ORIGINAL ARTICLE

De novo donor-specific HLA antibodies after combined intestinal and vascularized composite allotransplantation — a retrospective study

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SUMMARY

Combining vascularized composite allotransplantation (VCA) with intestinal transplantation to achieve primary abdominal closure has become a feasible procedure. Besides facilitating closure, the abdominal wall can be used to monitor intestinal rejection. As the inclusion of a VCA raises the possibility of an enhanced alloimmune response, we investigated the incidence and clinical effect of *de novo* donor-specific HLA antibodies (dnDSA) in a cohort of patients receiving an intestinal transplant with or without a VCA. The sequential clinical study includes 32 recipients of deceased donor intestinal and VCA transplants performed between 2008 and 2015; eight (25%) modified multivisceral transplants and 24 (75%) isolated small bowel transplants. A VCA was used in 18 (56.3%) cases. There were no episodes of intestinal rejection without VCA rejection. Fourteen patients (14 of 29; 48.3%) developed dnDSA. In the VCA group, fewer patients developed dnDSA; six of 16 (37.5%) VCA vs. eight of 13 (61.5%) non-VCA. There was no statistically significant difference in one- and 3-year overall graft survival stratified for the presence of dnDSA; $P = 0.286$. In the study, there is no evidence that the addition of a VCA increases the incidence of dnDSA formation compared to transplantation of the intestine alone.

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Key words
donor-specific antibodies, intestinal transplantation, rejection, vascularised composite allograft

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Introduction

The field of intestinal transplantation (ITx) is small with less than 200 transplants having been performed in the UK and less than 3 000 worldwide [1,2]. There is even less international experience of vascularized composite allotransplantation (VCA), which is the youngest field in transplantation, but one that has expanded the scope of reconstructive surgery. In the early days of transplantation, while VCA was viewed as a clinical option, the known high antigenicity of skin combined with evidence from experimental models suggested that the risk of rejection was too high to be translated into clinical practice [3]. Similar to ITx, reconstructive transplantation has evolved from being considered high risk to become a clinical reality in the past two decades. However, both functional and immunological outcomes have exceeded initial expectations, and for carefully

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selected patients, VCA is now seen as the best restorative option available.

Intestinal transplantation and VCA developed independently over the last 20 years, until Levi *et al.* [4] published 'Transplantation of the abdominal wall' in Lancet in 2003. They proposed abdominal wall transplantation (AWTx) as a solution for the problems of abdominal wall closure after ITx when the abdominal space is often severely contracted and the skin scarred due to multiple operations prior to the transplant [5,6].

Intestinal and composite transplants have reached a stage at which the investigation of long-term graft outcomes and immunological responses is becoming essential. A humoral immune response may be detrimental to a graft, leading to functional deterioration and the requirement for regular clinical follow-up and/or antirejection treatment. Transplants may be performed in the presence of pre-existing donor-specific HLA antibodies (DSA) in previously sensitized intestinal and VCA recipients (preformed DSA). Alternatively, DSA may develop after transplantation (*de novo* DSA; dnDSA) [7–10].

The effects of DSA/dnDSA on intestinal and vascularized composite allografts are largely unknown, but the majority of studies have reported that the presence of DSA is associated with rejection and graft impairment [10,11]. Even less is known of the occurrence and impact of dnDSA in combined ITx and VCA patients; especially whether a VCA, a graft with potentially high immunogenicity, increases the development of dnDSA. We have therefore investigated our cohort of recipients receiving an ITx graft [isolated small bowel transplant (SBTx) or modified multivisceral transplant (MMVTx)] performed with or without a full-thickness VCA to determine whether the addition of a VCA increased the incidence of dnDSA following transplantion and whether the development of dnDSA had an impact on graft survival.

Patients and methods

This is a retrospective study of 32 consecutive deceased donor intestinal transplants performed with or without a VCA at the Oxford Transplant Centre between October 2008 and December 2015. The combined intestinal and abdominal wall transplant programme is approved under the licence #40038 HTA (Human Tissue Authority):00041. Outcome variables were evaluated in all ITx (SBTx and MMVTx) and combined ITx + VCA transplants. The MMVTx comprised stomach, pancreaticoduodenal-complex, small bowel and the right hemicolon. None of the transplants included a liver allograft. The endpoints of this study were the development of dnDSA after transplantation and graft survival at one and 3 years after transplantation.

Immunosuppression

All recipients, intestinal transplant $^{+/-}$ VCA, received lymphocyte-depleting induction therapy with the CD52 antibody alemtuzumab (Campath®, 30 mg intravenous (iv) within 6 h of reperfusion and a second dose at 24 h), followed by tacrolimus monotherapy for maintenance immunosuppression. We aimed for tacrolimus trough levels of 10–12 ng/ml in the first 6 months and for 8–10 ng/ml thereafter.

Infection prophylaxis

Meropenem (500 mg TDS, iv) and micafungin (100 mg OD, iv) were given for 5 days. Cytomegalovirus (CMV) prophylaxis was administered in cases where either the donor or the recipient was CMV positive. It comprised of iv ganciclovir (5 mg/kg OD, iv), followed by valganciclovir (900 m OD, po) for 1 year. No CMV prophylaxis was used when both donor and recipient were negative for CMV IgG.

Clinical follow-up protocol

Intestine: The endoscopy and biopsy protocol for the clinical follow-up was the same in both groups. It comprised protocol –and indication-driven zoom endoscopies and mucosal biopsies. Protocol biopsies were performed twice a week for the first 3 months and per indication thereafter (deterioration of bowel function/suspicion of rejection). Biopsies were reviewed according to the grading system presented at the Eighth International Small Bowel Transplant Symposium [12].

Vascularized composite allotransplantation: Biopsies of the abdominal wall were all 4 mm-punch biopsies. Vascularized composite allotransplantation biopsies were performed when there was an indication either for the bowel (indication-driven intestinal biopsies) or the skin (rash).

It should be noted that early in the study, we were strictly adherent to the early, intensive intestinal biopsy protocol. However, with growing experience, we have become more reliant on utilizing the VCA skin as a surrogate marker [6] for intestinal rejection and therefore have significantly decreased the number of protocol biopsies.

Rejection treatment

The standard first-line treatment for intestinal and/or VCA rejection was high dose iv steroid; 500 mg bolus on 3 following days. Alemtuzumab was used in cases of steroid-resistant rejection episodes ($n = 2$).

Immunology assessment

HLA typing

All patients were typed for HLA-A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 loci using polymerase chain reaction sequence-specific primer (PCR-SSP) methods.

HLA antibody monitoring

Before transplant, 3-monthly serum samples (monthly if the patient was sensitized) were tested by solid-phase bead-based Luminex assays using LABScreen antibody screening (LSM12) and identification kits (LS1PRA, LS2PRA, LS1A04, LS2A01; One Lambda Inc. Canoga Park, CA, USA).

Post-transplant, routine HLA antibody monitoring was performed at 1, 3, 6, 9 and 12 months then annually thereafter, as well as at the time of clinical events. All DSA were identified by Single Antigen bead (SAB) assays.

All serum samples were tested undiluted and those requiring SAB testing for antibody specification were pretreated with 0.3% ethylene-diamine-tetra-acetic acid (EDTA) [13]. Following the treatment, 10 µl serum was incubated with 2.5 µl of beads for 30 min. Following washing, bound antibody was detected by incubating the beads with 100 µl phycoerythrin-conjugated goat antihuman IgG antibody (One Lambda Inc.) for 30 min in the dark. Samples were analyzed using a Luminex 100 IS fluorescence detector system (Luminex Corp. Austin, TX, USA).

Cross-match

At the time of transplantation, all patients were cross-matched against their donors by flow cytometry and complement-dependent cytotoxicity (CDC). In the CDC cross-match, patient sera were tested with and without 1,4-Dithiothreitol to distinguish between IgG and IgM antibodies.

Data assessment

In addition to donor, recipient- and surgery-related characteristics, the course of DSA binding was analyzed and recorded as the highest median fluorescence intensity (MFI). Median fluorescence intensity of class I and II antibodies, as well as the cumulative MFI of both antibody classes, was measured. A dnDSA was considered as positive with an MFI level >1 000. The antibodies were identified and analyzed for three different time periods: 0–6 months, 6–12 months and after 12 months.

Statistical analysis

Statistical analyses were performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) and GRAPHPAD Prism 6.0 (GRAPHPAD Software, La Jolla, CA, USA). Graft survival was calculated using Kaplan–Meier estimates. Graft loss was defined as either loss of the organ or patient death with a functioning organ. Differences between survival curves were tested for significance by the log-rank test. Cumulative incidence curves were performed for occurrence of dnDSA. Values if not otherwise indicated are means ± SD.

Results

Demographics

Recipient and donor demographics and all relevant surgical factors are shown in Table 1 for patients transplanted with or without a VCA.

In the overall cohort, the median recipient age was 39.5 (range 23–73) years and 56.3% were male. The mean recipient BMI was 20.8 ± 2.9 kg/m^2. The most common cause of intestinal failure was inflammatory bowel disease ($n = 8$), followed by visceral neuropathy ($n = 6$). Retransplantation of both intestine and abdominal wall was performed in 1 patient.

Twenty-four patients (24 of 32, 75%) received an isolated intestinal transplant. Twelve (12 of 24, 50%) had a concurrent VCA, including one small bowel and VCA retransplant. Eight recipients (8/32, 25%) received a MMVTx, including stomach, duodenum, pancreas, small bowel and right hemicolon; six (six of eight, 75%) transplants were combined with a VCA.

In 24 of 32 cases, systemic venous drainage was chosen. The mean number of blood units administered per patient was 7 ± 7.7. Mean cold ischaemia time (CIT) was 405 ± 93 min in the overall cohort.

In Table 1, HLA class I and II mismatches are shown for the overall cohort and the subgroups. In 21 of 32 (65.6%) recipients, there were 4–6 HLA mismatches and in 11 of 32 (34.4%) cases, 0–3 HLA mismatches.

Transplant International 2018; 31: 398–407

Table 1. Demographics of intestinal and modified multivisceral transplantations between 2008 and 2015 (n = 32).

Characteristics	Overall Cohort (n = 32)	VCA (n = 18)	No VCA (n = 14)	dnDSA + (n = 14)	dnDSA- (n = 15*)
Recipient age in years [median (min-max)]	39.5 (23–73)	37.5 (26–69)	42.5 (23–73)	35 (23–47)	44 (25–69)
Recipient BMI kg/m^2 (mean, SD)	20.8 ± 2.9	20.7 ± 2.7	21 ± 3.2	21.2 ± 3.6	20.3 ± 1.9
Recipient male gender (n, %)	18 (56.3%)	11 (61.1%)	7 (50%)	9 (64%)	8 (53%)
Prior Tx (n, %)	1 (3.1%)	1 (5.6%)	0	1/14 (7%)	0
Donor age in years [median (min-max)]	24.5 (8–51)	22.5 (8–49)	31.5 (10–51)	30.1 (10–51)	27 (8–46)
Donor BMI in kg/m^2 (mean, SD)	22.1 ± 2.2	22.1 ± 2.5	22.1 ± 2	22.2 ± 1.5	22 ± 2.8
Donor male gender (n, %)	17 (53%)	9 (50%)	8 (57.1%)	8 (57%)	7 (47%)
Inflammatory bowel disease (IBD)	8 (25%)	5 (27.8%)	3 (21.4%)	7 (50%)	1 (6.7%)
Enteritis/enterocolitis other than IBD	4 (12.5%)	3 (16.7%)	1 (7.1%)	1 (7.1%)	3 (20%)
Pseudomyxoma peritonei (PMP)	4 (12.5%)	4 (22.2%)	0	0	2 (13.4%)
Neoplasia other than PMP	5 (15.6%)	3 (16.7%)	2 (14.3%)	1 (7.1%)	4 (26.6%)
Mesenteric thrombosis	5 (15.6%)	1 (5.6%)	4 (28.6%)	3 (21.5%)	1 (6.7%)
Visceral neuropathy	6 (18.8%)	2 (11.1%)	4 (28.6%)	2 (14.3%)	4 (26.6%)
Isolated intestinal Tx (n, %)	24 (75%)	na	12 (85.7%)	12 (85.7%)	11 (73.3%)
Intestinal Tx with VCA (n, %)	12/24 (50%)	12 (66.7%)	na	5/12 (41.7%)	7/11 (63.6%)
Modified multivisceral Tx (n, %)	8 (25%)	na	2 (14.3%)	2 (14.3%)	4 (26.7%)
Modified multivisceral Tx with VCA (n, %)	6/8 (75%)	6 (33.3%)	na	1/2 (50%)	3/4 (75%)
Systemic versus portal venous drainage (n, %)	24 (75%)	12 (66.7%)	12 (85.7%)	12 (85.7%)	11 (73.3%)
Cold ischaemia time in h (mean, SD)	6.8 ± 1.5	6.6 ± 1.6	7 ± 1.5	6.5 ± 1.2	6.7 ± 1.6
Class I mm					
HLA A mm (mean, SD)	1.16 ± 0.68	1.11 ± 0.76	1.21 ± 0.56	1.25 ± 0.68	1.07 ± 0.73
0 and 1 (n, %)	22 (68.8%)	12 (66.7%)	10 (71.4%)	9 (64.3%)	11 (73.3%)
2 (n, %)	10 (31.2%)	6 (33.3%)	4 (28.6%)	5 (35.7%)	4 (26.7%)
HLA B mm (mean, SD)	1.41 ± 0.76	1.56 ± 0.62	1.21 ± 0.89	1.63 ± 0.62	1.07 ± 0.83
0 and 1 (n, %)	14 (43.8%)	7 (38.9%)	7 (50%)	4 (28.6%)	9 (60%)
2 (n, %)	18 (56.2%)	11 (61.1%)	7 (50%)	10 (71.4%)	6 (40%)
Class II mm					
HLA DR mm (mean, SD)	1.31 ± 0.74	1.28 ± 0.75	1.36 ± 0.75	1.44 ± 0.73	1.14 ± 0.77
0 and 1 (n, %)	17 (53.1%)	10 (55.6%)	7 (50%)	7 (50%)	9 (60%)
2 (n, %)	15 (46.9%)	8 (44.4%)	7 (50%)	7 (50%)	6 (40%)
Total number of mm [median (min-max)]	4 (0–6)	4 (1–6)	4 (0–6)	5 (1–6)	3 (0–6)
Total number of blood units	7 ± 7.7	8.9 ± 9.2	4.4 ± 3.9	4.9 ± 2.9	6.6 ± 7.4
(mean, SD, median, IQR)	4, 7	4, 8	3, 6	5, 5	3, 11
Pre-existing DSA (n, %)	2 (6.3%)	2/16* (11.8%)	0	0	2 (13.4%)
De novo DSA (n, %)		6/16* (35.3%)	8/13* (61.5%)	Na	0
Acute rejection intestinal Tx (n, %)	8/32 (25%)	3 (16.7%)	5 (35.7%)	6/14 (42.9%)	2/15 (13.3%)
Acute rejection VCA (n, %)	7/18 (38.9%)	7 (38.9%)	na	2/6 (33.3%)	5/10 (50%)

dnDSA, *de novo* donor-specific HLA antibodies; na, not applicable in this group; VCA, vascularized composite allotransplantation; TX, transplantation.

*no follow-up for dnDSA in three cases.

Immunosuppression

According to our protocol, all our recipients received alemtuzumab induction followed by tacrolimus monotherapy. In the longer follow-up period, immunosuppressive treatment was adjusted due to renal dysfunction. Prednisolone was commenced at a low dose (5 mg OD) for all patients with renal impairment. In six patients (six of 32, 18.8%), there was evidence of calcineurin inhibitor nephrotoxicity as demonstrated by a mean decline of 45 ml/min (range 25–70 ml/min) in eGFR from pretransplant values ($P < 0.001$). All six patients were switched from tacrolimus to belatacept; all had positive Epstein–Barr virus (EBV) serology at the time of the switch. Five patients (83.3%) demonstrated an immediate improvement in eGFR. One patient demonstrated a decrease in proteinuria without a significant improvement of the eGFR.

Rejection episodes

All rejection episodes (both intestine and skin) were biopsy proven, and both intestine and VCA were biopsied in all cases of rejection. Histology revealed T-cell-mediated rejection in all cases. Staining for C4d was not undertaken. Overall, eight of 32 (25%) patients experienced intestinal rejection episodes. Fewer patients developed intestinal rejection in the combined ITx + VCA group (three of 18, 16.7%) compared to the non-VCA group (five of 14, 35.7%). One of these five non-VCA group intestinal rejection episodes was graded as severe and one was moderate. In the patients receiving a VCA, seven of 18 (38.9%) had visible rejection of the skin (Table 1). In the combined ITx + VCA patient group, there was no evidence of rejection in the intestine without skin rejection. Moderate intestinal rejection was seen in two of three ITx + VCA patients; one of three was graded as mild. So far, there has been no evidence that the presence of the VCA leads to an increase in the frequency of intestinal rejection episodes ($P = 0.23$).

All rejection episodes (intestine or VCA) were treated with three pulses of methylprednisolone (500 mg). Alemtuzumab was used in two steroid-resistant rejection episodes.

Graft survival

The overall one- and 3-year graft survival rates are 86% and 65%. For the ITx group, this was 78% and 70% and for the ITx + VCA group 94% and 57%, respectively ($P = 0.67$). The reasons for graft loss and mortality are shown in Table 2.

Donor-specific antibodies

Before transplantation, HLA antibodies were detected in 13 of 32 (40.6%) patients using Luminex assays. Two (two of 32, 6.3%) transplants were performed in the presence of known DSA. Pretransplant CDC cross-matches were negative for all patients and in one of 32 transplants, the known DSA (HLA-Bw4 and DQ5) resulted in a positive flow cytometry cross-match. The other DSA-positive transplant was both CDC and flow cytometry cross-match negative (DSA: HLA-DPB1*04:01).

Post-transplant samples were available for 29 of 32 (90.6%) transplants. There was no change in the HLA antibody status following eight of 29 (27.6%) transplants. *De novo* HLA antibodies were detected following 21 of 29 (72.4%) transplants. In 14 of 29 (48.3%) transplants, the *de novo* HLA antibodies were directed against the donor (dnDSA) and in the remaining 24.1% transplants, the *de novo* antibodies were not directed against donor HLA mismatches. In the two transplants

Table 2. Reasons for graft loss and death after combined intestinal and abdominal wall transplant.

Characteristics	Intestinal Tx	Intestinal Tx + VCA
Graft loss ($n = 10$)		
Pancytopenia/Sepsis	1	0
Rejection	2	2
MOF/Liver ischaemia	1	0
Mesenteric thrombosis	1	0
Venous thrombosis	1	0
Duodenal leak	0	1
CMV enteritis	0	1
	6	4
Death ($n = 12$)		
Sepsis	2	1
Pulmonary embolism	1	0
MOF/Liver ischaemia	1	0
Brain abscess	1	0
Mesenteric thrombosis	1	0
CMV enteritis	0	1
Cerebral oedema/ Hyperammonemia	0	1
GVHD	0	1
Tx pancreatitis	0	1
Upper GI bleed	0	1
	6	6

CMV, cytomegalovirus; VCA, vascularized composite allotransplantation; TX, transplantation

Transplant International 2018; 31: 398–407

performed in the presence of known DSA, the DSA persisted post-transplant, but there were no dnDSA. In four of 14 (28.6%) cases, dnDSA developed against HLA class I, in three of 14 cases against HLA class II (21.4%) and in eight of 14 (50%) cases against both HLA class I and II.

The addition of a VCA did not increase the incidence of development of dnDSA post-transplant. Six of 16 (37.5%) VCA cases developed dnDSA compared to eight of 13 (61.5%) in the group without a VCA ($P = 0.198$). Furthermore, patients who received an MMVTx were not more likely to develop dnDSA than patients receiving an SBTx. Two of six (33.3%) MMVTx recipients developed dnDSA, whereas 12 of 23 (52.2%) SBTx recipients formed dnDSA ($P = 0.651$).

There was no significant association between the presence of HLA sensitization pretransplant and the development of HLA antibodies following transplant ($P = 0.549$). Also, the number of blood units received in the peri- and post-operative period did not correlate significantly with the development of *de novo* HLA antibodies ($P = 0.632$) or dnDSA ($P = 0.417$).

In the VCA group, three intestinal rejections were identified, whereas there were five confirmed intestinal rejections in the non-VCA group ($P = 0.730$). It should be noted that six of 14 cases (42.9%) with dnDSA had at least one episode of intestinal rejection. In four of six (66.7%) cases, dnDSA were detected before or around the occurrence of intestinal rejection. In one case (one of six, 16.7%), dnDSA appeared 1 year after intestinal rejection. In another case (one of six, 16.7%), dnDSA were detected after the change in immunosuppression (Fig. 1). Only two of 15 (13.3%) dnDSA-negative cases had an episode of intestinal rejection. The switch to a noncalcineurin inhibitor was not associated with higher incidence of dnDSA ($P = 0.311$).

De novo DSA, MFI levels and their development over time

The development of dnDSA over time was analyzed; in nine of 14 (64.3%) cases, dnDSA developed during the first 6 months; in one case (7.1%) between 6 and 12 months, and in four of 14 (28.6%) cases after 1 year. The MFI levels and the dnDSA for each patient are shown in Fig. 1.

0–6 months post-transplant

In the first 6 months after transplantation, dnDSA were detected in nine of 29 (31%) patients. Four of the nine cases belonged to the VCA group and five of nine to the non-VCA group. Four patients had class I antibodies, three class II and two had both class I and II dnDSA.

6–12 months post-transplant

Between 6 and 12 months after transplantation, there were five patients with dnDSA. Of the nine patients who had developed dnDSA within the first 6-month post-transplant, two of nine lost their grafts. Donor-specific HLA antibodies became undetectable in three of nine patients and persisted in four of nine patients.

>12-month post-transplant

In this group, there were eight patients with dnDSA. Four were in the VCA group and four in the non-VCA group. Two patients had class I, two class II and four both classes. Four of the eight cases in the >12 months group had persistent dnDSA, whereas the remaining four developed late dnDSA. A triggering factor could be found for all late dnDSA cases: #1 a change to the immunosuppressive therapy (switch from tacrolimus to belatacept); #2 dnDSA occurred after a Norovirus infection and in patents #3 and #4, dnDSA appeared after antirejection treatment and a consequent change in maintenance immunosuppression. It should be noted here that two patients with late persistent dnDSA were known to be nonadherent.

Impact of donor-specific antibodies

There was no statistically significant difference in one- and 3-year overall graft survival stratified for the presence of DSA; 85% and 51% in the DSA-positive group, respectively; 93% and 85% in the DSA-negative group, respectively ($P = 0.235$).

When stratified for the presence of dnDSA, the one- and 3-year overall graft survival was 85% and 54%, respectively, in the dnDSA-positive group; 93% and 85% graft survival in the dnDSA-negative group ($P = 0.286$); Fig. 2a. In addition, we analyzed cumulative graft survival in the dnDSA-positive group after the appearance of antibodies. The cumulative incidence curve is shown in Fig. 2b.

The cumulative incidence of dnDSA after transplantation is displayed in Fig. 3a. The cumulative incidence curve for occurence of dnDSA after transplantation stratified for inclusion of a VCA is shown in Fig. 3b.

The cumulative MFI levels for class I dnDSA were significantly higher in the patients who lost their graft; 17.384 ± 13.050 than in those with a functioning graft 4.038 ± 4.302, $P = 0.03$. The cumulative MFI levels for class II dnDSA were also higher in the graft loss group, but this was not statistically significant; 14.500 ± 21.022 vs. 3.520 ± 2.603, respectively ($P = 0.38$).

Discussion

This is the first analysis to explore the immunological impact of VCA in combination with solid organ transplantation. The addition of a VCA to an intestinal transplant raises the question of whether sensitization is increased by transplanting more tissue and especially skin.

Skin is regarded as the most immunogenic tissue [14,15], and this is potentially a benefit when transplanting along with a visceral transplant. As well as using the abdominal wall to achieve effective closure of the abdominal cavity after intestinal transplantation, detection of rejection in the skin from the same donor might provide some 'lead time' before rejection is apparent in the visceral organ. Furthermore, and a particular advantage in intestinal transplantation, it might also help to distinguish rejection from an infection. With longer follow-up and increasing experience, we can confirm our previous findings regarding the immunological significance of the abdominal wall VCA [6,16,17].

Most importantly, in this patient cohort, the presence of the VCA did not appear to increase sensitization or

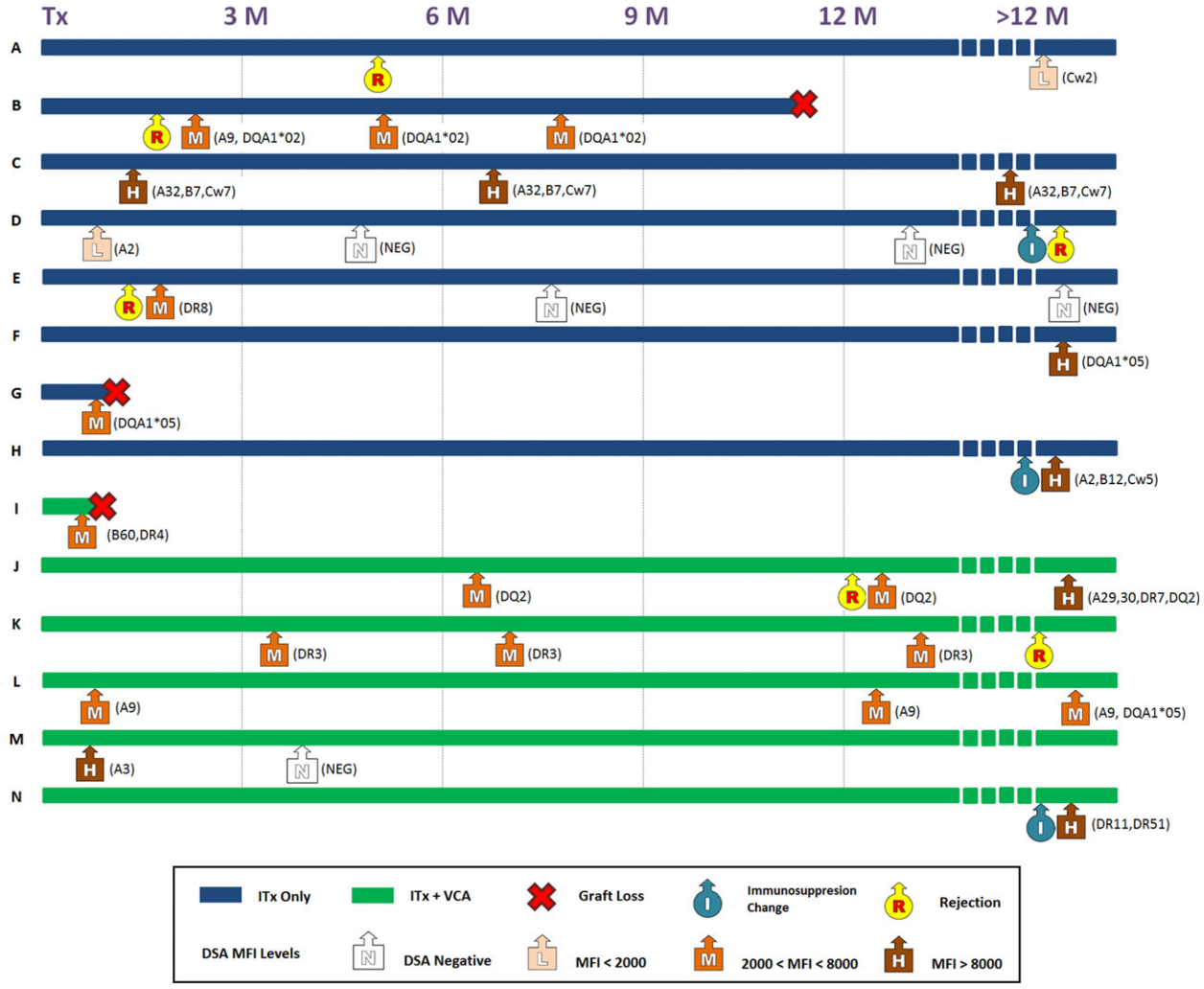

Figure 1 Timeline showing the development of *de novo* donor-specific HLA antibodies in the two patient cohorts (ITx and ITx + VCA), Median fluorescence intensity (MFI) levels and association with immunological events. ITx, intestinal transplantation; VCA, vascularized composite allotransplantation.

Transplant International 2018; 31: 398–407

the formation of dnDSA compared to transplants performed without a VCA. *De novo* DSA seems to be less frequent in the VCA inclusive group, albeit not statistically significantly. A potential explanation for this could be that rejection was diagnosed and treated earlier in these patients, before it caused significant tissue injury and formation of dnDSA. Rejection of the intestine in the absence of prior or concomitant skin rejection has yet to be seen, as there has been no single case where intestinal rejection was not accompanied by a skin rejection. Overall, these early data suggest that VCA provides a valuable immunological monitoring system and can be used as a sentinel [6,16,17].

Besides early dnDSA, we identified patients that developed late dnDSA (>12 months). With the exception of a single case of infective gastroenteritis, all other late dnDSA coincided with either changes in immunosuppression or nonadherence. As previously reported by other studies [18], calcineurin inhibitor minimization or cessation either through nonadherence or driven by physician (intended to reverse drug nephrotoxicity) was a major risk factor for development of dnDSA with subsequent allograft rejection and loss [19]. Changes in immunosuppression were found to trigger immunological events in our cohort, but this was not statistically significant.

This study also evaluated the impact of dnDSA on allograft survival. The one- and 3-year graft survival was inferior in the group of patients who developed dnDSA. The difference was not significant which could be a

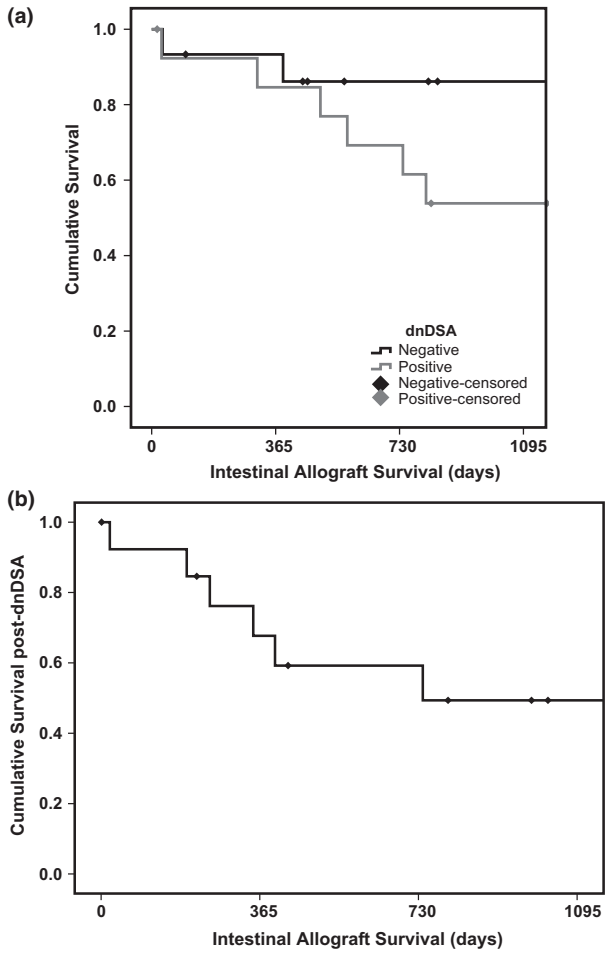

Figure 2 (a) Kaplan–Meier survival plot for intestinal allografts stratified by the presence of *de novo* donor-specific HLA antibodies (dnDSA) ($P = 0.286$). (b). Cumulative graft survival plot for survival post-dnDSA occurrence.

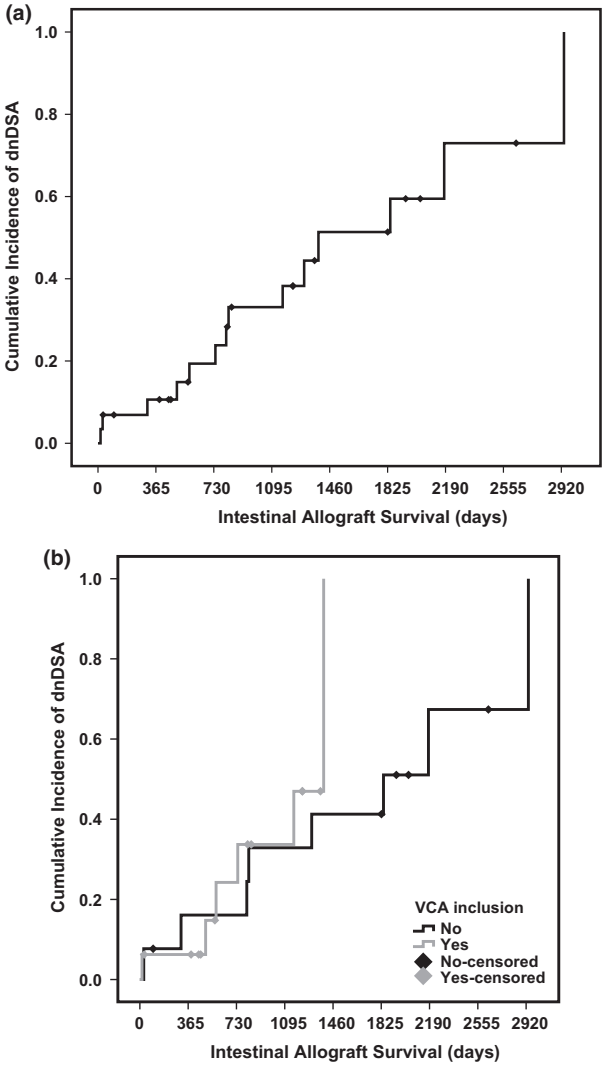

Figure 3 (a) Cumulative incidence curve for *de novo* donor-specific HLA antibodies (dnDSA) development after intestinal transplantation. (b). Cumulative incidence curve for dnDSA stratified for inclusion of vascularized composite allotransplantation after intestinal transplantation.

result of the small number of 14 dnDSA-positive patients in our cohort. This is consistent with previous studies regarding the role of DSA. The Miami group [20], who first described abdominal wall transplantation in visceral transplant patients, were also the first group to study the impact of DSA on intestinal allograft rejection. In their cohort of 15 grafts in 13 patients, clinical rejection episodes were significantly associated with the presence of DSA; reduction in MFI levels was associated with clinical resolution of the rejection. Recently, the UCLA group [21] has reported in a cohort of 109 intestinal transplants, an association between DSA and accelerated intestinal allograft failure. They showed that dnDSA against class II HLA antigens were persistent, which is consistent with our observation of persistent class II dnDSA.

A recent publication demonstrated that blood transfusion was an independent predictive factor for the development of dnDSA [22], but although antibody-mediated rejection was more likely in transfused patients, blood transfusion was not a significant independent factor. In contrast to these findings, our study did not show a correlation between total blood units transfusion and intestinal rejection episodes ($P = 0.756$) or dnDSA development ($P = 0.417$). However, further investigations are needed on the effect of blood transfusions early after ITx and VCA.

The incidence of dnDSAs has been found to be 48.3% in our cohort and is therefore higher when compared with data from other centres (18–40%) [8,9,20,23]. There are probably two main reasons to explain this higher incidence. Firstly, we utilize alemtuzumab as induction agent for all our transplants. Alemtuzumab induction has been associated with reduced acute rejection episodes as shown by the 3C study in renal transplantation [24]. However, on the other hand, it has been associated with a higher incidence of dnDSAs compared to Basiliximab or ATG [25]. Secondly and more importantly, our cohort does not include any liver grafts and we therefore lose the favourable liver effect [1,26].

There are some limitations to this study, as it is retrospective and single-centre. However, this is a unique topic given that there are not currently any other centres worldwide routinely performing the combination of ITx + VCA. Sample size is therefore limited and collaborations are yet to be sought.

In conclusion, the development of DSA in intestinal transplantation, as in other organ types, is detrimental to the long-term survival of the graft. Our data so far suggest that combining an abdominal wall VCA with an intestinal transplant does not increase the incidence of dnDSA.

Authorship

AW, GV, MC, PF, SF: participated in the research design, writing the paper, performing the research and in analyzing the data. SR: participated in the research design, writing the paper and in performing the research. PA, HG, MCNMB, AV: participated in writing the paper and in performing the research.

Conflict of interest

The authors declare no funding or conflict of interests.

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