



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Development of donor specific antibodies after SARS-CoV-2 vaccination in kidney and heart transplant recipients

Thomas R. McCune^{a,b,g,*}, Robert A. Bray^c, David A. Baran^{d,e,g}, Angela J. Toepp^{f,h}, Steven J. Forteⁱ, Lauren T. Gilgannonⁱ, Troy Williams^h, Shirui Chen^a, Hooman Sadr^b, Howard M. Gebel^c, John M. Herre^{d,e,g}

^a Eastern Virginia Medical School, Division of Nephrology, Norfolk, VA 23501-1980, USA

^b Sentara Norfolk General Hospital, Kidney/Pancreas Transplant Program, Norfolk, VA 23507, USA

^c Emory Univ Hosp, Dept of Pathology Rm F149, 1364 Clifton Rd NE, Atlanta, GA 30322, USA

^d Eastern Virginia Medical School, Division of Cardiology, Norfolk, VA 23501-1980, USA

^e Sentara Norfolk General Hospital, Advanced Heart Failure and Transplantation, Norfolk, VA 23507-1999, USA

^f Sentara Healthcare, Quality Research Institute, Virginia Beach, VA 23462, USA

^g Eastern Virginia Medical School, Department of Internal Medicine, Norfolk, VA 23501-1980, USA

^h Enterprise Analytics, Sentara Healthcare, Norfolk, VA 23501, USA

ⁱ Eastern Virginia Medical School, School of Medicine, Norfolk, VA 23501-1980, USA

ARTICLE INFO

Keywords:

SARS-CoV-2 vaccine
Covid-19 infection
DSA
Kidney transplant
Heart transplant
Acute rejection

ABSTRACT

This study examined the development of new or changes in donor specific antibodies (DSA) mean-fluorescence intensity (MFI) after SARS-CoV-2 vaccination in 100 kidney and 50 heart transplant recipients. The study was performed when the Center for Disease Control and Prevention (CDC) recommended two doses of Pfizer/BioNTech [BNT162b2] and Moderna [mRNA-1273 SARS-CoV-2] vaccine or 1 dose Johnson & Johnson/Janssen [Ad26.COV2-S] vaccines for full vaccination in transplant recipients. A novel assay bead-based platform for detecting antibodies against 4 domains of the SARS-CoV-2 spike protein to determine vaccine response (SA) and one nucleocapsid protein (NC) to determine prior SARS-CoV-2 infection was utilized. These assays were performed on the multiplex, bead-based platform utilized to assay DSA levels. 61/150 patients (40.7%) had successful vaccination. 18 patients had confirmed SARS-CoV-2 infection based on positive NC assay or previous Covid-19 oropharyngeal swab. 138 patients had no DSA prior to vaccination but 3 heart recipients developed new DSA's. Among 12 patients with known DSA prior to vaccination, 4 developed new DSA's or increased MFI. All 7 patients with new or increased DSA had stable graft function without rejection and had no changes in immunosuppression. All 8 patients with stable post vaccine DSA had stable graft function and immunosuppression was not changed. The presence of DSA before vaccination was associated with subsequent development of increased MFI or new DSA's ($p = 0.001$). There was no association between pre-vaccine DSA and positive vaccine response (NS). There was no association with successful vaccination or prior SARS-CoV-2 infection and DSA changes (NS).

1. Introduction

SARS-CoV-2 vaccines have been vital in decreasing the spread and

mortality of Covid-19 due to the high rates of antibody development in the general population [1–3]. Vaccination against SARS-CoV-2 for solid organ transplant candidates has been a high priority due to the risk of

Abbreviations: CDC, The Centers for Disease Control and Prevention; DSA, donor specific antibodies; H, heart transplant recipient; HLA, human lymphocyte antigens; JJ/J, Johnson & Johnson/Janssen [Ad26.COV2-S]; K, kidney transplant recipient; M, Moderna [mRNA-1273 SARS-CoV-2]; MFI, mean-fluorescence intensity; mRNA, messenger RNA; NC, SARS-CoV-2 nucleocapsid antibody; P/BNT, Pfizer/BioNTech [BNT162b2]; RBD, SARS-CoV-2 spike receptor-binding domain; SA, SARS-CoV-2 spike antibody as defined as RBD exceeded 5000 MFI PLUS one other spike protein [Full Spike and S2 MFI \geq 5000, S1 MFI \geq 7500].

* Corresponding author at: 301 Riverview Ave, Suite 600, Norfolk, VA 23510, USA.

E-mail addresses: mccunetr@evms.edu (T.R. McCune), rbray@emory.edu (R.A. Bray), ajtoepp@sentara.com (A.J. Toepp), fortesj@evms.edu (S.J. Forte), GilganLT@evms.edu (L.T. Gilgannon), WilliaT2@evms.edu (T. Williams), hgebel@emory.edu (H.M. Gebel), JMHERRE@sentara.com (J.M. Herre).

<https://doi.org/10.1016/j.trim.2022.101722>

Received 21 June 2022; Received in revised form 12 September 2022; Accepted 14 September 2022

Available online 22 September 2022

0966-3274/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

severe disease in immunocompromised patients post-transplant [4]. SARS-CoV-2 vaccination programs for transplant recipients have demonstrated the low incidence of development in SARS-CoV-2 spike protein antibody formation in these immunocompromised patients [5–7]. The novel messenger RNA (mRNA) vaccines against the spike protein of SARS-CoV-2 have not been fully evaluated for their potential to stimulate other immunologic responses such as the production of human leukocyte antigen (HLA) antibodies that may foster the development of acute cellular or humoral rejections when the antibodies are directed to donor HLA [8]. A previous pandemic of H1N1 influenza in 2009 was associated with widespread vaccinations of transplant patients which led to the development of anti-HLA antibodies and antibody mediated rejection in some patients. [9–11]

A multicenter of kidney transplant recipients failed to identify new or increased DSA titers or acute rejection after a second mRNA SARS-CoV-2 vaccine [12]. The largest US study to evaluate response to the two-dose mRNA SARS-CoV-2 vaccine of 741 solid organ transplant recipients reported an episode of acute rejection based on a patient report but the authors admitted that other similarly affected study participant may not have been able to return their study questionnaire [13]. Additionally, two recent case reports regarding alterations in HLA responsiveness after SARS-CoV-2 vaccination have suggested a potential relationship. In the first, a new positive crossmatch prior to a potential kidney transplant developed in a patient who had recently received the second Pfizer/BioNTech [BNT162b2] (P/BNT) SARS-CoV-2 vaccine that was negative prior to vaccination [14]. In the second case, acute cellular rejection with the development of a weak class II donor specific antibody (DSA) occurred after the second P/BNT vaccine [15]. These reports highlight the potential risk of allosensitization after SARS-CoV-2 vaccination.

The transplant programs at Sentara Norfolk General Hospital examined the response to SARS-CoV-2 vaccination and the incidence of changes in DSA in a cohort of fully vaccinated patients followed at the center. The SARS-CoV-2 mRNA vaccine technology had not been employed in this population previously; it was felt prudent to evaluate for this potential complication. The study was undertaken when the Centers for Disease Control and Prevention (CDC) determined that full vaccination against SARS-CoV-2 in transplant recipients was two doses of P/BNT and Moderna [mRNA-1273 SARS-CoV-2] (M) or 1 dose of Johnson & Johnson/Janssen [Ad26.COV2-S] (JJ/J) vaccines. In this study, the programs utilized a novel assay for detecting antibodies against SARS-CoV-2 antigens that has been developed on a multiplex, bead-based platform. This assay can evaluate antibodies against components of the SARS-CoV-2 spike protein including the full spike protein; the S-1 and S-2 sections; the spike receptor-binding domain (RBD) as well as the nucleocapsid protein (NC). This is the same platform used to detect DSA development after transplantation [16]. Presence of antibodies to the NC can indicate prior infection. Presence of antibodies against the SA (without NC) can indicate successful vaccination.

The goal of this study was to assess the incidence of DSA formation and possible acute humoral and cellular rejection following vaccination against SARS-CoV-2.

2. Methods and materials

The Eastern Virginia Medical School Institutional Review Board approved this study of heart, kidney and pancreas transplant recipients followed at the transplant programs of Sentara Norfolk General Hospital to evaluate the development of DSA after SARS-CoV-2 vaccinations (IRB # 21–04-FB-0111). Kidney transplant recipients were encouraged to start their SARS-CoV-2 vaccine series 3 months after transplantation if they were on stable immunosuppressive regimen. Heart transplant patients were encouraged to start their SARS-CoV-2 vaccine series 2 months after transplantation if their immunosuppressive regimen was stable. A sample of 150 patients from all the clinics was planned. Inclusion criteria included age 18 years or older, having received the final dose of the vaccine between 14 and 180 days from date of sample

collection and ability to give informed consent. Participants were recruited in May 2021 when full vaccination against SARS-CoV-2 was two doses of P/BNT and M or 1 dose of JJ/J vaccines. The last study sample was obtained by 1 July 2021 before the CDC recommended a third SARS-CoV-2 vaccine for immunocompromised transplant recipients for full vaccination on 13 August 2021 and SARS-CoV-2 vaccine booster dose was recommended on 21 October 2021. Written informed consent was obtained from each participant. A questionnaire was administered that included date of transplant and types of organs transplanted, Covid-19 symptoms, a prior positive Covid-19 nasal swab, hospitalization due to Covid-19, type of vaccine received, dates of vaccine administration, and immunosuppressive regimen at time of vaccination. A single sample, 10 cc, of blood was obtained. Serum samples were batched and assessed for both DSA and antibodies against SARS-CoV-2. SARS-CoV-2 antibody testing was performed using the COVID Plus Assay (One Lambda, Inc). This test is a semi-quantitative assay and evaluates antibodies against 4 spike proteins (Full spike, S-1, S-2 and RBD) and the NC. HLA antibodies (Class I and Class II) were assessed using the LABScreen Single Antigen Bead assays (One Lambda, Inc). Results of HLA assay and were compared to donor antigens and reported if a DSA was identified with the value of mean-fluorescence intensity (MFI). All MFI DSA's were reported as positive, and results were clinically assessed for each patient. Any identified SARS-CoV-2 associated antibodies were reported as MFI. Results of DSA obtained after vaccination were compared to the most recent DSA results prior to vaccination. Kidney transplant recipients had routine DSA assessed at month 1,3,6,9,12 and 24. Heart transplant recipients had routine DSA assessed at month 3, 6 and 12. A positive antibody response to the SARS-CoV-2 vaccination was determined when the MFI of the RBD-SARS-CoV-2 exceeded 5000 MFI and there was at least one other spike protein with adequate MFI (SA) [Full Spike and S2 MFI \geq 5000; S1 MFI \geq 7500] [13]. Positivity antibody development against the NC protein was determined by an MFI \geq 5000 [16].

The subjects' electronic health records were reviewed for induction immunosuppression at transplantation and any recent immunosuppressive therapies used to treat acute cellular and/or antibody mediated rejection.

Categorical data for SARS-CoV-2 spike protein antibody production (positive/negative) and NC identification (positive/negative) were assessed using two-sided Chi-Square test or Fisher's exact test where appropriate. The Haldane-Anscombe correction was applied when required. Odds Ratios were also calculated. *P*-values less than 0.05 were considered statistically significant. All analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC) and GraphPad Prism 9.3.1 (GraphPad Software Inc., La Jolla, CA).

This study was funded by the Sentara Clinical Research Institute, Sentara Healthcare, Norfolk Virginia.

3. Results

Patients followed in the transplant clinics that had previously expressed interest in having post vaccination SARS-CoV-2 antibody titers measured were invited to participate in the study. The transplant clinic enrolled 100 kidney and 50 heart transplant recipients (Table 1). Sixty patients received the P/BNT, 89 received M and 1 received JJ/J. Successful vaccination with the development of adequate MFI values against the SARS-CoV-2 spike protein (SA) was observed in 61 of the 150 participants (40.7%). Covid-19 infection was observed in 16 of the 150 patients (10.7%) based on Covid-19 nasopharyngeal swab or positive NC antibody. Covid-19 infection was identified in 13 patients with no changes in DSA. Prior to vaccination 138 (43 heart and 95 kidney) patients had no pre vaccine identified DSAs. Of these patients 135 (97.1%) had no new DSA's identified after vaccination. A DSA was identified prior to vaccination in 12 patients to complete the study population.

After vaccination, Two of the 100 kidney transplant patients and 5 of 50 heart transplant patients had observed increases in MFI or new DSA

Table 1
Demographics of study participants by organ type (n = 150).

	Kidney (n = 100)	Heart (n = 50)
Vaccine Type		
Moderna	64	26
Pfizer/BioNTech	36	23
Johnson & Johnson	0	1
Successful vaccination*		
Yes	32	29
No	68	21
Previous Covid-19 infection**		
Yes	14	2
No	86	48
Pre-vaccination DSA		
Yes	5	7
No	95	43

* Successful vaccination: RBD-SARS-CoV-2 MFI \geq 5000 MFI with one other spike protein [Full Spike and S2 MFI \geq 5000 S1 MFI \geq 7500].

** Covid-19 infection as defined by: positive Covid-19 nasopharyngeal swab Or Nucleocapsid Antibody MFI \geq 5000.

($p = 0.02$) (Table 2) Three heart transplant patients without a previously identified DSA developed new DSA (3/43; 6.9%) while none of the kidney transplant patients without previously identified DSA developed new DSA (0/95; 0%) ($p = 0.03$). After vaccination two heart transplant patients with a previously identified DSA developed increased DSA (2/7; 28%). After vaccination two kidney transplant patients with a previously identified DSA developed increased DSA (2/5; 40%). Within the pre-sensitized patient populations there was no difference in the change in DSA between the two transplant populations ($p = 0.67$). Overall, 143 patients (95.3%) had no change in their DSA identified after SARS-CoV-2 vaccination.

Within the total population of patients with pre-vaccine DSA, 8 of the 12 patients remained stable or had decreases in DSA MFI (66.6%). In the group of 8 patients with stable or decreasing DSA MFI, five of these patients were heart transplant recipients and 3 were kidney recipients (Table 3). Two patients had Covid-19 infection prior to vaccine. Three patients developed adequate anti-SARS-CoV-2 antibodies to confirm vaccination.

New DSA or increased DSA MFIs were identified in 7 of the 150 patients of the combined population (4.7%) (Table 4). In the population without DSA prior to vaccination 3 of the 138 patients had new DSA identified after vaccinations. Four of the 12 patients with preexisting DSA's either showed increases in their MFI values or development of a new DSA. These new, previously unidentified DSAs or increased MFI results were observed in 5 heart recipients and 2 kidney recipients. One patient had Covid-19 infection prior to vaccination. Four patients with new DSA or changes in DSA had successful SARS-CoV-2 vaccination. All 7 patients were evaluated by their treatment teams and were determined clinically or through allograft biopsy [2] to be immunologically stable.

Table 2
DSA recognized after vaccination and the correlation of pre-DSA with successful vaccination.

	Kidney (n = 100)	Heart (n = 50)
New DSA or Increased MFI post vaccine		
Yes	2	5
No	98	45
P = 0.02		
New DSA post-vaccination with no prior DSA		
Yes	0	3
No	95	40
P = 0.03		
Elevated DSA pre-vaccination	5	7
Increased post vaccine	2	2
Remained stable	3	5
P = 0.67		

No changes in immunosuppression medications were made on any of the patients (Supplement 1).

To evaluate the association of changes in DSA after vaccination with prior Covid-19 infection and successful vaccination, the group with new DSA or increases in MFI identified after vaccination was compared to the remaining population (Table 5). One of the 7 with an increased MFI DSA identified had a prior Covid-19 infection while 15 of the 143 patients without changes in post vaccine DSA had evidence of a Covid-19 infection. Prior Covid-19 infection was not associated with increased in DSA identified after vaccination ($p = 0.75$). The association of post-vaccine changes in DSA was compared to the successful SARS-CoV-2 vaccination. Four of the seven patients with DSA identified after vaccination had been successfully vaccinated compared to 57 successfully vaccinated in the remaining 143 patients. Development of SA after SARS-CoV-2 vaccine was not associated with changes in post-vaccine new DSA's or increase in MFI ($p = 0.36$).

To evaluate the association of DSA prior to vaccination with successful vaccination and changes in DSA after vaccination, the population with prior DSA ($n = 12$) was compared to the population without DSA prior to vaccination (Table 6). Six patients with DSA prior to vaccine developed SA while 55 patients without prior DSA developed SA. There was no association between DSA prior to SARS-CoV-2 vaccination and subsequent development of SA ($p = 0.49$). The association of prior DSA on new and increased MFI after SARS-CoV-2 vaccination was also evaluated. Four of the 12 patients with DSA prior to SARS-CoV-2 vaccine had increased DSA recognized, while only 3 of the remaining 138 patients developed DSA after vaccination. There was an association between prior DSA and subsequent changes in DSA after vaccination ($p = 0.001$).

4. Discussion

This study was able to successfully employ a multiplex bead-based assay technique to determine levels of DSA, evidence of prior SARS-CoV-2 infection and response to SARS-CoV-2 vaccination with spike protein development using the same blood sample. This technique allowed the team to assess changes in DSA that could be related to SARS-CoV-2 vaccination and previous Covid-19 infection. This study evaluated a large group of heart transplant recipients for DSA formation while other studies focused on kidney transplant recipients [12,17].

There were no observed changes in DSA after SARS-CoV-2 vaccination in 143 of the 150 heart and kidney transplant recipients in this study. There was an association of new DSA after vaccination in the heart transplant patients compared to the kidney transplant patients. This may be related to the different immunosuppression utilized between populations. The heart transplant patients in this population were less likely to be on prednisone than the patients with kidney transplants [18]. These results differ from a multicenter observational study of 58 kidney transplant recipients that failed to observe any cases of increases in DSA or acute rejection after a two-dose mRNA SARS-CoV-2 vaccine course [12]. Additionally, a study of 148 kidney transplant recipients failed to observe the development of new DSA 2 weeks after the second M vaccine [17]. One heart and one kidney recipient each had graft biopsies that found no evidence of acute humeral or cellular rejection. Acute rejection was evaluated on clinical course in the remaining 148 patients. None of the patients in this study had immunosuppression altered after vaccination. None of the patients in this study had evidence of acute rejection related to changes in DSA.

Previous Covid-19 infection based a positive Covid-19 nasopharyngeal swab or positive NC assay was not found to be associated with changes in DSA after vaccination. Prior to this study, the transplant programs had not identified any episodes of changes in DSA or acute rejection in heart or kidney transplant patients related to Covid-19 infections. Additionally, the authors could not find case reports of acute rejection or changes in DSA related to Covid-19 infection. The multicenter study that evaluated DSA changes related to SARS-CoV-2

Table 3

DSA assay pre and post SARS-CoV-2 vaccination demonstrating stability in anti-donor HLA antibodies. This demonstrates the association between successful vaccination and previous COVID-19 infection.

Patient Number	Organ*	Vaccine**	SARS-CoV-2 Spike Antibodies	Covid-19 infection***	Months since Previous DSA	DSA	Previous MFI	Post-Vaccine MFI
1	H	M	Neg	Neg	3	B45 DQ4 DP01:01	9840 3414 2476	1870 1420 1985
2	K	P/BNT	Neg	Neg	4	B50 DR8	9980 1730	7502 1677
3	H	M	Pos	Pos	24	Cw12 DR53 DQ8	3146 1433 8424	1150 500 5900
4	H	M	Pos	Neg	36	DR53 DQ2 DQA01	4442 19,384 12,900	5885 20,460 0
5	K	P/BNT	Neg	Neg	22	DQA05:01	4981	4201
6	H	M	Neg	Neg	23	DQ7 DP04:01 DP04:02	1538 2913 2683	1905 2737 3218
7	K	M	Pos	Pos	83	DQ6	2900	2250
8	H	P/BNT	Neg	Net	4	DR7 DQA1*02 DQB02	1221 2267 1000	650 1000 3237

* Organ Type H=Heart transplant recipient, K=Kidney transplant recipient.

** Vaccine Type M = Moderna (mRNA-1273 SARS-CoV-2), P/BNT = Pfizer/BioNTech (BNT162b2), JJ/J = Johnson & Johnson/Janssen (Ad26.COV2.S).

*** Covid-19 infection as defined by positive Covid-19 nasopharyngeal swab or Nucleocapsid Antibody MFI >=5000.

Table 4

New or increased DSA MFI post SARS-CoV-2 vaccination demonstrating alterations in anti-donor HLA antibodies. This demonstrates the association between successful vaccination and previous COVID-19 infection.

Patient Number	Organ*	Vaccine**	SARS-CoV-2 Spike Antibodies	COVID-19 Infection***	Months since Previous DSA	DSA	Previous MFI	Post-Vaccine MFI
1	H	P/BNT	Neg	Neg	11	A2 B62 DR15 DQ4 DQ6		2800 2700 3400 7800 2500
2	H	P/BNT	Neg	Neg	48	DR53		2700
3	H	P/BNT	Pos	Neg	21	B7 DR7 DQ2		9500 5600 17,000
4	H	M	Pos	Pos	20	A29 B51 DR53 DQ8 DR12 A31	1977 1624 30,106 32,296 3798	8700 6600 40,000 31,000 1800 4200
5	H	M	Pos	Neg	41	A2 B45	1476	2400
6	K	P/BNT	Pos	Neg	73	A23 A3 DR53 DR51 DQ6 DQA1*02	5500	6971 12,051 6485 2676 2793 16,700
7	K	P/BNT	Neg	Neg	132	DQ6	4169	18,000

* Organ Type H=Heart transplant recipient, K=Kidney transplant recipient.

** Vaccine Type M = Moderna (mRNA-1273 SARS-CoV-2), P/BNT = Pfizer/BioNTech (BNT162b2), JJ/J = Johnson & Johnson/Janssen (Ad26.COV2.S).

*** Covid-19 infection as defined by positive Covid-19 nasopharyngeal swab or Nucleocapsid Antibody MFI >=5000.

vaccination in 58 patients included 2 patients with previous Covid-19 infections but no changes in DSA [12]. Within the entire population of this study, only 16 of 150 patients (5.33%) were identified as having had Covid-19 by the report of a positive SARS-CoV-2 nasopharyngeal swab or positive NC. In the group of 7 patients with changes in DSA after vaccine only one patient had evidence of a prior Covid-19 infection. In the 8 patients with stable DSA, two had recovered from a Covid-19 infection. The remaining 13 patients with Covid-19 infections of this population had no previously identified DSA and remained negative after vaccination. Previous Covid-19 infection was not associated with

new DSA's or changes in DSA MFI.

Successful vaccination as defined by the development of SA after two doses of P/BNT or M and one dose of JJ/J and this was evaluated to identify an associated with new DSA or an increase in DSA MFI. The multicenter study of 58 kidney transplant patients fail to identify a single case of new or increased DSA [12]. The larger study of 148 patients after completing the initial M vaccine course failed to identify an increase in DSA despite a vaccine response rate of 69% [17]. The incidence of successful vaccination within that study was higher than the 40.7% vaccination rate reported in this population. This study found no

Table 5

Association of DSA identified after SARS-CoV-2 vaccination with prior Covid-19 infection and successful vaccination.

	New DSA after vaccination	No new DSA after vaccination
Prior Covid-19 infection		
**		
Yes	1	15
No	6	128
P = 0.75		
Successful vaccination*		
Yes	4	57
No	3	86
P = 0.36		

* Successful vaccination: RBD-SARS-CoV-2 MFI \geq 5000 MFI with one other spike protein [Full Spike and S2 MFI \geq 5000; S1 MFI \geq 7500].** Covid-19 infection as defined by: positive Covid-19 nasopharyngeal swab Or Nucleocapsid Antibody MFI \geq 5000.**Table 6**

Association of DSA prior to SARS-CoV-2 vaccination with successful vaccination and identified changes in DSA after vaccination.

	DSA prior to vaccination	No DSA prior to vaccination
Successful vaccination*		
Yes	6	55
No	6	83
P = 0.49		
Increases in DSA after vaccination		
Yes	4	3
No	8	135
P = 0.001		

* Successful vaccination: RBD-SARS-CoV-2 MFI \geq 5000 MFI with one other spike protein [Full Spike and S2 MFI \geq 5000 S1 MFI \geq 7500].

association with successful SARS-CoV-2 vaccination and changes in DSA after vaccination.

This study observed that patients with DSA prior to SARS-CoV-2 vaccination were more likely to have post vaccine changes in DSA. Increases in MFI values were identified in 4 of the 12 patients with known DSA prior to vaccination, but only 3 patients without DSA before vaccination. A study during the H1N1 pandemic observed that preformed anti-HLA predicted the development of new anti-human leukocyte antibodies after H1N1 vaccination [19]. The authors of H1N1 vaccination study could not conclusively associate H1N1 vaccination and changes in DSA with the speculation that other sensitizing effect could have occurred [19]. Infections have been shown to increase anti-HLA antibodies in patients awaiting kidney transplant [20]. In the multicenter SARS-CoV-2 vaccine study, none of 5 patients with previously identified DSA had changes in MFI observed after vaccination [12]. The changes in DSA after vaccination in the previously sensitized group of this study could be related to sensitizing events other than Covid-19 infection or SARS-CoV-2 vaccination because the period from prior DSA identification ranged from 20 to 132 months.

It is uncertain if the presence of DSA without humeral rejection is a marker of lower levels of immunosuppression. It has been shown that early steroid discontinuation results in the development of new Class II DSA in kidney transplant recipients [21]. This study also observed new DSA formation in previously heart transplant patients who had early steroid discontinuation (Forte). This finding was observed primarily in the heart transplant populations with no DSA prior to vaccination. The fact that the previously sensitized heart transplant population did not observe a similar increase in DSA number or MFI suggests that this finding needs further investigation.

The findings of this study echoes the experience with the H1N1 influenza pandemic of 2009. Early reports after the influenza pandemic

of 2009 suggested an alloreactivity to HLA from the H1N1 vaccine, subsequent review of a large cohort of solid organ transplant recipients failed to observe any association [22,23]. The vaccine employed for the H1N1 pandemic was an inactivated H1N1 virus while SARS-CoV-2 vaccines available in the United States use mRNA to produce the SARS-CoV-2 spike protein which results in antibody formation [1–3]. Since the H1N1 and SARS-CoV-2 vaccines are so different, direct comparisons are limited. Further research into the potential for alloreactivity of mRNA vaccines is needed as the potential for additional vaccines using mRNA technology.

There are limitations to this study related to the severity of the pandemic on solid organ transplant recipients and the rapidly evolving vaccination requirements for this population. The urgency to vaccinate immunosuppressed patients during the Covid-19 pandemic did allow time to develop a comprehensive study to capture DSA immediately prior to vaccination. This failure to measure DSA prior to vaccination occurred in other studies that commented on DSA and acute rejection after SARS-CoV-2 vaccination [12,13]. This study was also completed before the CDC recommended a third SARS-CoV-2 vaccine for immunocompromised transplant recipients for full vaccination on 13 August 2021. Therefore, reproducing this study will be difficult. This review did not evaluate for non-donor HLA and non-HLA antibody formation therefore the full effect of mRNA vaccine associated sensitization cannot be determined. Among the other limitations of this study, the reliance on clinical course and not mandating protocol biopsies in the presence of post-vaccination DSA recognition limited the conclusion that newly developed DSA were not associated with sub-clinical rejection. Finally, the small sample size of only heart and kidney transplant recipients does not allow for a full picture of the association of SARS-CoV-2 vaccination and DSA in the immunocompromised transplant patient.

5. Conclusion

The ability to assay HLA, prior Covid-19 infection and SARS-CoV-2 vaccine responsiveness via the same sample allows transplant centers using a multiplex bead-based assay platform allows transplant programs the ability to monitor their transplant population for immunity related to Covid-19.

This study of kidney and heart transplant recipients failed to identify any episodes of clinically evident acute cellular or antibody mediated rejection following SARS-CoV-2 vaccination. SARS-CoV-2 vaccination was not associated with changes in DSA. Infections with Covid-19 was not associated with changes in DSA in this population. This study observed that patients with pre-vaccine DSA were more likely to have post-vaccine changes in DSA. The presence of pre-vaccination DSA was not associated with increased rates of vaccine response. Based on these results the transplant programs have determined that further testing after SARS-CoV-2 initial vaccine course or any boosters for DSA or organ function was not warranted.

Additional studies should be undertaken to completely understand the potential that the mRNA SARS-CoV-2 vaccines may have to cross-react with the HLA system as it relates to successful organ transplantation.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.trim.2022.101722>.

References

- [1] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E.D. Moreira, C. Zerbini, R. Bailey, K.A. Swanson, S. Roychoudhury, K. Koury, P. Li, W.V. Kalina, D. Cooper, R.W. Frenck Jr., L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D.B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K.U. Jansen, W.C. Gruber, C4591001 Clinical Trial Group, Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine, *N. Engl. J. Med.* 383 (27) (2020 Dec 31) 2603–2615, <https://doi.org/10.1056/NEJMoa2034577>. Epub 2020 Dec 10. PMID: 33301246; PMCID: PMC7745181.
- [2] L.R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Rouphael, C.B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Broz, C. Ferro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B.S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, COVE Study Group, Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine, *N. Engl. J. Med.* 384 (5) (2021 Feb 4) 403–416, <https://doi.org/10.1056/NEJMoa2035389>. Epub 2020 Dec 30. PMID: 33378609; PMCID: PMC7787219.
- [3] J. Sadoff, G. Gray, A. Vandebosch, V. Cárdenas, G. Shukarev, B. Grinsztajn, P. A. Goepfert, C. Truyers, H. Fenneema, B. Spiessens, K. Offergeld, G. Scheper, K. L. Taylor, M.L. Robb, J. Treanor, D.H. Barouch, J. Stoddard, M.F. Ryser, M. A. Marovich, K.M. Neuzil, L. Corey, N. Cauwenberghs, T. Tanner, K. Hardt, J. Ruiz-Guiñazú, M. Le Gars, H. Schuitemaker, J. Van Hoof, F. Struyf, M. Douoguih, ENSEMBLE Study Group, Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19, *N. Engl. J. Med.* 384 (23) (2021 Jun 10) 2187–2201, <https://doi.org/10.1056/NEJMoa2101544>. Epub 2021 Apr 21. PMID: 33882225; PMCID: PMC8220996.
- [4] M. Negahdaripour, M. Shafiekhani, S.M.I. Moezzi, S. Amiri, S. Rasekh, A. Bagheri, P. Mosaddeghi, A. Vazin, Administration of COVID-19 vaccines in immunocompromised patients, *Int. Immunopharmacol.* 99 (2021 Oct) 108021, <https://doi.org/10.1016/j.intimp.2021.108021>. Epub 2021 Jul 28. PMID: 34352567; PMCID: PMC8316069.
- [5] A. Grupper, L. Rabinowich, D. Schwartz, I.F. Schwartz, M. Ben-Yehoyada, M. Shashar, E. Katchman, T. Halperin, D. Turner, Y. Goykhman, O. Shibolet, S. Levy, I. Houry, B. Baruch, H. Katchman, Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus, *Am. J. Transplant.* 21 (8) (2021 Aug) 2719–2726, <https://doi.org/10.1111/ajt.16615>. Epub 2021 May 7. PMID: 33866672; PMCID: PMC8250589.
- [6] V.G. Hall, V.H. Ferreira, M. Ierullo, T. Ku, T. Marinelli, B. Majchrzak-Kita, A. Yousef, V. Kulasingam, A. Humar, D. Kumar, Humoral and cellular immune response and safety of two-dose SARS-CoV-2 mRNA-1273 vaccine in solid organ transplant recipients, *Am. J. Transplant.* 21 (12) (2021 Dec) 3980–3989, <https://doi.org/10.1111/ajt.16766>. Epub 2021 Aug 4. PMID: 34347934; PMCID: PMC8441872.
- [7] B.J. Boyarsky, T.P. Chiang, M.T. Ou, W.A. Werbel, A.B. Massie, D.L. Segev, J. M. Garonzik-Wang, Antibody response to the Janssen COVID-19 vaccine in solid organ transplant recipients, *Transplantation.* 105 (8) (2021 Aug 1) e82–e83, <https://doi.org/10.1097/TP.0000000000003850>. PMID: 34098566; PMCID: PMC8298284.
- [8] B.J. Boyarsky, W.A. Werbel, R.K. Avery, A.A.R. Tobian, A.B. Massie, D.L. Segev, J. M. Garonzik-Wang, Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients, *JAMA.* 325 (17) (2021 May 4) 1784–1786, <https://doi.org/10.1001/jama.2021.4385>. PMID: 33720292; PMCID: PMC7961463.
- [9] S. Candon, E. Thervet, P. Lebon, C. Suberbielle, J. Zuber, C. Lima, D. Charron, C. Legendre, L. Chatenoud, Humoral and cellular immune responses after influenza vaccination in kidney transplant recipients, *Am. J. Transplant.* 9 (10) (2009 Oct) 2346–2354, <https://doi.org/10.1111/j.1600-6143.2009.02787.x>. Epub 2009 Jul 28. PMID: 19656126.
- [10] I. Katerinis, K. Hadaya, R. Duquesnoy, S. Ferrari-Lacraz, S. Meier, C. van Delden, P. Y. Martin, C.A. Siegrist, J. Villard, De novo anti-HLA antibody after pandemic H1N1 and seasonal influenza immunization in kidney transplant recipients, *Am. J. Transplant.* 11 (8) (2011 Aug) 1727–1733, <https://doi.org/10.1111/j.1600-6143.2011.03604.x>. Epub 2011 Jun 14. PMID: 21672157.
- [11] S. Brakemeier, B. Schweiger, N. Lachmann, P. Glander, R. Schönemann, F. Diekmann, H.H. Neumayer, K. Budde, Immune response to an adjuvanted influenza A H1N1 vaccine (Pandemrix®) in renal transplant recipients, *Nephrol. Dial. Transplant.* 27 (1) (2012 Jan) 423–428, <https://doi.org/10.1093/ndt/gfr278>. Epub 2011 May 25. PMID: 21613386.
- [12] A. Al Jurdi, R.B. Gassen, T.J. Borges, Z. Solhjoui, F.E. Hullekes, I.T. Lape, O. Efe, A. Alghamdi, P. Patel, J.Y. Choi, M.T. Mohammed, B. Bohan, V. Pattanayak, I. Rosales, P. Cravedi, C.N. Kotton, J.R. Azzi, L.V. Riella, Non-invasive monitoring for rejection in kidney transplant recipients after SARS-CoV-2 mRNA vaccination, *Front. Immunol.* 13 (2022 Feb 25), 838985, <https://doi.org/10.3389/fimmu.2022.838985>. PMID: 35281011; PMCID: PMC8913529.
- [13] M.T. Ou, B.J. Boyarsky, J.D. Motter, R.S. Greenberg, A.T. Teles, J.A. Ruddy, M. R. Krach, V.S. Jain, W.A. Werbel, R.K. Avery, A.B. Massie, D.L. Segev, J. M. Garonzik-Wang, Safety and reactogenicity of 2 doses of SARS-CoV-2 vaccination in solid organ transplant recipients, *Transplantation.* 105 (10) (2021 Oct 1) 2170–2174, <https://doi.org/10.1097/TP.0000000000003780>. PMID: 33859151; PMCID: PMC8487696.
- [14] A. Del Bello, O. Marion, A. Delas, N. Congy-Jolivet, M. Colombat, N. Kamar, Acute rejection after anti-SARS-CoV-2 mRNA vaccination in a patient who underwent a kidney transplant, *Kidney Int.* 100 (1) (2021 Jul) 238–239, <https://doi.org/10.1016/j.kint.2021.04.025>. Epub 2021 Apr 28. PMID: 33932459; PMCID: PMC8080493.
- [15] Q. Xu, P. Sood, D. Helmick, J.S. Lomago, A.D. Tevar, A. Zeevi, Positive flow cytometry crossmatch with discrepant antibody testing results following COVID-19 vaccination, *Am. J. Transplant.* 21 (11) (2021 Nov) 3785–3789, <https://doi.org/10.1111/ajt.16753>. Epub 2021 Jul 19. PMID: 34241963; PMCID: PMC8441686.
- [16] R.A. Bray, J.H. Lee, P. Brescia, D. Kumar, T. Nong, R. Shih, E.S. Woodle, J. S. Maltzman, H.M. Gebel, Development and validation of a multiplex, bead-based assay to detect antibodies directed against SARS-CoV-2 proteins, *Transplantation.* 105 (1) (2021 Jan 1) 79–89, <https://doi.org/10.1097/TP.0000000000003524>. PMID: 33273320.
- [17] D. Cucchiari, N. Egri, M. Bodro, S. Herrera, J. Del Risco-Zevallos, J. Casals-Urquiza, F. Cofan, A. Moreno, J. Rovira, E. Banon-Maneus, M.J. Ramirez-Bajo, P. Ventura-Aguilar, A. Pérez-Olmos, M. Garcia-Pascual, M. Pascal, A. Vilella, A. Trilla, J. Ríos, E. Palou, M. Juan, B. Bayés, F. Diekmann, Cellular and humoral response after MRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients, *Am. J. Transplant.* 21 (8) (2021 Aug) 2727–2739, <https://doi.org/10.1111/ajt.16701>. Epub 2021 Aug 4. PMID: 34036720; PMCID: PMC8222867.
- [18] S.J. Forte, A.J. Toepp, R.A. Bray, D.A. Baran, T.R. McCune, et al., The efficacy of SARS-CoV-2 antibody response after two dose mRNA vaccination in kidney and heart transplant recipients using a multiplex bead-based assay: evaluating the factors affecting vaccine response, *Arch. Organ Transplant* 7 (1) (2022) 001–008, <https://doi.org/10.17352/2640-7973.000019>.
- [19] L. Marino, J. Alberú, L.E. Morales-Buenrostro, Influenza immunization and the generation of anti-HLA and anti-MICA antibodies in patients with renal failure and in kidney transplant recipients, *Clin. Transpl.* 32 (2016) 161–171 (PMID: 28564534).
- [20] J.E. Locke, A.A. Zachary, D.S. Warren, D.L. Segev, J.A. Hou, R.A. Montgomery, M. S. Leffell, Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody, *Am. J. Transplant.* 9 (9) (2009 Sep) 2136–2139, <https://doi.org/10.1111/j.1600-6143.2009.02764.x>. Epub 2009 Jul 30. PMID: 19663896.
- [21] E. Cordero, A. Bulnes-Ramos, M. Aguilar-Guisado, F. González-Escribano, I. Olivias, J. Torre-Cisneros, J. Gavaldá, T. Aydllo, A. Moreno, M. Montejo, M.C. Fariñas, J. Carratalá, P. Muñoz, M. Blanes, J. Fortún, A. Suárez-Benjumea, F. López-Medrano, C. Roca, R. Lara, P. Pérez-Romero, Effect of influenza vaccination inducing antibody mediated rejection in solid organ transplant recipients, *Front. Immunol.* 11 (2020 Oct 6) 1917, <https://doi.org/10.3389/fimmu.2020.01917>. PMID: 33123119; PMCID: PMC7574595.
- [22] W.R. Mulley, C. Dendle, J.E.H. Ling, S.R. Knight, Does vaccination in solid-organ transplant recipients result in adverse immunologic sequelae? A systematic review and meta-analysis, *J. Heart Lung Transplant.* 37 (7) (2018 Jul) 844–852, <https://doi.org/10.1016/j.healun.2018.03.001>. Epub 2018 Mar 10. PMID: 29609844.
- [23] J. Andrade-Sierra, A.M. Cueto-Manzano, E. Rojas-Campos, E. Cardona-Muñoz, J. I. Cerrillos-Gutiérrez, E. González-Espinoza, L.A. Evangelista-Carrillo, M. Medina-Pérez, B. Jalomo-Martínez, J. Nieves Hernández, L. Pazarín-Villaseñor, C. A. Mendoza-Cerpa, B. Gómez-Navarro, A.G. Miranda-Díaz, Donor-specific antibodies development in renal living-donor receptors: effect of a single cohort, *Int. J. Immunopathol. Pharmacol.* 35 (2021 Jan-Dec), <https://doi.org/10.1177/20587384211000545>, 20587384211000545. PMID: 33787382; PMCID: PMC8020398.