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Clinical Infectious Diseases

MAJOR ARTICLE

Relationship between immune response to SARS-cov2 vaccines and development of breakthrough infection in solid organ transplant recipients: the CONTRAST cohort

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DOI: 10.1093/cid/ciad016

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Background: SARS-CoV-2 vaccination in solid organ transplant (SOT) is associated with poorer antibody response (AbR) compared to non-SOT recipients. However, its impact on the risk of breakthrough infection (BI) should yet be assessed.

Methods:. Single-center prospective longitudinal cohort study enrolling adult SOT recipients who received SARS-CoV2 vaccination during 1-year period from February 2021, and followed-up to April 30th 2022. Patients were tested for AbR at multiple timepoints. Primary endpoint was BI (laboratory confirmed SARS-CoV2 infection ≥14 days after 2nd dose). Immunization (positive AbR) was considered an intermediate state between vaccination and BI. Probabilities of being in vaccination, immunization and BI states were obtained for each type of graft and vaccination sequence with multistate survival analysis, then multivariable logistic regression was performed to analyse the risk of BI in AbR levels.

Results.: 614 SOT (275 kidney, 163 liver, 137 heart, 39 lung) recipients were included. Most patients (84.7%) received three vaccine doses, the first two consisted of BNT162b2 and mRNA-1273 in 73.5% and 26.5% of cases, respectively; while at the third dose mRNA-1273 was administered in 59.8% of patients. Overall, 75.4% of patients reached immunization and 18.4% developed BI. Heart transplant recipients showed lowest probability of immunization (0.418) and highest of BI (0.323), all-mRNA-1273 vaccine-sequence showed higher probability of immunization (0.732) and lowest of BI (0.098). Risk of BI was higher for non-high-level AbR, younger age and shorter time from transplant.

Conclusions*:* SOT patients with non-high-level AbR, shorter time from transplantation, and heart recipients are at highest risk of BI.

Keywords: SARS-CoV2 infection, COVID-19, vaccination, mRNA vaccines, serology, antibody-response, solid organ transplantation, multistate model

INTRODUCTION

Immune response to SARS-CoV-2 vaccines, as well as their clinical effectiveness, have been shown to be lower in solid organ transplant (SOT) recipients compared to general population (1–4). Indeed, immune monitoring in this setting has been proposed by some experts (5) in order to identify patients at higher risk of infection in which other preventive strategies (i.e. pre-exposure monoclonal antibodies, booster dosages associated with temporary reduction of immunosuppressive treatment) could be implemented.

However, several controversies surround the practice of antibody testing in SOT recipients including the lack of an established antibody threshold associated with protection in patients on

long-term immunosuppression, and the potential role of cellular response (5). Indeed, studies investigating the relationship between the presence and level of antibody response with the rate and severity of SARS-CoV-2 infection in SOT recipients are limited.

To fill this gap, in the current prospective longitudinal study we aimed to assess the rate and severity of SARS-CoV2 breakthrough infections (BIs) and their relationships with antibody response (AbR) in fully vaccinated SOT recipients.

MATERIAL AND METHODS

Study design

CONTRAST is a single-center prospective longitudinal cohort study of SOT recipients who underwent SARS-CoV2 vaccination within the Horizon 2020 ORCHESTRA project (https://orchestra-cohort.eu/).

Recruitment period was of one year starting from February 1st 2021 corresponding to the onset of vaccination campaign in fragile patients in the Emilia-Romagna (Italy) region. All patients were followed-up until April 30th 2022, and the minimum follow-up period per patient was of 1 month after the last vaccination dose.

Clinical charts and hospital electronic records were used as data sources. Data were recorded anonymously and managed using REDCap electronic data capture tools hosted at University of Bologna (6). The study database was locked on May 15th 2022 after a careful revision of missing and/or incongruent data.

The study was approved by the local institutional review board (n° 167/2021/Oss/AOUBo on March 12th, 2021). Informed consent was obtained before patients were enrolled.

Setting

The IRCCS Azienda Ospedaliero-Universitaria di Bologna is a 1400-bed tertiary teaching hospital with four active transplant programs: kidney, liver, heart and lung with an average volume of transplantation of 120, 90, 25 and 10 per year, respectively.

Participants

All adult (≥18 years) SOT recipients who received ≥2 dosages of SARS-CoV2 vaccines, or 1 dosage in patients with prior history of infection during the recruitment period and provided consent to participate in the study.

Variables

Primary endpoint was diagnosis of breakthrough infection (BI) defined as the detection of SARS-CoV-2 RNA or antigen in a respiratory specimen collected ≥14 days after the administration of second vaccine dosage (https://www.cdc.gov/coronavirus/2019-ncov/vaccines/effectiveness/why-measure-effectiveness/breakthrough-cases.html). Diagnostic testing for SARS-Cov2 infection was performed according to local policy (generally active infection was ruled out in all patients before being visited at day care or outpatient facilities, and twice weekly in hospitalized patients) and clinical judgment and was not dictated by study protocol. Information about BI was retrieved during the routine hospital visits and in periodical phone and/or email interviews, when patients were asked about self-administered tests, symptoms, or exposure to positive subjects which occurred since the last contact. Data on clinical severity according to WHO criteria (https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum), hospitalization and death were collected for all patients diagnosed with BI.

AbR was assessed at multiple timepoints scheduled from, and including, the administration of first dosage (t_0), second dosage (t_1), 3±1 months after first dosage (t_2), and 6±2 months after first dosage (t_3). Since, during the study period, administration of booster dosages in SOT recipients was recommended, and most patients received a third dosage within 4-5 months after first dosage, t_3 included also patients assessed at 1 month after third dosage. AbR was determined by Elecsys® Anti-SARS-CoV2 ECLIA assay (Roche Diagnostics AG, Rotkreuz, Switzerland). Minimum and maximum thresholds for detection of anti-RBD antibody levels were 0.4 and 2500 UI/mL, respectively. Positive AbR was defined as an anti-rapid binding domain (RBD) titer of ≥5 UI/ml, as previously described (7). The positive AbR was stratified into very low (5.58-<45 UI/mL), low (45-204), medium (205-<817 UI/mL), and high level (>817 UI/mL) following the WHO reference panel for anti-SARS-Cov2 immunoglobulin (WHO/BS/2020.2403). The last available determination of AbR before the diagnosis of BI or at the end of follow-up was considered.

Data was also collected on: sex, age, comorbidities according to Charlson score (active underlying diseases, other than that related to the end-stage-organ failure requiring transplantation, were considered as active comorbidities), type of transplant and time from transplant to first vaccine dosage administration, basal (t_0 or t_1) immune parameters including lymphocyte subpopulations and IgG levels, type of each vaccine dosage administration, exposure to an induction regimen within 6 months before the administration of the last vaccine dosage, immunosuppressive drugs (calcineurin inhibitors, anti-metabolites, mTOR inhibitors, steroids) at the time of the last vaccine dosage administration, and graft function.

Statistical analysis

Patients' characteristics represented by categorical variables were described as absolute numbers and percentages, continuous variables were presented as mean ± standard deviation (SD) if normally distributed, or as median and interquartile range (IQR) if non-normally distributed.

Multistate survival analysis was conducted considering AbR as an intermediate state between vaccination and BI, thus building separate models for each one of the three transitions: vaccination-immunization, vaccination-infection, immunization-infection. For immunization, the outcome was the time from the first vaccine dose administration to the first time that a positive antibody response was found. For breakthrough infection, the outcome was the time from the first vaccine dose administration or from the first positive antibody response to the time of the first BI. In the case that no event occurred, patients remained in the vaccination state and were censored at the date of their last follow-up. The best fitting survival model was chosen among Cox, exponential, Gompertz, gamma, loglogistic, lognormal, Weibull, and Royston-Parmar models, based on the lowest values of AIC and BIC. Multivariable models with type of graft or vaccination pattern as exposure were built, including as covariates the main predictors of immunization and BI, and as time-varying variables those that did not satisfy the proportionality of hazards assumption. The probabilities of being in each state during one year of follow-up since vaccination were then obtained for each type of graft and vaccination sequence. Finally, a multivariable logistic regression was carried out to identify the prediction role of AbR, type of graft and vaccination sequence on breakthrough infection, adjusting for clinical covariates. Multistate and logistic regression analyses were conducted on the same subset of patients who were administered three vaccine dosages and with complete data on all covariates. Stata v.17 was used for all analyses; specifically, the merlin package was used for multistate modeling (8).

RESULTS

Characteristics of study cohort

Overall, 614 patients were analyzed (Strobe Flow Chart at Supplementary Figure 1), of them 213 (34.7%) were female, mean (±SD) age was 57.3±13.6 years (Table 1). The distribution of SOT type was kidney (n=275, 44.8%), liver (n=163, 26.5%), heart (n=137, 22.3%) and lung (n=39, 6.4%). All patients received mRNA-based vaccines. The median time from SOT to the first dosage was 6.82 [IQR: 3.12-12.89] years. Primary vaccine series consisted of BNT162b2 and mRNA-1273 in 73.5% and 26.5% of patients, respectively. The median duration of follow-up was 260 days (interquartile range: 226-281 days), and the 5th percentile was of 82 days. At the end of follow-up, the majority of patients (n=520, 84.7%) had received three vaccine dosages. In these patients, mRNA-1273 was frequently used for the third dosage (n=307, 59.8%). History of SARS-CoV2 infection prior to vaccination or between the first two vaccine dosages was present in 19 patients, additionally 12 had positive serology at t_0.

Antibody response assessment

In 94 patients (15.3%) the last available AbR assessment was after 2 dosages and in 520 (84.7%) after three dosages. Mean titers of antibody levels at each time point are shown in supplemental Figure 2. Overall, positive AbR was observed in 463 (75.4%) patients. In patients with positive AbR, anti-RBD levels were classified as very low, low, medium and high level in 47 (10.1%), 50 (10.6%), 51 (10.8%) and 317 (68.5%), respectively. The comparison of patients with positive and negative AbR showed that positive AbR rates were significantly higher in patients with longer time from transplantation, liver transplant recipients, and in those who received third dosage. Conversely, positive AbR rates were significantly lower in heart transplant recipients, in patients with comorbidities, and in those receiving mycophenolate and/or steroids (Table 1). In patients with available baseline immune parameters, mean CD4 count and mean IgG level were significantly higher among patients with positive AbR (Table 1).

Description of breakthrough infections

Overall, 113 (18.4%) patients were diagnosed with BI within a median of 294 (IQR 273-325) days after t_0. Of them, 92 (81.4%) had received three vaccine dosages, and 80 (70.8%) had positive AbR.

Infection was classified as asymptomatic/mild, moderate, and severe/critical in 80.2%, 14.3% and 5.5% of cases, respectively. Hospitalization occurred in 15/113 (16.5%) patients, death in 3/113 (1.9%). Admitted patients were older (63.7±9.3 vs. 50.9±14.1, p=0.001), reported a higher rate of comorbidities (60.0% vs. 32.9%, p=0.047), had received third dosage less frequently (66.7% vs. 86.8%, p=0.054) and showed a lower rate of positive AbR (33.3% vs. 77.6%, p=0.001) compared with non-hospitalized patients (data shown in supplementary Table 1).

Patients who developed BI compared to those without BI (Table 2) were younger (52.4±14.1 vs 58.4±12.7 years, p<0.001) and showed a shorter median time from transplantation (4.69 vs. 7.22 years, p<0.001). They were also administered at higher rates mycophenolate (63.7% vs. 50.7%, p=0.012) and steroids (74.3% vs. 63.9%, p=0.034). A non-significant lower proportion of positive AbR (70.8% vs 76.4%, p=0.208) was observed among patients with BI. In patients with available immune parameters, mean CD4 count was not significantly lower in patients with BI (434±255 vs. 604±413, p=0.051), while mean IgG levels were higher in BI compared to non-BI patients (1123±366 vs. 989±268, p=0.040).

Multistate analysis

The multistate analysis showed that, starting from the 614 vaccinated patients, 463 were found to have a positive AbR, 33 recorded BI without being previously AbR positive, and 118 did not experience any event; 80 AbR positive patients developed a subsequent BI. Thus, at the end of follow-up, immunization was the most frequent state, including 383/614 patients (Supplementary Figure 3).

The multistate survival models were carried out on 505 patients, after excluding 94 patients who did not receive the third vaccine dosage, 13 patients who were found AbR positive at or before the date of the first vaccination, and 2 who had missing data on the type of vaccine administered. In addition to the exposure (type of graft or type of vaccine) the following covariates were included: age at first vaccination, gender, time from transplant to first vaccination, and the indicators of use of mycophenolate, steroids and calcineurin inhibitors. With regards to the type of graft, patients with heart transplant were estimated having the lowest probability of being in the immunized state (0.418) and, conversely, with the highest probabilities of being in the vaccination (0.259) and in the BI (0.323) states. The lowest probability of BI was estimated for patients with liver transplant (0.093), who had the highest probability of being in the immunization status (0.729) (Figure 1, panel A; supplementary Table 2). The state occupation probabilities were less differentiated among vaccine sequence groups: patients who received mRNA-1273 at all three doses showed the highest probability of being in the immunization state (0.732) and the lowest probability of being in the BI state (0.098) (Figure 1, panel B; supplementary Table 2).

Multivariable logistic regression analysis

From multivariable logistic regression, patients who achieved medium level antibody response resulted at higher risk of BI than patients with high response (OR=2.447, p=0.040, Table 3). High risk of BI was also observed in patients classified as having very low (OR=1.971, p=0.164) and low (OR=1.849, p=0.176) AbR. Possibly, the OR of these groups did not reach statistical significance because of the low number of patients (further reduced to n=37 and n=38, respectively, in this multivariable model because of missing data in the covariates). Other factors associated with BI were younger age (OR=0.970, p=0.004) and shorter time elapsed from transplant to vaccination (OR=0.946, p=0.014).

DISCUSSION

This is the first study assessing the relationship between antibody response and breakthrough infection after three dosages of mRNA SARS-CoV-2 vaccines in a large prospective longitudinal cohort of SOT recipients using a multistate model analysis. The rates of positive AbR and of BI were of 75.4% and 18.4%, respectively. Patients with heart transplant were estimated to have the lowest probability of reaching positive AbR and the highest of developing BI. For the type of vaccine manufacturer, patients who received mRNA-1273 at all three dosages showed a slightly higher probability of reaching immunization and the lowest of developing BI. Surprisingly, younger patients resulted at increased risk of BI; while, as expected, compared to high levels, very low, low and medium levels of AbR were associated with increased risk of BI, as well as shorter time from transplant to vaccination onset.

In a multicentre longitudinal cohort study of 1467 SOT recipients from United States, 150 (10.2%) patients reported a BI during the Omicron wave, with 11 (7.3%) hospitalizations and 2 (1.3%) deaths (9). Among SOT recipients with serological available data, 96 of 666 (14.4%) patients were seronegative, 24% had medium-level AbR (anti-RBD 250–2500 U/mL) and 47% had high-level AbR (anti-RBD >2500 U/mL). Rates of seronegative status were higher, although at not significant level, among patients with BI (21.6% vs. 14%), as well as that of high-level AbR response were lower in BI patients (29.7% vs. 48%) compared to patients without BI; in addition, there was a statistically significant relationship between increasing titer categories and proportion with confirmed infection (Wilcoxon rank-sum, P = 0.006) (9). It's also worth mentioning that in other cohort of SOT recipients with BI, patients with negative or non-high-level AbR had a higher risk of unfavourable outcomes in terms of mortality or hospitalization compared to patients with high-level AbR (10, 11, 12).

In our analysis, in the assessed exposures (type of graft and type of vaccine manufacturer), as expected, the higher the probability of achieving immunization the lower that of developing BI and *vice versa*. As already reported in the literature, liver recipients were confirmed as the group more likely to have positive AbR and with the lowest probability of BI, while heart transplant patients were those with the lowest probability of achieving immunization and the highest of BI. The highest incidence of BI in heart recipients compared to other types of SOT has been already reported in a study including 4776 SOT recipients with BI from US (13). These findings could be explained by the different distribution of age, gender, comorbidities, and immunosuppressive regimens administered in heart transplant patients compared to the other SOT recipients (Supplementary table 3).

The independent risk factors for BI were younger age, shorter time from transplant to vaccination onset, and medium level of antibody response. The younger age underlines an issue rarely explored in clinical studies on the risk of BI in in fragile patients, that is the role of social distancing, occupational activities as well as the compliance with mask wearing and hand hygiene (14). However, it should be underlined that older age was associated with an increased risk of severe BI requiring hospitalisation. Surprisingly, at multivariate analysis immunosuppressive drugs were not associated with an increased risk of BI, as reported from other authors mainly for the use of mycophenolate and steroids (13). Finally, confirming the increased risk of BI among patients with poor AbR in SOT recipients, we bring an argument in favour of the controversial role of serology monitoring in this setting (5).

Some limitations of our study should be considered. First, the single centre design may limit generalizability of our results. This is primarily true for lung transplantation, due to low number of patients included with such type of transplant. Second, due to the censored structure of the detection thresholds of antibody levels, we refrained from investigating antibody level as quantitative variable. Third, we did not assess cellular immune response which may play a role in this framework. Furthermore, genotype analysis of the viral variant was not performed in all enrolled patients during the study period, thus we could not assess if it had a role in the transition

from vaccination to immunization and/or BI. However, as the majority of BI events occurred between December 2021 and April 2022, when the Omicron variant started to predominate in our Region, we may consider our findings as mostly applicable in SOT recipients exposed to Omicron variants. Since breakthrough infections in patients' subgroups were limited to few cases, some estimates may lack robustness, thus further studies on larger numbers of patients are advocated to corroborate our findings. Finally, we were able to collect baseline immune parameters in a limited number of patients. Our findings that low CD4 count could be associated to poorer AbR and to increased susceptibility to BI, while lower IgG levels were associated with poorer AbR but not with an increased rate of BI should be corroborated by further larger studies.

To conclude, our results support the practice of immune-monitoring in SOT recipients in order to identify patients at highest risk of BI, and confirm the need of adjunctive strategies in heart transplant recipients, patients recently undergoing SOT and in those with evidence of low/medium level AbR. Further studies are needed to confirm our results, and in particular to link the role of social behaviors in the transition from vaccination to immunization and/or BI.

ACKNOWLEDGEMENTS: CONTRAST study group: Michela Di Chiara, Maria Eugenia Giacomini, Oana Vatamanu, Clara Solera Horna, Caterina Campoli, Michele Bartoletti, Linda Bussini, Fabio Trapani, Luciano Attard, Milo Gatti, Antonio Gramegna, Gaetano La Manna, Valeria Grandinetti, Marcello Demetri, Simona Barbuto, Chiara Abenavoli, Giovanni Vitale, Laura Turco, Matteo Ravaioli, Matteo Cescon, Valentina Bertuzzo, Paola Messina, Alessandra Trombi, Marco Masetti, Paola Prestinenzi, Mario Sabatino, Laura Giovannini, Aloisio Alessio, Antonio Russo, Maria Francesca Scuppa, Giampiero Dolci, Gianmaria Paganelli, Liliana Gabrielli, Matteo Pavoni, Marta Leone, Federica Lanna.

FUNDING SOURCES: The study was funded within the ORCHESTRA project from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167 and from the Ricerca Corrente 2022 funding programme of the Italian Ministry of Health (N. RC-2022-2774290). Natascia Caroccia also reports support for this work in the form of a research grant for management and coordination of the project activities such as scheduling the patient visits and the blood withdrawals for the study-specific bio-sampling, coordinating the personnel (clinicians, nurses and biologists) involved in the study and data management (data collection, data entry, data query resolution, data export). Chiara Gamberini also reports support for this work in the form of a research grant for performing of serological tests. Francesca Fanì reports support for this work in the form of management and coordination of the project activities such as scheduling the patient visits, the blood withdrawals for the study-specific bio-sampling and coordinating the personnel (clinicians,nurses and biologists) involved in the study. Data management (data collection, data entry, data query resolution, data export).

CONFLICT OF INTERESTS: Natascia Caroccia also reports grants or contracts unrelated to this work for management and coordination of the project activities such as scheduling the patient visits and the blood withdrawals for the study-specific bio-sampling, coordinating the

personnel (clinicians, nurses and biologists) involved in the study and data management (data collection, data entry, data query resolution, data export). Giorgia Comai reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Alexion, Novartis, and Hansa. Chiara Gamberini also reports grants or contracts unrelated to this work in the form of a research grant for performing of serological tests. Maddalena Giannella reports participation in advisory boards on Nirmatrelvir/Ritonavir (Pfizer). Luigia Scudeller reports support for attending meetings and/or travel from ESCMID for ECCMID 2018-ECCMID 2022. Pierluigi Viale reports consulting fees from Biomerieux, Mundipharma, Astrazeneca, Tillots Pharma, Gilead, Shionogi, Sobi, Advanzpharma, MSD, Angelini, Pfizer. The authors have no other conflicts of interest regarding the subject of this manuscript.

REFERENCES

1. Manothummetha K, Chuleerarux N, Sanguankeo A, Kates OS, Hirankarn N, Thongkam A, et al. Immunogenicity and Risk Factors Associated With Poor Humoral Immune Response of SARS-CoV-2 Vaccines in Recipients of Solid Organ Transplant: A Systematic Review and Meta-Analysis. JAMA Netw Open. 2022 Apr 1;5(4):e226822.
2. Kwon JH, Tenforde MW, Gaglani M, Talbot HK, Ginde AA, McNeal T, et al. mRNA Vaccine Effectiveness Against COVID-19 Hospitalization Among Solid Organ Transplant Recipients. J Infect Dis. 2022 Apr 6;jiac118.
3. Giannella M, Pierrotti LC, Helanterä I, Manuel O. SARS-CoV-2 vaccination in solid-organ transplant recipients: What the clinician needs to know. Transpl Int. 2021 Oct;34(10):1776–88.
4. Galmiche S, Luong Nguyen LB, Tartour E, de Lamballerie X, Wittkop L, Loubet P, et al. Immunological and clinical efficacy of COVID-19 vaccines in immunocompromised populations: a systematic review. Clin Microbiol Infect. 2022 Feb;28(2):163–77.
5. Werbel WA, Segev DL. SARS-CoV-2 antibody testing for transplant recipients: A tool to personalize protection versus COVID-19. Am J Transplant. 2022 May;22(5):1316–20.
6. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019 Jul;95:103208.
7. Giannella M, Righi E, Pascale R, Rinaldi M, Caroccia N, Gamberini C, et al. Evaluation of the Kinetics of Antibody Response to COVID-19 Vaccine in Solid Organ Transplant Recipients: The Prospective Multicenter ORCHESTRA Cohort. Microorganisms. 2022 May 12;10(5):1021.
8. Crowther MJ. merlin—A unified modeling framework for data analysis and methods development in Stata. The Stata Journal. 2020 Dec 1;20(4):763–84.
9. Alejo JL, Chiang TPY, Bowles Zeiser L, Kim J, Mitchell J, Avery R, et al. Incidence and Severity of COVID-19 Among Vaccinated Solid Organ Transplant Recipients During the Omicron Wave. Transplantation. 2022 Sept; 106(9) p e413-e415.
10. Biagio P, Rosa C, Nicola SM, Fabrizio S, Amerigo P, Giulia Z, et al. Serological Response and Clinical Protection of Anti-SARS-CoV-2 Vaccination and the Role of Immunosuppressive Drugs in a Cohort of Kidney Transplant Patients. Viruses. 2022 Sep 2;14(9):1951.
11. Malahe SRK, Hoek RAS, Dalm VASH, Broers AEC, den Hoed CM, Manintveld OC, et al. Clinical characteristics and outcome of immunocompromised patients with COVID-19 caused by the Omicron variant: a prospective observational study. Clin Infect Dis. 2022 Jul 23:ciac571.

12. Hamm SR, Rezahosseini O, Møller DL, Loft JA, Poulsen JR, Knudsen JD, Pedersen MS, Schønning K, Harboe ZB, Rasmussen A, Sørensen SS, Nielsen SD. Incidence and severity of SARS-CoV-2 infections in liver and kidney transplant recipients in the post-vaccination era: Real-life data from Denmark. Am J Transplant. 2022 Jul 8:10.1111/ajt.17141. doi: 10.1111/ajt.17141. Epub ahead of print. PMID: 35801693; PMCID: PMC9349423.
13. Vinson AJ, Anzalone AJ, Sun J, Dai R, Agarwal G, Lee SB, et al. The risk and consequences of breakthrough SARS-CoV-2 infection in solid organ transplant recipients relative to non-immunosuppressed controls. Am J Transplant. 2022 Jun 8;
14. De Lucas Ramos P, García-Botella A, García-Lledó A, Gómez-Pavón J, González Del Castillo J, Hernández-Sampelayo T, et al. Actions and attitudes on the immunized patients against SARS-CoV-2. Rev Esp Quimioter. 2022 Feb;35(1):7–15.

FIGURE LEGEND

Figure 1: Probability of Breakthrough Infection

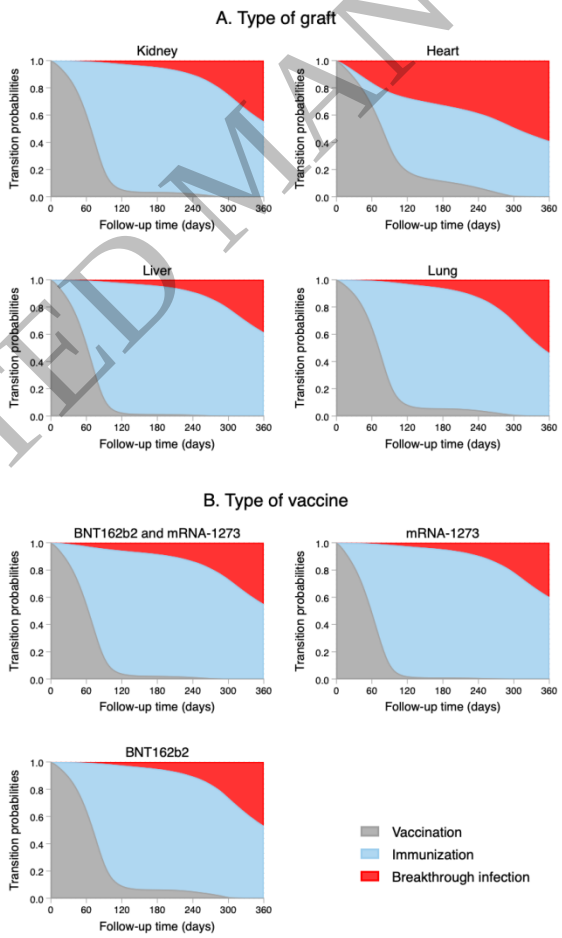

Table 1: Characteristics of the study population and comparison of patients with positive and negative antibody response

N=614 (except where otherwise stated)	Overall	positive (n=463, 75.4%)	negative (n=151, 24.6%)	test; p-value
Age at first vaccine dose (mean ± SD)	57.3±13.6	57.0±13.6	58.1±11.9	0.88; 0.380^
Females	213(34.7)	153(33.1)	60(39.7)	2.25; 0.134
Time from transplant to vaccine 1st dose, median [IQR] (years)	6.82 [3.12-12.89]	7.51 [3.78-13.77]	4.82 [1.68-9.14]	**-4.8; <0.001§**
Type of graft				**46.0; <0.001**
kidney	275(44.8)	205(44.3)	70(46.4)	
liver	163(26.5)	150(32.4)	13(8.6)	
heart	137(22.3)	80(17.3)	57(37.8)	
lung	39(6.4)	28(6.1)	11(7.3)	
Comorbidities	215(35.0)	152(32.8)	63(41.7)	**3.96; 0.047**
Booster	520(84.7)	401(86.6)	119(78.8)	**5.34; 0.021**
Vaccine sequence (n=513)				4.53; 0.104
BNT162b2 - BNT162b2 - BNT162b2	70(13.7)	47(11.9)	23(19.5)	
mRNA-1273 - mRNA-1273 - mRNA-1273	136(26.5)	108(27.3)	28(23.7)	
BNT162b2 - BNT162b2 - mRNA-1273	307(59.8)	240(60.8)	67(56.8)	
Graft function (n=561)				1.68; 0.195
Good	500(89.1)	382(90.1)	118(86.1)	
Impaired and/or failure	61(10.9)	42(9.9)	19(13.9)	
Induction regimen in the last 6 months	9(1.5)	5(1.1)	4(2.7)	0.234*
Immunosuppressive drugs				
Calcineurin inhibitors	592(96.4)	443(95.7)	149(98.7)	2.96; 0.086
Mycophenolate	326(53.1)	213(46.0)	113(74.8)	**38.0; <0.001**
mTOR inhibitors	81(13.2)	64(13.8)	17(11.3)	0.65; 0.419
Steroids	404 (65.8)	277(59.8)	127(84.1)	**29.8; <0.001**
Baseline immune parameters				
CD4 (n=199)	584±401	630±405	420±343	**-3.13; 0.002^**
CD4 >500 (n=199)	101(50.7)	89(57.4)	12(27.3)	**12.5; <0.001**
CD8 (n=209)	556±339	581±331	475±357	-1.90; 0.058^
IgG (n=179)	1004±283	1040±265	881±314	**-3.21; 0.002^**
IgG >600 (n=179)	171(95.5)	137(98.6)	34(85.0)	**0.002***

chi-square test except
* Fisher's exact test
^ t-test

§ Mann-Whitney test

Abbreviations

SD: standard deviation

IQR: interquartile range

mTOR: mammalian target of rapamycin

Table 2. Comparison of patients with and without diagnosis of breakthrough infection

N=614 (except where otherwise specified)	yes (n=113, 18.4%)	no (n=501, 81.6%)	test; p-value
Age at first vaccine dose, mean ± SD	52.4±14.1	58.4±12.7	**4.44; <0.001^**
Females	37 (17.4)	176 (82.6)	0.23; 0.630
Time from transplant to vaccine 1st dose, median [IQR)] (years)	4.69 [2.11-9.24]	7.22 [3.45-13.57]	**3.50; <0.001§**
Type of graft			5.49; 0.139
kidney	60 (21.8)	215 (78.2)	
heart	20 (14.6)	117 (85.4)	
liver	24 (14.7)	139 (85.3)	
lung	9 (23.1)	30 (76.9)	
Comorbidities	40 (18.6)	175 (81.4)	0.01; 0.925
Booster	92 (17.7)	428 (82.3)	1.15; 0.285
Vaccine sequence (n=513)			**0.37; 0.830**
BNT162b2 - BNT162b2 - BNT162b2	13 (18.6)	57 (81.4)	
mRNA-1273 - mRNA-1273 - mRNA-1273	21 (15.4)	115 (84.6)	
BNT162b2 - BNT162b2 - mRNA-1273	53 (17.3)	254 (82.7)	
SARS-CoV-2 infection preceding vaccination	2 (10.5)	17 (89.5)	0.550*
Positive serum test at first vaccine	1 (8.3)	11 (91.7)	0.705*
Positive serum test at last serology test	80 (17.3)	383 (82.7)	1.59; 0.208

Graft function (n=561)			0.04; 0.840
Good	85 (17.0)	415 (83.0)	
Impaired and/or failure	11 (18.0)	50 (82.0)	
Induction regimen in the last 6 months	2 (22.2)	7 (77.8)	0.674*
Immunosuppressive drugs			
Calcineurin inhibitors	107 (18.1)	485 (81.9)	0.267*
Mycophenolate	72 (22.1)	254 (77.9)	6.27; 0.012
mTOR	14 (17.3)	67 (82.7)	0.08; 0.780
Steroids	84 (20.8)	320 (79.2)	4.49; 0.034
Baseline immune parameters			
CD4 (n=199)	434±255	604±413	1.97; 0.051^
CD4 >500 (n=199)	9(37.5)	92(52.6)	1.92; 0.166
CD8 (n=209)	466±221	568±351	1.43; 0.154^
IgG (n=179)	1123±366	989±268	**-2.07; 0.040^**
IgG >600 (n=179)	21(100.0)	150(94.9)	0.599*

chi-square test except
* Fisher's exact test
^ t-test

§ Mann-Whitney test

Abbreviations

SD: standard deviation

IQR: interquartile range

mTOR: mammalian target of rapamycin

Table 3: Multivariable logistic regression analysis of breakthrough infection (n=505)

	OR (95%CI)	p-value
Antibody Response		
negative (<5 lU/mL)	1.053 (0.523-2.118)	0.884
very low (5-44 lU/mL)	1.971 (0.757-5.132)	0.164
low (45-204 lU/mL)	1.849 (0.75-4.504)	0.176
medium (205-817 lU/mL)	**2.447 (1.039-5.763)**	**0.040**
high (>817 lU/mL)	ref.	

Type of graft		
Kidney	ref.	
Liver	0.505 (0.218-1.172)	0.112
Heart	0.666 (0.277-1.601)	0.364
Lung	0.78 (0.305-2.017)	0.616
Vaccine sequence		
BNT162b2 - BNT162b2 - mRNA-1273	ref.	
mRNA-1273 - mRNA-1273 - mRNA-1273	0.608 (0.330-1.11)	0.110
BNT162b2 - BNT162b2 - BNT162b2	1.456 (0.643-3.300)	0.367
Age at first vaccine dose	**0.969 (0.950-0.98)**	**0.003**
Time from transplant to vaccine 1st dose	**0.945 (0.904-0.988)**	**0.013**
Female gender	1.083 (0.634-1.849)	0.770
Calcineurin inhibitors	0.777 (0.165-3.647)	0.750
Mycophenolate	1.335 (0.704-2.532)	0.375
mTOR inhibitors	0.755 (0.294-1.933)	0.558
Steroids	1.186 (0.599-2.350)	0.624
Comorbidities	0.969 (0.560-1.677)	0.913
SARS-CoV-2 infection preceding vaccination	0.404 (0.037-4.388)	0.457
constant	2.055 (0.249-16.959)	0.503

Abbreviations

OR: odds ratio

CI: confidence interval

IU/mL: International Units per milliliter

mTOR: mammalian target of rapamycin