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Prevalence and Clinical Impact of Donor-Specific Alloantibody Among Intestinal Transplant Recipients

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Background. Rejection remains the leading cause of allograft loss, and a major barrier to improving long-term outcomes after intestinal transplantation. Our aim is to define the prevalence and investigate the role of donor-specific antibody (DSA) on intestinal graft outcomes. **Methods.** The study includes 109 transplants performed in 95 recipients at a single center. Patients were screened for DSA pretransplant, monitored regularly posttransplant and when clinically indicated using the single-antigen bead Luminex assay. Standard induction immunosuppression was with interleukin-2 receptor antagonists, and antithymocyte globulin in high-risk recipients. Maintenance regimens were tacrolimus-based. **Results.** Pretransplant DSA was detected in 12 (11%) recipients with 50% continuing to have circulating antibodies posttransplant. An additional 24 (25%) patients developed de novo DSA, and of these, 71% had persistent antibodies. Recipients with preformed DSA demonstrated elevated risks of early graft failure, whereas those with de novo DSA experienced accelerated graft loss once DSA was detected, reaching a 28% failure rate within 2 years. HLA-DQ mismatch is a significant risk factor for de novo DSA emergence, whereas the persistence of antibodies is predicted by DSA strength and specificity. Although inclusion of the liver in the intestinal allograft imparts an immunological advantage against rejection-related graft loss, this protective effect was lost among recipients with persistent DSA. **Conclusions.** The presence of DSA is associated with inferior graft outcomes among intestinal transplant recipients. An enhanced understanding of the mechanisms by which DSA causes allograft injury, and effective strategies targeting humoral immune reactivity are needed to improve long-term intestinal graft outcomes.

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The presence of posttransplant antibodies directed at HLA, and in particular donor-specific antibodies (DSA), has been associated with rejection and allograft loss in solid-organ transplantation.[1] Donor-specific antibodies in kidney transplant recipients are linked with acute antibody-mediated rejection (AMR), progression to chronic rejection (CR), late graft dysfunction, and allograft failure.[2-4] Similarly, heart transplant recipients harboring DSA demonstrate higher incidences of vasculopathy and rejection, as well as inferior patient and graft survivals.[5,6] Donor-specific antibody is also an independent predictor of the occurrence and severity of bronchiolitis obliterans in lung transplant recipients.[7] In liver transplantation, persistent DSA increases the risk of allograft immunologic injury, and may be associated with fibrosis, ductopenia, and biliary strictures.[8]

The field of intestinal transplantation (ITx) has witnessed dramatic improvements in short-term patient and graft survival over the past 3 decades. However, the potent immunogenicity of the intestinal graft remains a formidable challenge to its long-term success.[9] Acute rejection (AR) continues to affect 45% of ITx recipients within the first posttransplant year, and graft survival rates beyond 1 year have not improved over time.[9-11] These observations highlight the need for better monitoring and treatment of intestinal graft rejections, and a thorough understanding of the immune mechanisms contributing to allograft loss.

The few published reports on HLA antibodies in ITx have suggested an association between circulating DSA and clinical rejection episodes. Donor-specific antibodies may contribute to AR by acting synergistically with cell-mediated mechanisms,[12] and likely plays a role in the progression to CR and graft failure.[13] Thus, the presence of DSA can

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potentially serve as a noninvasive biomarker for impending graft injury and may guide decisions regarding immunosuppression strategies to improve long-term ITx outcomes.[14]

The aim of the current study is to define the prevalence of preformed, de novo, and persistent DSA in ITx recipients. Risk factors associated with the appearance and elimination of antibodies are explored, and the clinical impact of DSA is discussed. Our findings contribute to the growing field of knowledge pertaining to HLA antibodies in ITx and provide justification for antibody-reduction treatments to improve posttransplant survival outcomes.

MATERIALS AND METHODS

Study Design

A retrospective review using a prospectively maintained database of all ITx recipients transplanted between November 1991 and February 2015 was performed. The collection and analysis of data was approved by the institutional review board at University of California, Los Angeles, Protocol 12-000867.

Study Population

During the study period, a total of 139 visceral transplants were performed in 117 recipients. HLA-A, -B, and DRB1 typing of all donors and recipients were achieved using DNA-based methods; routine HLA-DQ and DRB3/4/5 typing were added in 2006. Pretransplant and posttransplant testing for HLA antibodies was available for 109 transplants in 95 recipients which were included in the analysis. The standard pretransplant evaluation includes determination of panel-reactive antibodies (PRA) initially by complement-dependent cytotoxicity (CDC) techniques in the early era, but is now measured by flow cytometry (see **SDC**, http://links.lww.com/TP/B322). Since 2010, candidates with positive PRA underwent further testing with the solid-phase based single-antigen Luminex technique to determine antibody specificities and strengths. Individual antibodies were considered positive by Luminex if the mean fluorescence intensity (MFI) value exceeded 1000. A transient DSA is defined as an antibody with MFI values below 1000 on the 2 most recent samples. All other DSA with MFI of 1000 or greater on repeated testing are considered persistent.

Transplantation

The conduct of the operation has been described elsewhere.[15,16] The following definitions of allograft types are used: (1) isolated intestine—all or part of the jejuno-ileum placed orthotopically; (2) liver-intestine—either en bloc liver-intestine graft with the donor pancreas removed (old technique), or en bloc liver-pancreas-intestine graft (new technique) placed orthotopically, of note, the native foregut is retained; (3) multivisceral—liver-pancreas-intestine graft placed orthotopically, with removal of the native foregut; (4) modified multivisceral—pancreas and intestine graft placed orthotopically or heterotopically. Additional organs, such as stomach, colon, or kidney, are transplanted on a case-by-case basis. Beginning in 2000, the donor spleen was removed at 1-hour postreperfusion for all multivisceral, modified multivisceral, and combined liver-intestine grafts. Removal of the recipient spleen was determined on a case-by-case basis.

Donor-recipient B and T cell CDC crossmatches (XM) were performed for all transplants. Since 2006, the flow cytometry XM was routinely used in addition to cytotoxic assays. A mean channel fluorescence shift more than 50 channels for the T-cell peak, or more than 150 channels for the B-cell peak, constituted a positive flow cytometry XM.

Immunosuppression

Our protocol for induction therapy was initiated in 1999 and consists of an IL2 receptor antagonist (IL2RA) for 6 to 8 weeks posttransplant in normal-risk recipients and rabbit antithymocyte globulin (rATG) or alemtuzumab in high-risk recipients, that is, liver-free allografts, presensitized and retransplant patients. IL2RA was used in the following manner with serum CD25 levels monitored by flow cytometry: daclizumab (Zenapax; Hoffmann-La Roche, Switzerland) on days 0, 4, 14, 28, 42, and 56; or basiliximab (Simulect; Novartis, East Hanover, NJ) on days 0, 4, 21, 42, and 63. Rabbit ATG (Thymoglobulin; Genzyme, Boston, MA) was given on days 0 to 5 for a total dose of 10 mg/kg. Alemtuzumab (Campath; Genzyme) was administered as a single 30-mg dose before ITx. Patients with positive XMs were additionally treated with IVIg (Privigen; CSL Behring, King of Prussia, PA), plasmapheresis, and/or rituximab (Rituxan; Biogen, Cambridge, MA). Maintenance immunosuppression is tacrolimus (Prograf; Astellas, Deerfield, IL) – based and also includes mycophenolate mofetil (Cellcept; Genentech, San Francisco, CA) since 1999. All recipients are given corticosteroids which are weaned off in 1 to 3 years.

Anti-HLA IgG Antibody Monitoring

Since 2012, patients were monitored with the single-antigen bead Luminex technique at 1, 3, 6, 12 months after ITx, and semiannually thereafter, or when clinically indicated. Before that time, monitoring was initiated only when clinical suspicion was present. High-risk recipients were tested more frequently within the first posttransplant year according to an institutional protocol. The decision to treat persistent DSA was made on a case-by-case basis and consisted of 1 or a combination of the following: high-dose IVIg (2 g/kg), plasmapheresis, rituximab, and bortezomib (Velcade; Millenium, Cambridge, MA).

Intestinal Graft Surveillance

Graft surveillance by endoscopy and mucosal biopsies are performed weekly in the initial posttransplant period, with decreasing frequency thereafter. The diagnosis of AR was made based on established pathologic criteria.[17] Histologically confirmed cases of AR were treated with pulse steroids, typically consisting of methylprednisolone 1 g daily (20 mg/kg daily for pediatric patients) for 2 days and a taper. Severe refractory cases of rejection were additionally treated with rATG or alemtuzumab.

Statistical Analysis

All statistical analyses were performed using STATA/MP version 13.1 (StataCorp, College Station, TX). Data were grouped according to the type of DSA present (preformed vs. de novo) and whether DSA persisted on subsequent testing. Between-group comparisons were made using the Kruskal-Wallis test for continuous and Fisher exact test for categorical variables. A Kaplan-Meier failure function was calculated for the time to first biopsy-proven AR episode.

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Allograft outcomes were plotted as Kaplan-Meier survival curves, and groups compared using log-rank tests or Cox proportional hazards models.

RESULTS

The schema of the entire study cohort, according to the presence of pretransplant and posttransplant DSA, is shown in Figure 1. Pretransplant DSA was detected in 12 (11%) cases. Adjunctive antibody-directed strategies with high-dose IVIg, rituximab, plasmapheresis, and/or bortezomib were given to 9 of these cases in the peritransplant period. In total, 6 (50%) recipients with preformed DSA cleared circulating antibodies after transplant. Four of these 6 recipients cleared DSA immediately after implantation of the allograft along with rATG induction, with or without adjunctive antibody-directed treatments. The 2 remaining patients underwent induction with IL2RA, and antibodies were eradicated after multiple rounds of high-dose IVIg at 6 and 17 months posttransplant, respectively.

Among cases without pretransplant antibodies, 24 (25%) went on to develop de novo DSA (dnDSA). In 7 patients (29%), the appearance of dnDSA was transient, whereas 17 patients (71%) formed persistent antibodies. Ten recipients were given antibody-directed therapies but all retained persistent antibodies.

Recipient Demographics

Baseline characteristics of the study population are listed in Table 1 and stratified by groups based on the presence or absence of preformed and dnDSA. There were no statistically significant differences in age, sex, ABO blood group, or the etiology of intestinal failure between groups. Transplant characteristics are also presented in Table 1. No differences were found in the percentage of retransplants, inclusion of the liver or kidney in the allograft, incidence of recipient splenectomy, or ischemic times between patients with or without DSA. Recipients of the multivisceral graft were less likely to develop dnDSA ($P = 0.03$). Median follow-up duration was 50 months, but only 14 months for the subgroup with preformed DSA due to a high rate of early graft failure.

Immunologic Data

Immunologic characteristics are shown in Table 2, stratified by groups based on DSA status. All transplants performed were ABO-identical or compatible, and most recipients had 4 to 6 HLA mismatches (HLA-A, -B, and -DR) with their respective donors. Recipients with preformed DSA showed a higher degree of presensitization, as evidenced by a larger proportion of patients with PRA levels 20% or higher at the time of transplant. Accordingly, these recipients were more likely to exhibit positive CDC and flow cytometry XMs. Because patients with preformed DSA are thought to have a high immunologic risk, their induction immunosuppression regimens more frequently incorporated the use of lymphocyte-depleting agents.

For patients who developed dnDSA, we found no significant differences in the peritransplant immunologic characteristics presented in Table 2. Most recipients in this group were not sensitized before transplantation. The degree of ABO and HLA matching, and the induction agent employed did not affect the risk of subsequent dnDSA emergence.

Routine HLA-DQ typing of the recipient and donor was instituted in 2006 and testing was available for 54 cases. The number of DQ mismatches was found to be a significant risk factor for dnDSA development (Figure 2). For recipients with 2-antigen mismatches at the DQ locus, the incidence of dnDSA was 52%, as compared with 25% for 0-antigen mismatch and 16% for 1-antigen mismatch ($P = 0.008$). Of the 11 recipients with 2-antigen mismatches at the DQ locus who developed dnDSA, all but 1 harbored DQ-specific DSA. The 1 remaining patient developed dnDSA against HLA-DRB1, which has a high degree of allelic association with the DQB1 locus.

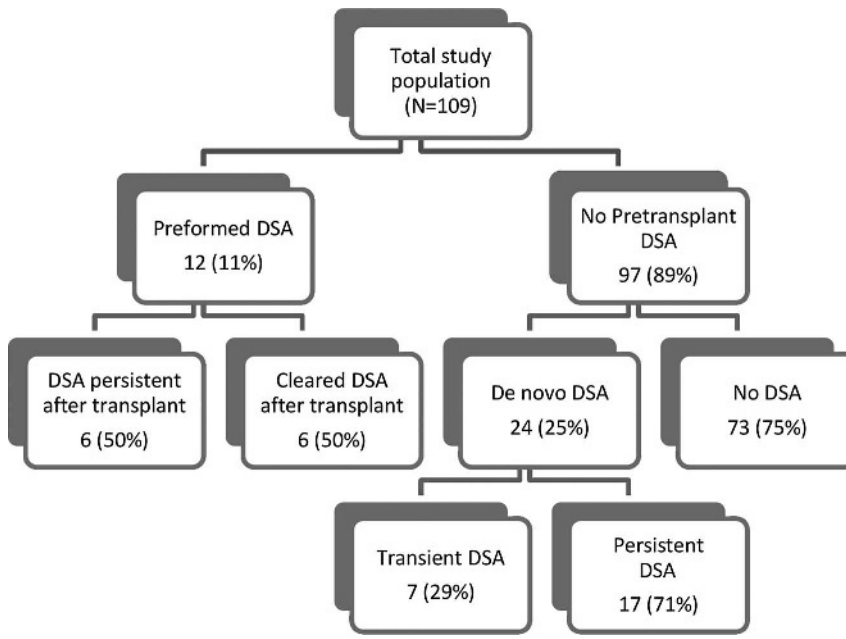

FIGURE 1. Schema of the entire study population according to DSA status. The study includes 109 transplants performed in 95 recipients.

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TABLE 1.

Recipient and transplant characteristics, stratified by DSA type

	Total (n = 109)	No DSA (n = 73)	Preformed DSA (n = 12)	De novo DSA (n = 24)
Median age at transplant, y	6.0 (2.0-22.0)	5.0 (2.0-18.0)	8.0 (3.0-42.5)	8.0 (3.5-24.5)
Pediatric, no. (%)	75 (69)	54 (74)	7 (58)	14 (58)
Female sex	49 (45)	35 (48)	5 (42)	9 (38)
Recipient ABO				
A	39 (36)	30 (41)	3 (25)	6 (25)
B	14 (13)	11 (15)	1 (8)	2 (8)
AB	7 (6)	4 (5)	1 (8)	2 (8)
O	49 (45)	28 (38)	7 (58)	14 (58)
Etiology of intestinal failure				
Gastroschisis	27 (25)	14 (19)	4 (33)	9 (38)
Intestinal atresia	12 (11)	10 (14)	0	2 (8)
Necrotizing enterocolitis	11 (10)	9 (12)	0	2 (8)
Vascular thrombosis	10 (9)	6 (8)	2 (16)	2 (8)
Chronic pseudo-obstruction	7 (6)	6 (8)	0	1 (4)
Retransplants	18 (17)	12 (16)	2 (17)	4 (17)
Transplant technique				
Combined liver-intestine	57 (52)	38 (52)	5 (42)	14 (58)
Multivisceral	23 (21)	17 (23)	5 (42)	1 (4)[a]
Isolated intestine	21 (19)	13 (18)	2 (17)	6 (25)
Modified multivisceral	8 (7)	5 (7)	0	3 (13)
Simultaneous renal transplant	7 (6)	6 (8)	1 (8)	0
Recipient splenectomy	28 (26)	20 (29)	5 (42)	3 (13)
Median operative time, h	7.6 (6.5-8.9)	7.6 (6.3-8.9)	7.4 (6.4-10.6)	7.4 (6.6-8.9)
Median cold ischemia time, h	6.7 (5.5-8.1)	6.5 (5.3-7.9)	7.0 (5.3-9.4)	7.3 (5.9-8.2)
Median warm ischemia time, h	0.61 (0.55-0.72)	0.62 (0.54-0.72)	0.57 (0.54-0.62)	0.62 (0.55-0.79)
Median follow-up duration, m	50 (14-105)	50 (14-71)	14 (4-38)[a]	71 (36-123)

[a] $P < 0.05$ vs "no DSA" group.
Continuous variables are presented as the median with interquartile range in parentheses. Categorical variables are presented as the number of observations with percentage in parentheses.

Preformed HLA Antibodies

Of the 12 recipients with preformed DSA, 10 (83%) harbored antibodies directed at class I HLA antigens. Class I antibodies were cleared in 6 (60%) patients posttransplant. In contrast, among 6 recipients who had detectable class II HLA antibodies pretransplant, all demonstrated persistent

TABLE 2.

Immunological data, stratified by DSA type

	Total (n = 109)	No DSA (n = 73)	Preformed DSA (n = 12)	De novo DSA (n = 24)
ABO match				
Identical	87 (80)	55 (75)	12 (100)	20 (91)
Compatible	20 (18)	18 (25)	0	2 (9)
Incompatible	0	0	0	0
No. HLA mismatches (HLA-A, -B, -DR)				
1	1 (1)	1 (1)	0	0
2	3 (3)	2 (3)	1 (8)	0
3	8 (8)	6 (9)	0	2 (8)
4	21 (20)	13 (19)	3 (25)	5 (21)
5	46 (43)	30 (43)	6 (50)	10 (42)
6	27 (25)	18 (26)	2 (17)	7 (29)
Class I PRA ≥20%, no. (%)	12 (13)	4 (13)	8 (67)[a]	0
Class II PRA ≥20%, no. (%)	12 (13)	3 (5)	8 (67)[a]	1 (5)
Positive CDC XM	7 (8)	5 (8)	2 (20)	0
Positive flow cytometry XM	7 (10)	2 (4)	4 (40)[a]	1 (8)
Induction immunotherapy				
IL2 receptor antagonist	64 (59)	44 (63)	3 (25)[a]	17 (71)
rATG/alemtuzumab	42 (39)	26 (37)	9 (75)[a]	7 (29)

[a] $P < 0.05$ vs "no DSA" group.
Categorical variables are presented as the number of observations with percentage in parentheses.

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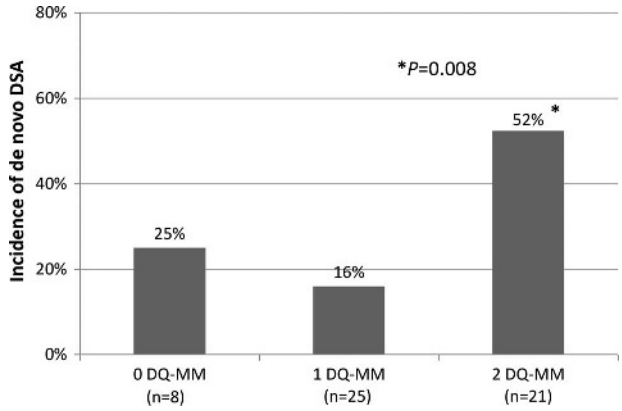

FIGURE 2. Incidence of de novo DSA development according to the degree of DQ mismatch (DQ-MM). A 2-antigen MM at the HLA-DQ locus is associated with de novo DSA emergence (P = 0.008 vs 0-antigen mismatch and 1-antigen mismatch).

antibodies. Therefore, the eradication of preformed DSA was more likely for class I than for class II antibodies (P = 0.01; Figure 3A).

The clearance of preformed HLA antibodies was independent of recipient age, sex, ABO group, number of HLA mismatches, XM status, inclusion of the liver, and induction agent used. There was a trend toward persistence of preformed DSA with the degree of class II presensitization, and with higher MFI values at the time of transplant (Table 3).

De Novo HLA Antibodies

De novo antibodies were more commonly directed against HLA class II antigens—21 of 24 recipients (88%) with dnDSA harbored class II antibodies, whereas only 8 (33%) showed class I antibodies (Figure 3B). Class II HLA antibodies were more likely to persist in the circulation (81%) when compared with class I (50%; P = 0.09).

Characteristics of recipients with persistent versus transient dnDSA are shown in Table 3. There was a trend toward DSA persistence in younger and pediatric recipients, but no association between the rate of DSA clearance with the use of liver-inclusive grafts, retransplantation, degree of HLA mismatch, or induction therapy. There was a strong correlation between DSA strength—both the initial MFI value at first DSA detection (P = 0.01) and the peak MFI (P = 0.001)—with the persistence of circulating antibodies (Table 4). Patients harboring persistent DSA were at elevated risk for rejection, with 14 (82%) being diagnosed with 1 or more episodes of AR.

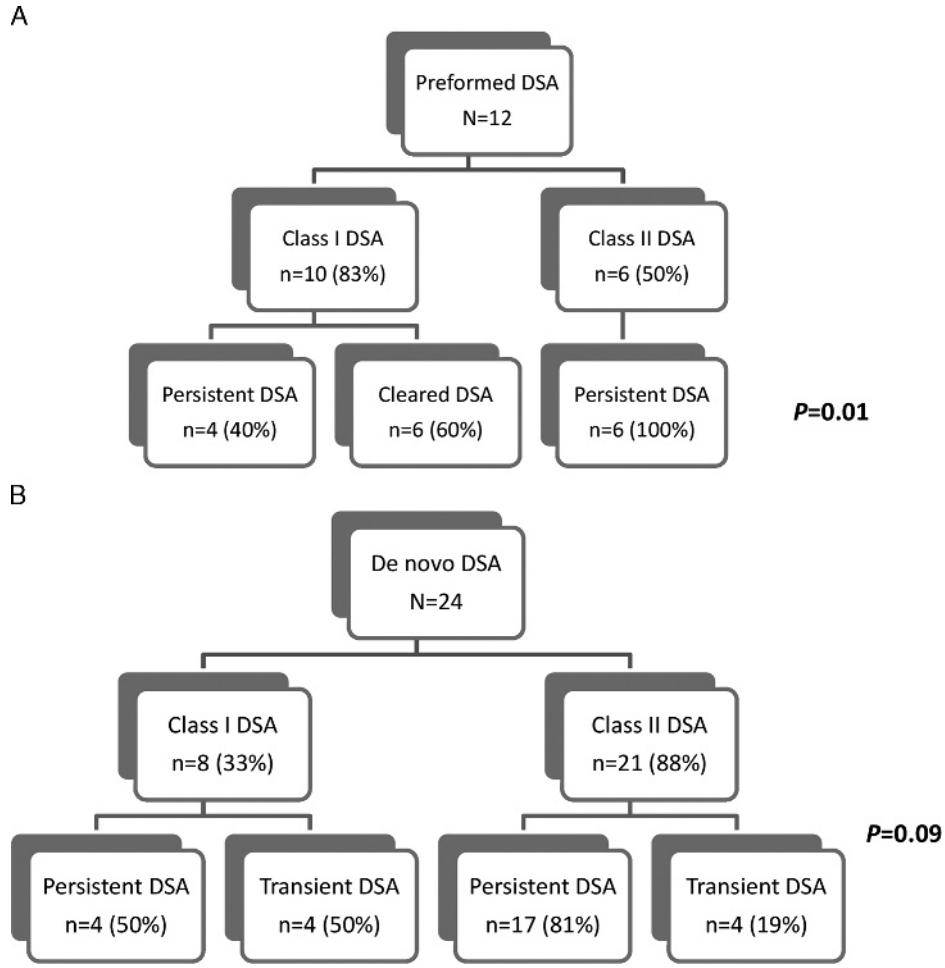

FIGURE 3. Percentage of class I vs. class II DSA in relation to DSA persistence. A, Preformed DSA show a high proportion of class I antibodies which are preferentially cleared posttransplant. B, De novo DSA are commonly directed against class II antigens and likely to persist.

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TABLE 3.

Characteristics of recipients with preformed and de novo DSA, stratified by the persistence of circulating antibodies posttransplant

	Preformed DSA			De Novo DSA		
	Persistent (n = 6)	Cleared (n = 6)	*P*	Persistent (n = 17)	Transient (n = 7)	*P*
Median age at transplant, y	16.0 (3.0-53.0)	6.5 (3.0-32.0)	0.81	6.0 (2.0-19.0)	23.0 (7.0-28.0)	0.05
Pediatric, no. (%)	3 (50)	4 (67)	1.00	12 (71)	2 (29)	0.08
Female sex	1 (17)	4 (67)	0.24	6 (35)	3 (43)	1.00
Recipient ABO			0.11			0.53
A	0	3 (50)		4 (24)	2 (29)	
B	0	1 (17)		1 (6)	1 (14)	
AB	1 (17)	0		1 (6)	1 (14)	
O	5 (83)	2 (33)		11 (65)	3 (43)	
Liver-inclusive grafts	4 (67)	6 (100)	0.45	11 (65)	4 (57)	1.00
Retransplants	2 (33)	0	0.45	3 (18)	1 (14)	1.00
Recipient splenectomy	1 (17)	4 (67)	0.24	2 (12)	1 (14)	1.00
ABO match			1.00			1.00
Identical	6 (100)	6 (100)		13 (87)	7 (100)	
Compatible	0	0		2 (13)	0	
No. HLA mismatches (A, B, DR)			1.00			0.67
1-2	0	1 (17)		0	0	
3-4	2 (33)	1 (17)		4 (23)	3 (43)	
5-6	4 (67)	4 (67)		13 (76)	4 (57)	
Class I PRA ≥20%, no. (%)	4 (67)	4 (67)	1.00	0	0	1.00
Class II PRA ≥20%, no. (%)	6 (100)	2 (33)	0.06	0	1 (7)	1.00
Positive CDC XM	2 (40)	0	0.44	0	0	1.00
Positive flow XM	3 (60)	1 (20)	0.52	0	1 (12)	1.00
Induction immunotherapy			1.00			0.13
IL2 receptor antagonist	1 (17)	2 (33)		14 (82)	3 (43)	
rATG/alemtuzumab	5 (83)	4 (67)		3 (18)	4 (57)	
Median DSA MFI at transplant	16 612 (9644-22 172)	8016 (5717-10 314)	0.16	n/a	n/a	
Median time to first detection of de novo DSA, y	n/a	n/a		6.1	3.1	0.73
Median de novo DSA initial MFI	n/a	n/a		7974 (2468-14 072)	2280 (1321-2446)	0.01[a]
Median de novo DSA peak MFI	n/a	n/a		15 676 (4721-20 695)	2280 (1321-2446)	0.001[a]
Biopsy-proven AR, no. (%)	5 (83)	3 (50)	0.22	14 (82)	4 (57)	0.19

[a]$P < 0.05$

Continuous variables are presented as the median with interquartile range in parentheses. Categorical variables are presented as the number of observations with percentage in parentheses. n/a, not applicable.

Clinical Impact of DSA

Acute Rejection

Biopsy-proven AR was diagnosed in 50 recipients (46%) within the first posttransplant year, with a median of 41 days to the first episode of AR. The incidence of AR did not differ significantly among groups with preformed or dnDSA and recipients without alloantibodies (Figure 4A). Recipients with persistent antibodies, regardless of whether the antibodies first appeared pre or posttransplant, demonstrated a higher cumulative incidence of AR compared to patients without circulating DSA ($P = 0.03$, Figure 4B).

Graft Survival

Rejection was the cause of graft failure in 23 (79%) of 29 cases. The probability of graft failure attributable to rejection according to DSA types are shown in Figure 5A. Recipients with preformed DSA experienced higher risks of early graft failure and demonstrated inferior survival compared to patients with no DSA ($P = 0.001$). Patients with dnDSA showed poor graft survival rates once antibodies were detected (Figure 5B), with 1- and 2-year failure rates of 10% and 28%, respectively. The persistence of DSA was also associated with an elevated risk of allograft loss to rejection ($P = 0.01$, Figure 5C).

Effect of the Liver Graft

In the overall study population, inclusion of the liver was protective against rejection-related allograft loss with a hazard ratio of 0.35 (95% confidence interval, 0.15-0.79; $P = 0.01$), though AR occurrence was not reduced ($P = 0.27$). In the subgroup of patients without circulating DSA, inclusion of the liver was associated with improved allograft survival (hazard ratio = 0.26; 95% confidence interval, 0.09-0.76; $P = 0.01$). However, the protective effect of the liver graft seems to disappear among patients with persistent DSA ($P = 0.85$; Figure 6).

Protocol Monitoring for DSA Posttransplant

To mitigate the effects of the evolution of antibody detection techniques over time, we performed a subgroup analysis of recipients transplanted from late 2011 onward, all of whom have been subjected to routine Luminex-based single-antigen bead testing starting from the time of transplantation. Of the 22 patients in this subgroup, 6 (27%) harbored preformed DSA and 4 (25%) additional patients developed dnDSA. Five of these 10 recipients had persistent

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TABLE 4.

DSA specificity and peak MFI values for recipients who developed de novo DSA

Patient	Class I DSA	Class II DSA
Transient DSA		
1	None	DQ7 (1413)
2	B63 (1157)	None
3	Cw10 (1321)	None
4	None	DQ7 (2280)
5	None	DQ7 (3359), DQ9 (2065)
6	Cw6 (2446)	None
7	Cw7 (1183)	DQ5 (2380)
Persistent DSA		
8	None	DR52 (6077), DQ5 (20695), DQ6 (18182)
9	A3 (7112), B7 (15746), B8 (7564)	DQ5 (20226), DQ7 (17281)
10	None	DR4 (1886), DR53 (5266)
11	None	DR51 (9226), DQ5 (21363)
12	A26 (14985), A30 (13536), B18 (4634), B27 (14336)	DR4 (23106), DR7 (21858), DR53 (21557), DQ2 (15183), DQ7 (19328)
13	None	DQ6 (18191), DQ8 (7917)
14	None	DQ7 (20325)
15	None	DQ7 (1759)
16	None	DR7 (2100), DR51 (3002)
17	None	DR53 (21522)
18	None	DQ4 (5673), DQ7 (14072)
19	Cw7 (4721)	DR17 (2079)
20	A68 (1408), Cw4 (1745)	DR4 (1929), DR11 (1377), DR53 (1969), DQ8 (3317)
21	Cw12 (3541)	DR4 (11156), DR53 (1053), DQ8 (14751)
22	A68 (4757), B58 (11584)	DR52 (8710)
23	None	DQ4 (17334), DQ8 (18419)
24	None	DQ2 (9765), DQ6 (2929)

DSA, all of whom demonstrated DSA specific for class II antigens. Persistent DSA was associated with an increased risk of early graft failure ($P = 0.005$, Figure 5D)—of the 5 recipients with persistent DSA, 2 patients lost their grafts to rejection within the first posttransplant year. On the other hand, all patients without circulating DSA had functioning grafts at the 2-year mark.

DISCUSSION

This report represents the largest study of the incidence and impact of DSA on ITx recipients using the sensitive Luminex-based single-antigen bead method and establishes a connection between circulating alloantibodies and rejection-related graft loss. Our study is the first to report accelerated rates of intestinal graft loss after dnDSA development—these antibodies are likely to persist in the circulation and the risk of allograft failure approaches 10% by 1 year and 28% by 2 years after dnDSA detection. We describe for the first time in ITx that a 2-antigen DQ mismatch is a significant risk factor for the emergence of dnDSA, and that antibodies directed at class II antigens are likely to persist. In contrast, preformed DSA show a higher rate of clearance after transplantation,

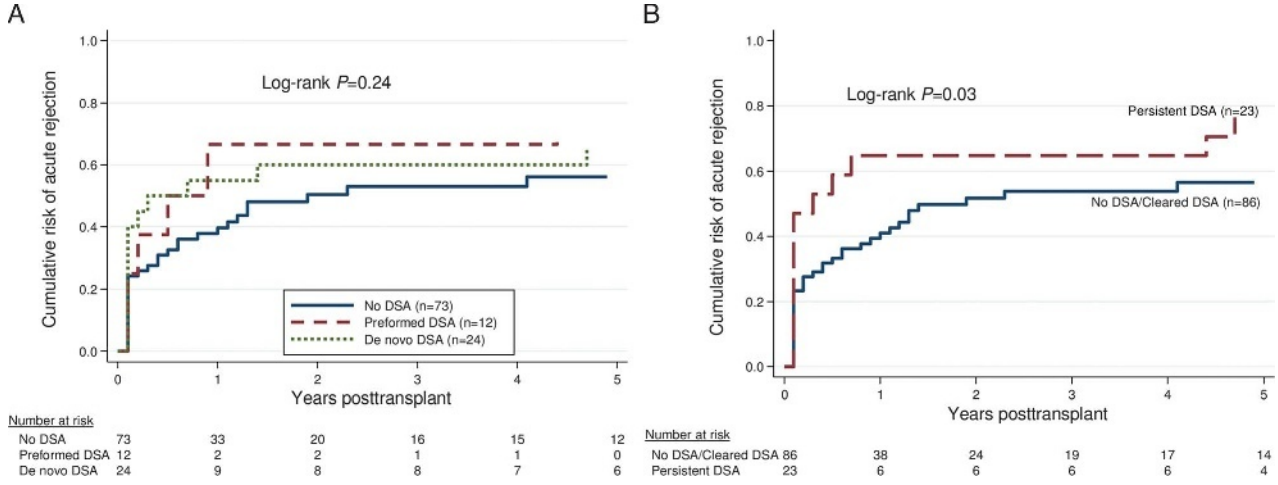

FIGURE 4. Cumulative incidence of acute rejection by A, type of DSA and B, persistent DSA.

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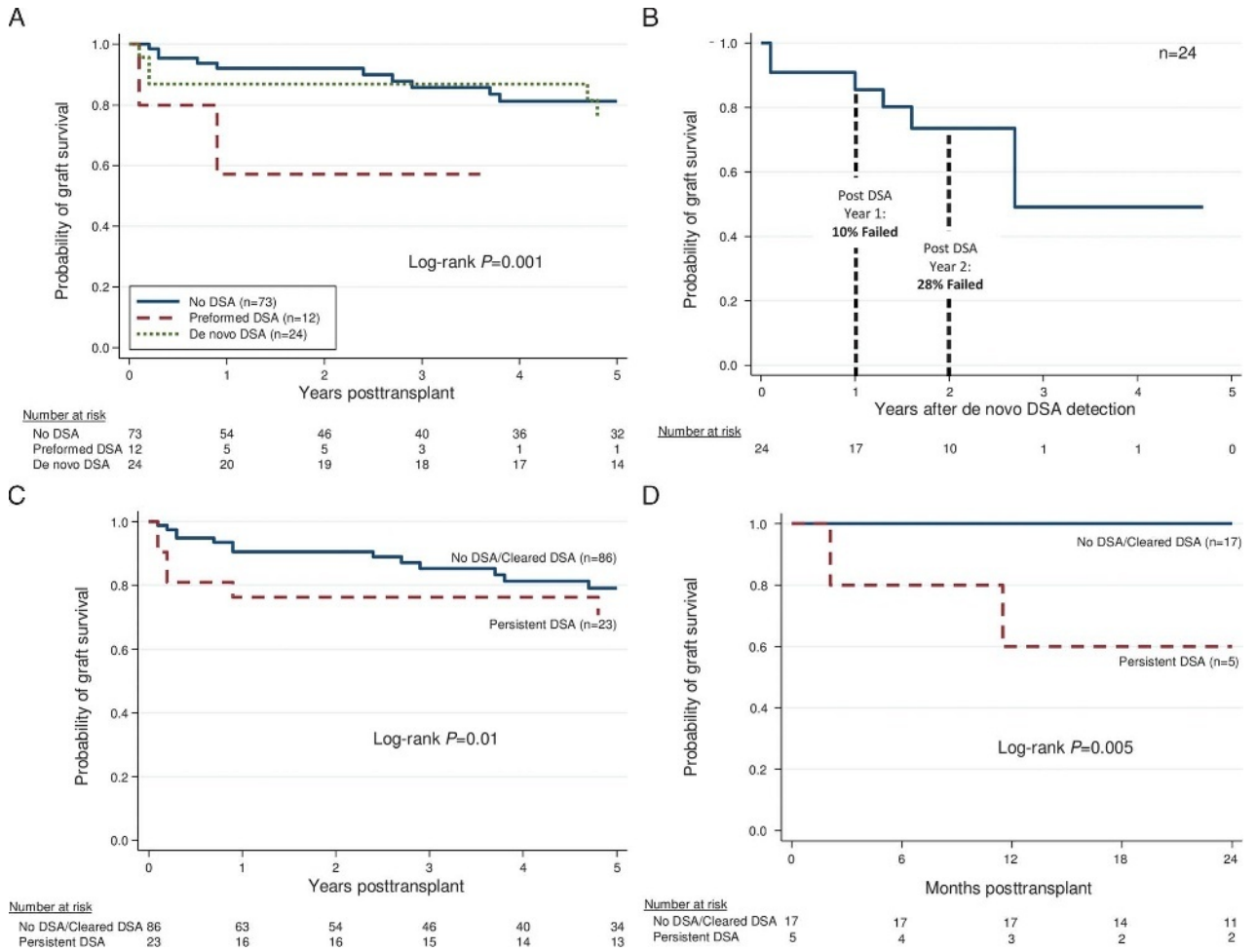

FIGURE 5. Death-censored graft survival A, from the time of transplantation stratified by DSA type. Preformed DSA was associated with early allograft failure due to rejection. There were no significant differences in graft survival between recipients with no DSA and those with de novo DSA. B, Accelerated graft loss was observed from the time of de novo DSA detection, with a 10% failure rate at 1-year and 28% at 2-years. C, Persistent DSA is associated with inferior posttransplant graft survival. D, Subgroup analysis of 22 recipients who underwent protocol monitoring of DSA in the early posttransplant period, showing that persistent DSA is associated with an increased risk of accelerated graft failure.

particularly for antibodies against class I HLA antigens. Nevertheless, the mere presence of pretransplant DSA predisposes to early allograft failure and should not be overlooked.

Our results complement the existing literature on the effects of HLA antibodies in solid-organ transplantation. There is increasing evidence linking DSA to AR and CR, which leads to allograft dysfunction in kidney,[2-4] heart,[5,6] lung,[7,18] liver,[8,19,20] pancreas,[21] and islet[22,23] transplantation. The prevalence of dnDSA in this study was 25%, commensurate with the 5% to 28% seen among kidney transplant recipients.[24] There are few published reports specifically addressing the incidence and impact DSA among ITx recipients. Abu-Elmagd et al[13] identified dnDSA in 18% of recipients and found that persistent DSA was associated with AR and CR as well as graft failure. On the other hand, Kubal et al[25] reported a 28% incidence of dnDSA emergence. In contradiction to the earlier study, these authors did not observe any significant adverse clinical outcomes with the presence of circulating DSA.

The favorable effect of the liver graft on ITx outcomes has been described in single-center and registry reports.[9,11,15] In the present study, we found an interaction between DSA and the effect of the liver graft—inclusion of the liver confers an immunological advantage against rejection-related graft loss, but this protective effect was lost among recipients with persistent DSA. Abu-Elmagd et al[13] have also observed the detrimental effects of persistent DSA on the survival of liver-free intestinal allografts, and inclusion of the liver only

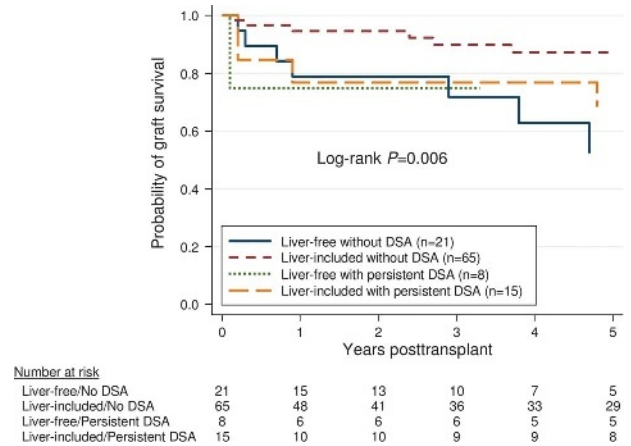

FIGURE 6. Allograft survival in relation to inclusion of the liver and DSA persistence.

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modestly improved 5-year survival rates. The liver is capable of eliminating or neutralizing HLA antibodies, particularly class I antibodies, which may in part account for its tolerogenic properties.[26-28] In the current study, we report that class II DSA is much more likely to persist when compared with class I. The differential clearance of class I versus class II antibodies has also been reported among simultaneous liver-kidney transplant recipients, and the presence of class II DSA is associated with increased risks of AMR and inferior survival outcomes.[29,30]

HLA-DQ mismatch was found to be a significant risk factor for dnDSA emergence, with greater than 50% of recipients with a 2-antigen mismatch developing posttransplant alloantibodies. The enhanced immunogenicity associated with DQ mismatch has been reported in renal transplant patients, and DSA directed against DQ antigens are independently associated with increased risks of AMR, transplant glomerulopathy, and renal allograft loss.[31,32] For these reasons, HLA-DQ matching strategies are now being proposed for kidney transplant recipients to minimize dnDSA development and improve outcomes.[33]

Our analysis of baseline immunologic data at the time of transplantation failed to reveal additional risk factors for dnDSA. Rather, the emergence of dnDSA may be more closely related to posttransplant events. For instance, we noted in this study that dnDSA commonly appeared shortly after AR episodes, suggesting that humoral and cell-mediated immune mechanisms may act in concert during allograft rejection.[34] Another interesting observation relates to patients with posttransplant lymphoproliferative disease, who appear to be at increased risk for dnDSA emergence long after the diagnosis of malignancy is made and immunosuppression reduced (data not shown). These observations support that dnDSA development may be a result of underimmunosuppression, as is seen among nonadherent kidney transplant patients who are predisposed to AMR and allograft loss.[35,36]

The elevated risks of rejection and inferior graft outcomes associated with persistent DSA justifies the use of antibody reduction strategies. It has been shown that kidney transplant patients who promptly respond to DSA reduction strategies experience improved allograft survival compared to nonresponders.[37,38] However, existing antibody reduction protocols have failed to demonstrate durable eradication of DSA posttransplant.[39] In our experience, the clearance of antibodies appears to be dependent on antibody specificity and strength, regardless of the treatments administered. Our data indicate that DSA directed at HLA class I antigens is preferentially cleared in comparison to class II. Antibody strength as measured by MFI values appears to correlate with the likelihood of persistence—whereas weaker DSA (MFI value <2000) are likely to be transient and may disappear spontaneously without treatment, strong DSA with MFI values greater than 10 000 are rarely eliminated. However, with the recent demonstration of the "prozone" effect,[40] MFI values should be interpreted with caution in the absence of corresponding C1q or titration studies.

Existing evidence has linked AMR and CR to the presence of HLA antibodies in other forms of solid-organ transplantation, especially kidney and heart. Establishing the diagnoses of AMR and CR with certainty has remained challenging for ITx recipients. C4d staining has not been shown to be a reliable marker,[41,42] and the histologic appearance of AMR in intestinal biopsies has not been adequately described for the diagnosis to be made reproducibly.[43] The accurate diagnosis of CR often requires a full-thickness biopsy which is not feasible during routine graft surveillance.[44] For these reasons, both AMR and CR were excluded as endpoints in the current study. Future research is needed to elucidate the relationship between DSA and antibody-mediated graft injury, both in the acute and chronic setting. With improved understanding of the mechanisms leading to allograft dysfunction, the presence of DSA may be applied as a noninvasive biomarker for ongoing graft injury,[14] and serve as the basis for initiating therapy to prevent intestinal graft loss.

The limitations of the current study relate to the evolving nature of antibody monitoring in transplantation. First, our study spans over 2 decades and patients who received ITx in the earlier years did not necessarily undergo antibody testing in the peritransplant period. However, our findings are supported by a subgroup analysis of the recipients from late 2011 onward, who are routinely monitored for the presence of antibodies by single-antigen bead testing from the time of transplantation. Second, Luminex single-antigen bead testing is currently the most sensitive method for DSA detection. An MFI value of 1000 or greater has been conventionally used as the definition for DSA positivity, but the clinical relevance of this threshold has yet to be determined. Third, our analysis is limited by the small number of DSA-positive patients. To evaluate additional risk factors for DSA and the variables associated with progression to graft failure, larger series of patients are required. With the infrequency of ITx at any given institution, a multicenter collaborative effort would be necessary to generate adequate samples for future studies.

In summary, our data indicate an association between DSA and accelerated intestinal allograft failure. Approximately 1 in 4 patients will develop dnDSA after ITx, and the probability of graft loss approaches 30% within 2 years after DSA detection. DQ mismatch was identified as a significant risk factor for posttransplant DSA development. De novo DSA are likely to persist, particularly for antibodies directed against class II HLA antigens and those with high MFI values. Further insight is needed to elucidate mechanisms by which alloantibodies cause allograft injury to facilitate the development of treatment strategies aimed at improving survival outcomes after ITx.

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