the graft was biopsy-proven. There were also 9 patients where post-transplant complications that were not caused by rejection (graft dysfunction, recurrence of hepatitis C). The incidence of rejection in patients with complement-binding and non-complement-binding DSA is shown in Fig. 1. All three patients with pretransplant complement-binding DSA developed severe antibody-mediated rejection, furthermore, two grafts in this cohort failed due to immunological reasons (Fig 1.). Three patients out of four, who produced de novo complement-fixing DSA, had AMR (two mild form and one plasma-rich rejection). In the non-complement-fixing cohort (21 patients), there were 6 episodes of AMR – five in patients with preformed antibodies and one in a patient with de

novo produced antibodies. Our study had a certain limitation – 29 patients did not have graft biopsy, due to the technical complexity of the procedure.

In conclusion, analysis of alloantibodies by the single antigen bead technique and their definition with respect to complement-fixing activity may provide clinically relevant information about the risk of antibody-mediated rejection after liver transplantation.

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Table 1: Demographic characteristics in patients with biopsy-proven rejection and without rejection.

	AMR[1] (n=28)	CR[2] (n=21)	AMR + CR (n=8)	Biopsy-proven absence of rejection (n=28)
Age (years)	61.5 (15 – 73)	58 (38 – 71)	58 (40 – 73)	50.5 (6 – 69)
PRA[3] (>10%)	16 (57%)	18 (85%)	4 (50%)	19 (68%)
PRA (≤10%)	5 (18%)	2 (10%)	2 (25%)	4 (14%)
PRA (>50%)	1 (4%)	1 (5%)	2 (25%)	0 (0%)
PRA (10-50%)	2 (7%)	0 (0%)	0 (0%)	2 (7%)
HLA mismatches	5 (3 – 6)	5 (4 – 6)	5 (4 – 6)	5 (2 – 6)

[1] AMR – antibody-mediated rejection

[2] CR – cellular rejection

[3] Panel-reactive antibodies (PRA) before transplantation were defined by the complement-dependent cytotoxicity test.

Table 2. Analysis of HLA antibodies - DSA-positive, DSA-negative, preformed and *de novo* antibody positive patients.

	Patients (n) 123
DSA-positive	27 (21%)
- Pre-formed	19 (15%)
- De novo	8 (6%)
Non-DSA positive	30 (25%)
- Pre-formed	17 (14%)
- De novo	13 (11%)
Negative	66 (54%)

Table 3. Complement-binding vs. non-complement binding antibodies, comparison of the C1q and C3d techniques.

	Patients (n)		Patients (n)
C1q DSA-positive	7 (5.7%)	C3d DSA-positive	5 (4.1%)
- Preformed	3 (2.4%)	- Preformed	3 (2.4%)
- De novo	4 (3.3%)	- De novo	2 (1.6%)
C1q non-DSA-positive	7 (5.7%)	C3d non-DSA-positive	4 (3.3%)
- Preformed	4 (3.3%)	- Preformed	3 (2.4%)
- De novo	3 (2.4%)	- De novo	1 (0.8%)
C1q-negative	109 (88.6%)	C3d-negative	114 (92.7%)

Fig. 1. Complement-binding, non-complement-binding DSA and incidence of rejection.