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SARS-CoV-2 anti-spike antibodies after a fourth dose of COVID-19 vaccine in adult solid-organ transplant recipients

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ABSTRACT

Background: A fourth dose of SARS-CoV-2 vaccine is recommended in solid-organ transplant (SOT) recipients, but the immunogenicity is poorly known.
Methods: We conducted a retrospective, observational, monocentric study between the 1st January 2021 and 31st March 2022 of the anti-Spike antibody titers after one to four doses of vaccine in SOT.
Results: 825 SOT were included. Median age at first vaccine injection was 61.2 (IQR 50.9–69.3) years; 66.7 % were male; 63.4 % had received four vaccine doses. The proportion of participants with a strong humoral response (>260 BAU/mL) increased with the number of vaccine doses: 10.6 % after the 1st dose (D1), 35.1 % after the 2nd (D2), 48.5 % after the 3rd (D3), and 65.1 % after the 4th (D4) ($p < 0.001$). Among the tested patients, the proportion with a detectable humoral response was significantly higher after D4 than after D3 (47 % vs 22 %, $p = 0.01$). Liver transplant recipients had more frequently a strong humoral response after D2, D3 and D4 (OR = 5.3, 3.7 and 6.6 respectively when compared with other organ transplant recipients, $p < 0.001$). In kidney transplant recipients, belatacept-containing regimen was associated with a lower rate of detectable humoral (9 % vs 40 %, $p = 0.025$) after D3, but there was no statistical difference after D4.
Conclusion: A fourth dose should be proposed to SOT recipients who did not developed an immune response after 3 doses. Kidney transplant recipients receiving belatacept have a poorer, although frequently detectable response.

1. Introduction

The current COVID-19 pandemic has affected solid-organ transplantation (SOT) recipients on several levels. Firstly, it caused temporary suspension of transplant programs in 2020; secondly, SARS-CoV-2 infection is associated in SOT recipients with higher morbidity and mortality compared to the general population [1,2]. Thus, for this at-risk population and others, the elaboration and validation of vaccines brought a lot of hope. In the European Union, between December 2020 and March 2021, four COVID-19 vaccines have received marketing authorization: BNT162b2 from Pfizer-BioNTech; mRNA-1273 from Moderna; AZD1222 from Oxford-AstraZeneca; and Ad26COV2-S from Janssen. In the initial clinical trials, these vaccines demonstrated great efficacy at reducing symptomatic infection by 66–95 % in healthy volunteers after

Abbreviations: D1, First dose; D2, Second dose; D3, Third dose; D4, Fourth dose; SOT, Solid-organ transplant; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

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two doses [3–5], and "real-life" data confirmed they efficiently prevented severe forms by 90–95 %; later, a booster dose showed an efficacy of ~90–95 % for maintaining this protection over time (for mRNA platforms) [6]. During the year 2021, both the emergence of variants of concern (Delta then Omicron) and the waning of antibody titers after two doses, resulted in decreased effectiveness against SARS-CoV-2 infection; however, vaccine-induced protection against severe forms of COVID-19 is still high. Recently, a study conducted in Israel on patients aged > 60 years reported the benefits of a fourth vaccine dose in reducing the risk of severe COVID-19 [7].

It is well established that lower antibody titers are observed in immunocompromised patients after vaccination (e.g., for hepatitis B virus or pneumococcal vaccines [8,9]). Unfortunately, SOT recipients were excluded from the pivotal trials of the COVID-19 vaccines. In 2021, several studies reported a decreased antibody response to COVID-19 vaccines after one [10] or two [11–14] doses in SOT recipients. To overcome this limitation, a third dose (D3) was recommended for immunocompromised patients, such as transplant recipients (before it was also recommended for immunocompetent subjects) and, in some countries (including France), even a fourth dose (D4). Higher seroconversion rates were reported after D3 (67.9 % vs 41.4 % after D2) in 396 SOT recipients vaccinated by the Pfizer-BioNTech vaccine [15]. More recently, some teams have reported case series (37 patients [16], 18 patients [17], 67 patients [18], 49 patients [19], 92 patients [20] and 188 patients [21]) of SOT recipients where D4 slightly improved the antibody response.

We aimed to assess the immunogenicity of COVID-19 vaccines in a larger population of SOT recipients after one to four doses of COVID-19 vaccine.

2. Materials and methods

2.1. Data collection

We conducted a retrospective, observational, monocentric study. We included all adults from our center (Grenoble-Alpes University Hospital, France) that had received a heart, kidney, liver, or lung transplantation, and that had received at least one dose of COVID-19 vaccine, and with at least one measure of serum SARS-CoV-2 anti-spike antibodies after a vaccine dose. Patients with a past documented SARS-CoV-2 infection were excluded from the study, as patient with detectable SARS-CoV-2 anti-spike antibodies before the first dose of vaccine. Data were collected between the 1st January 2021 and 31st March 2022. All patients had previously given their consent for the retrospective use of their hospital-care data. This study falls within the scope of the French Reference Methodology MR-004 according to the 2016–41 law dated 26 January 2016.

2.2. Anti-spike antibody level

Due to the prolonged period considered (from January 2021 to March 2022), the quantification of anti-SARS-CoV-2 Spike antibodies was obtained using diverse immunoassays using the receptor binding domain (RBD) as target antigen, and available in different city and hospital laboratories: the Wantai SARS-CoV-2 Ab ELISA detecting total antibodies (Beijing Wantaï Biological Pharmacy Enterprise), the VIDAS SARS CoV-2 IgG II ELFA assay (Biomérieux), the Alinity i SARS-CoV-2 IgG II Quant assay (Abbott), the Elecsys anti-SARS CoV-2 S assay detecting total antibodies (Roche Diagnostics) and the Atellica sCOVG IgG assay (Siemens Healthineers). The Hospital virology laboratory used the Wantai ELISA with a TECAN Evolyser device. A linear relationship between sample-to-cutoff (S/CO) and antibody concentration was evaluated for samples in the 1.25 to 15 S/CO range. Samples with S/CO over 15 were diluted 1/40 in phosphate-buffered saline containing 7.5 % bovine serum albumin. We used the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human) as reference for anti-SARS-CoV-2 Ab titers (NIBSC code: 20/136). This standard is supplied as a vial containing 250 IU for neutralizing antibody activity equivalent to 250 binding antibody units (BAU) for binding antibody assays. Using the Wantai ELISA on the TECAN platform, we found a conversion factor of 0.75 between the results expressed in S/CO and BAU (1 S/CO = 0.75 BAU) which was similar as previous reports using the same ELISA reagent [22]. All results of SARS-CoV2 anti-spike antibody levels were expressed as binding antigen units (BAU)/mL, according to the manufacturers' (Biomérieux, Abbott, Roche, Siemens) and WHO recommendations [23]. All these assays exploring anti-Spike (RBD) antibody levels showed a good correlation with the titers of anti-SARS-CoV-2 neutralizing antibodies [22,24,25].

2.3. Anti-spike serological profile

A threshold of 260 BAU/mL has been proposed by the French health authorities to classify the patients as responders or low responders to COVID-19 vaccines, in reference to a study assessing the correlates of protection in immunocompetent patients against the Alpha B.1.1.7 strain [26]. In France this threshold still defines the immunocompromised patients eligible to a treatment with anti-SARS-CoV-2 neutralizing monoclonal antibodies. Therefore, the serological status of patients was classified as 1) **seronegative** if the anti-spike antibody level was below the limit of detection in the assay, 2) a **weak humoral response** if antibodies were detectable but under the threshold of 260 BAU/mL, or 3) **strong humoral response** if antibody level was > 260 BAU/mL.

2.4. Immunosuppressive regimen

For kidney-transplant recipients, all patients received 1 g/day of mycophenolate mofetil (MMF) and, prednisolone was given at the dose of 10 mg/day and stopped at 30 post-transplant. If kidney allograft surveillance biopsy at 3 months post-transplant was normal prednisone was stopped. Tacrolimus was adjusted to achieve residual levels of 8–12 ng/mL the first month, and then 4–8 ng/mL. Moreover, in case of nephrotoxicity, late-seroconversion to belatacept was possible. Finally, the immunosuppressive regimen at the time of vaccination was recorded as receiving or not receiving belatacept.

For liver-transplant recipients, all patient received 2 g/day of MMF and in absence of allograft rejection, it was stopped at 6 months post-transplant. Prednisolone was given at the dose of 10 mg/day and stopped at 60 days post-transplant. Tacrolimus was adjusted to achieve residual levels of 8–12 ng/mL the first month, 6–9 ng/mL the first year, 3–5 ng/mL the second year and finally 2–5 ng/mL five years post-transplant. Moreover, in case of nephrotoxicity, adjunction of everolimus could be possible in order to reduce the dose of tacrolimus.

For heart-transplant recipients, all patient received 2 g/day of MMF to achieve an area under curve at 45 during the first year post transplant and 35 after. Prednisolone was given 10 mg/day and stopped between 12 and 18 months post-transplant. Cyclosporine A was adjusted to achieve residual levels of 250–300 ng/mL the first two months, 200–250 ng/mL the first year, and then 150–200 ng/mL. In case of toxicity cyclosporine A could be switch by tacrolimus that was adjusted to achieve residual levels of 10–15 ng/mL the first two months, 8–10 ng/mL the first year, and then 5–8 ng/mL. Moreover, in case of nephrotoxicity, adjunction of everolimus could be possible in order to reduce the dose of tacrolimus.

For lung-transplant recipients, all patient received 2 g/day of MMF and prednisolone 0,1 mg/kg/day. Tacrolimus was adjusted to achieve trough levels of 10–15 ng/mL for the first three month, and then 5–10 ng/mL. Moreover, in case of nephrotoxicity, adjunction of everolimus could be possible in order to reduce the dose of tacrolimus.

2.5. Statistical analyses

Categorical variables are expressed as their frequency (and percentages); quantitative variables are expressed as the median (with interquartile ranges). Patient characteristics were compared using ANOVA for continuous data (or non-parametric tests when normality was not met) and the chi-square test for categorical data (or Fisher's exact test if theoretical n < 5). The factor and covariates associated with the outcomes were subsequently tested using multivariate logistic regression. The odds ratio and the associated 95 % confidence intervals are reported for these variables. *P*-values < 0.05 were considered significant. Analyses were performed with JAMOVI® software, version 1.6.23 (The Jamovi Project, 2020) and with Prism Software v7 (GraphPad).

3. Results

3.1. Study population

A total of 825 SOT recipients were included (Table 1): 46.2 % had received a kidney transplantation, 35.3 % a liver transplantation, 10.8 % a heart transplantation, and 7.7 % a lung transplantation. Median age at the time of the first vaccination (D1) of SARS-CoV-2 vaccine was 61.2 (IQR 50.9–69.3) years; median time since receiving a transplant was 6.7 years (IQR 3.3–11.9); 66.7 % of patients were male. Regarding the number of doses of SARS-CoV-2 vaccine, 94.6 % of patients received three doses or more, including 63.4 % of patients with four doses or more. More than 97 % of the patient received BNT162b2 vaccine (as a result, we did not explore the impact of the type of vaccine received). The median delay between two consecutive doses was 28 days between D1 and D2, 46.5 days between D2 and D3, and 201 days between D3 and D4 (Table 1).

3.2. Anti-SARS-CoV-2 spike antibody response

Among the 825 SOT recipients, 1083 anti-spike serological tests were carried out, including 66 after D1, 244 after D2, 538 after D3, and 235 after D4 (Fig. 1). The median delay between the dose of vaccine and the serology was the shortest after D1 (28 days) and after D4 (31.5 days), then after D2 (68 days), and the longest after D3 (122 days) (Table 2). The proportion of participants with a strong humoral response (>260 BAU/mL) increased with the number of doses: 10.6 % after D1, 35.1 % after D2, 48.5 % after D3, and 65.1 % after D4 ($p < 0.001$). However, 23.8 % of SOT recipients remained seronegative after D4 (Fig. 1).

Similar results were observed when considering each type of SOT recipient separately (Table 2): the proportion of patients with a detectable humoral response increased after each dose of vaccine, even though between 4.3 % and 43.3 % remained seronegative after D4 (depending of organ transplanted). A higher proportion of strong humoral responders was observed for liver transplant recipients whatever the number of SARS-CoV-2 vaccine injections received (Table 2). Liver transplant recipients (Table 3) compared to other transplant recipients were older at the time of transplantation (57.4 vs 58.7 years-old, $p < 0.001$), at the time of D1 (63.7 vs 58.7 years-old, $p < 0.001$), higher proportion of male (73.5 % vs 62.9 %, $p = 0.002$), had higher delay between D3-D4 (213 vs 195 days, $p < 0.001$) and higher delay between dose of vaccine and serology than other transplant recipients.

The multivariate analysis model included after checks of cofounders: type of transplant, age at D1, sex, and the delay between doses of vaccine and the serology. After D2 and D3, liver transplantation and young age were independently associated with a strong humoral response (Table 4). After D4, only liver transplantation was independently associated with a strong humoral response (Table 4).

3.3. Follow-up of the humoral response

For 219 patients, at least two determinations of the anti-spike antibody titers were available (after D2 and D3 for 44, and after D3 and D4 for 175; Table 5), and only nine patient had three determination of the anti-spike antibody titers (as the number is low, no analysis has been made regarding the evolution of titer between D2 then D3 then D4).

Table 1
Characteristics of solid-organ transplant recipients.

Characteristic	All patients ($n = 825$)		Kidney transplant ($n = 381$)		Liver transplant ($n = 291$)		Heart transplant ($n = 89$)		Lung transplant ($n = 64$)	
Gender, *n* (%)										
Male	550	(66.7)	235	(61.7)	214	(73.5)	59	(66.3)	42	(65.6)
Female	275	(33.3)	146	(38.3)	77	(26.5)	30	(33.7)	22	(34.4)
Age at transplantation, median (IQR) years	53.0	(42.5–61.9)	51.2	(41.3–62.4)	57.4	(48.5–62.4)	45.1	(35.1–54.1)	53.6	(36.2–62.8)
Age at D1 of vaccination, median (IQR) years	61.2	(50.9–69.3)	59.4	(49.3–69.0)	63.7	(57.1–70.6)	53.6	(47.0–64.0)	62.0	(47.9–69.6)
Time between transplant and D1 of vaccination, median (IQR) years	6.7	(3.32–11.9)	6.3	(3.3–11.7)	6.7	(3.0–11.2)	7.9	(3.8–16.1)	7.9	(4.4–11.8)
Number of SARS-CoV-2 vaccine doses received, *n* (%)										
1 dose	4	(0.5)	3	(0.8)	1	(0.3)	–	–	–	–
2 doses	41	(5.0)	19	(5.0)	18	(6.2)	1	(1.1)	3	(4.7)
3 doses	257	(31.2)	152	(39.9)	85	(29.2)	8	(9.0)	12	(18.8)
4 doses and more	523	(63.4)	205	(54.3)	187	(64.3)	80	(89.9)	49	(76.6)
Time between 1st and 2nd dose, median (IQR) days	28	(27–30)	28	(26–29)	28	(27–32)	28	(28–29)	28.5	(28–31)
Time between 2n and 3rd dose, median (IQR) days	46.5	(31–75)	47	(30–77.5)	42	(31–74.3)	58	(40.5–74.3)	42	(31–72)
Time between 3rd and 4th dose, median (IQR) days	201	(173–221)	199	(179–216)	213	(191–231)	160	(142–203)	187	(171–214)

n (%) or median delay (IQR).

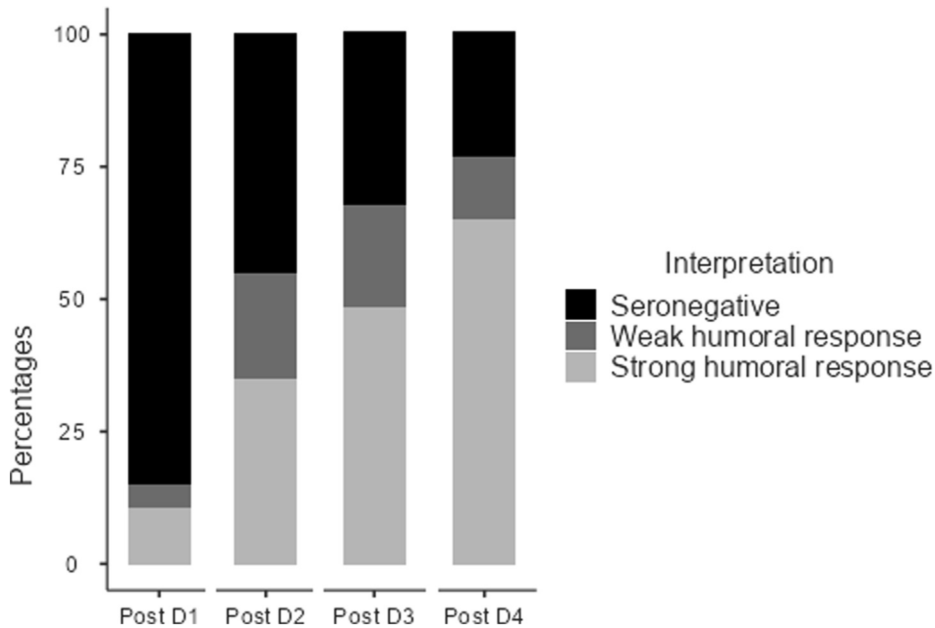

Fig. 1. Level of anti-SARS-CoV-2 antibody response in transplant recipients according to the number of vaccine doses. Post D1 (n = 66) corresponds to the distribution of patients' serology profiles after a first dose of COVID-19 vaccine. Post D2 (n = 244) corresponds to the repartition of patients' serology profiles after a second dose of COVID-19 vaccine. Post D3 (n = 538) corresponds to the repartition of patients' serology profiles after a third dose of COVID-19 vaccine. Post D4 (n = 235) corresponds to the repartition of patients' serology profiles after a fourth dose of COVID-19 vaccine. Seronegative profile corresponds to patients with no detectable antibodies. Weak humoral-response profile corresponds to patients with a detectable anti-spike antibody level below 260 BAU/mL. Strong humoral-response profile corresponds to patients with an anti-spike antibody level >260 BAU/mL. Serology profiles differed regarding the number of doses of vaccine ($p < 0.001$, chi-squared test).

Table 2
Serology profiles according to the number of vaccine doses and the type of organ transplant.

Transplant	Serology profile	Post D1		Post D2		Post D3		Post D4	
Kidney (n = 516)	Delay between vaccine and serology (days)	28	(24–28)	44	(28–81)	84	(42.5–125)	35	(23.8–52.5)
	Seronegative	54	(88.5)	105	(62.9)	117	(49.8)	23	(43.3)
	Weak humoral response	3	(4.9)	24	(14.4)	32	(13.6)	8	(15.1)
	Strong humoral response	4	(6.7)	38	(22.8)	86	(36.6)	22	(41.5)
Liver (n = 361)	Delay between vaccine and serology (days)	221	(169–240)	151	(92.3–193)	152	(121–173)	29	(21–36)
	Seronegative	1	(25.0)	9	(13.6)	24	(10.9)	3	(4.3)
	Weak humoral response	–	–	14	(21.2)	52	(23.5)	6	(8.6)
	Strong humoral response	3	(75.0)	43	(65.2)	145	(65.6)	61	(87.1)
Heart (n = 108)	Delay between vaccine and serology (days)	5	–	82.5	(48.8–116)	127	(35.5–156)	31	(28–37)
	Seronegative	1	(100)	–	–	14	(50.0)	20	(26.0)
	Weak humoral response	–	–	2	(100)	3	(10.7)	8	(11.6)
	Strong humoral response	–	–	–	–	11	(39.3)	49	(63.6)
Lung (n = 98)	Delay between vaccine and serology (days)	–	–	153	(142–169)	121	(84.3–146)	55	(31–80.8)
	Seronegative	–	–	2	(22.2)	19	(35.2)	10	(28.6)
	Weak humoral response	–	–	3	(33.3)	16	(29.6)	4	(11.4)
	Strong humoral response	–	–	4	(44.4)	19	(35.2)	21	(60.0)
Global (n = 1083)	Delay between vaccine and serology (days)	28	(24.3–28)	68	(32–147)	122	(72–160)	31.5	(28–54.3)
	Seronegative	56	(84.8)	116	(47.9)	174	(32.3)	56	(23.8)
	Weak humoral response	3	(4.5)	41	(16.9)	103	(19.1)	26	(11.1)
	Strong humoral response	7	(10.6)	85	(35.1)	261	(48.5)	153	(65.1)

n (%) or median delay (IQR), Post D1: After the first dose of COVID-19 vaccine, Post-D2: After the second dose of COVID-19 vaccine, Post-D3: After the third dose of COVID-19 vaccine, Post D4: After the fourth dose of COVID-19 vaccine; Seronegative profile corresponds to patients with no detectable anti-spike antibodies, Weak humoral-response profile corresponds to patients with detectable anti-spike antibodies <260 BAU/mL, Strong humoral-response profile corresponds to patients with an anti-spike antibody level >260 BAU/mL.

Among the 37 seronegative patients before D3, 22 % developed a weak (n = 1) or strong (n = 7) humoral response after D3, whereas 78 % remained seronegative. Among the four patients with a weak humoral response before D3, two developed a strong humoral response and two a weak response after D3 (Fig. 2).

Among the 73 patients with a seronegative status before D4, 47 % developed a weak (n = 15) or a strong (n = 19) humoral response after D4, whereas 53 % remained seronegative. Among the 34 patients with a weak humoral response before D4, 29 developed a strong humoral response and five retained a weak response after D4.

The proportion of patients with a detectable humoral response was significantly higher after D4 than after D3 (47 % vs 22 %, p = 0.01).

Table 3
Strong humoral response regarding liver and other transplant recipient.

Characteristics	Liver transplant		Other transplant		*p*-value
Gender, *n* (%)					
Male	214	(73.5)	336	(62.9)	0.002
Female	77	(26.5)	198	(37.1)	
Age at transplantation, median (IQR) years	57.4	(48.5–62.4)	50.4	(39.4–60.8)	< 0.001
Age at D1 of vaccination, median (IQR) years	63.7	(57.1–70.6)	58.7	(48.5–68.6)	< 0.001
Time between transplant and D1 of vaccination, median (IQR) years	6.7	(3.02–11.2)	6.67	(3.43–12.3)	0.974
Time between 1st and 2nd dose, median (IQR) days	28	(27–32)	28	(27–29)	0.136
Time between 2n and 3rd dose, median (IQR) days	42	(31–74.3)	48	(31–75.3)	0.993
Time between 3rd and 4th dose, median (IQR) days	213	(191–231)	195	(164–214)	0.001
Delay between D2 and serology post D2 (days)	151	(66–193)	48.5	(28–94.5)	0.001
Delay between D3 and serology post D3 (days)	152	(121–173)	91	(45–134)	0.001
Delay between D4 and serology post D4 (days)	89	(70–95)	37	(29–67)	0.001
Strong humoral responders after D2, *n* (%)	43	(65.2)	42	(23.6)	0.001
Strong humoral responders after D3, *n* (%)	145	(65.6)	116	(36.6)	0.001
Strong humoral responders after D4, *n* (%)	61	(87.1)	92	(55.8)	0.001

P-value was determined by the chi-square test for categorical data (or Fisher's exact test if theoretical n < 5) and for continuous data the ANOVA Welch's test (or Fisher's test if variances were assume equal).

Table 4
Multivariate analysis for strong humoral responders regarding transplant recipient and number of doses of vaccine received.

Parameters	*p*-value	Odds ratio	95 % Confidence Interval
Post D2 analysis			
Liver vs other transplant	< 0.001	5.316	[2.648–10.67]
Age at D1 of vaccination	0.103	0.981	[0.959–1.00]
Delay between D2 and serology post D2	0.205	1.003	[0.998–1.01]
Male vs Female	0.255	0.705	[0.386–1.29]
Post D3 analysis			
Liver vs other transplant	<0.001	3.677	[2.421–5.584]
Age at D1 of vaccination	0.002	0.977	[0.963–0.992]
Delay between D3 and serology post D3	0.936	1.00	[0.996–1.004]
Male vs Female	0.110	1.373	[0.931–2.024]
Post D4 analysis			
Liver vs other transplant	<0.001	6.608	[2.887–15.126]
Age at D1 of vaccination	0.049	0.979	[0.958–1.00]
Delay between D4 and serology post D4	0.497	0.997	[0.988–1.006]
Male vs Female	0.149	1.563	[0.852–2.868]

P-value was determined by multivariate logistic regression.

3.4. Effect of Belatacept on anti-SARS-CoV-2 spike antibody responses in kidney-transplant recipients

Belatacept was given to 40.5 % ($n = 209$) of kidney transplant recipients. Fewer of the patients receiving belatacept had a strong humoral response after D2 (6.5 % with belatacept vs 21.4 % with no belatacept) and after D3 (32.4 % with belatacept vs 45.1 % with no belatacept (Supplementary Table 1).

In 96 kidney transplant recipients, at least two determinations of the anti-spike antibody levels were available (after D2 and D3 for 44, and after D3 and D4 for 52) (Supplementary Table 2). Before D3, 22 patients receiving belatacept and 14 patients without belatacept were seronegative. Fewer of the patients receiving belatacept developed a detectable humoral response after D3 compared to those not receiving belatacept (9 % vs 40 %, $p = 0.025$); 91 % of those receiving belatacept remained seronegative after D3. Moreover, patients under belatacept less frequently developed a strong humoral response (12 % vs 44 %, $p = 0.021$) (Fig. 3). Before D4, 16 patients receiving belatacept and 20 not receiving belatacept were seronegative. The same proportions of patients either receiving ($n = 6$) or not receiving belatacept ($n = 7$) had developed a detectable humoral response (38 % vs 35 %, $p = 0.88$). Moreover, among patients without a strong humoral response before D4, of the 18 that were receiving belatacept and the 24 not receiving belatacept, four and eight patients, respectively, developed a strong humoral response (22 % vs 33 %, $p = 0.43$). No difference in humoral response was observed after D4 between those receiving or not receiving belatacept.

4. Discussion

Herein, we have reported on the humoral immune responses of a large population of SOT recipients that received one to four doses

Table 5
Follow-up of the humoral response after three and four doses of vaccine.

Serology profile after D2	Serology profile after D3	Number of patients	Serology profile after D3	Serology profile after D4	Number of patients
Seronegative ($n = 37$)	Seronegative	29	Seronegative ($n = 73$)	Seronegative	39
	Weak humoral response	–		Weak humoral response	15
	Strong humoral response	8		Strong humoral response	19
Weak humoral response ($n = 4$)	Seronegative	–	Weak humoral response ($n = 34$)	Seronegative	
	Weak humoral response	2		Weak humoral response	5
	Strong humoral response	2		Strong humoral response	29
Strong humoral response ($n = 3$)	Seronegative	–	Strong humoral response ($n = 68$)	Seronegative	2
	Weak humoral response	–		Weak humoral response	2
	Strong humoral response	3		Strong humoral response	64

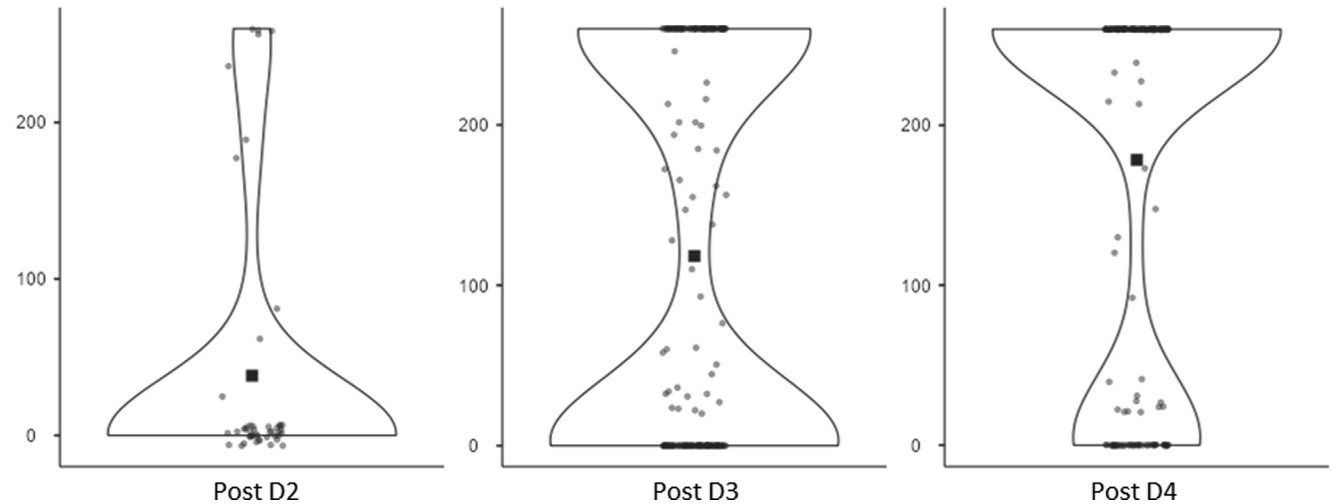

Fig. 2. Anti-spike antibody level after two, three, or four vaccine doses given to SOT recipients. All results for antibody levels were capped at 260 BAU/mL. The square represented the mean value of anti-spike antibody level. Evolution of serology between D2 and D3 was evaluated for 44 patients, and for 175 patients between D3 and D4. P-value was determined by the chi-square test.

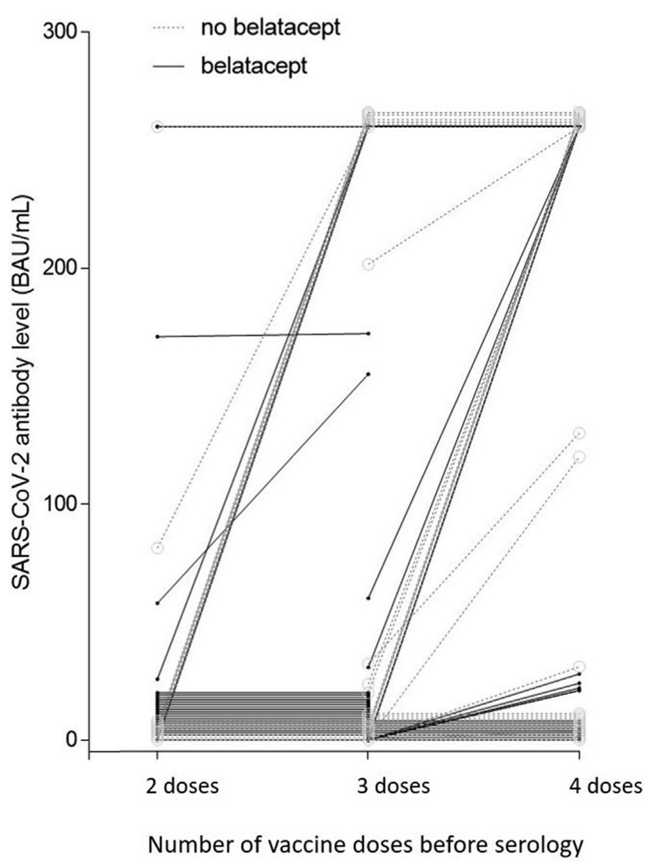

Fig. 3. All anti-spike antibody levels after two, three, or four vaccine doses given to kidney recipients according to immunosuppressive regimen. All results for antibody levels were capped at 260 BAU/mL. Evolution of serology between D2 and D3 was evaluated for 44 patients, and for 52 patients between D3 and D4. Stacked line on the graft signified that several patient were no responders (bottom of the graft) or strong responders (top of the graft). P-value was determined by the chi-square test.

of COVID-19 vaccine. Our primary aim was to assess the benefits of the different doses.

As immunocompromised patients were excluded from the initial clinical trials that led to marketing authorization for the COVID-19 vaccine, their immune responses after vaccination had to be assessed. Several trials have been conducted regarding immunogenicity after one or two doses of vaccine [10–14]: these have reported a detectable humoral response of 17 % after one dose, and responses ranging from 34.5 % to 62.0 % after two doses. More recently, a third dose has been shown to increase the proportion of patients with a detectable anti-spike antibodies by 26.5 % [15] to 49 % [27], which is close to the increase we reported in our study (18.1 %). Moreover, we observed that 32.3 % of patients remained seronegative after D3, which is similar to that previously reported (33.7 % in 872 SOT recipients [28] and 23.1 % in 396 SOT recipients [15]).

Only a few reports are available regarding the effect of a D4 in SOT recipients. Kamar et al. [16] reported that 41.0 % of their 32 seronegative patients after D3 had a humoral response after a D4, for Alejo et al. [17] it was 57.0 % of their patients, for Karaba et al. [29] 62.5 %, and for Masset et al. [19] 42.8 %, with only 8.1 % of patients with a strong humoral response. In addition, Benotmane et al. [18] reported that 81 % of patients with a weak immune response after the third dose displayed a strong anti-RBD IgG response after the fourth injection. Overall, these results are close to our observations: on the 73 patients seronegative after D3, 47 % had developed humoral response after D4. Taken together, these previous results and ours should encourage the use of a fourth dose given to all SOT recipients that have not responded after a D3.

Some teams previously reported better humoral response in liver transplant recipient. First, Nazaruk et al. reported higher humoral response in liver versus kidney transplant dependent of age, type of transplant et immunosuppression [30]. Second, Balsby et al. reported that liver transplant, age and immunosuppression were associated with antibody response to the BNT162b2 vaccine [31]. Our results suggest a better humoral response to COVID-19 vaccine in liver transplant recipient, as the highest proportion of strong humoral responders after each dose of COVID-19 vaccine was observed in this population. This is likely to result from immunosuppressive regimen differences between the different types of transplants, with a less heavy immunosuppressive treatment in liver transplant. Indeed, the immunosuppression protocol used in liver transplantation is routinely composed of anticalcineurins alone quickly after transplantation, whereas the protocols classically used in other types of transplantation are based on a combination of anticalcineurins, antimetabolic and low-dose corticosteroid therapy.

Belatacept use in kidney-transplant recipients appears to improve glomerular-filtration rates through an absence of nephrotoxicity and possibly graft survival. However, belatacept is associated with others issues: e.g., humoral responses to seasonal influenza vaccination in patients under a belatacept-containing regimen are impaired [32]. This is in line with our observation that kidney-transplant recipients have less frequently a detectable humoral response after D3 if they have received belatacept (9 %) compared to patients on a belatacept-free regimen (40 %). A previous study conducted on kidney-transplant recipients reported similar results [33], with 20 % of patients receiving belatacept developing a vaccine humoral response after a third dose of vaccine compared to 68 % of those not receiving belatacept. Another study showed an even lower rate of vaccine humoral response after a third dose (6.4 %) for kidney-transplant recipients receiving belatacept [34]. These results suggest that patients receiving belatacept should be informed early-on of to the potential use of prophylactic neutralizing antibodies in case of weak vaccine response.

Our study has several limitations. Firstly, its retrospective design may have led to heterogeneous data, although we could include a relative high number of patients in the study. Second, the delay between vaccine injection and serology testing was heterogeneous. Third, all anti-Spike antibody level determination were not conducted within the same laboratory; however, the results could be compared after adjustment to the same WHO international standard and the calibration controls provided by the manufacturers, which limits the heterogeneity of our results [35,36]. It should also be noted that a threshold of 260 BAU/mL was used to classify our patients (weak/strong humoral response to COVID-19 vaccines), but this is disputable. Indeed, this threshold was considered potentially protective against Alpha VOC, and it is highly likely that the protective values against Delta and Omicron VOC are higher due to the immune escape they displayed [21,37]. Moreover, this threshold has been obtained in a study analyzing immunocompetent subjects, and may have a different, higher value in immunocompromised patients. Therefore, we cannot conclude that the patients we classified as having a strong immune response are efficiently protected during the current Omicron circulation; our conclusions could have differed on this point if he had assessed the neutralizing antibody titers. Moreover, it could also be of interest to explore anti-SARS-CoV-2 cellular immune response which is an effector for preventing severe disease [38]. Finally, we cannot rule out reinfection events, which may interfere with the kinetic of the patients' humoral response. However, this study represents the largest cohort of SOT recipients analyzed for their immune responses after receiving between one and four dose(s) of COVID-19 vaccine.

5. Conclusion

This study shows that the proportion of SOT recipients with a strong humoral response increase with the number of doses of COVID-19 vaccine received, including the fourth vaccination, even for patients receiving belatacept. Thus, a fourth dose of vaccine should be systematically proposed to patients that did not develop a strong immune response with the previous doses.

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CRediT authorship contribution statement

Quentin Perrier: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Julien Lupo:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Théophile Gerster:** Data curation, Writing – review & editing. **Caroline Augier:** Data curation, Writing – review & editing. **Loïc Falque:** Data curation, Writing – review & editing. **Lionel Rostaing:** Data curation, Writing – review & editing. **Laurent Pelletier:** Data curation, Writing – review & editing, Investigation. **Pierrick Bedouch:** Data curation, Writing – review & editing. **Myriam Blanc:** Data curation, Writing – review & editing. **Christel Saint-Raymond:** Data curation, Writing – review & editing. **Aude Boignard:** Data curation, Writing – review & editing. **Agnès Bonadona:** Data curation, Writing – review & editing. **Johan Noble:** Data curation, Writing – review & editing, Validation. **Olivier Epaulard:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.08.065.

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