ORIGINAL ARTICLE

Association between post-transplant donor-specific antibodies and recipient outcomes in simultaneous liver–kidney transplant recipients: single-center, cohort study

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SUMMARY

There is a dearth of published data regarding the presence of post-transplant donor-specific antibodies (DSA), especially C1q-binding DSA (C1q+DSA), and patient and kidney allograft outcomes in simultaneous liver–kidney transplant (SLKT) recipients. We conducted a retrospective cohort study consisted of 85 consecutive SLKT patients between 2009 and 2018 in our center. Associations between presence of post-transplant DSA, including persistent and/or newly developed DSA and C1q+DSA, and all-cause mortality and the composite outcome of mortality, allograft kidney loss, and antibody-mediated rejection were examined using unadjusted and age and sex-adjusted Cox proportional hazards and time-dependent regression models. The mean age at SLKT was 56 years and 60% of the patients were male. Twelve patients (14%) had post-transplant DSA and seven patients (8%) had C1q+DSA. The presence of post-transplant DSA was significantly associated with increased risk of mortality (unadjusted model: Hazard Ratio (HR) = 2.72, 95% confidence interval (CI): 1.06–6.98 and adjusted model: HR = 3.20, 95% CI: 1.11–9.22) and the composite outcome (unadjusted model: HR = 3.18, 95% CI: 1.31–7.68 and adjusted model: HR = 3.93, 95% CI: 1.39–11.10). There was also higher risk for outcomes in recipients with C1q+DSA compared the ones without C1q+DSA. Post-transplant DSA is significantly associated with worse patient and kidney allograft outcomes in SLKT. Further prospective and large cohort studies are warranted to better assess these associations.

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Key words
C1q binding donor-specific antibodies, *de novo* donor-specific antibodies, donor-specific antibodies, simultaneous liver–kidney transplantation

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Introduction

The indication for simultaneous liver–kidney transplantation (SLKT) is based on the need for liver transplantation (LT), as prioritized by the model for end-stage liver disease (MELD) score, which mainly evaluates the 3-month mortality of patients with end-stage liver disease (ESLD) [1,2]. Unlike kidney transplantation alone (KTA), pretransplant sensitized status is not a contraindication regardless of receipt of desensitization treatment before SLKT [3,4]. This decision is based on clinical observations made in LT and SLKT [5–9], and in several experimental studies, which suggests that the liver allograft would protect the kidney allograft from the same donor by absorbing and neutralizing alloantibodies [10–13]. These observations have supported current clinical practice and there are currently no clinical practice guidelines for evaluation of immunological risk in SLKT candidates [4]. Therefore, about 35% of all of SLKT procedures were performed in sensitized patients with no prophylactic measures [8,14].

On the other hand, unexpected rejection can happen after SLKT. Pretransplant sensitized status, especially a high quantity of preformed donor-specific antibody (DSA), might contribute to antibody-mediated rejection (ABMR) in SLKT. An earlier study suggested that class II DSAs are less likely to be absorbed by the allograft liver compared to class I DSAs [7]. High levels of pretransplant DSA, which may not be completely absorbed after SLKT (persistent DSA) could theoretically affect the kidney allograft. Furthermore, newly developed DSA after transplantation (*de novo* DSA), which could exceed the absorptive capacity of the liver allograft might also be facilitating ABMR. Only one cohort study has investigated the relationship between any pretransplant DSA or *de novo* DSA and outcomes, and showed that the presence of DSA, especially class II DSA, was associated with significantly higher risk of death, and liver allograft loss [8]. As far as we know, no clinical study has investigated the specific relationship between post-transplant DSA (persistent- and/or *de novo* DSA and class I and/or II DSA) and patient and also kidney allograft outcomes.

One of the most discussed topics in the KTA literature is the pathophysiological role played by different types of DSA in antibody-mediated rejection. Recent studies have shown that complement binding IgG (especially C1q binding) DSA (C1q positive DSA) may play an important role in allograft and patient survival in solid organ transplantation. The first evidence of a pathophysiological role of C1q-positive DSA came from pediatric heart transplantation [15]. Loupy *et al.* [16] showed that C1q-positive DSA (pretransplant and *de novo*) is a strong predictor for allograft loss and patient mortality in KTA recipients. In addition, *de novo* C1q-positive DSA was more strongly associated with increased risk of allograft loss compared to pretransplant or persistent C1q positive DSA [16], therefore C1q-positive DSA, especially *de novo* DSA, may be a potential new target of immunologic risk assessment in SLKT. However, no study examined whether the presence of C1q-positive DSA is associated with worse outcomes in SLKT.

The goal of this retrospective, single-center study was to assess the association of post-transplant (persistent or *de novo*) DSA and C1q-positive DSA with recipient outcomes in SLKT. Our hypothesis was that post-transplant DSA (persistent or *de novo* DSA) and C1q-positive DSA are associated with higher risk of mortality and composite outcome of death, allograft loss and antibody-mediated rejection in SLKT. In order to test this hypothesis, we conducted a cohort study in a relatively high-volume SLKT cohort in the MELD and modern immunosuppressant era.

Materials and methods

Cohort definition and data source

This single-center, retrospective cohort was comprised of 85 consecutive SLKT patients transplanted from April 1, 2009, to February 28, 2018, at Methodist University Hospital in Memphis, Tennessee, USA. No patients were excluded from this study even though exclusion criteria were established (those less than 18 years old and those who did not have outcome data). We created a control group that included adult patients who underwent first-time deceased donor kidney transplantation alone (KTA) in our center between January 1, 2014, and December 31, 2015 ($N = 197$).

We retrieved the information from the local electronic medical record (EMR) until February 9, 2019 for SLKT patient information, from the UNOS database for deceased donor information, and from the Transplant Immunology Laboratory, DCI Inc. HLA laboratory database for immunologic information. Detailed information regarding initiation of dialysis therapy was retrieved from the Medicare 2728 form. We captured all data into a Research Electronic Data Capture (REDCap) system, which is an electronic data capturing tool hosted at the Center for Biomedical Informatics, the University of Tennessee Health Science Center [17].

Immunosuppression protocol

The applied immunosuppression protocol was similar for all patients [18]. As induction therapy, all patients received intravenous methylprednisolone (500 mg) on day 0, and rabbit anti-thymocyte globulin (1.5 mg/kg) on day 0 and again on postoperative day (POD) 2. Mycophenolate mofetil (MMF) or equivalent mycophenolic acid was started immediately postoperatively and continued until month three. Tacrolimus was started after improvement in kidney function, usually between POD 3–7, and target trough range was 6–8 ng/ml until 3 months post-transplantation and 3–5 ng/ml thereafter. No patients received pretransplant desensitization therapy before SLKT. All patients were maintained on a steroid-free protocol.

All KTA recipients received rabbit anti-thymocyte globulin induction therapy with a planned cumulative dose of 4.5 mg/kg divided into three doses and a triple immunosuppressive regimen consisting of tacrolimus, MMF, and prednisone. Steroid remained on maintenance dose of prednisone 5 mg daily indefinitely.

Exposure

We assessed the presence and relative strength of post-transplant DSA including persistent and/or newly developed (*de novo*) DSA in each patient after SLKT and KTA. HLA specificities were identified using a solid-phase single antigen bead platform (SAB; One Lambda Inc, a division of Thermo-Fisher, Canoga Park, CA, USA) combined with Luminex xMAP technology (Luminex Corporation., Northbrook, IL, USA).

Patients with any observed class DSA were categorized as post-transplant DSA (+), while those negative for identified DSA were classified DSA (−). Persistent DSA was defined as DSA presence before and after SLKT, while *de novo* DSA was defined as newly developed DSA following SLKT. Additionally, we performed C1q analysis (One Lambda Inc, a division of Thermo-Fisher) to investigate the complement binding ability of detected DSA. The results were divided into positive post-transplant C1q-binding DSA (C1q+DSA) and negative post-transplant C1q-binding DSA (C1q−DSA). A mean fluorescence intensity (MFI) value of ≥2000 was used as a positive threshold for IgG DSA assignment pretransplant and an MFI threshold of 1000 was used to assign C1q+DSA. However, in the post-transplant setting, we defined post-DSA that has an MFI that is elevated compared to pre-transplant levels or has an MFI of greater than 1000.

Covariates

We retrieved data about recipients' baseline characteristics including age, sex, race (African-American, Caucasian, and other), body mass index (BMI), marital status, insurance (Medicare, Medicaid, and private), cause of ESLD (hepatitis C (HCV), alcoholic hepatitis, HCV and alcoholic hepatitis, nonalcoholic steatohepatitis (NASH), and other), cause of chronic kidney disease (CKD)/end-stage renal disease (ESRD) (hypertension, diabetes, glomerular nephritis, cystic disease, metabolic/inherited disease, and other), pre-SLKT dialysis information including length and type (maintenance dialysis was defined as dialysis for ≥3 months; acute dialysis initiation was defined as dialysis for <6 weeks; while subacute dialysis was defined as dialysis for ≥6 weeks to <3 months before transplantation), comorbid conditions, the MELD score at SLKT, the number of HLA mismatches, calculated panel reactive antibody (cPRA), and cold-ischemic time (CIT) of donated kidney from the abovementioned sources. Delayed graft function (DGF) was defined as need for at least one dialysis session within a week after SLKT. Deceased donor information included age, sex, race, cause of death, history of hypertension and diabetes, and expanded criteria donor (ECD) status. All donations occurred after brain death. Same covariates have been captured in the KTA cohort.

Outcomes

The primary endpoint was time to all-cause death. The date of death was based on declaration to UNOS. The secondary endpoint was the composite of time to death or kidney allograft loss or antibody-mediated rejections (ABMR). The tertiary endpoint was the kidney allograft outcomes such as allograft loss, ABMR, or the combination of these as composite kidney outcome (allograft loss and/or ABMR). Kidney allograft loss was defined as requirement for renal replacement therapy (re-kidney transplantation or return to dialysis). ABMR was diagnosed by indication biopsy and/or any treatment for ABMR including plasmapheresis, intravenous immunoglobulin (IVIG), and rituximab. Two investigators (MY and OC) who are familiar with kidney transplantation carried out EMR reviews for information about historic biopsy findings, which were diagnosed by pathologists based on Banff criteria at the time of diagnoses (from 2009 to 2017) [19–23]. The cause of kidney allograft loss was extracted from the EMR.

Statistical analysis

Baseline characteristics were described for the entire cohort and for groups categorized based on the presence or absence of post-transplant DSA and the presence or absence of post-transplant C1q DSA, and presented as mean ± standard deviation (SD) or median and interquartile range (IQR) for continuous variables and percent for categorical variables. Differences between groups were assessed by the Student *t*-test or Mann–Whitney test for continuous variables and chi-square-test (or Fisher's exact test) for categorical variables.

The start of the follow-up period was the date of SLKT, and patients were followed up until the date of respective endpoints (death or composite outcome event), or other censoring events including loss to follow-up or the end of the follow-up period (February 9, 2019). For the KTA cohort, the start and end of follow-up period were the date of KTA and July 25, 2019, respectively.

We used the Kaplan–Meier method and Cox proportional hazards regressions as well as time-dependent Cox regression model. We used log-rank tests for statistical comparisons. We tested proportional hazards assumptions using scaled Schoenfeld residuals. All covariates were tested for multi-collinearity; the highest variance inflation factor (VIF) was 1.02 (mean VIF = 1.01).

We examined the association in the entire cohort using unadjusted and multivariable-adjusted models. In our multivariable-adjusted models, we only adjusted for age and sex as we had only 22 events for mortality and 24 events for the composite outcome. However, we only had few events for kidney allograft outcomes, described using Kaplan–Meier curve and log-rank test. We also performed interaction analysis to assess whether type of transplantation (SLKT versus KTA) is effect modifier using the merged SLKT and KTA cohort. *P* values were two-sided and significance level was set at less than 0.05 for all analyses. All analyses were conducted using STATA version 13 (STATA Corporation, College Station, TX, USA). This study was approved by the Institutional Review Committee of The University of Tennessee Health Science Center (18-06146-XP).

Results

Baseline characteristics

Twelve patients (14.1%) had any class post-transplant DSA after SLKT. Six patients had persistent DSA and eight patients developed *de novo* DSA (four patients with persistent DSA only, six patients *de novo* DSA only, and two patients with both persistent and *de novo* DSA). The median time to any post-transplant DSA measurement after SLKT was 22 days (IQR: 8–281) in 12 patients and the median time to *de novo* DSA measurement after SLKT was 53.0 (14.0–280.5) in eight patients who had *de novo* DSA. A total of seven patients (8.2%) were identified with any class post-transplant C1q+DSA (one patient had persistent C1q+DSA; six patients developed *de novo* C1q+DSA). No patients were identified with both persistent and *de novo* C1q+DSA (Fig. 1).

As shown in Table 1, the mean age at SLKT was 56 ± 10 years, 62% were male, and 26% were African-American. Most patients had chronic kidney disease (CKD) before SLKT. The two major causes of ESLD were HCV (28%) and alcoholic hepatitis (26%) and the

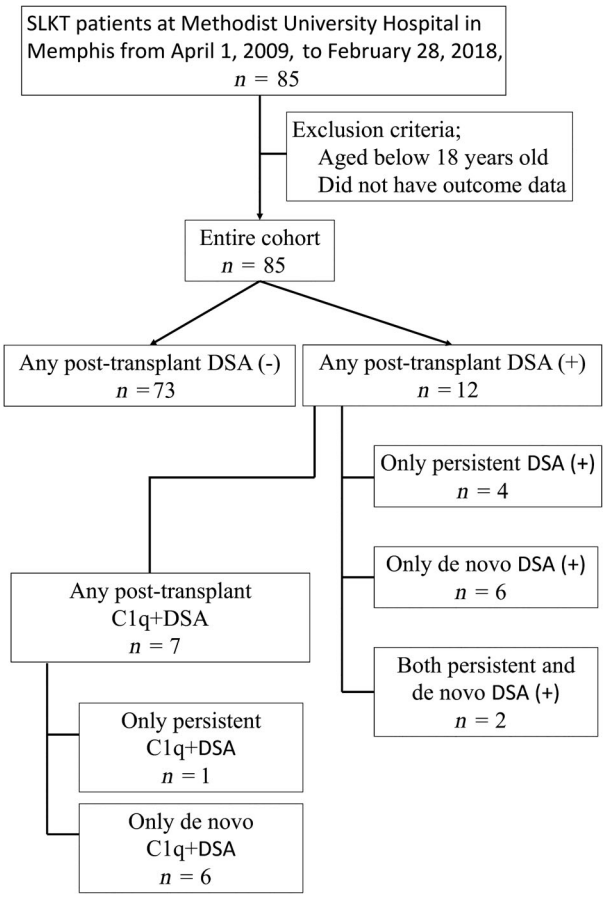

Figure 1 Flowchart of patient selection. C1q+DSA, positive post-transplant C1q binding DSA; C1q−DSA, negative post-transplant C1q binding DSA; *de novo* DSA, newly developed DSA; DSA, donor-specific antibody; *N*, number; SLKT, simultaneous liver–kidney transplantation.

Table 1. Baseline characteristics of the entire cohort and comparison between with and without of any post-transplant DSA.

Baseline characteristics	Entire cohort *N* = 85	Post-transplant DSA (+) group *N* = 12	Post-transplant DSA (−) group *N* = 73	*P* value*
Recipient information				
Age, years, mean ± SD	55.5 ± 10.1	47.3 ± 14.0	56.9 ± 8.7	0.002
Sex, male, *n* (%)	53 (62.4)	5 (41.7)	48 (65.8)	0.110
BMI, kg/m^2, mean ± SD	28.4 ± 6.4	28.7 ± 5.5	28.3 ± 6.6	0.862
Race, *n* (%)				
African-American	22 (25.9)	7 (58.3)	15 (20.5)	0.029
Caucasian	51 (60.0)	3 (25.0)	48 (65.8)	
Other	12 (14.1)	2 (16.7)	10 (13.7)	
Marital status, married, *n* (%)	52 (61.2)	3 (25.0)	49 (67.1)	0.018
Insurance, *n* (%)				
Private	31 (36.5)	5 (41.7)	26 (35.6)	0.290
Medicaid	6 (7.1)	2 (16.7)	4 (5.5)	
Medicare	48 (56.5)	5 (41.7)	43 (58.9)	
Presence of pre-existing CKD, *n* (%)	74 (87.1)	9 (75.0)	65 (89.0)	0.179
Cause of CKD, *n* (%)				
Hypertension	8/74 (10.8)	1/9 (11.1)	7/65 (10.8)	0.182
Diabetes	14/74 (18.9)	0	14/65 (21.5)	
Glomerular nephritis	4/74 (5.4)	2/9 (22.2)	2/65 (3.1)	
Cystic disease	3/74 (4.1)	1/9 (11.1)	2/65 (3.1)	
Metabolic/inherited disease	2/74 (2.7)	0	2/65 (3.1)	
Other/unknown	43/74 (58.1)	5/9 (55.6)	38/65 (58.5)	
Dialysis status before SLKT, *n* (%)				
Maintenance dialysis, *n* (%)	38 (44.7)	6 (50.0)	32 (43.8)	0.337
Sub-acute dialysis, *n* (%)	9 (10.6)	1 (8.3)	8 (11.0)	
Acute dialysis initiation before SLKT, *n* (%)	16 (18.8)	4 (33.3)	12 (16.4)	
Length of dialysis before SLKT (maintenance and sub-acute dialysis group), months, median (IQR)	8.9 (3.9, 36.8)	93.4 (5.0, 142.3)	8.8 (3.7, 20.9)	0.100
Length of acute dialysis before SLKT (acute dialysis group only), days, median (IQR)	13.5 (6.5, 24.5)	10.5 (9.0, 22.5)	17.0 (4.0, 24.5)	1.0
Cause of ESKD (maintenance and sub-acute group), *n* (%)				
Acute on CKD	12/47 (25.5)	1/7 (14.3)	11/40 (27.5)	0.819
Same as CKD	35/47 (74.5)	6/7 (85.7)	29/40 (72.5)	
Cause of ESLD, *n* (%)				
HCV	24 (28.2)	2 (16.7)	22 (30.1)	0.533
Alcoholic hepatitis	22 (25.9)	3 (25.0)	19 (26.0)	
HCV and alcoholic hepatitis	3 (3.5)	0	3 (4.1)	
NASH	16 (18.8)	2 (16.7)	14 (19.2)	
Other	20 (23.5)	5 (41.7)	15 (20.5)	
Comorbidity—diabetes, *n* (%)	35 (41.2)	5 (41.7)	30 (41.1)	0.970
Comorbidity—hypertension, *n* (%)	61 (71.8)	10 (83.3)	51 (69.9)	0.337
HLA mismatches locus A, *n*, mean ± SD	1.6 ± 0.6	1.8 ± 0.5	1.5 ± 0.6	0.191
HLA mismatches locus B, *n*, mean ± SD	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	0.642
HLA mismatches locus DR, *n*, mean ± SD	1.5 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	0.673
Total HLA mismatches, *n*, mean ± SD	4.8 ± 1.0	4.9 ± 1.1	4.8 ± 1.0	0.729
cPRA, %, median (IQR)	0 (0, 8)	10 (0, 86)	0 (0, 2)	0.081
Cold-ischemic time of donated kidney, minutes, mean ± SD	496.5 ± 114.6	519.0 ± 121.2	492.2 ± 113.9	0.465
MELD score, mean ± SD	28.3 ± 6.5	29.5 ± 6.8	28.1 ± 6.4	0.501

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Table 1. Continued.

Baseline characteristics	Entire cohort *N* = 85	Post-transplant DSA (+) group *N* = 12	Post-transplant DSA (−) group *N* = 73	*P* value*
Length of stay for SLKT admission, days, median (IQR)	15.0 (9.0, 30.0)	27.0 (23.0, 116.0)	13.0 (8.0, 28.0)	0.002
Delayed graft function (kidney), *n* (%)	15 (17.6)	3 (25.0)	12 (16.4)	0.471
Donor information				
Age, years, mean ± SD	28.9 ± 11.1	29.6 ± 13.0	28.8 ± 10.8	0.824
Gender, male, *n* (%)	47 (55.3)	8 (66.6)	39 (53.4)	0.393
Donor race, *n* (%)				
Caucasian	65 (76.5)	10 (83.3)	55 (75.3)	0.760
African-American	18 (21.2)	2 (16.7)	16 (21.9)	
Hispanic	2 (2.4)	0	2 (2.7)	
Donation after brain death, *n* (%)	85 (100%)	12 (100)	73 (100)	1.0
Cause of death, *n* (%)				
Anoxia	27 (31.8)	6 (50.0)	21 (28.8)	0.525
Cerebrovascular/stroke	18 (21.2)	3 (25.0)	15 (20.5)	
Head trauma	33 (38.8)	3 (25.0)	30 (41.1)	
Central nerve system tumor	1 (1.2)	0	1 (1.4)	
Other	5 (5.9)	0	5 (6.8)	
Comorbidity—diabetes, *n* (%)	1 (1.2)	0	1 (1.4)	0.683
Comorbidity—hypertension, *n* (%)	11 (12.9)	2 (16.7)	9 (12.3)	0.678
Expanded criteria donor, *n* (%)	1 (1.2)	0	1 (1.4)	0.683

BMI, body mass index; CKD, chronic kidney disease; cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; ESKD, end-stage kidney disease; ESLD, end-stage liver disease; HCV, hepatitis C; HLA, human leukocyte antigen; IQR, interquartile range; MELD, model of end-stage liver disease; NASH, nonalcoholic steatohepatitis; SLKT, simultaneous liver–kidney transplantation.

*Compared between post-transplant DSA (−) and (+) groups. *P* values for continuous variables with mean ± SD are result of *t*-test and with median (IQR) are result of Mann–Whitney test, and categorical variables are chi-square test.

mean MELD score at SLKT was 28. In terms of immunological risk, the mean number of HLA mismatches was 4.8 ± 1.0, median cPRA was 0% (IQR: 0–8), and mean CIT of kidney was approximately 8.3 h. The prevalence of dialysis therapy before SLKT was 74% (63/85), and the median length of dialysis therapy in those with maintenance or sub-acute dialysis was 8.9 months. Acute dialysis initiation <6 weeks before SLKT occurred in 19% and the median length of acute dialysis therapy was 13.5 days.

The post-transplant DSA (+) group was significantly younger and had a twofold higher length of hospital stay after SLKT, more likely to be African-American, have longer dialysis duration in maintenance and sub-acute dialysis, and also have higher cPRA compared to the post-transplant DSA (−) group. Donor characteristics were similar between the two groups (Table 1). Table S1 shows the baseline characteristics stratified by post-transplant C1q+DSA status. The post-transplant C1q+DSA group was significantly younger, but the other variables were similar between the two groups. Baseline characteristics of KTA are shown in Table S2.

Detailed information of post-transplant DSA and C1q+DSA

Table 2 shows the detailed information of pre- and post-transplant DSA including C1q+DSA. Seven patients had only class I DSA and five patients had both class I and II DSA (totally 12 patients with class I DSA) before SLKT. Of these, only one patient (8.3%) with class I DSA persisted after SLKT. In terms of class II DSA before SLKT, we had the same proportion with class I DSA, but five out of 12 patients (41.7%) persisted their class II DSA after SLKT. Only one out of 12 recipients with post-transplant DSA had class I DSA, the others had only class II DSA (8/12) or both classes I and II DSA (3/12). We found persistent or *de novo* C1q+DSA in 7 out of 85 patients (8.2%). Of these, six patients (85.7%) developed *de novo* C1q+DSA and most of these (5/6) had only class

Table 2. All information about classes and antigenes of DSA according to each persistent- and de novo DSA including C1q+DSA as well as pretransplant DSA.

Patient	Pretransplant DSA (+)	Pretransplant C1q+DSA	Post-transplant persistent DSA (+)	Post-transplant persistent C1q+DSA	Post-transplant *de novo* DSA (+)	Post-transplant *de novo* C1q+DSA
1	Both Class I (B) and II (DQ, DR)					
2					Only Class II (DQ)	Only Class II (DQ)
3	Both Class I (A, B) and II (DR)					
4	Only Class I (B)					
5	Only Class II (DQ, DR)		Only Class II (DQ, DR)		Only Class I (B)	Only Class II (DQ, DR)
6	Both Class I (A, B) and II (DR)					
7					Only Class II (DR)	Only Class II (DR)
8	Only Class I (A)		Only Class I (A)			
9	Only Class I (B, C)	Only Class I (B, C)			Only Class II (DQ)	
10	Only Class II (DR)					
11	Only Class II (DQ)					
12	Only Class II (DP, DQ, DR)	Only Class II (DP, DQ, DR)				
13					Both Class I (B, C) and II (DQ, DR)	Only Class II (DQ)
14	Only Class I (B)					
15	Only Class I (A)					
16	Only Class I (A, B)					
17	Only Class II (DR)		Only Class II (DR)		Both Class I (B) and II (DR)	Both Class I (B) and II (DR)
18					Only Class II (DQ, DR)	Only Class II (DQ, DR)
19	Both Class I (A, B, C) and II (DQ, DR)	Only Class II (DQ, DR)	Only Class II (DQ, DR)	Only Class II (DR)		
20	Only Class I (B)				Only Class II (DQ)	
21	Both Class I (A, B) and II (DQ)	Only Class II (DQ)				
22	Only Class II (DQ, DR)		Only Class II (DQ)			
23	Only Class II (DQ, DR)		Only Class II (DQ, DR)			

C1q+DSA, positive post-transplant C1q binding DSA; C1q−DSA, negative post-transplant C1q binding DSA; DSA, donor-specific antibody.

II C1q+DSA, and the remaining one had both *de novo* class I and II C1q+DSA (Table 2).

Association between presence of post-transplant DSA and all-cause mortality

The median follow-up time was 32.0 (12.1–54.8) months. The incidence of all-cause mortality was 22 (83/1000 person-years, 95% CI: 55–126). The leading causes of death were infection (50%), followed by others (32%), cardiovascular death (9%), malignancy (4.6%), and liver failure (4.6%). The incidence rate was 204/1000 person-years (95% CI: 92–454) in the post-transplant DSA (+) group and 68/1000 person-years (95% CI: 42–111) in the DSA (−) group (Fig. 2a, log-rank test $P = 0.030$). The post-transplant DSA (+) group had significant higher risk of death in the unadjusted (HR = 2.72, 95% CI: 1.06–6.98) and in the age- and gender-adjusted model (HR = 3.20, 95% CI: 1.11–9.22; Table 3) compared to the post-transplant DSA (−) group. Qualitative similar result was found using time-dependent Cox regression model (Table 3).

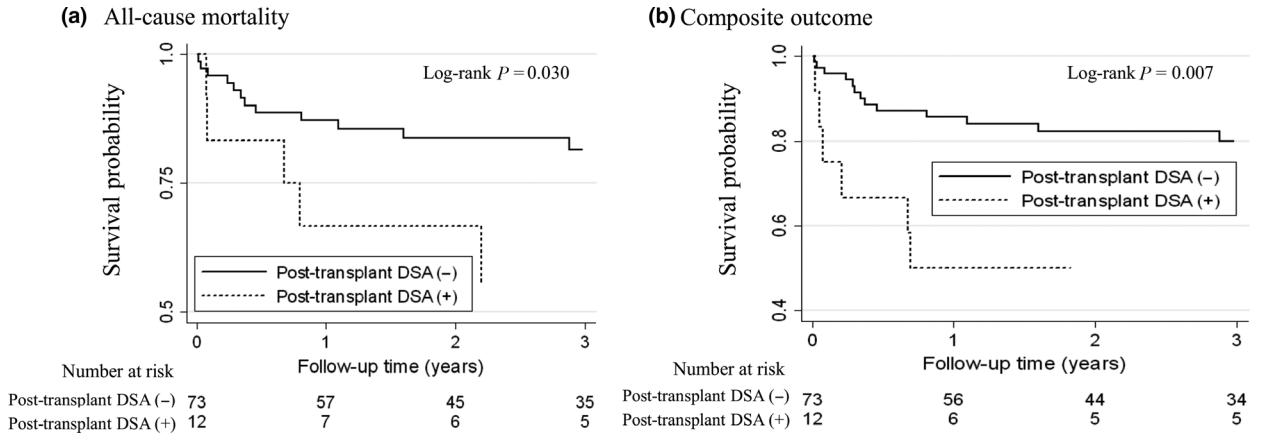

Figure 2 Probability of all-cause mortality (panel a) and composite outcome (panel b) of simultaneous liver–kidney transplant recipients with and without post-transplant DSA. Composite outcome consisted of death, allograft kidney loss, and antibody-mediated rejection. DSA, donor-specific antibody.

Table 3. Association between presence of any post-transplant DSA and all-cause mortality and composite outcome using univariate and adjusted Cox proportional model and time-dependent Cox model.

	All-cause mortality			Composite outcome		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Cox proportional model						
Univariate analysis						
Post-transplant DSA (+) [versus DSA (–)]	2.72	1.06–6.98	0.037	3.18	1.31–7.68	0.010
Multivariate analysis						
Post-transplant DSA (+) [versus DSA (–)]	3.20	1.11–9.22	0.031	3.93	1.39–11.10	0.010
Age (each 1 year)	1.01	0.96–1.06	0.696	1.01	0.97–1.05	0.776
Female gender (versus male gender)	0.79	0.31–1.99	0.612	0.71	0.28–1.80	0.474
Time-dependent Cox model						
Univariate analysis						
Post-transplant DSA (+) [versus DSA (–)]	4.92	1.90–12.75	0.001	5.44	2.24–13.21	<0.001
Multivariate analysis						
Post-transplant DSA (+) [versus DSA (–)]	5.15	1.92–13.77	0.001	5.95	2.30–15.39	<0.001
Age (each 1 year)	1.00	0.96–1.05	0.921	1.00	0.96–1.04	0.870
Female gender (versus male gender)	0.85	0.35–2.09	0.714	0.75	0.31–1.81	0.526

95% CI, 95% confidence interval; DSA, donor-specific antibody; HR, hazard ratio.

Composite outcome consisted of death, allograft kidney loss, and antibody-mediated rejection.

Association between presence of post-transplant DSA and the composite outcome

The median follow-up time was 31.5 (9.7–54.4) months and the composite outcome occurred in 24 patients (incidence rate: 93 cases/1000 person-year, 95% CI: 63–139). The incidence rate was 270/1000 person-years (95% CI: 129–565) in the post-transplant DSA (+) group and 74/1000 person-years (95% CI: 46–118) in the DSA (–) group (Fig. 2b, Log-rank test $P = 0.007$). The post-transplant DSA (+) group had significant higher risk of the composite outcome in the unadjusted (HR = 3.18, 95% CI: 1.31–7.68) and in the age- and gender-adjusted model (HR = 3.93, 95% CI: 1.39–11.10) (Table 3) compared to post-transplant DSA (–) group. Qualitative similar result was found using time-dependent Cox regression model (Table 3).

Association between presence of post-transplant DSA and the kidney allograft outcomes

There were only four kidney allograft losses, five ABMRs, and seven composite kidney outcome events. The probability rate of all of the kidney allograft

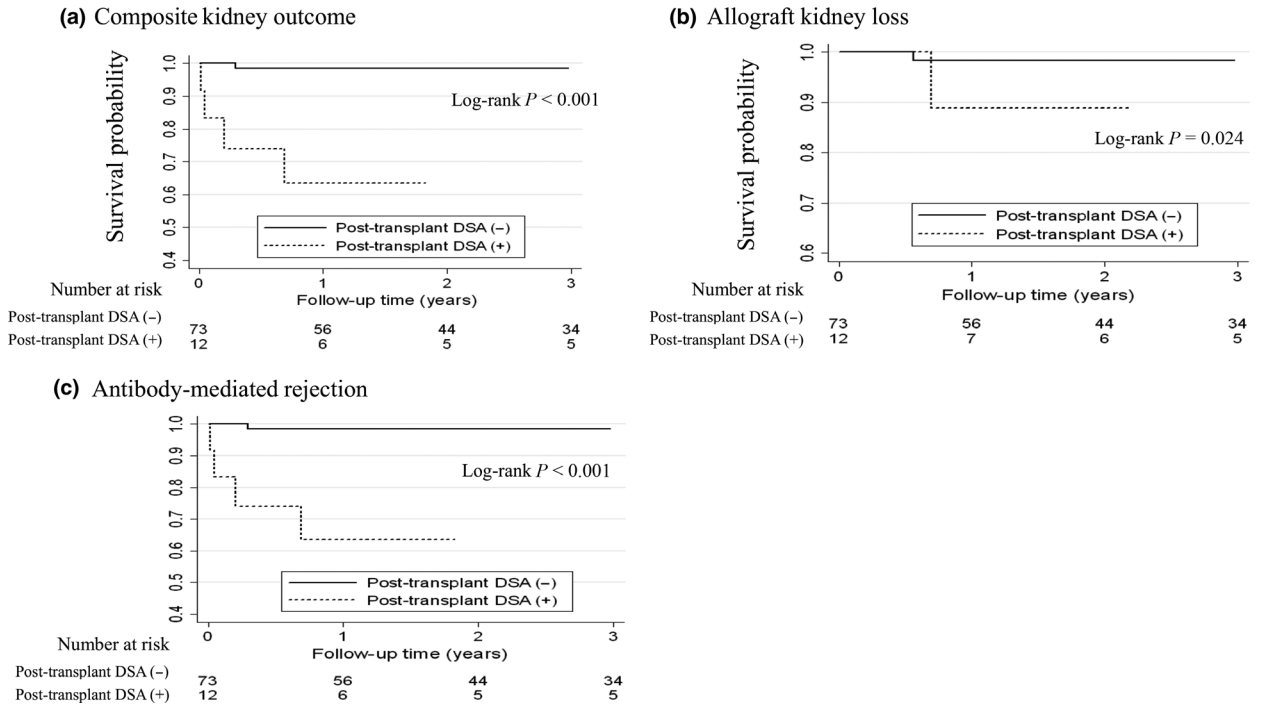

Figure 3 Probability of composite kidney outcome (panel a), allograft kidney loss (panel b), and antibody-mediated rejection (panel c) of simultaneous liver–kidney transplant recipients with and without post-transplant DSA. Composite kidney outcome consisted of allograft kidney loss and antibody-mediated rejection. DSA, donor-specific antibody.

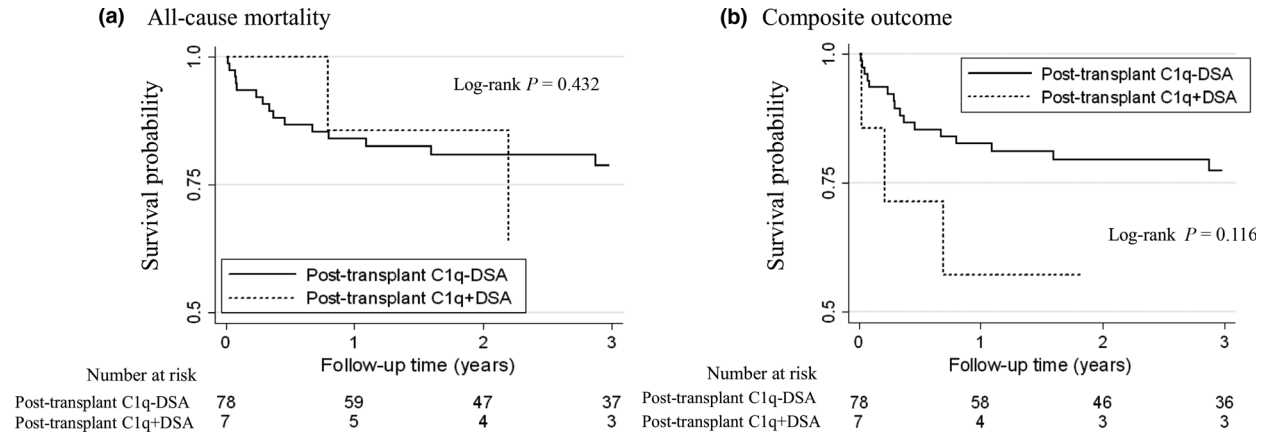

Figure 4 Probability of all-cause mortality (panel a) and composite outcome (panel b) of simultaneous liver–kidney transplant recipients with and without post-transplant C1q DSA. Composite outcome consisted of death, allograft kidney loss, and antibody-mediated rejection. C1q+DSA, positive post-transplant C1q binding DSA; C1q−DSA, negative post-transplant C1q binding DSA; DSA, donor-specific antibody.

outcomes was significantly higher in the post-transplant DSA (+) group than in the post-transplant DSA (−) group as shown in the Kaplan–Meier curves (Fig. 3).

Association between positive post-transplant C1q binding DSA and all-cause mortality

The all-cause mortality rate was 138/1000 person-years (95% CI: 45–429) in the post-transplant C1q+DSA group and 78/1000 person-years (95% CI: 50–122) in the C1q−DSA group (Fig. 4a, log-rank test $P = 0.432$). The post-transplant C1q+DSA group showed a similar risk for all-cause mortality in the unadjusted (HR = 1.62, 95% CI: 0.48–5.49) and in the age- and gender-adjusted model (HR = 1.67, 95% CI: 0.43–6.45; Table 4) as the post-transplant C1q−DSA group using Cox proportional regression model. However, the post-transplant C1q+DSA

Table 4. Association between positive post-transplant C1q binding DSA and all-cause mortality and composite outcome using univariate and adjusted Cox proportional model and time-dependent Cox model.

	All-cause mortality			Composite outcome		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Cox proportional model						
Univariate analysis						
Post-transplant C1q+DSA (versus C1q−DSA)	1.62	0.48–5.49	0.437	2.31	0.79–6.79	0.127
Multivariate analysis						
Post-transplant C1q+DSA (versus C1q−DSA)	1.67	0.43–6.45	0.457	2.61	0.70–9.75	0.155
Age (each 1 year)	1.00	0.95–1.04	0.957	1.00	0.96–1.04	0.911
Female gender (versus male gender)	0.92	0.36–2.34	0.864	0.81	0.31–2.21	0.663
Time-dependent Cox model						
Univariate analysis						
Post-transplant C1q+DSA (versus C1q−DSA)	3.50	1.02–11.95	0.046	4.68	1.57–13.94	0.006
Multivariate analysis						
Post-transplant C1q+DSA (versus C1q−DSA)	3.62	1.03–12.76	0.045	5.63	1.65–19.21	0.006
Age (each 1 year)	0.99	0.95–1.04	0.804	0.99	0.95–1.03	0.525
Female gender (versus male gender)	0.89	0.36–2.19	0.803	0.73	0.29–1.88	0.519

95% CI, 95% confidence interval; C1q+DSA, positive post-transplant C1q binding DSA; C1q−DSA, negative post-transplant C1q binding DSA; DSA, donor-specific antibody; HR, hazard ratio.

Composite outcome consisted of death, allograft kidney loss, and antibody-mediated rejection.

group showed higher risk for all-cause mortality in the unadjusted (HR = 3.50, 95% CI: 1.02–11.95) and in the age- and gender-adjusted model (HR = 3.62, 95% CI: 1.03–12.76; Table 4) as the post-transplant C1q−DSA group using time-dependent Cox regression model.

Association between positive post-transplant C1q binding DSA and the composite outcome

The incidence rate of the composite outcome was 219/1000 person-years (95% CI: 82–583) in the post-transplant C1q+DSA group and 84/1000 person-years (95% CI: 54–130) in the C1q-DSA group (Fig. 4b, log-rank test $P = 0.116$). Although there was a trend toward higher risk, the post-transplant C1q+DSA group did not show significantly increased risk for the composite outcome in the unadjusted (HR = 2.31, 95% CI: 0.79–6.79) and in the age- and gender-adjusted model (HR = 2.61, 95% CI: 0.70–9.75; Table 4) compared to the post-transplant C1q−DSA group using Cox proportional regression model. Moreover, the post-transplant C1q+DSA group showed higher risk for composite outcome in the unadjusted (HR = 4.68, 95% CI: 1.57–13.94) and in the age- and gender-adjusted model (HR = 5.63, 95% CI: 1.65–19.21; Table 4) as the post-transplant C1q−DSA group using time-dependent Cox regression model.

Kidney transplantation alone cohort

Table S2 shows baseline characteristics of this cohort. Twenty-four out of 197 KTA recipients (12.2%) had post-transplant DSA. Of those, 21 recipients newly developed *de novo* DSA (10.7%) after KTA. Given the low incidence of kidney allograft outcomes, these were only analyzed by Cox regression analysis adjusted for age and gender. Figure S1, Tables S3 and S4 show the association between post-transplant DSA and outcomes in the KTA cohort.

Merged SLKT and KTA cohort

We also repeated our analysis in a merged SLKT and KTA cohort as shown in Table S5 and found similar result. We also examined whether the type of transplantation (SLKT versus KTA) has any effect modification in the merged cohort to perform direct comparison of KTA and SLKT. The p value of the interaction terms are as follows: mortality-unadjusted model: $P = 0.108$; mortality-adjusted model: $P = 0.113$; composite outcome-unadjusted model: $P = 0.971$; and composite outcome-adjusted model: $P = 0.863$. This interaction analysis indicates KTA vs SLKT is not an effect modifier.

Discussion

In this single-center, retrospective cohort study the presence of post-transplant DSA (persistent and/or *de novo*) was significantly associated with increased risk of all-cause mortality and worse kidney allograft outcomes. There was also higher risk for outcomes in recipients with C1q+DSA compared the ones without C1q+DSA. As far as we know, this is the first report using a relatively high-volume cohort study to investigate the association between only post-transplant DSA including C1q+DSA and outcomes among SLKT patients in the MELD- and modern immunosuppressant regimen era. These results might have an impact on patient care and monitoring strategies with regard to immunological risk in postoperative SLKT patients.

Previous cohort studies investigating the relationship between immunological risk and outcomes in SLKT have focused on pretransplant sensitized status with no methodological consistency regarding the measurement of sensitized status [8,14,24,25]. Furthermore, results have been controversial regarding the risk of pretransplant sensitized status on allograft kidney outcomes. Some studies used complement-dependent cytotoxicity test (CDC) or flow-cytometric cross-match test [24,25], while others used PRA as a measurement method of sensitized status [14]. All of these immunological risk assessment methods have a severe limitation, namely they are unable to assess donor-specific antibodies, which serve as the basis of immunological risk assessment in the modern era. Currently, we routinely have information not only about DSA but also the class and intensity of HLA antibodies from commercially available solid-phase assays. Our study used only post-transplant DSA measured by solid-phase assay as exposure. However, we could not perform separate analyses for each class I and II DSA since most post-transplant DSA were categorized as class II DSA. This is likely due to the physiologic effect of the liver allograft, which is more likely to absorb class I DSA than class II DSA [10,26]. Examining the effect of class I DSA in postoperative SLKT, patients would require much larger cohort studies.

The timing of DSA measurement in SLKT may also be important. As the liver graft can absorb DSAs, pretransplant DSA may not portend significant physiological risk for the risk of long-term outcomes [8,14,24,25]. In fact, only one-third (6 out of 19 patients, 32%) of the recipients with pretransplant DSA had persistent DSAs after SLKT, while in two-thirds of the recipients the pretransplant DSA disappeared (Table 2). Therefore, the results of prior studies using only pretransplant DSA status may have underestimated the impact of DSA on long-term outcomes [8,14,24,25].

Simultaneous liver–kidney transplant is carried out based on medical urgency depending on the MELD score and only ABO blood type compatibility, regardless of the pretransplant immunological risk. This clinical practice has been based on the absorptive ability of the liver allograft, called the "protective phenomenon" [5–8]. This "protective phenomenon" would be definitely complete, if the liver allograft could absorb all pretransplant DSA and no *de novo* DSA would be formed. However, our results suggest that DSA are not completely absorbed and *de novo* DSA formation is also possible, indicating that providers should reassess the immunological risk by measuring DSAs after SLKT.

The incidence of *de novo* C1q+DSA in our cohort is comparable with that of *de novo* C1q+DSA in KTA (7.6%) published by Loupy *et al.* [16]. There are several interesting implications of these observations; (i) although the liver allograft could absorb alloantibodies, the incidence of developing *de novo* C1q+DSA was similar to KTA, which does not have an absorptive pool, and a nearly 10% incidence should not be ignored in the clinical setting, (ii) most of the post-transplant C1q+DSA were *de novo* DSA; of these, most were class II DSA. Our study shows higher risk of all-cause mortality and composite outcome in recipients with C1q binding DSA. According to the unfavorable results described in the KTA literature [16,27,28] and for other solid organ [29], the circulating C1q+DSA can theoretically cause kidney allograft injury. Hence, C1q+DSA, especially its post-transplant form, might be a new target of immunologic risk assessment in SLKT.

Length of hospital stay in the post-transplant DSA (+) group was significantly longer than in the DSA (−) group and infection was the leading cause of death in this cohort. Longer hospital stay is associated with higher rates of infection [30], more blood transfusions [31], and early allograft liver dysfunction [32] which all can cause the development of *de novo* DSA by sensitization or reduced immunosuppressive medications. Another potential explanation is that the development of *de novo* DSA might have therapeutic consequences such as liver and kidney graft rejections, early liver allograft dysfunction, delayed allograft (liver/kidney) function, which all result in intensification of immunosuppressive treatments, which might lead to more complications such as infections and eventually death. In other words, the development of *de novo* DSA might be a mediating factor between clinical complications and outcomes, not a

proximal cause of poorer outcomes. Unfortunately, our current sample size and available data are insufficient to conclusively answer these questions. Further prospective studies with more detailed data collection are needed to clarify this question.

Our study has several limitations. First, this study was conducted in a single-center and in a relatively small number of patients. However, the incidence of *de novo* DSA (14%) and baseline characteristics in our cohort were comparable to previous cohort studies [8,14,25]. Thus, our study might be generalizable. Second, our study was a retrospective cohort study, therefore we cannot conclude a causal relationship between post-transplant DSA (+)/C1q+DSA and outcomes. We can only conclude that there is an association between post-transplant DSA (+) and outcomes. Third, we could adjust for only two confounders in our multivariable analysis because of the low number of events. Potential confounders, such as delayed graft function, cold-ischemic time, adherence, and socio-economic status could not be taken into account in this analysis. Fourth, biopsies and DSA measurements were performed according to clinical indication, which may have resulted in confounding by indication and a potential overestimation of the risk associated with post-transplant DSA (+). Fifth, we could not determine the exact dates when *de novo* DSA developed after SLKT, therefore the date of cohort entry was uniformly set at the time of SLKT, which may have led to the underestimation of the risk associated with *de novo* DSA (+) in time to event analyses (immortal time bias) [33]. The landmark analysis, introduced by Gleiss *et al.* [33], could address the immortal time problem, but in our cohort the observation and event numbers were too low to apply this approach [33]. However, we performed time-dependent survival analysis to address this limitation. Finally, although we performed the same analysis for KTA cohort as a control group and the result of composite outcome was comparable with SLKT recipients, it is important to notice KTA and SLKT recipients had quite different characteristics regarding comorbidities and immunosuppression protocol. Prospective studies using protocol biopsies and protocol-led measurement of post-transplant DSA may be warranted in order to solve this problem.

Strengths of our study include the assessment of the association between C1q+DSA and outcomes in SLKT and the description of their prevalence before SLKT and incidence after SLKT. Moreover, we could collect covariates, exposures, and outcomes reliably due to complete access to patient records. In order to clarify the association between only post-transplant DSA and outcomes, we completely distinguished post-transplant DSA as exposure from any DSA throughout SLKT period.

In conclusion, the presence of post-transplant DSA, which develop beyond the absorptive capacity of the liver allograft, was significantly associated with patient and kidney allograft outcomes. The presence of post-transplant DSA should not be ignored in routine patient care after SLKT even though pretransplant sensitized status is usually neglected at the time of SLKT. Further prospective and large cohort studies including protocol biopsies and routine measurement of post-transplant DSA are warranted to better assess the association between post-transplant DSA and outcomes.

Authorship

All authors contributed to conception and design of the study and approved the final version of the manuscript. MY, OC, PSBP, and MZM: collected the clinical data. SF: examined all donor-specific antibodies. MY and MZM: performed the analysis and contributed to interpretation of results. MY, MZM, and UAA: wrote the manuscript. MZM: takes responsibility for the data and analysis accuracy and all other aspect of the work.

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Conflict of interest

The authors have declared no conflicts of interest.

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The results of this paper have not been published previously in whole or part.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Probability of the composite kidney outcome (panel a), graft loss (panel b), and antibody-mediated rejection (panel c) in kidney transplantation alone recipients with and without post-transplant DSA.

Table S1. Baseline characteristics of the entire cohort and comparison between with and without of post-transplant C1q DSA.

Table S2. Baseline characteristics of the cohort of kidney transplantation alone, entire cohort of SLKT and comparison between with and without of any post-transplant DSA in SLKT.

Table S3. Association between presence of any post-transplant DSA and all-cause mortality and composite outcome using univariate and adjusted Cox proportional model in cohort of kidney transplantation alone (N = 197).

Table S4. The incidence numbers and rate of composite kidney outcome, antibody-mediated rejection, and graft loss in the cohort of kidney transplantation alone (N = 197).

Table S5. Association between presence of any post-transplant DSA and all-cause mortality and composite outcome using univariate and adjusted Cox proportional model in kidney transplant alone and simultaneous liver-kidney transplantation recipients (n = 282).

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