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SARS-CoV-2 vaccine antibody response and breakthrough infections in transplant recipients

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ABSTRACT

Introduction

Rates and modulators of SARS-CoV-2 vaccine non-response and breakthrough infections remain unclear in serially vaccinated transplant recipients.

Materials and methods

In a prospective, mono-centric, observational study, 1878 adult solid organ and hematopoietic cell transplant recipients, with prior SARS-CoV-2 vaccination, were included between March 2021 and February 2022. SARS-CoV-2 anti-spike IgG antibodies were measured at inclusion and details on SARS-CoV-2 vaccine doses and infection were collected.

Results

No life-threatening adverse events were reported after a total of 4039 vaccine doses. In transplant recipients without prior SARS-CoV-2 infection (n=1636), antibody response rates ranged widely, from 47% in lung transplant to 90% in liver transplant and 91% in hematopoietic cell transplant recipients after third vaccine dose. Antibody positivity rate and levels increased after each vaccine dose in all types of transplant recipients. In multivariable analysis, older age, chronic kidney disease and daily dose of mycophenolate and corticosteroids were negatively associated with antibody response rate. Overall rate of breakthrough infections was 25.2% and mainly (90.2%) occurred after third and fourth vaccine dose. Lung transplant recipients had the highest rates of severe breakthrough infection (10.5%) and death (2.5%). In multivariable analysis, older age, daily dose of mycophenolate and corticosteroids were associated with severe breakthrough infection. Transplant recipients with infection prior to first vaccine dose (n=160) had higher antibody response rates and levels after each vaccine dose, and a

significantly lower overall rate of breakthrough infections compared to those without prior infection.

Discussion

Antibody response after SARS-CoV-2 vaccination and rate of severe breakthrough infections vary largely between different transplant types and are modulated by specific risk factors. The observed heterogeneity supports a tailored approach against COVID-19 in transplant recipients.

Registration

The study was registered on Clinicaltrials.gov (NCT04579471).

Key words

SARS-CoV-2, COVID-19, transplantation, transplant, vaccination, infection

INTRODUCTION

Transplant recipients are at increased risk for severe COVID-19 compared to the general population and are prioritized for SARS-CoV-2 vaccination.[1,2] However, the humoral response after SARS-CoV-2 vaccination is suboptimal in this population, with pooled antibody response rates of 45% to 49% after the second dose and 56% to 63% after the third dose respectively.[3-7] But the range of reported antibody responses across studies is high due to variations in their design. Most studies included a limited number of patients, often selected patients based on antibody response to prior vaccination or focused on a specific type of organ transplantation, with kidney transplant recipients being the most studied population.[6,7] In addition, the total number of published studies on the immunogenicity and its modulating factors after the third-dose vaccination remains limited, hampering meaningful conclusions.[6-8]

The rate of breakthrough infections and clinical outcomes in transplant recipients following third and fourth vaccine dose are underreported.[6,9] Risk factors for severe disease, such as increased age and co-morbidities, were identified in studies that were mainly performed in unvaccinated patients, and the number of these studies reporting on non-hospitalized patients with only mild or asymptomatic infection is low.[9,10] Therefore, risk factors for SARS-CoV-2 infection and severe COVID-19 in serially vaccinated transplant recipients remain unclear. In particular, inconsistent data have been published on the potential role of different types of baseline immunosuppressive therapy as a risk factor for vaccine non-response and severe COVID-19.[6,7, 9, 11-16] However, immunosuppressive therapy is the main discriminating factor from non-transplanted individuals, and is a modifiable risk factor.

Adequate insight in the rate and modulators of vaccine non-response and breakthrough infections is essential to tailor strategies aimed at prevention of severe COVID-19 in transplant recipients, such as the use of extra vaccine doses, monoclonal antibodies or antiviral therapy. Importantly, these strategies may differ between different types of solid organ transplant (SOT)

or hematopoietic cell transplant (HCT) recipients given differences in immunosuppressive therapy and co-morbidities which may impact vaccine response and risk for severe COVID-19. In this study, we analysed the antibody response and safety of SARS-CoV-2 vaccination in a large single centre cohort of all types of SOT and HCT recipients, and assessed risk factors associated with vaccine non-response, with a focus on immunosuppressive therapy. Finally, we analysed the rate and severity of SARS-CoV-2 breakthrough infections and associated risk factors in this population.

MATERIALS AND METHODS

Study population and definitions

Transplant recipients were consecutively included between March 1st 2021 and February1st 2022, at the routine outpatient visit to their transplant physician in University Hospitals Leuven. All adult patients (≥ 18 years) who had at least one SARS-CoV-2 vaccine dose and who underwent any SOT or HCT before their first vaccine dose were eligible. After written informed consent, a questionnaire regarding dates and types of SARS-CoV-2 vaccination, local and systemic adverse events per vaccine dose and prior SARS-CoV-2 infection (confirmed with a test) was collected. In all included patients, SARS-CoV-2 anti-spike (anti-S) IgG antibody and SARS-CoV-2 anti-nucleocapsid (anti-N) IgG antibody measurements were performed at the time of inclusion.

In line with the general population in Flanders - Belgium, SARS-CoV-2 vaccination of our transplant population was coordinated and administered out-of-hospital by the governmental health authorities. Time periods per vaccine dose, the evolution of SARS-CoV-2 variants of concern in Belgium since January 2021 and the study milestones are presented in Fig. 1. Reported dates and types of the different doses of SARS-CoV-2 vaccination of every included patient were cross-checked via *Vaccinnet*. This is a registration tool developed by the Flemish government where dates and types of all administered vaccines are automatically documented and linked to the hospital electronic medical file of the patient (https://www.vaccinnet.be).

Patient demographics, transplant history and following comorbidities were collected from their electronic medical file: diabetes mellitus (patient under glucose lowering treatment), chronic kidney disease (CKD) (eGFR of < 60 mL/min/1·73m^2 during > 6 months), cirrhosis, human immunodeficiency virus infection, and solid and/or hematological malignancies at the time of

inclusion (ongoing chemo- or immunotherapy or malignancy in a non-curable stage). Type, dose and trough levels of immunosuppressive therapy were collected from the electronic medical file and were measured at each patient visit to the hospital in the context of standard care.

Breakthrough SARS-CoV-2 infections were defined as infections confirmed with a positive RT-PCR (via nasopharyngeal swab and/or bronchoalveolar lavage (BAL)) and/or positive SARS-CoV-2 anti-N IgG antibody and/or self-reported positive antigen self-test after the first vaccine dose and were included till May 1st, 2022. In case of hospitalization due to COVID-19, the need for oxygen supplementation, admission to the intensive care unit and death were collected as outcome parameters. Severe COVID-19 was defined as the need for oxygen supplementation (oxygen saturation < 90% on room air) [17], intensive care unit admission or death. SARS-CoV-2 infection *before* SARS-CoV-2 vaccination was defined as documentation of any positive RT-PCR test (via nasopharyngeal swab and/or via BAL) and/or any positive SARS-CoV-2 IgG antibody (anti-S and/or anti-N) and/or self-reported positive antigen-self-test prior to first vaccine dose. The majority (n=1407) of included transplant recipients had at least one SARS-CoV-2 anti-N and/or anti-S IgG antibody analysis in the pre-vaccination period in the context of our epidemiological COVID-19 study in unvaccinated transplant recipients (in preparation). Results of prior SARS-CoV-2 RT-PCR and antibody analyses were collected from the electronic medical file.

The study was conducted at the University Hospitals of Leuven, approved by the Ethics Committee of University Hospitals Leuven (S64036) and registered on Clinicaltrials.gov (NCT04579471). The study was conducted in accordance with the ethical principles of the declaration of Helsinki.

Antibody assays

SARS-CoV-2 anti-N and anti-S IgG analyses were performed directly after blood collection by the department of Laboratory Medicine of the University Hospitals Leuven using Abbot's SARS-CoV-2 IgG anti-N and IgG II Quant anti-S chemiluminescent immunoassays (CLIA) on the Architect I2000SR (Abbot, USA). In line with the manufacturer's recommendations, for anti-S IgG a value of ≥ 50 arbitrary units per ml (AU/ml) and for anti-N IgG signal / cut off (S/CO) value of ≥ 1.40 were considered positive.

Statistical analysis

All analyses were performed and presented separately for transplant recipients who were infected by SARS-CoV-2 or not before the first vaccine dose. Continuous variables are presented as mean and standard deviation. Categorical variables are presented as numbers and percentages. The marginal proportion of positive anti-S IgG (≥ 50 AU/ml) measurements after each vaccine dose was determined from a random intercept logistic regression model with the vaccine dose number (1, 2 or 3) as categorical variable in the model.[18] In case of several measurements in the same patient between two vaccine doses, the measurement with the highest level of antibodies was taken for the analysis. Measurements after a breakthrough infection were no longer taken into account in the antibody response analyses. The relation of several co-variates with a positive anti-S IgG measurement after second and third vaccine dose was analyzed using a logistic random effects model. The relation of co-variates with breakthrough infections and severe COVID-19 were analyzed using a logistic regression model. Detailed description can be found in the Method section in the supplemental material. All statistical analyses were performed using SAS/STAT software, version 9.4 for Windows.

Role of the funding source

The study was supported by an internal COVID-19 fund of the University Hospitals Leuven (KOOR). The funding source had no involvement in the study process and the decision to submit the paper for publication.

RESULTS

Study population

A total of 1878 transplant recipients were included. Patients with transplantation after receiving a first vaccine dose (n=67), unclear timing of SARS-CoV-2 infection in relation to vaccination (n=10), age < 18 years (n=3) and isolated small bowel transplantation (n=2) were excluded from analyses.

Characteristics of transplant recipients without a SARS-CoV-2 infection before first vaccine dose (n=1636) can be found in Table 1. Co-morbidities were more frequent in SOT recipients (57% CKD and 28.9% diabetes mellitus) than in HCT recipients. Tacrolimus, followed by mycophenolate, were the most commonly used immunosuppressive agents. Amongst SOT recipients, liver transplant recipients had the lowest tacrolimus trough levels and lowest overall use and daily dose of mycophenolate (Table 1). The rate of corticosteroid use highly varied between different transplant types: 99.2% in lung and 63.3% in kidney transplant recipients and only 16.4% in heart and 8.3% in liver transplant recipients.

At the first and second dose respectively, BNT162b2 (Pfizer BioNTech) (1085/1636 (66.3%) and 1121/1630 (68.8%)) and ChAdOx1 (AstraZeneca) (373/1636 (22.8%) and 364/1630 (22.3%)) were the most administered vaccine types. The majority of transplant recipients received BNT162b2 (Pfizer BioNTech) as the third and fourth vaccine dose (1495/1573 (95.0%) and 1232/1356 (90.9%)) respectively) (see supplemental Table S2).

Characteristics of the 160 transplant recipients who had a SARS-CoV-2 infection before first vaccine dose are listed in Table S4 in the supplemental material.

Adverse events after SARS-CoV-2 vaccine doses

Local pain at the injection site was the most frequent adverse event after each vaccine dose (total n=4039), followed by fatigue, myalgia and headache (see supplemental Table S5). After first vaccine dose, two transplant recipients were admitted to the hospital because of nausea (n=1) and generalized weakness (n=1). After second vaccine dose, one patient was admitted to the hospital because of cough (CT lung negative) and asthenia (without neurological deficit) that quickly resolved spontaneously. We did not observe any life-threatening adverse events associated with SARS-CoV-2 vaccination.

Antibody response after SARS-CoV-2 vaccine doses

In transplant recipients without prior infection, median interval between the first, second and third vaccine dose and the corresponding post-vaccine antibody analysis were 23, 39 and 51 days, respectively (see supplemental Fig. S1 & supplemental Table S6). Rates of positive antibody response after each vaccine dose per transplant type are presented in Fig. 2A. Lung transplant recipients demonstrated the lowest antibody response rate (22% and 47% after second and third dose, respectively) and liver transplant recipients had the highest antibody response rate (77% and 90% after second and third dose, respectively) amongst SOT recipients. HCT recipients had antibody response rates of 78% and 91% after second and third dose, respectively. The antibody positivity rate increased after each vaccine dose in all types of transplant recipients. In the subgroup of transplant recipients who had no antibody response after the first vaccine dose and had an antibody measurement after the second dose, 33.3% became antibody positive after the second dose. In the subgroup of transplant recipients who had no antibody response after the second vaccine dose and had an antibody measurement after the third dose, 43.4% became antibody positive after the third dose (see supplemental Table S7).

In line with antibody positivity rates, antibody levels increased after each vaccine dose and varied between different types of transplant recipients, with the lowest antibody levels in lung transplant recipients (Fig. 2B).

Transplant recipients who had a SARS-CoV-2 infection prior to the first vaccine dose had higher pooled antibody positivity rates of 86% after the second vaccine dose ($P < 0.0001$) and 88% after the third vaccine dose ($P = 0.0008$), and higher absolute antibody levels ($P < 0.0001$ after each dose) compared to transplant recipients without prior infection (see supplemental Fig. S2).

Risk factors SARS-CoV-2 vaccine non-response

In transplant recipients without SARS-CoV-2 infection prior to first vaccine dose, univariable analyses of factors associated with antibody response after the second and after the third vaccine dose are listed in Table 2. In the multivariable analysis, number of vaccine doses and interval between last transplant and first vaccine dose were positively associated with antibody response, while older age, CKD, and daily dose of mycophenolate and daily dose of corticosteroids were negatively associated with antibody response (Table 2). No independent role of the other immunosuppressive drugs was found.

Timing, rate and severity of breakthrough infections and associated risk factors

In transplant recipients without prior SARS-CoV-2 infection, overall rate of breakthrough infections was 25.2% during the study period (Fig. 3A) and mainly occurred after the third (46.7%) or fourth (43.5%) vaccine dose (see supplemental Table S8 and Fig. 1). Liver transplant recipients had a relatively lower breakthrough infection rate of 10.1% compared to other types of transplant recipients. Univariable and multivariable analyses of factors associated

with breakthrough infection are listed in supplemental Table S9. Transplant type, younger age, daily dose of corticosteroids and shorter interval between last transplant and first vaccine dose were independent risk factors for breakthrough infection.

In transplant recipients without prior SARS-CoV-2 infection, overall rate of severe breakthrough infection/COVID-19 was 5.5% (Fig. 3B). However, lung transplant recipients had the highest rates of severe breakthrough infection and death (10.5% and 2.5%, respectively) (Fig. 3B-3D). Univariable and multivariable analyses of factors associated with severe breakthrough infection are listed in Table 3. Older age, daily dose of mycophenolate and daily dose of corticosteroids were independent risk factors for severe breakthrough infection.

In transplant recipients with a SARS-CoV-2 infection before first vaccine dose (n=160), overall rate of breakthrough infection was 12.5% during the study period, which is significantly lower than in transplant recipients without prior infection (P = 0.0002). Severe breakthrough infection occurred in only 4/160 (2.5%) of these recipients, including three lung transplant recipients and one combined heart-kidney transplant recipient, but not in other transplant types (see supplemental Table S10).

DISCUSSION

We found that antibody response after SARS-CoV-2 vaccination varies largely between different types of transplant recipients without prior infection. Rate of antibody response increased after the second and third vaccine dose in every transplant type, while older age, presence of CKD and both daily dose of mycophenolate and corticosteroids were risk factors for lack of antibody response. No independent association between other types of immunosuppressive drugs and antibody response was found. Lung and kidney transplant recipients had poor antibody response rates (47% and 62%, respectively, after three vaccine doses) and have lower antibody levels compared to other transplant types. This might be explained by their higher prevalence of CKD, frequent use of mycophenolate in relatively high doses and their corticosteroid use. Nearly all lung transplant recipients and around 60% of kidney transplant recipients received corticosteroids as baseline immunosuppressive therapy, while this was only the case in a minority of other transplant types. The association between corticosteroid use and lack of antibody response was found despite a low mean daily dose of 4 mg methylprednisolone in our study population. Therefore, discontinuation of low dose corticosteroids in order to increase the antibody response rate after SARS-CoV-2 vaccination in SOT recipients may be worthwhile to study. Liver transplant recipients (who have the lowest level of immunosuppressive drugs amongst SOT recipients) and HCT recipients (of whom only 25% was still under immunosuppressive drugs) had the highest antibody response rates. After 3 vaccine doses, both groups had positive antibody response rates of 90% and 91%, respectively.

We did not observe life-threatening adverse events associated with SARS-CoV-2 vaccination in our study, providing further evidence for the safety of SARS-CoV-2 vaccination in transplant recipients.[12]

The ultimate goal of SARS-CoV-2 vaccination is to reduce the rate and in particular the severity of SARS-CoV-2 infections. Our study shows that despite serial doses of vaccination, breakthrough infections are prevalent in transplant recipients and occurred mostly (in more than 90% of all infections) after the third and fourth vaccine dose. Contributing factors to breakthrough infections are the high transmissibility of the omicron variant carrying multiple spike-protein mutations that allow for immune escape from neutralizing antibodies[19-21], the risk compensation behavior amongst transplant recipients fueled by the feeling of protection after vaccination and less strict measures by Belgian authorities compared to the first waves in 2020.[9,22] Younger age, daily dose of corticosteroids and shorter interval between last transplantation and first vaccine dose were independent risk factors for breakthrough infections. These factors might partly explain the lower prevalence of breakthrough infections in liver transplant recipients who were slightly older and were infrequently treated with corticosteroids (8.1% of all liver transplant recipients) compared to other types of SOT recipients. The fact that younger age is associated with an increased risk for breakthrough infection is in line with the study of Bell et al. in kidney transplant recipients and might be related to lower adherence to protective measures and social distancing than older people.

Knowledge of risk factors for severe breakthrough infection/COVID-19 (need for oxygen, intensive care unit admission or death) in serially vaccinated transplant recipients is key to develop preventive and therapeutic strategies. We identified older age, daily dose of mycophenolate and daily dose of corticosteroids as independent risk factors for severe breakthrough infection in our transplant population. While the rate of severe breakthrough infection in liver transplant recipients was the lowest, lung transplant recipients had the highest risk for severe breakthrough infection and subsequent death. This is in line with their intensive immunosuppressive therapy and observed low humoral response after serial vaccination. Other

possible contributing factors are that SARS-CoV-2 is primarily a respiratory virus and increased vulnerability of the transplanted lungs by local changes in their immune defense. Potential differences between T-cell response (cellular immunity) after SARS-CoV-2 vaccination between different transplant types and their impact on protection against severe breakthrough infections should be the topic of further study.

Hybrid immunity resulting from prior infection and recent booster vaccination is known to confer the strongest protection against symptomatic SARS-CoV-2 infection in the general population.[23] Accordingly, vaccinated transplant recipients with infection prior to first vaccine dose had higher antibody positivity rates and antibody levels, and a lower rate and severity of breakthrough infections, compared to vaccinated transplant recipients without prior infection. These data further indicate the heterogeneity amongst transplant recipients.

Our study results support a tailored approach for sub-populations with a higher risk for severe COVID-19. Non-responders to vaccination should be prioritized for additional vaccine doses in order to obtain humoral response, supported by the observed increasing antibody response after each cumulative vaccine dose. An important note is that the T-cell response (cellular immunity) induced by SARS-CoV-2 vaccination is another important aspect of protection against severe COVID-19, which however is not feasible to measure on large scale in order to guide additional measures on individual level. Vaccine non-responders and recipients with high risk for severe COVID-19 can be prioritized for administration of pre-exposure monoclonal antibodies or for clinical trials investigating their efficacy.[24] The disease course in infected transplant recipients with high risk for severe COVID-19 should be followed closely. These patients should be prioritized for post-exposure monoclonal antibodies or antiviral therapy, while these therapies might be of limited benefit against severe COVID-19 in lower risk

transplant recipients.[25-27] From an investigational perspective, clinical trials on therapies against severe COVID-19 should focus on high-risk transplant recipients. Our study clearly demonstrated that lung transplant recipients have the highest risk for severe COVID-19 amongst transplant recipients and therefore deserve special attention in all initiatives regarding COVID-19 care. Finally, our data give context and grip to the individual transplant recipient concerning safety and efficacy of SARS-CoV-2 vaccination and risk estimation for severe disease.

Although we provide a comprehensive analysis on SARS-CoV-2 vaccination and breakthrough infections, in the largest study in transplant recipients to date, our study does have limitations. We did not assess SARS-CoV-2 anti-S IgG antibody level after the fourth vaccine dose due to time and cost restrictions. Although BNT162b2 (Pfizer BioNTech) was the dominant vaccine type in our study, more than one vaccine type was used and we did not assess the effect of a specific vaccine type or the effect of heterologous vaccination. The latter has been demonstrated to increase immunogenicity in immune-competent individuals.[28,29] Neutralizing capacity of SARS-CoV-2 antibodies, cellular immunity and their potential waning were not analyzed. In case of breakthrough infection, we do not have an anti-S IgG antibody analysis after the last vaccine dose before infection of every patient due to the study set-up with out-of-hospital vaccine administration and consecutive inclusion of transplant recipients visiting the hospital within standard care.

In conclusion**,** we provide an extensive framework for all stakeholders to tailor strategies against COVID-19 in the highly heterogeneous group of transplant recipients.

Contributor statement

All authors contributed to the study design. JV conceptualized and supervised the study. BV and CV accessed and verified the data and did the data linkage. KB and GM developed the statistical analysis plan and performed the formal analysis. All authors contributed to data interpretation. KB created the figures. JV wrote the first draft of the manuscript. All authors critically revised and edited the manuscript and approved the final version for submission. All authors (as joint team, coordinated by JV) obtained funding for the study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

None of the authors declares to have a conflict of interest directly relevant to the submitted work. JV received research grants, speaker fee and travel fees from Gilead, travel fees from AbbVie and Dr. Falk Pharma, and consultancy fee from Eisai. KL received consultancy fees from MRM Health, MSD and Gilead, speaker fees from FUJIFILM WAKO, Pfizer and Gilead and a service fee from Thermo fisher Scientific and TECOmedical. FR received an unrestricted research grant from Medtronic and consultancy fees from AtriCure and Corsym. LC received a consultancy fee from Medtronic and a Medtronic university chair on lung volume reduction surgery. DVR is supported by a grant from the Broere Charitable Foundation. IJ is listed as co-inventor of a patent application on methods and applications of analysing the perfusate of an *ex situ* perfused kidney (EP 22155190.6) and received speaker fees from XVIVO Perfusion, paid to her institution. JP is the Chair of the Institut Georges Lopez foundation. TV reports financial support for research from Danone, MyHealth, Takeda and VectivBio, has served on the speaker bureau for Abbott, Falk Pharma, Fresenius Kabi, Menarini, Remedus, Takeda, Truvion, VectivBio, Ipsen, Baxter, MyHealth and Microbiotica, and received consultancy fees from

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TABLES

Table 1. Characteristics of transplant recipients without prior SARS-CoV-2 infection. *(continued on p 25)*

	Kidney	Heart	Liver	Lung	HCT	Combined liver-kidney	Combined other*	Total
Male – n/N (%)	219/365 (60.0%)	211/292 (72.3%)	134/218 (61.5%)	273/517(52.8%)	64/98 (65.3%)	18/43 (41.9%)	61/103 (59.2%)	980/1636 (59.9%)
Age (years) – [n] Mean (SD)	[365] 58 (13)	[292] 57 (16)	[218] 63 (13)	[517] 59 (12)	[98] 58 (14)	[43] 63 (10)	[103] 53 (14)	[1636] 59 (13)
BMI (kg/m²) – [n] Mean (SD)	[339] 25 (5)	[289] 26 (5)	[208] 27(5)	[501] 25 (5)	[96] 25 (4)	[41] 66 (12)	[101] 69 (13)	[1574] 25 (5)
Obesity (BMI>=30 kg/m²) – n/N (%)	39/339 (11.5%)	47/289 (16.3%)	50/208 (24.0%)	69/501 (13.8%)	8/96 (8.3%)	1/40 (2.5%)	11/101 (10.9%)	225/1574 (14.3%)
Co-morbidities at 1st SARS-CoV-2 vaccine dose								
DM – n/N (%)	102/365 (27.9%)	70/292 (24.0%)	76/218 (34.9%)	152/517 (29.4%)	12/97 (12.4%)	7/43 (16.3%)	38/103 (36.9%)	457/1635 (28.0%)
CKD – n/N (%)	240/365 (65.8%)	137/292 (46.9%)	95/218 (43.6%)	300/517 (58.0%)	13/98 (13.3%)	24/43 (55.8%)	53/103 (51.5%)	862/1636 (52.7%)
CKD requiring dialysis – n/N (%)	3/365 (0.8%)	8/292 (2.7%)	2/218 (0.9%)	13/517 (2.5%)	0/98 (0.0%)	0/43(0.0%)	2/103(1.9%)	28/1636 (1.7%)
Cirrhosis – n/N (%)	3/365 (0.8%)	2/292 (0.7%)	6/218 (2.8%)	5/517 (1.0%)	0/98 (0.0%)	0/43 (0.0%)	0/103 (0.0%)	16/1636 (1.0%)
HIV – n/N (%)	2/365 (0.5%)	0/292 (0.0%)	1/218 (0.5%)	0/517 (0.0%)	0/98 (0.0%)	0/43 (0.0%)	0/103 (0.0%)	3/1636 (0.2%)
Malignancy – n/N (%)	6/365 (1.6%)	2/292 (0.7%)	5/218 (2.3%)	6/517 (1.2%)	3/98 (3.1%)	0/43 (0.0%)	6/103 (5.8%)	28/1636 (1.7%)
Immunosuppressive therapy at 1st SARS-CoV-2 vaccine dose								
Tacrolimus – n/N (%)	302/365 (82.7%)	250/292 (85.6%)	200/218 (91.7%)	471/517(91.1%)	9/98 (9.2%)	39/43 (90.7%)	94/103 (91.3%)	1365/1636 (83.4%)
Tacrolimus trough level (µg/L) – [n] Mean (SD)	[302] 7 (2)	[250] 7 (2)	[199] 5 (2)	[470] 7 (2)	[8] 5 (3)	[39]7(1)	[94]7(2)	[1362] 7 (2)
Cyclosporin – n/N (%)	45/365 (12.3%)	36/292 (12.3%)	12/218 (5.5%)	42/517(8.1%)	3/98 (3.1%)	4/43 (9.3%)	6/103 (5.8%)	148/1636 (9.0%)
Cyclosporin trough level (µg/L) – [n] Mean (SD)	[45] 109 (34)	[36] 125 (28)	[12] 53 (20)	[42]139(56)	[1] 137 (NA)	[4] 80 (30)	[6] 112 (32)	[146] 117 (45)

Mycophenolate –n/N (%)	259/365 (71.0%)	234/292 (80.1%)	95/218 (43.6%)	268/516(51.9%)	1/98 (1.0%)	34/43 (79.1%)	61/103 (59.2%)	952/1635 (58.2%)
Mycophenolate daily dose (mg) – [n] Mean (SD)	[259] 957 (382)	[234] 1254 (579)	[95] 611 (218)	[268]1111(610)	[1] 2000 (NA)	[34]676 (292)	[61]939 (438)	[952] 1029 (534)
mTOR inhibitors – n/N (%)	7/365 (1.9%)	21/292 (7.2%)	38/218 (17.4%)	16/517(3.1%)	0/98 (0.0%)	1/43 (2.3%)	10/103 (9.7%)	93/1636 (5.7%)
mTOR inhibitors through level (µg/L) – [n] Mean (SD)	[7] 7 (2)	[21] 6 (2)	[38] 3 (1)	[16]5(1)	[0]	[1] 5 (NA)	[10] 6 (8)	[93] 5 (3)
Corticosteroids – n/N (%)	231/365 (63.3%)	48/292 (16.4%)	18/218 (8.3%)	513/517(99.2%)	24/98 (24.5%)	13/43 (30.2%)	77/103 (74.8%)	924/1636 (56.5%)
Corticosteroids daily dose (mg) – [n] Mean (SD)	[231] 4 (2)	[48] 3 (2)	[18] 5 (4)	[513]5(3)	[24] 5 (5)	[13]3(1)	[77]4(1)	[924] 4 (3)
Azathioprine – n/N (%)	23/365 (6.3%)	9/292 (3.1%)	8/218 (3.7%)	129/517(25.0%)	0/98 (0.0%)	0/43 (0.0%)	13/103 (12.6%)	182/1636 (11.1%)
Other immunosuppressive medication** – n/N (%)	11/365 (3.0%)	2/292 (0.7%)	8/218 (3.7%)	7/517(1.4%)	19/98 (19.4%)	1/43 (2.3%)	3/103 (2.9%)	51/1636 (3.1%)
Number of received SARS-COV-2 vaccine doses								
1 – n/N (%)	1/365 (0.3%)	0/292 (0.0%)	0/218 (0.0%)	3/517 (0.6%)	1/98 (1.0%)	0/43 (0.0%)	1/103 (1.0%)	6/1636 (0.4%)
2 – n/N (%)	13/365 (3.6%)	11/292 (3.8%)	2/218 (0.9%)	20/517 (3.9%)	2/98 (2.0%)	0/43 (0.0%)	3/103 (2.9%)	51/1636 (3.1%)
3 – n/N (%)	52/365 (14.2%)	38/292 (13.0%)	24/218 (11.0%)	73/517 (14.1%)	28/98 (28.6%)	3/43 (7.0%)	11/103 (10.7%)	229/1636 (14.0%)
4 – n/N (%)	299/365 (81.9%)	243/292 (83.2%)	192/218 (88.1%)	421/517 (81.4%)	67/98 (68.4%)	40/43 (93.0%)	88/103 (85.4%)	1350/1636 (82.5%)

* The types of transplantation within the group *Combined other transplant recipients* can be found in supplemental Table S1.
Immunosuppressive drugs after 2nd and 3rd vaccine dose can be found in supplemental Table S3.
** Other immunosuppressive medication: medication against auto-immune and neoplastic disorders.
BMI body mass index, CKD chronic kidney disease, DM diabetes mellitus, HIV human immunodeficiency virus, mTOR mammalian target of rapamycin, NA: not applicable, SD standard deviation.

Table 2. Univariable and multivariable analyses for positive SARS-CoV-2 anti-S IgG antibody response in transplant recipients without prior SARS-CoV-2 infection. *(continued on p 27)*

	Univariable models						Multivariable model	
	After 2nd vaccine dose		After 3rd vaccine dose		Average effect$			
Variable	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Transplant type (7 categories)	**NR**	**<.001**	**NR**	**<.001**	**NR**	**<.001**		**@**
Number of vaccine doses; dose 3 vs. 2	NA	NA	NA	NA	NA	NA	**4.05 (1.97;8.31)**	**<.001**
Time from last transplant to first vaccine dose (year)	**1.04 (1.02;1.06)**	**<.001**	**1.05 (1.03;1.08)**	**<.001**	**1.04 (1.02;1.06)**	**<.001**		**@**
Interaction between transplant type and time from last transplant to first vaccine dose	NA	NA	NA	NA	NA	NA	**NR**	**0.07**
Gender (Male versus female)	0.94 (0.73;1.22)	0.64	0.87 (0.67;1.14)	0.32	0.91 (0.73;1.13)	0.38		
Age (years)	**#**	**<.001**	**#**	**<.001**	**#**	**<.001**	**0.96 (0.94;0.98)**	**<.001**
Obesity& (yes vs no)	1.21 (0.84;1.77)	0.31	1.45 (0.96;2.18)	0.08	1.32 (0.95;1.82)	0.10		
Comorbidities								
DM (yes vs no)	0.89 (0.67;1.18)	0.42	0.84 (0.63;1.13)	0.26	0.87 (0.68;1.11)	0.25		
CKD (yes vs no)	**0.36 (0.24;0.55)**	**<.001**	**0.44 (0.30;0.64)**	**<.001**	**0.39 (0.27;0.57)**	**<.001**	**0.49 (0.31;0.76)**	**0.001**
Cirrhosis (yes vs no)	2.53 (0.63;10.2)	0.19	**5.64 (1.05;30.4)**	**0.04**	**3.59 (1.02;12.6)**	**0.046**		
Malignancy (yes vs no)	1.49 (0.59;3.77)	0.40	0.42 (0.14;1.24)	0.12	0.87 (0.38;1.97)	0.74		
Immunosuppressive therapy								
Tacrolimus (yes vs no)	**0.59 (0.40;0.86)**	**0.007**	**0.50 (0.32;0.78)**	**0.002**	**0.55 (0.38;0.78)**	**<.001**		
Tacrolimus trough level (8 categories)	**NR**	**<.001**	**NR**	**<.001**	**NR**	**<.001**		
Cyclosporin (yes vs no)	1.22 (0.79;1.89)	0.37	1.33 (0.83;2.14)	0.23	1.27 (0.87;1.85)	0.22		

Cyclosporin through level (4 categories)	NR	0.12	**NR**	**0.02**	**NR**	**0.006**		
Mycophenolate (yes vs no)	**0.33 (0.21;0.51)**	**<.001**	**0.41 (0.27;0.61)**	**<.001**	**0.36 (0.24;0.54)**	**<.001**		
Mycophenolate daily dose (g)	**#**	**<.001**	**#**	**<.001**	**#**	**<.001**	**#**	**<.001**
mTOR inhibitor (yes vs no)	1.71 (0.98;3.01)	0.06	**2.50 (1.18;5.31)**	**0.02**	**1.95 (1.16;3.28)**	**0.01**		
Corticosteroids (yes vs no)	**0.29 (0.18;0.49)**	**<.001**	**0.29 (0.17;0.49)**	**<.001**	**0.29 (0.18;0.48)**	**<.001**		
Corticosteroid daily dose (mg)	**#**	**<.001**	**#**	**<.001**	**#**	**<.001**	**#**	**<.001**
Azathioprine (yes vs no)	1.20 (0.80;1.78)	0.38	1.06 (0.70;1.60)	0.80	1.13 (0.80;1.58)	0.48		

$: When no significant interaction between vaccine dose number and variable of interest was found, the model was refitted using only the variable of interest.
@: P-value not reported because interaction between transplant type and time from last transplant to first vaccine dose is in the model.
NR: not reported due to the large amount of pairwise comparisons.
NA: Not applicable.
#: Age was univariably modeled using a 3rd order polynomial. Mycophenolate daily dose was univariably and multivariably modelled using a 3rd order polynomial. Corticosteroid daily dose was univariably and multivariably modelled using a spline.
&: Obesity is defined as BMI>=30 kg/m².
The univariable p-value indicates whether or not there is a statistically significant relationship between each predictor variable and the response variable. The multivariable p-value does the same but after correction for the other predictor variables in the model. The effect of a predictor then has to be interpreted given values for the other predictors.
BMI body mass index, CKD chronic kidney disease, DM diabetes mellitus, mTOR mammalian target of rapamycin, OR odds ratio.

Table 3. Univariable and multivariable analysis for severe breakthrough infection in transplant recipients without prior SARS-CoV-2 infection. *(continued on p 29)*

	Univariable models		Multivariable model	
Variable	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**
Transplant type (7 categories)$		**<.001**		0.10
Heart vs liver	**4.63 (1.02;20.9)**	**0.047**		
Heart vs lung	**0.37 (0.20;0.71)**	**0.003**		
Liver vs kidney	**0.21 (0.05;0.94)**	**0.041**		
Liver vs lung	**0.08 (0.02;0.33)**	**<0.001**		
Lung vs kidney	**2.65 (1.47;4.78)**	**0.001**		
Lung vs combined other	**17.0 (2.33;124)**	**0.005**		
Gender (female vs male)	0.68 (0.43;1.07)	0.10		
Age (years)	#	0.08	**1.03 (1.01;1.05)**	**0.003**
Obesity[&] (yes vs no)	1.20 (0.67;2.17)	0.54		
Time from last transplant to first vaccine dose (years)	**0.94 (0.91;0.98)**	**0.002**		
Comorbidities				
DM (yes vs no)	0.95 (0.59;1.53)	0.83		
CKD (yes vs no)	**1.57 (1.00;2.46)**	**0.048**		
Cirrhosis (yes vs no)	1.15 (0.15;8.84)	0.89		
Malignancy (yes vs no)	0.64 (0.09;4.74)	0.66		
Immunosuppressive therapy				
Tacrolimus (yes vs no)	1.14 (0.63;2.09)	0.66		
Tacrolimus trough level (µg/L)	1.05 (0.98;1.13)	0.16		
Cyclosporin (yes vs no)	1.17 (0.57;2.38)	0.67		
Cyclosporin through level (µg/L)	1.00 (1.00;1.01)	0.30		

Mycophenolate (yes vs no)	**1.95 (1.21;3.14)**	**0.006**		
Mycophenolate daily dose (mg)	**1.75 (1.30;2.35)**	**<.001**	**1.71 (1.25;2.33)**	**<.001**
mTOR inhibitors (yes vs no)	0.20 (0.03;1.48)	0.12		
Corticosteroids (yes vs no)	**4.49 (2.52;8.03)**	**<.001**		
Corticosteroid daily dose (mg)	**#**	**<.001**	**#**	**<.001**
Azathioprine (yes vs no)	1.05 (0.55;2.02)	0.87		

Transplant type was forced in the multivariable model.
BMI body mass index, CKD chronic kidney disease, DM Diabetes Mellitus, mTOR mammalian target of rapamycin, OR odds ratio.
$: only significant pair wise comparisons are shown.
#: Age was univariably modelled using a second order polynomial. Corticosteroid daily dose was univariably modelled using a 3rd order polynomial and multivariably using a spline
&: Obesity is defined as BMI>=30 kg/m².
The univariable p-value indicates whether or not there is a statistically significant relationship between each predictor variable and the response variable. The multivariable p-value does the same but after correction for the other predictor variables in the model. The effect of a predictor then has to be interpreted given values for the other predictors.

FIGURES

Figure 1. Time periods per vaccine dose, the evolution of SARS-CoV-2 variants of concern in Belgium since January 2021 and study milestones. Figure from Genomic surveillance report, Update for Belgium, Laenen et al., May 10, 2022 https://www.uzleuven.be/nl/media/b0c6797a-6191-43f2-bd59-3df521d3b919/genomic_surveillance_update_220510.pdf and adapted with permission.

Figure 2. Antibody response rate and levels per SARS-CoV-2 vaccine dose and per transplant type in transplant recipients without prior infection. A. SARS-CoV-2 anti-S IgG antibody response rates. Positive anti-S IgG antibody response is defined as antibody level ≥ 50 AU/ml. Error bars depict 95% confidence intervals. Numbers on top of error bars represent the total number of patients within transplant type. **B.** Absolute SARS-CoV-2 anti-S IgG antibody levels. Box is drawn from first to third quartile. Whiskers extend to 1.5 times interquartile range. Outliers are depicted as circles. Horizontal black line in box is the median. HCT hematopoietic cell transplant.

Figure 3. Prevalence and severity of breakthrough infections per transplant type in transplant recipients without prior infection. A. Rate of breakthrough infection within study period. **B.** Rate of severe breakthrough infection/COVID-19 (defined as the need for oxygen supplementation (oxygen saturation < 90% on room air), intensive care unit (ICU) admission or death). **C**. Rate of ICU admission. **D.** Mortality rate. Percentages are calculated as number of affected transplant recipients within the total number of included recipients of the respective transplant type group. HCT hematopoietic cell transplant.