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ORIGINAL ARTICLE

Exploring pre-surgery donor-specific antibodies in the context of organ shortage in liver transplant

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Abstract

Background There is a growing disparity between the number of liver transplant (LT) candidates and availability of suitable liver allografts. Antibody-mediated rejection (AMR), secondary to positive donor-specific antibodies (DSA), remains a concern in liver transplantation. This study aimed to correlate expression of DSA on pre-transplant screening and outcomes of LT, specifically development of AMR in liver allografts and liver function profile in the post-operative period.

Methods Data of consecutive patients undergoing orthotopic LT (OLT) at the South Australian Liver Transplant Unit was analysed. All patients underwent DSA testing pre-transplant.

Results Within a cohort of 96 patients, over a post-OLT median follow-up of 849 days, only 2 patients (2%) developed AMR. While both patients had a positive DSA test preoperatively, overall DSA positivity was noted in 31% patients, with a specificity for prediction of AMR of 0.708. No significant association was noted between AMR (p = 0.092), T cell–mediated rejection/TCMR (p = 0.797) or late hepatic artery thrombosis/LHAT (p = 0.521). There was no significant interaction effect between DSA positivity and serum bilirubin or transaminases over a period of 100 days.

Conclusion AMR following LT is uncommon. A positive DSA pre-transplant does not imply a definite risk of AMR. Also, there does not exist a significant interaction in time between DSA expression and serum bilirubin or transaminase levels. Until there emerges evidence to the contrary, it appears reasonable to consider DSA-positive donors within the broad context of marginal donors in the context of a worldwide shortage of LT donor allografts.

Keywords Rejection · Thrombosis · Outcomes

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Introduction

There is a growing disparity in the number of liver transplant (LT) candidates and corresponding availability of suitable liver allografts around the world [1]. This dilemma has spawned the concept of extending the criteria for selection of organ donors. The limits for organ donation have stretched from using allograft donations after cardiac death (DCD) [2] to the use of marginal donors such as from the elderly [3], and even the use of split grafts, steatotic livers or even donors with viral hepatitis and malignancy [4]. The harsh reality is that such efforts are not without an attendant risk of graft failure and reduced patient survival even in high-volume centres [5]. Thus, it is of paramount importance to achieve a better understanding of the implications of using marginal donors [6].

The pioneering work of Patel and Terasaki [7] on the humoral theory of transplantation [8] demonstrated that a positive crossmatch for donor-specific antibodies (DSA) was associated with a propensity for hyperacute rejection of the kidney allograft through an antibody-mediated pathway. The incidence of antibody-mediated rejection (AMR) varies depending on the transplanted organ [9]. The kidneys and heart are more susceptible to AMR than the lungs which, in turn, are more often affected than the liver [10, 11]. The relative resistance of the liver to AMR has been postulated by Demetris and colleagues to be a result of the fact that the 'liver is an immunoregulatory organ in which a tolerogenic microenvironment mitigates the relative "strength" of local immune responses' [11].

Despite the relative 'resistance' of the liver to AMR, there continue to be reports of such occurrences [12, 13]. The EASL Clinical Practice Guidelines have indicated that the correlation between the cut-off of DSA and the outcome of LT is unclear [14]. At a time when the criteria for selection of donors for LT are truly being stretched with an inherent risk of compromising patient survival and graft function, understanding the significance of a positive DSA test in the context of development of AMR, as well as the use of such patients as donors, seems pertinent.

The aim of the present study was therefore to determine the correlation between expression of DSA on pre-transplant screening and the outcomes of LT, more specifically the development of AMR in liver allografts and liver function profile in the post-operative period.

Methods

Patient population

The data of consecutive patients who underwent an orthotopic LT (OLT) at the South Australian Liver Transplant Unit between January 2014 and December 2017 was obtained from a prospectively maintained database. All transplants were performed using organs from deceased donors.

Study design

The study is a retrospective cohort study based on patients whose data is maintained in a prospective surgical database. Some secondary data was additionally collected from records.

Immunosuppression protocols

The standard approach is triple therapy (tacrolimus/azathioprine/prednisolone). Azathioprine use is reviewed at 12 months and is often ceased in patients with good liver function, no prior rejection, and less immunogenic causes (non-primary biliary cirrhosis/autoimmune hepatitis / primary sclerosing cholangitis). Steroids are tapered usually by the 3-month mark, except in autoimmune patients where we continue low-dose steroids in the long-term. The detailed protocol is provided as Supplementary file 1.

Diagnosis of AMR

For the current study, AMR and acute cellular rejection (ACR)/T cell-mediated rejection (TCMR) were defined as per the Banff Working Group proposal [9]. To diagnose AMR, the patient must have had (a) pre-transplant positive human leucocyte antigen (HLA) DSAs; (b) evidence of graft dysfunction proven on biopsy in the form of microvascular inflammation (portal microvascular endothelial hypertrophy, portal capillary and inlet venule dilatation) and immune cell (monocytic, eosinophilic and/or neutrophilic) infiltration; and (c) reasonable exclusion of other causes for liver dysfunction, supplemented by clinical suspicion of AMR. The diagnosis was strengthened in the presence of C4d staining (performed using rabbit polyclonal antibodies supplied by Cell Marque 404A, Sigma-Aldrich, CA, USA) on the Roche Ventana Benchmark Ultra platform. Late hepatic artery thrombosis (LHAT) was defined as a thromboembolic occlusion of the hepatic artery that occurred after 2 months of LT [15]. The rationale for studying LHAT stems from the fact that most of our understanding of AMR is derived from knowledge gained with renal transplant. Central to the pathophysiology of AMR is injury to the vascular endothelium. AMR has a significantly higher risk of vascular rejection than TCMR [16]. Besides, the early clinical pictures of LHAT and AMR are similar in terms of transaminase profile [17]. Thus, to understand the contribution of DSA to vascular rejection in the liver—an entity that has not been previously studied—we chose to analyse LHAT and not early HAT, which is generally perceived as a technical problem.

We do not perform protocol biopsies. The specific clinical indication for a biopsy is significant, unexplained liver

function derangement. All suspected cases of rejection are proven on biopsy before treatment.

Antibody testing

Solid phase assays were used for donor-specific antibody testing and these were performed at the South Australia Transplantation and Immunogenetics Service, Australian Red Cross Blood Service, Adelaide. The Luminex ® platform was used for analysis of the fluorescent-conjugated antigen bound antibody. We perform tissue typing workup testing with initial samples. This includes HLA typing and HLA antibody testing. Once the recipient is activated on the transplant waiting list, we perform regular HLA antibody screening every 6 months (or until the recipient is transplanted). The crossmatch is performed with the most recent serum received in the laboratory and is by CDC (complement dependent cytotoxicity) methods. Results were interpreted using raw mean fluorescence intensity (MFI) values. The positive cut-off is 500 MFI. Below are the ranges used to compare strength.

Immucor (Tepnel): weak 300–1499; moderate 1500–3999; strong 4000+
OneLambda: weak 500–1999; moderate 2000–7999; strong: 8000+

Follow-up

All patients are regularly followed up in the LT outpatient clinics at the Flinders Medical Centre, Adelaide. In addition, the LT nurse coordinators make regular phone calls to the patients to ensure that they are symptom-free and are compliant with their immunosuppressant medication.

Ethics

All procedures performed in this study were in accordance with the ethical standards of the Institute and with the 1964 Helsinki declaration.

Statistical analysis

Statistical analysis was performed using the statistical program IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). A p value of < 0.05 was considered statistically significant. Variables were summarised using the number of observations and percent, mean and standard deviations, median and range. For analysis, strong and moderate donor antibody strengths were considered positive while weak or absent were considered negative. For T and B cells, equivocal and negative were considered negative while any level of positive expression was considered positive.

For survival, the period from the day of the OLT to 16 July, 2018, was calculated.

Fisher's exact test was used to determine the strength of association between DSA test results on the one hand, and results for AMR, TCMR and LHAT on the other hand. Ranges have been expressed as interquartile range (IQR).

We also investigated whether the DSA-positive group and the DSA-negative group exhibited significant differences in their time course of test results for each of bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), measured at 1, 7, 30 and 100 post-operative days following OLT, using a mixed analysis of variance with time and DSA group as fixed effects, and patient as a random effect. The rationale for studying this time course stems from our larger experience with DSA and AMR in renal transplant (as compared with the meagre experience in liver transplant). Subclinical AMR is a proven entity in renal transplant and is associated with poor graft survival and increased risk of allograft loss [18, 19]. In renal transplant patients, unlike liver transplant, protocol biopsies are performed to help detect rejection. We thus wanted to study if there exists an objective trend in alteration of liver function post-transplant that would help identify if subclinical AMR also exists in liver transplant. This would also be helpful to guide future research. A significant interaction between time and DSA would indicate that the DSA-positive and DSA-negative groups followed different trajectories over time. Analysis was done in R, using the lme4, car and lsmeans packages.

Results

Patient demographics

Ninety-six patients (65 males, 31 females) were included in the study. Two patients underwent re-transplantation accounting for a total of 98 cases of OLT included in the study. The median age in the study cohort was 57 years (IQR 50.75 to 62.0 years). The indications for OLT are listed in Table 1. The median model for end-stage liver disease (MELD) and sodium MELD (Na MELD) scores at the time of OLT were 17 (range 6–40) and 18 (range 6–40), respectively.

Surgical factors

Whole-organ transplants were performed in 90 patients (92%) with 8 patients receiving split (right lobe) livers. These organs were retrieved from 93 donors after brain death and 5 DCD. The median (total) ischaemic time was 5 h and 30 min (IQR, 4 h 23 min to 7 h 53 min). The splenic patch or artery to hepatic bifurcation (34%; 33 patients) and the celiac artery

Table 1 Indications for liver transplantation

Indication	Number of patients $N = 96$
Alcohol	19
Hepatitis C virus (HCV)/hepatocellular carcinoma (HCC)	12
HCC/alcohol	8
Alcohol/HCV/HCC	8
HCV/alcohol	7
Primary sclerosing cholangitis	6
Non-alcoholic fatty liver disease (NAFLD)	6
HCV	6
Seronegative subacute liver failure	2
Cryptogenic cirrhosis	3
Hepatitis B virus (HBV)/HCC	2
Primary biliary cirrhosis	2
Hereditary tyrosinaemia/HCC	1
Autoimmune hepatitis	2
Fulminant hepatic failure (HBV)	1
Post-transplant biliary strictures with recurrent cholangitis	1
Secondary biliary cirrhosis	1
Recurrent HCV (graft failure)*	1
Autoimmune hepatitis/hepatopulmonary syndrome (HPS)	1
Graft failure with outflow obstruction	1
NAFLD/HPS	1
Type 1 primary hyperoxaluria	1
Alcohol/HPS	1
Congenital hepatic fibrosis	1
HBV/alcohol	1
Granulomatous hepatitis	1
Glycogen storage disorder	1
Hepatic artery thrombosis*	1

*Indications for re-transplantation in 2 patients

to hepatic bifurcation (36%; 35 patients) were the most common modalities of arterial reconstruction. An aortic patch to hepatic bifurcation or an aortic patch to aortic jump graft were only utilised in 4 patients and 1 patient, respectively. Biliary anastomosis was performed as a duct-to-duct anastomosis in 91 (93%) patients with 7 patients requiring a Roux-en-Y biliary anastomosis. Median operative duration was 386 min (range 271–540 min) with a median blood loss of 1430 ml (range 100–10,000 ml). Standard post-operative immunosuppression protocol consisted of calcineurin inhibitors (tacrolimus), azathioprine and steroids (3-month course).

Perioperative morbidity and mortality

The overall morbidity rate was 11% (11 patients) with a perioperative mortality rate of 2% (2 patients). The 90-day reoperation rate was 8% (8 patients) for bleeding in 6 patients and for a hepatic artery clot and biliary anastomotic leak in 1 patient each. The median length of stay in hospital was 15 days (IQR, 12.0 to 21.0 days).

Follow-up

No patients were lost to follow-up. The median follow-up was 849 days (IQR, 508.8 to 1147.0 days). Seven patients, in total, died since the time of OLT. The Kaplan Meier survival curve for all patients is shown in Fig. 1.

Post-OLT rejection and DSA as a predictor of AMR, TCMR and LHAT

The overall incidence of AMR was low in this study, occurring in only 2 (2%) of the 96 cases. The details of these 2 patients are highlighted in Table 2 (Figs. 2 and 3). One patient had DSA directed at Cw4 while the other had DSA directed at DQ. Patient no. 2 is currently on the waiting list for a re-

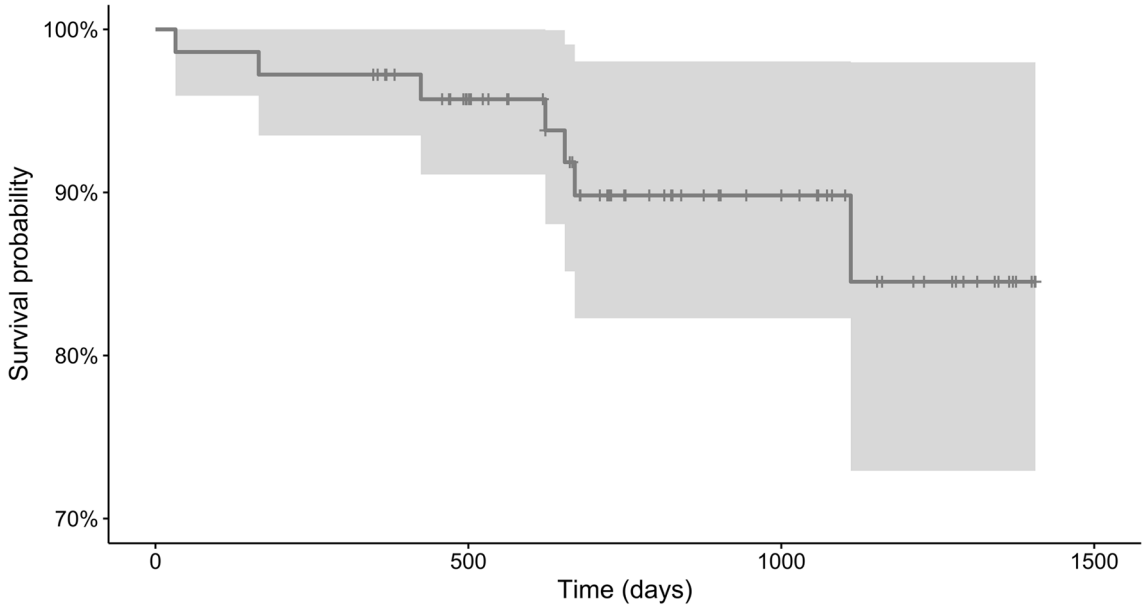

Fig. 1 Kaplan-Meier survival curve for the cohort of OLT patients

transplant. TCMR was diagnosed in 23 (23.5%) patients while only 2 (2%) patients developed LHAT. DSA tests returned a positive result for 30 (31%) of all cases. Five patients had a positive (including weakly positive) B cell crossmatch while 5 patients had a positive (including weakly positive) B and T cell crossmatch. Patient no. 1 with AMR had a weakly positive B cell crossmatch. Detailed pre-transplant DSA MFI values for all patients are provided in Supplementary file 2.

Both cases of positive AMR co-occurred with positive DSA results, so that for these data, sensitivity of DSA for AMR was equal to 1.0; however, it was not the case that a positive DSA result was predictive of a positive AMR result, as specificity of DSA for AMR was only 0.708. This conclusion was confirmed by statistical testing, with no significant association found between DSA and AMR (Fisher's exact test, $p = 0.092$).

DSA had low sensitivity (0.261) and moderate specificity (0.68) for TCMR and there was no significant association between DSA and TCMR (Fisher's exact test, $p = 0.797$).

DSA also had low sensitivity (0.5) and moderate specificity (0.698) for LHAT and there was no significant association between DSA and LHAT (Fisher's exact test, $p = 0.521$).

Table 2 Clinical details of patients developing antibody-mediated rejection (*AMR*)

	Patient no. 1	Patient no. 2
Age/sex	64/female	60/male
Indication for OLT	Seronegative subacute liver failure	Alcohol
Recipient blood group	A negative	A positive
Donor blood group	O positive	A positive
Donor age	38	22
Graft	Whole liver	Whole liver
Positive DSA (MFI)	Strong Cw4 (9428) Weak B53 (1293)	Moderate DQB1*06:03 (2967) Weak: B58 (559)
C4d immunostaining	Staining noted at the following sites: • Portal venous endothelium • Within intima of small portal branches of hepatic artery Endothelium of terminal hepatic venules	No labelling of portal veins or capillaries (C4d score = 0). Possibly a sampling issue since only 4 (instead of 10) portal tracts available or assessment in the biopsy or C4d-negative AMR [30]
Total ischaemic time	7 h	5 h
Treatment used	Plasmapheresis Rituximab	Plasmapheresis Intravenous immunoglobulin Rituximab Bortezomib Candesartan

(*Abbreviations*: *OLT*, orthotopic liver transplantation; *DSA*, donor-specific antibody; *MFI*, mean fluorescence intensity)

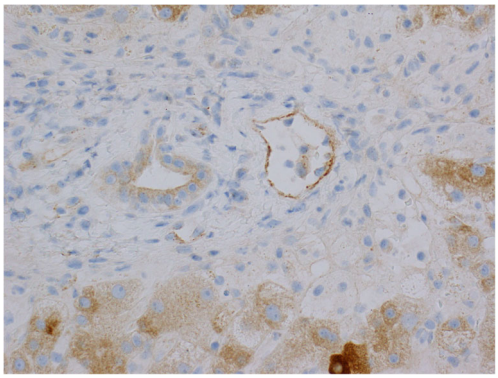

Fig. 2 Photomicrograph of liver biopsy immunostained for C4d (× 40 magnification) from patient no. 1 with AMR demonstrating circumferential labelling of portal venule with venulitis (C4d score = 2, focal (10–50% portal tracts) C4d deposition in > 50% of the circumference of portal microvascular endothelia (portal veins and capillaries)—usually without extension into periportal sinusoids [9])

DSA as a predictor of post-operative time course of bilirubin, AST, ALT and ALP

Bilirubin

The time course of post-operative bilirubin results at 1, 7, 30 and 100-day follow-up is shown in Fig. 4. Bilirubin values declined steadily following OLT for both the positive and negative DSA groups. There was a significant effect of time on bilirubin ($F = 31.870$, df = 3, $p = 3.69e^{-14}$), but no significant effect of DSA ($F = 1.273$, df = 1, $p = 0.262$). There was no significant interaction between DSA and time ($F = 0.748$, df = 3, $p = 0.526$), showing that the positive and negative DSA groups did not exhibit significantly different patterns of change in bilirubin values over time after OLT.

ALT

Figure 5 shows the time course of ALT values at 1, 7, 30 and 100 days follow-up after OLT. ALT values declined steadily from days 1 to 30, but appeared to remain relatively similar between the last two measurements. As with bilirubin, there was a significant effect of time ($F = 38.990$, df = 3, $p = 2.69e^{-16}$), and no significant interaction effect between time and DSA ($F = 0.823$, df = 3, $p = 0.484$). For DSA, the effect on ALT was not significant at the level $p = 0.05$ ($F = 3.902$, df = 1, $p = 0.051$), but as the obtained significance value was close to the chosen threshold, a post hoc analysis was conducted to compare the pairwise differences between positive and negative DSA over time. This analysis showed that ALT was significantly higher in the DSA-negative group compared with the DSA-positive group, on day 7 ($p = 0.028$), but not on any of days 1, 30 or 100 ($p > 0.05$).

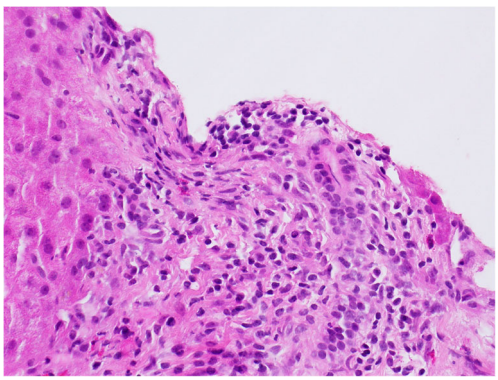

Fig. 3 Photomicrograph of liver biopsy stained with haematoxylin and Eosin (× 40 magnification) from patient no. 2 with AMR demonstrating oedema, portal microvascular endothelial cell enlargement and monocytic vasculitis ($h = 2$, histopathological score [9])

AST

Figure 6 shows AST results at days 1, 7, 30 and 100 post-OLT. As with ALT, AST values decline over the first three measurements, but remain approximately similar between day 30 and day 100. There was a significant main effect of time on AST ($F = 15.785$, df = 3, $p = 2.39e^{-8}$), no significant effect of DSA ($F = 0.455$, df = 1, $p = 0.502$) and no significant interaction effect between time and DSA ($F = 0.475$, df = 3, $p = 0.700$).

Discussion

In the present study, over a post-OLT median follow-up of 849 days, only 2 patients (2%) developed AMR. While both patients had a positive DSA test preoperatively, overall DSA positivity was noted in 31% patients with a specificity for prediction of AMR of 70.8%. No significant association was noted between AMR ($p = 0.092$), TCMR ($p = 0.797$) or LHAT ($p = 0.521$). There was no significant interaction effect between DSA positivity and serum bilirubin or transaminases over a period of 100 days.

The effect of pre-existent DSA on outcomes following LT have been suggested in the past with the spectrum of involvement varying from the clearly defined entity of AMR [9], to unexplained graft failure [20] and decreased allograft survival [21], to pathological appearances of ductopenia and fibrosis, plasma cell hepatitis, biliary strictures and accelerated fibrosis associated with recurrent liver disease [22]. DSAs have been noted to be associated not only with acute AMR, but also with a chronic form characterised by progressive fibrosis [23]. However, in a majority of these studies, it appears that the contribution of DSAs has been suggested after excluding other causes including HAT and TCMR [17]. Moreover, chronic AMR has been shrouded in lack of clarity as to whether the entity is purely a result of DSA or a co-existent component of cell-mediated rejection [24]. Finally, the time frames for development of acute and chronic AMR have not been adequately elucidated.

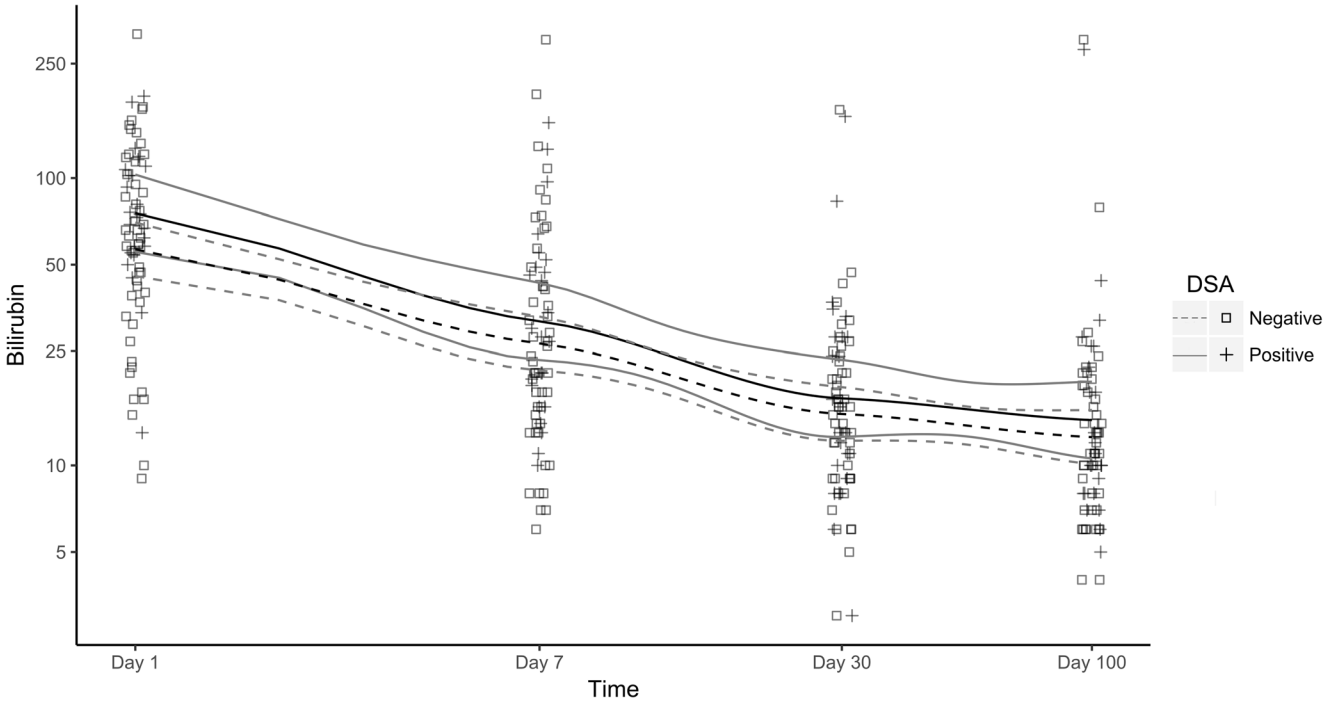

Fig. 4 The time course of bilirubin test results at 1, 7, 30 and 100 days post-operative follow-up, for positive (solid line) versus negative (dashed line) pre-operative DSA results. Predicted values using loess estimation are shown in black, with 95% confidence intervals in grey. Both axes are displayed on a logarithmic scale. Individual data points are shown overlaid, with a plus symbol indicating a positive result and a square symbol a negative result

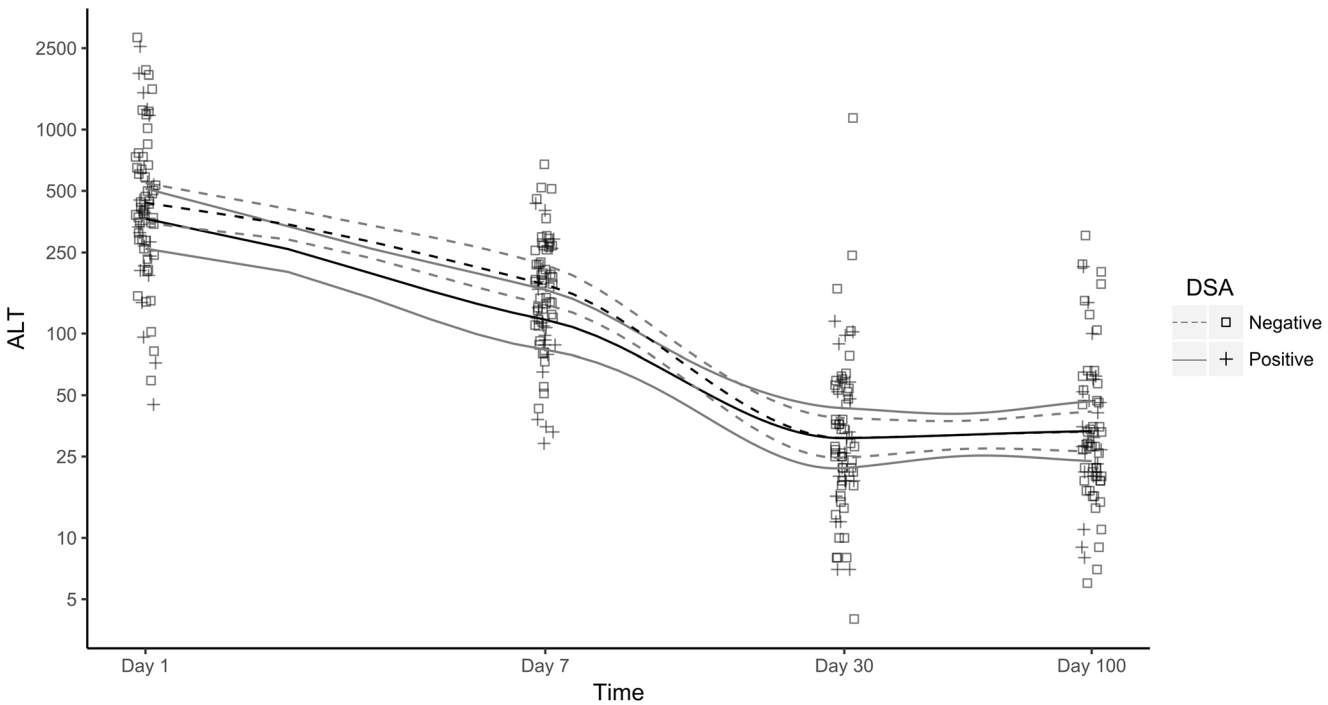

Fig. 5 The time course of ALT test results at 1, 7, 30 and 100 days post-operative follow-up, for positive (solid line) versus negative (dashed line) pre-operative DSA results. Predicted values using loess estimation are shown in black, with 95% confidence intervals in grey. Both axes are displayed on a logarithmic scale. Individual data points are shown overlaid, with a plus symbol indicating a positive result and a square symbol a negative result

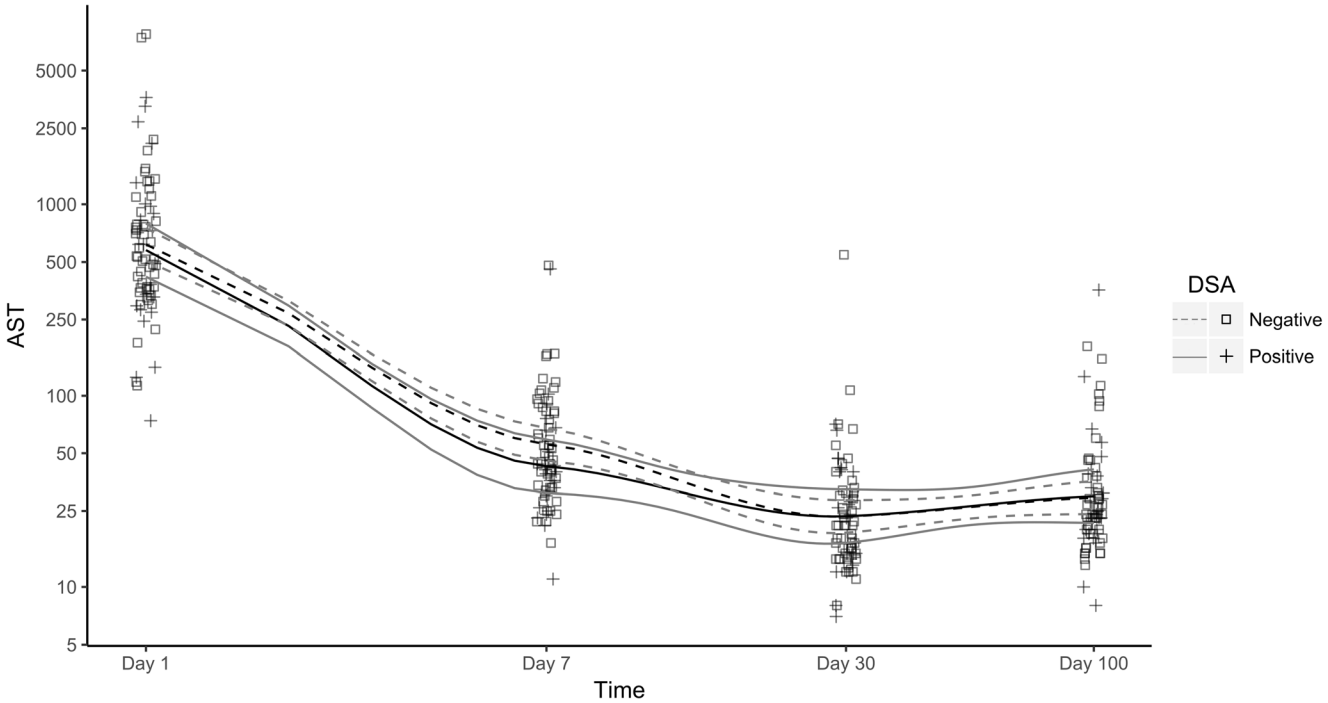

Fig. 6 The time course of AST test results at 1, 7, 30 and 100 days post-operative follow-up, for positive (solid line) versus negative (dashed line) pre-operative DSA results. Predicted values using loess estimation are shown in black, with 95% confidence intervals in grey. Both axes are displayed on a logarithmic scale. Individual data points are shown overlaid, with a plus symbol indicating a positive result and a square symbol a negative result

On account of this conundrum, the present study adopted a step-wise approach, first addressing the incidence of AMR ensuring an adequate follow-up period. Next, the association between a positive DSA result and AMR, TCMR and LHAT was analysed. Finally, taking into account the non-specific clinical presentations of AMR, an interaction between DSA expression and a time trend of liver functions over 100 days was assessed.

The overall incidence of AMR in the present study was 2%, similar to other reports in the literature [9]. In literature, AMR has often been reported to occur following the development of DSA directed at HLA class II, especially DQ [25, 26]. However, in our study, of the 2 patients with AMR, only 1 patient had DSA directed at DQB1, the other patient had DSA directed against Cw4 (Table 2). Importantly, the majority of patients with any DSA also had a negative T and B cell CDC crossmatch. The DSA, although detectable by Luminex, may therefore not be complement binding. The requirement for a negative T and B CDC crossmatch, or borderline positive crossmatch, in this cohort, may in part explain the specificity of 8% for positive DSA to predict AMR. The specificity of 70.8% for positive DSA to predict AMR appears to be a testament to the relative resistance of the liver to AMR [9]. Potential mechanisms may include Kupffer cell DSA clearance of activated complement, platelet aggregates, variable hepatic microvascular class II expression, the ability of the liver on account of its large size to dilute antibody-binding across a larger endothelial cell surface, the Fc receptor expression and phagocytic activity of Kupffer and hepatic sinusoidal endothelial cells and the capability of the liver to heal either without fibrosis or reverse fibrosis.

While the contribution of DSA to pathological changes noted in patients with delayed rejection secondary to other obvious causes, such as the above, has been suggested in literature [22], the present study did not support such a hypothesis.

The Banff Working Group update [9] has provided rough time frames for the occurrence of acute AMR (first several weeks after LT) and late-onset AMR (> 6 months). The biochemical findings of post-transplant hyperbilirubinaemia [20, 27] and transaminasaemia are more commonly observed as early features of acute AMR rather than the florid rapid allograft failure [9]. However, in the present study no significant interaction effect between DSA positivity and serum bilirubin ($p = 0.262$) or transaminases (ALT, $p = 0.051$; AST, $p = 0.700$) over a period of 100 days was noted.

An area of recent focus in liver transplantation has been the appreciation of the significant impact of biopsy-proven acute rejection (bpAR) on graft failure, all-cause mortality and graft failure-related death [28]. While it has been postulated that DSAs may contribute to an increased risk of graft failure, especially in patients undergoing deceased donor transplants (DDLT) compared with living donor liver transplants (LDLT) [29], we were

unable to address this aspect as all our patients underwent DDLTs. Another area worth investigating in the future is the influence of positive pre-OLT DSAs on graft survival in the setting of tapering of immunosuppression [28]—a practice aimed at reducing complications of immunosuppression in OLT patients.

On the balance, the data thus suggests that in the current clinical dilemma of shortage of donor allografts, DSA-positive donors do not appear to result in reduced patient survival or increased graft failure, and should therefore be considered routinely for donation in non-HLA-matched recipients.

The strengths of the present study include a consecutive cohort of patients in whom DSA expression data, available for all patients, was measured using one of the most reliable current assays. In addition, detailed follow-up data was available for all patients with a long median follow-up.

Unfortunately, the rarity of AMR means it is difficult to draw firm conclusions about the lack of correlation. It does demonstrate however that incorporating DSA measurement into the routine allocation algorithm in relatively low-volume centres is unlikely to be helpful for organ allocation. Larger cohorts would allow other factors to be examined such as whether marginal grafts impact the resistance of the liver to AMR. Additional retrospective data was required for this study as the importance of AMR was not considered when the prospective database was constructed and a longer follow-up period would be ideal. While Luminex is one of the most reliable assays for DSA today, the ideal assay and MFI cut-off for LT are not known.

Conclusions

AMR following LT is uncommon. A positive DSA pre-transplant does not imply a definite risk of AMR. Also, there does not exist a significant interaction in time between DSA expression and serum bilirubin or transaminase levels. Until there emerges evidence to the contrary, it appears reasonable to consider DSA-positive donors within the broad context of marginal donors in the context of a worldwide shortage of LT donor allografts.

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Author's contributions Conceptualisation and design: JWC, SGB. Data collection: SGB, TE. Data analysis: SGB, RL, RC. Drafting manuscript: SGB. Editing the manuscript for intellectual content: JWC, AW, MBS, ELN, PD, RC. Final approval of the manuscript: all authors

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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