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Serological Response to Three, Four and Five Doses of SARS-CoV-2 Vaccine in Kidney Transplant Recipients

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Abstract: Mortality from COVID-19 among kidney transplant recipients (KTR) is high, and their response to three vaccinations against SARS-CoV-2 is strongly impaired. We retrospectively analyzed the serological response of up to five doses of the SARS-CoV-2 vaccine in KTR from 27 December 2020 until 31 December 2021. Particularly, the influence of the different dose adjustment regimens for mycophenolic acid (MPA) on serological response to fourth vaccination was analyzed. In total, 4277 vaccinations against SARS-CoV-2 in 1478 patients were analyzed. Serological response was 19.5% after 1203 basic immunizations, and increased to 29.4%, 55.6%, and 57.5% in response to 603 third, 250 fourth, and 40 fifth vaccinations, resulting in a cumulative response rate of 88.7%. In patients with calcineurin inhibitor and MPA maintenance immunosuppression, pausing MPA and adding 5 mg prednisolone equivalent before the fourth vaccination increased the serological response rate to 75% in comparison to the no dose adjustment (52%) or dose reduction (46%). Belatacept-treated patients had a response rate of 8.7% (4/46) after three vaccinations and 12.5% (3/25) after four vaccinations. Except for belatacept-treated patients, repeated SARS-CoV-2 vaccination of up to five times effectively induces serological response in kidney transplant recipients. It can be enhanced by pausing MPA at the time of vaccination.

Keywords: kidney transplantation; COVID-19; vaccination; immunosuppression

1. Introduction

At the beginning of 2021, the successful vaccine campaign against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started, offering protection against hospitalization and death from coronavirus disease 2019 (COVID-19) for most patient groups, irrespective of emerging variants [1]. Despite this success in the general population, solid organ transplant (SOT) recipients had a poor response to vaccination and a limited benefit from the initially recommended two vaccinations [2]. At the same time, the mortality of SOT recipients acquiring COVID-19 is unacceptably high, with rates up to 20% reported in registries [3,4]. Recent data from the United Kingdom collected between September 2020

and August 2021 show an unadjusted COVID-19 case fatality rate of 9.8% in SOT. The vaccination with two doses did not prevent infections in SOT recipients and increased 28-day survival in COVID-19 infected SOTs only marginally from 88.8% to 91.8%. This is equivalent to a 20% reduction in risk of death in vaccinated SOT recipients, as compared to a 68-fold reduction of death in the general population [5].

Early in 2021, it was recognized that SOT, and especially kidney transplant recipients (KTR), show a diminished serological response compared to healthy individuals and hemodialysis patients [6–8]. Subsequent studies revealed that the level of antibodies correlates with protection from disease [9,10], arguing for serological response controls in SOT recipients.

After three vaccinations, which were early recommended for SOT recipients and later for the general population, serological vaccine response was inadequately low in at least 40% [11]. T cell response over time only changes at a functional level, never reaching the level of healthy individuals [12,13]. In summary, the poor T cell response in combination with an impaired humoral response offered only a limited protection from infection and a severe course of COVID-19 [14]. Consistent with the observation that mycophenolic acid treatment impairs B-cell proliferation and plasmablast proliferation, experimental data have suggested that a short-term MPA pause can improve serological response to the fourth dose of the SARS-CoV-2 vaccination [13].

The question therefore arises about optimal management of non-responding patients, and, in particular, whether repeated vaccinations increase cumulative serological response rates. In the current study, we provide the first systematic investigation analyzing the serological response to up to five repeated vaccinations against SARS-CoV-2 in non-responding COVID-19-naïve KTR. In particular, we report the response rates of KTR after basic immunization, three, four, and five vaccinations, and the predictors of serological response after three and four vaccinations as well as the effects of different immunosuppressive reduction regimes on the serological response.

2. Methods

Study Population

At our institution, basic immunization against SARS-CoV-2 was performed with two doses of one of the following vaccines in different combinations—BNT162b2 (Comirnaty, BioNTech/Pfizer, Mainz, Germany), mRNA-1273 (Spikevax, Moderna Biotech, Madrid, Spain), ChAdOx1-S (AZD1222, AstraZeneca, Södertälje, Sweden), or Ad26.COV2.S (Johnson & Johnson, Janssen, Beerse, Belgium). Sustained non-responders received up to five doses of SARS-CoV-2 vaccines. A detailed institutional protocol is provided in Item S1 and Figure S1. All patients provided written and informed consent into off-label use for vaccine doses four and five. For the current analysis, we included adult kidney transplant recipients who received SARS-CoV-2 vaccinations from 27 December 2020, until 31 December 2021. Serological response to vaccination, demographic data, transplantation data, and medication, as well as routine laboratory data, were analyzed retrospectively. The ethics committee of Charité—Universitätsmedizin Berlin approved this study (EA1/030/22).

3. Outcome

The primary outcome was serological response to immunization, defined as the maximum serological response after a minimum of 14 days after each immunization, i.e., after basic immunization, three, four, and five doses of SARS-CoV-2 vaccines.

We used an anti-SARS-CoV-2 enzyme-linked immunosorbent assays (ELISA) for the detection of IgG antibodies against the S1 domain of the SARS-CoV-2 spike (S) protein in serum according to the instructions of the manufacturer (Anti-SARS-CoV-2-ELISA (IgG), EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) [15,16]. Processing and measurement were done using the fully automated "Immunomat" (Institut Virion\Serion GmbH, Würzburg, Germany). Results were determined by comparing the

obtained signals of the patient samples with the previously obtained cut-off value of the calibrator. As suggested by the manufacturer, samples with a cut-off index $\geq$ 1.1 were considered to be positive. Alternatively, the electrochemiluminescence immunoassay (ECLIA, Elecsys, Anti-SARS-CoV-2, Roche Diagnostics GmbH, Mannheim, Germany) was used either alone or in parallel detecting human immunoglobulins, including IgG, IgA, and IgM against the spike receptor binding (RBD) domain protein. Results were determined by comparing the obtained signals of the patient samples with the previously obtained cut-off value of the calibrator. As suggested by the manufacturer, samples with a cut-off index $\geq$ 264 U/mL were considered to be positive as recommended by Caillard et al. [17].

Any non-negative titer below the cut-off in each test was defined as low-positive. Accordingly, response to immunization was categorized as a sufficient serological response (responders) in the case of a positive SARS-CoV-2 antibody titer or as an insufficient serological response (non-responders) in case of a negative or low-positive SARS-CoV-2 antibody titer. The serological response rate was calculated as the rate of responders after each basic immunization, three, four, and five doses.

In order to exclude patients with a history of COVID-19, we simultaneously measured antibodies against the nucleocapsid (N) protein with an electrochemiluminescence immunoassay (ECLIA, Elecsys Anti-SARS-CoV-2, Roche Diagnostics GmbH). As before, results were determined by comparing the obtained signals of the patient samples with the previously obtained cut-off value of the calibrator. As suggested by the manufacturer, samples with a cut-off index $\geq$ 1.0 were considered to be positive.

We included immunization from all adult KTR at our institution within the study period. Immunizations were excluded from analysis when they occurred before transplantation, when no medication data were available, or in case of ineligible serological data (see Table 1). The latter occurred if patients received additional vaccination doses without the assessment of the SARS-CoV-2 IgG titer before and after, mostly when performed outside the transplant center.

Table 1. Inclusion and exclusion criteria. Since main data analysis was performed at the vaccination level, all SARS-CoV-2 vaccinations for patients meeting the inclusion criteria were included, while vaccinations were excluded based on the exclusion criteria.

Inclusion Criteria per Patient
• Kidney transplantation
• Age of 18 years or older
• At least one SARS-CoV-2 vaccination after kidney transplantation
Exclusion Criteria per Vaccination
• SARS-CoV-2 vaccinations, which were performed before transplantation
• SARS-CoV-2 infection before vaccination or before measurement of the respective serological response as defined by ◦ Positive SARS-CoV-2 RNA PCR ◦ Positive anti-SARS-CoV-2-N-protein antibodies
• Missing data about medication at the time of vaccination
• Sufficient serological response before respective SARS-CoV-2 vaccination
• Monoclonal anti-SARS-CoV-2-S-protein antibody therapy before measurement of the respective serological response
• Missing data about serological response before respective SARS-CoV-2 vaccination (does not apply for basic immunization)
• Missing data about serological response after respective SARS-CoV-2 vaccination

The cumulative serological response was calculated using the Kaplan–Meier method with the number of vaccinations as the time variable and the first positive serological response as the event of interest. Patients, who remained sustained non-responders after their last vaccination, were treated as censored at this point [18].

3.1. Multivariable Analysis of Predictors of Serological Response

The influence of 8 variables on the primary outcome after the third vaccination dose and the influence of 5 variables on the primary outcome after the fourth vaccination dose were examined in two separate multivariable analyses using logistic regression. Candidate variables included basic patient demographics, transplantation data, vaccination characteristics, the latest immunosuppressive medication, and routine laboratory parameters (detailed variable definition provided in Table S1). Immunizations with missing candidate variable data were excluded from multivariable analysis. No imputation methods were used. Adjusted p-values according to Holm's correction for multiple comparisons are reported.

Since not all patients received calcineurin inhibitors (CNI), the influence on CNI through levels on serological response rate was studied in two separate multivariable analyses, which included % deviance from the target CNI-through level (6 ng/mL for tacrolimus, and 80 ng/mL for cyclosporine) as an additional predictor variable [19].

3.2. Comparison of Mycophenolic Acid (MPA) Dose Adjustment Regimens

Due to the important role of MPA with regard to the response to immunization, different approaches for MPA dose adjustment before fourth SARS-CoV-2 immunization were followed at our institution according to the patients' individual risk factors, such as previous rejection episodes, anti-HLA antibodies, previous response to SARS-CoV-2 immunization, and based on the physicians' and patients' shared-decision making. In CNI-treated patients, MPA was reduced (reduction of 25–50% compared to the maintenance dose) or paused from one week before immunization until four weeks after immunization. Steroids were maintained at 5 mg prednisolone equivalent. In case of steroid-free treatment, 5 mg prednisolone equivalent was added for the time of MPA reduction, which was discontinued after restart of MPA.

To examine MPA related effects, patients on CNI-based immunosuppression receiving a fourth dose of a SARS-CoV-2 vaccine were assigned to three groups according to their change in MPA dose in relation to the MPA dose before their third SARS-CoV-2 vaccination: (1) steady MPA dose, (2) reduced MPA dose, and (3) paused MPA. Serological response rates between the 3 groups were compared using the Kruskal–Wallis test, and pairwise comparisons were performed using Mann–Whitey U test with Holm's correction for multiple comparisons.

3.3. Serological Response in Patients with CNI and Belatacept Maintenance Immunosuppression

The serological response rate in patients on CNI-based as well as belatacept-based immunosuppression was calculated separately. Additionally, the cumulative serological response rate was described as stated above. For patients with belatacept-based immunosuppression, no cumulative serological response rate is shown after 5 vaccinations, due to the low patient count in this group. Serological responders receiving belatacept-based immunosuppression were further analyzed on the patient-level.

Statistical analysis was performed using R studio v.1.2.5042 (Boston, MA, USA) and R version 4.0.2 (Vienna, Austria) (22 June 2020).

4. Results

4.1. Serological Response to Immunization against SARS-CoV-2

A total of 4277 vaccinations against SARS-CoV-2 in 1478 patients were evaluated. The distribution of included and excluded immunizations, the reasons for exclusion and the resulting serological response is shown in the patient flow diagram (Figure 1). Demographic, clinical, and vaccination data for patients receiving three, four, and five doses of SARS-CoV-2 vaccines are summarized in Table 2.

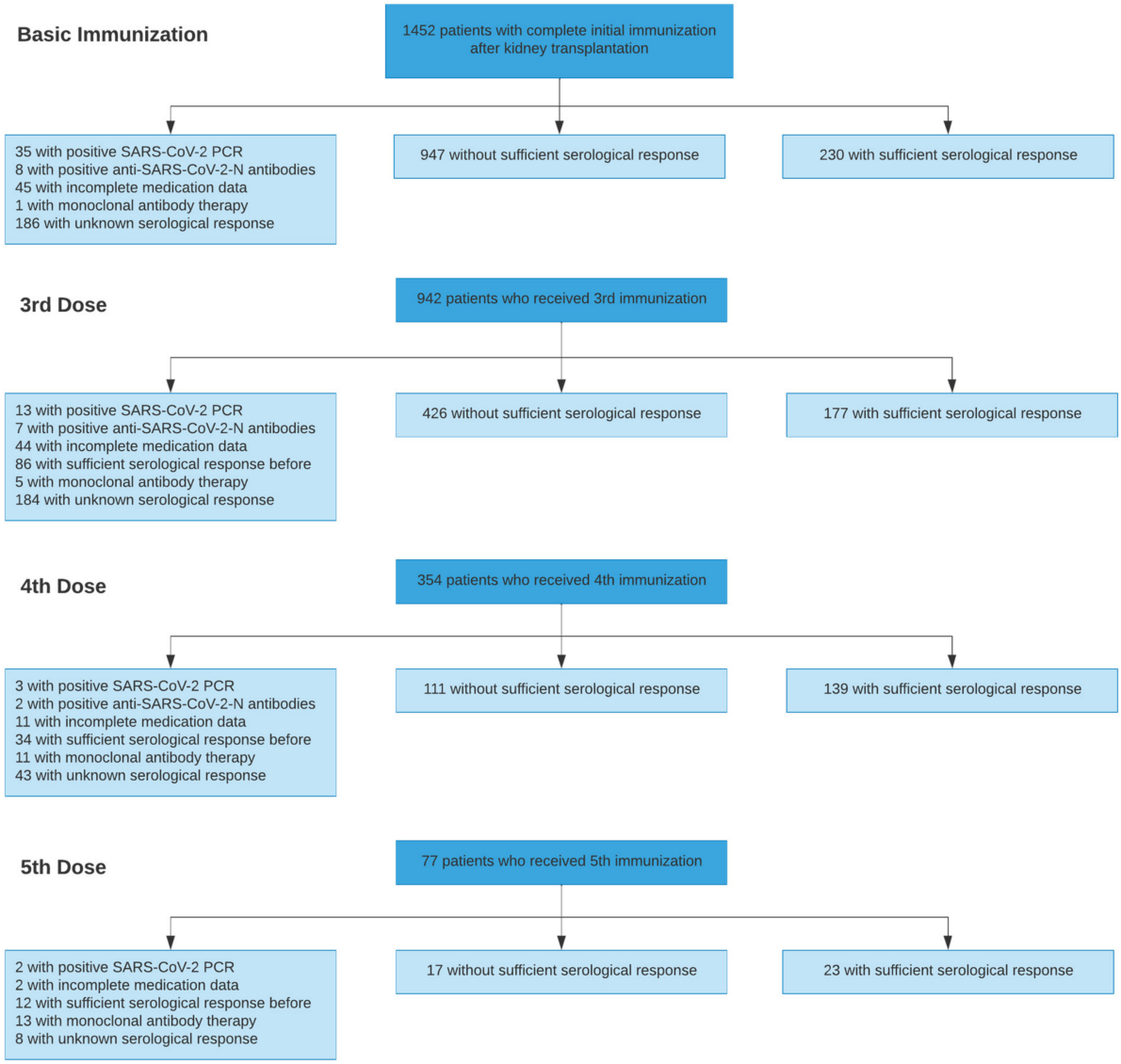

Figure 1. Patient flow diagram showing the number of patients included into each analysis and the number of excluded patients and the respective reasons for exclusion.

Table 2. Baseline characteristics of patients who received three, four, and five doses of SARS-CoV-2 vaccines. Continuous variables are presented as mean (±standard deviation) unless stated otherwise. IQR—interquartile range. BMI—body mass index. IS—immunosuppression. mTORi—mammalian target of rapamycin inhibitor. eGFR—estimated glomerular filtration rate.

Vaccination Number	3	4	5
Total Patients	603	250	40
Demographics and Comorbidities			
Female/male patients	38%/62%	33%/67%	45%/55%
Median age in years (IQR)	59 (48–68)	61 (51–70)	63 (56–72)
BMI in kg/m^2	25.4 ± 4.7	25.0 ± 4.4	24.76 ± 4.77
Diabetes	21.7%	21.8%	18.0%
Transplantation			
Median transplant age in years (IQR)	8.2 (3.1–13.5)	7.7 (3.0–12.7)	7.2 (2.2–11.5)
Tacrolimus-based IS	73.6%	73.6%	77.5%
Ciclosporin-based IS	16.8%	16.4%	12.5%
Belatacept-based IS	7.6%	10%	12.5%
Patients with MPA	93.7%	50.4%	42.5%
Patients with mTORi	1.0%	-	-
Patients with Azathioprine	0.8%	-	-
Patients with more than 2 immunosuppressive drugs	60.7%	39.6%	32.5%
Laboratory Values			
Baseline eGFR in mL/min/1.73 m^2	51.2 ± 20.0	47.5 ± 19.6	43.35 ± 18.68
Urine albumin creatinine ratio in g/g	0.14 ± 0.45	0.19 ± 0.62	0.44 ± 1.45
Hemoglobin in g/dL	12.68 ± 1.65	12.56 ± 1.52	12.73 ± 1.69
Leukocyte count in /nL	7.33 ± 2.38	7.41 ± 2.53	7.86 ± 2.44
Vaccination			
Baseline SARS-CoV-2 IgG low positive	3.5%	11.6%	45%
mRNA Vaccination	72.8%	86.8%	100%
Median time since previous vaccination in days (IQR)	71 (53–102)	64 (55–84)	62 (46–70)

The vaccination-specific rate of serological response to 1.203 basic immunizations that met the inclusion/exclusion criteria was 19.5%. The rate increased to 29.4%, 55.6%, and 57.5% in response to 603 third, 250 fourth, and 40 fifth vaccinations against SARS-CoV-2, respectively (Figure 2A). Correspondingly, the cumulative serological response increased from 19.1% after two vaccinations to 42.0% after three, 74.2% after four, and 88.7% after five vaccinations (Figure 2B). No serious adverse events were found in patients receiving the fourth or fifth vaccination dose.

4.2. Predictors of Serological Response to Immunization against SARS-CoV-2

We performed multivariable analysis using logistic regression for 574 patients with third vaccination and 226 patients with fourth vaccination separately to identify factors that influence serological response. We found any previous low positive anti-SARS-CoV-2-S-protein IgG titer, younger age, higher BMI, higher transplant age, higher estimated glomerular filtration rate (eGFR), and higher hemoglobin levels to be associated with improved serological response after three doses of SARS-CoV-2 vaccine. Any previous low positive anti-SARS-CoV-2-S-protein IgG titer, younger age, higher transplant age and mRNA-based vaccination were associated with improved serological response after four doses of SARS-CoV-2 vaccine (Tables 3 and 4).

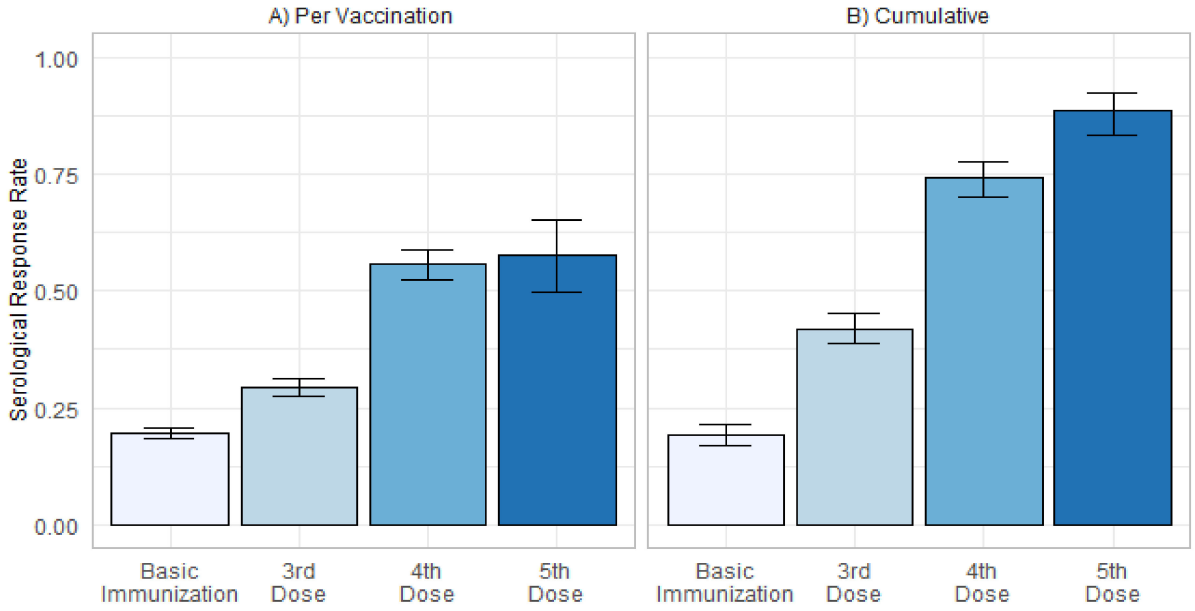

Figure 2. (**A**) Serological response rate (±standard deviation) per vaccination after basic immunization, three, four, and five doses of SARS-CoV-2 vaccines in kidney transplant recipients without sufficient serological response before the latest vaccination. (**B**) Cumulative serological response rate (±95% confidence interval) after up to five doses of SARS-CoV-2 vaccines in all kidney transplant recipients with at least one vaccination meeting the inclusion and exclusion criteria.

Table 3. Predictors of serological response after three doses of SARS-CoV-2 vaccines identified in multivariable analysis. Adjusted (adj.) *p*-value according to Holm's correction for multiple comparisons.

Variable	Odds Ratio (95% CI)	Adj. *p*-Value
Low positive anti-SARS-CoV-2-S-protein IgG before vaccination	28.5 (7.18–201)	<0.001
Age	0.98 (0.96–0.99)	0.016
BMI	1.06 (1.01–1.11)	0.012
Transplant age	1.06 (1.03–1.09)	<0.001
Belatacept	0.15 (0.03–0.45)	0.008
MPA dose in MMF equivalent in g	0.29 (0.20–0.43)	<0.001
eGFR in mL/min/1.73 m^2	1.02 (1.01–1.04)	<0.001
Hemoglobin	1.29 (1.13–1.49)	0.001

Table 4. Predictors of serological response after four doses of SARS-CoV-2 vaccines identified in multivariable analysis. Adjusted (adj.) *p*-value according to Holm's correction for multiple comparisons.

Variable	Odds Ratio (95% CI)	Adj. *p*-Value
Low positive anti-SARS-CoV-2-S-protein IgG before vaccination	18.7 (4.68–134)	0.001
Age	0.96 (0.94–0.99)	0.004
Transplant age	1.09 (1.04–1.16)	0.002
Belatacept	0.03 (0.004–0.13)	<0.001
MPA dose in MMF equivalent in g	0.34 (0.18–0.59)	0.001

Belatacept treatment and higher MPA dose were associated with reduced serological response after three doses and four doses. Hence, both are the two major modifiable risk factors found in our analysis apart from repeated vaccination.

For patients with CNI-based immunosuppression, deviance from a target CNI-through level did not show significant influence on serological response rate (Tables S2 and S3).

4.3. Change in MPA Dose as Predictor of Serological Response after Four Doses of SARS-CoV-2 Vaccine

Next, we analyzed how MPA dose adjustment affects the serological response to a fourth vaccination in patients receiving CNI and MPA as maintenance immunosuppression. Among the 200 patients receiving a fourth dose of a SARS-CoV-2 vaccine, 33 patients maintained a steady MPA dose, 63 received a reduced MPA dose, and 104 patients had paused MPA before immunization. Baseline characteristics are summarized in Table 5.

Table 5. Baseline characteristics of patients in the steady MPA group, the reduced MPA group, and the paused MPA group receiving a fourth dose of a SARS-CoV-2 vaccine. Continuous variables are presented as mean (±standard deviation) unless stated otherwise.

	MPA Pause	MPA Reduction	MPA Continuation
Number of Patients	104	63	33
Median age in years (IQR)	60 (51–70)	63 (47–70)	64 (55–71)
Female	33.7%	25.4%	33.3%
BMI in kg/m^2	25.5 ± 4.3	24.2 ± 4.3	24.6 ± 4.2
Median transplant Age in years (IQR)	7.6 (3.0–7.5)	6.8 (2.1–11.4)	4.9 (2.3–9.6)
eGFR in mL/min/1.73 m^2	47.6 ± 19.0	55.1 ± 17.8	44.7 ± 8.1
Change in eGFR in mL/min/1.73 m^2	−1.09 ± 6.73	−2.47 ± 9.30	0.53 ± 5.96
ACR in g/g	0.18 ± 0.77	0.11 ± 0.21	0.13 ± 0.23
Change in ACR in g/g	0.02 ± 0.21	0.03 ± 0.30	−0.05 ± 0.16
mRNA Vaccination	96.2%	76.2%	75.8%
IgG low positive before vaccination	12.5%	7.9%	24.3%
MPA dose in g MMF equivalent	0	0.87 ± 0.25	1.18 ± 0.43
Patients with steroid treatment	100%	76.2%	57.6%
Steroid dose in mg methylprednisolone equivalent	4.1 ± 0.8	2.8 ± 1.8	1.8 ± 1.9
Median time since last vaccination in days (IQR)	65 (56–83)	65 (58–94)	65 (48–87)

The serological response rate in the paused MPA group was 75%, which was significantly higher than in the reduced MPA group (46%, adjusted $p < 0.001$) and the steady MPA group (52%, adjusted $p = 0.02$) (Figure 3), with no significant difference between the latter two groups (adjusted $p = 0.61$).

In the paused MPA group, 1/104 patients (1%) developed de-novo DSA, and 1/104 patients (1%) developed an episode of acute T cell mediated rejection (TCMR) requiring intermittent dialysis, which could be terminated after steroid pulse therapy and adaption of immunosuppressive therapy. In the latter case, TCMR was further precipitated by two factors: first, the MPA pause was extended, since the patient received abdominal wall hernia repair in another hospital, which was complicated by a superinfected hematoma; second, low tacrolimus levels of 2.59 ng/mL were found when the patient was transferred to our clinic. In the reduced MPA group, 1/63 patients (1.6%) developed de-novo DSA and 1/63 patients (1.6%) developed an episode of chronic active antibody-mediated rejection.

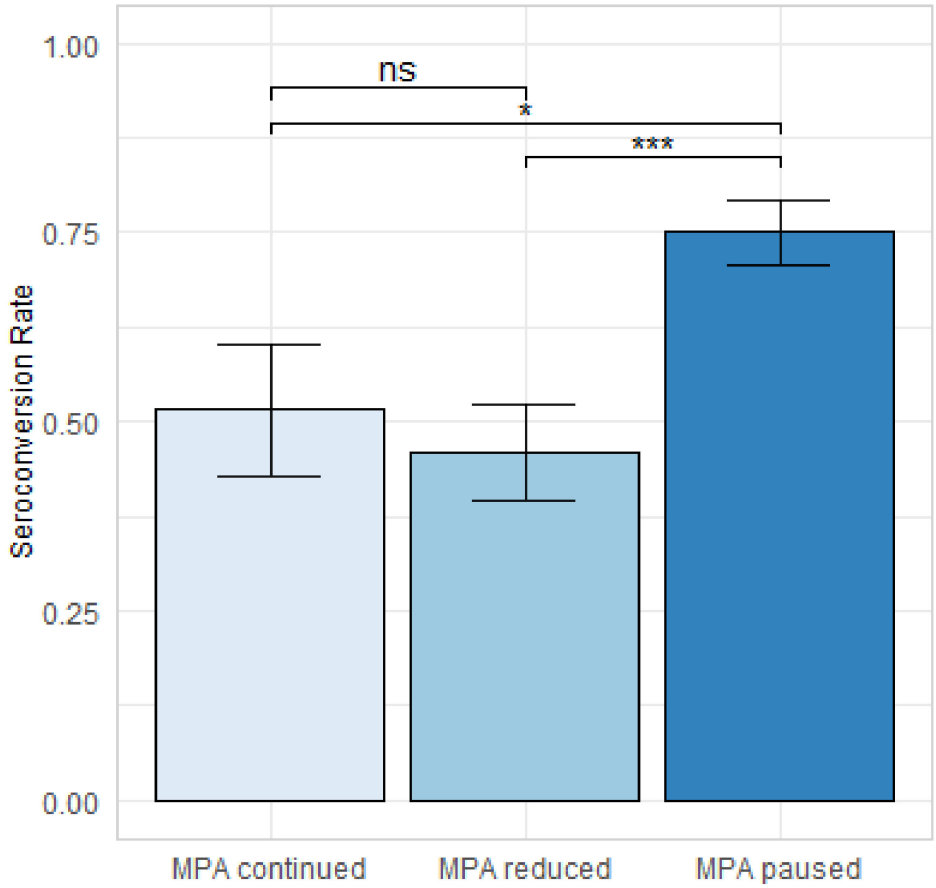

Figure 3. Serological response rates after four doses of SARS-CoV-2 vaccines in kidney transplant recipients with steady MPA dose (n = 33), reduced MPA dose (n = 63), and paused MPA (n = 103). * adjusted $p < 0.05$, *** adjusted $p < 0.001$.

4.4. Belatacept-Based Immunosuppression as Predictor of Serological Response

Multivariable analysis revealed that patients who received belatacept immunosuppression at the time of the third vaccination have strongly reduced serological response. Still, we found 3 out of 63 patients (4.8%) responded after the second vaccination, 4 out of 46 patients (8.7%) responded after the third vaccination, 3 out of 25 patients (12%) after the fourth vaccination, and 2 out of 5 patients (40%) after the fifth vaccination. A detailed analysis revealed special immunological circumstances or reduced immunosuppressive medication in 8 out of these 9 patients with a serological response, which might explain why these patients developed serological responses despite belatacept treatment (Table S4). Conversely, patients treated with belatacept and full dose MPA are highly unlikely to show serological response even with repeated vaccination.

In summary, patients with belatacept-based immunosuppression show impaired cumulative serological response (4.4%, 12.4%, and 16.4%) in comparison to patients with CNI-based immunosuppression (19.1%, 37.6%, and 70.1%) after basic immunization, three, and four vaccinations (Figure 4).

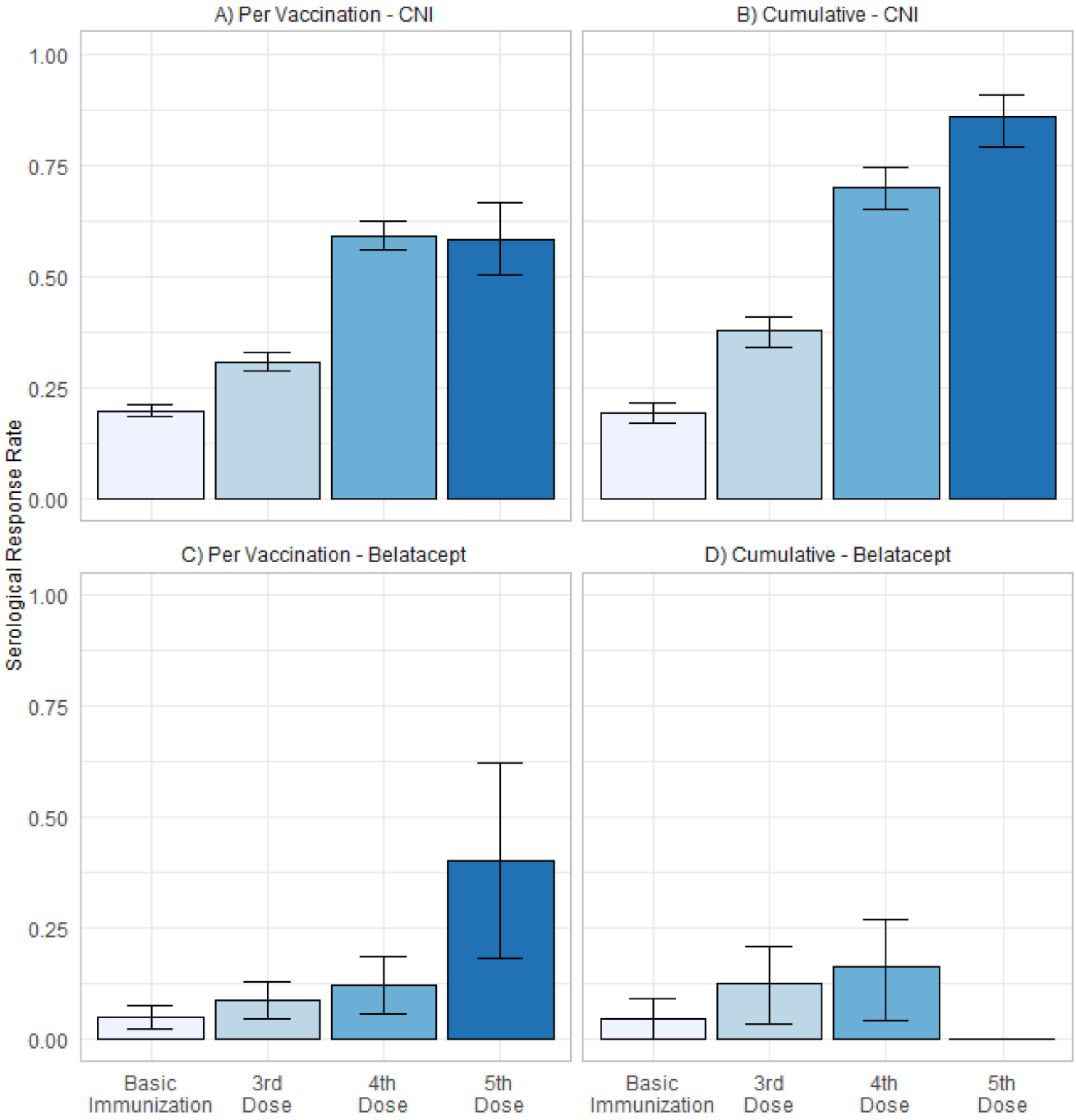

Figure 4. (**A**) Serological response rate per vaccination (±standard deviation) and (**B**) cumulative serological response rate (± 95% confidence interval) after up to 5 vaccinations in patients with CNI-based immunosuppression, as well as (**C**) serological response rate per vaccination (±standard deviation) and (**D**) cumulative serological response rate (±95% confidence interval) after up to 4 vaccinations in patients with belatacept-based immunosuppression. Cumulative response rate after fifth vaccination is not shown for patients with belatacept, due to low patient count of 5 patients receiving fifth vaccination.

5. Discussion

We provide the first systematic investigation analyzing the serological response to up to five repeated vaccinations against SARS-CoV-2 in a closely monitored cohort of adult KTR. This includes the largest reported cohort of KTR receiving four doses as well as the first reported cohort of KTR receiving five doses of a SARS-CoV-2 vaccine.

Our data indicate that repeated vaccination of up to five times is safe and induces sufficient serological response in patients who did not respond after two or three vaccinations and achieves satisfactory antibody titers in most patients.

Contrary to other previously reported case series that supported the administration of a fourth dose of vaccine [20,21], we were able to also compare different approaches to the reduction of immunosuppression and their effects on serological response. In CNI-treated non-responders after three vaccinations, serological response was improved by pausing MPA and adding 5 mg prednisolone equivalent for 4 to 8 weeks at the time of fourth vaccination. A mere partial reduction of MPA, however, did not lead to an improved response rate.

In patients treated with belatacept, additional immunizations have only a limited effect on a serological response, in particular, if treated with full-dose MPA—a result that complements previous descriptions of poor serological response to three doses of vaccine in KTR under belatacept immunosuppression [22,23]. It is obvious that these patients require different approaches.

We were able to show that several factors reported to affect the response to basic immunization against SARS-CoV-2 are also predictors of serological response after three and four doses of the vaccine [24,25]. Multivariable analysis revealed that MPA dose and belatacept treatment are the most important modifiable risk factors of impaired serological response, while non-modifiable factors include younger age, higher BMI, and years after transplantation. These observations are consistent with the concept that increased immunosenescence in elderly individuals leads to a diminished vaccine response in general [26–28], and especially in KTR [24–29]. A longer time after transplantation goes along with a general reduction of immunosuppression from waning steroids over a reduction in CNI levels [30]. Conflicting data exist concerning the BMI of patients. On the one hand, it has been shown that convalescent plasma donors with higher BMI had higher and more stable antibody titers [31], while a direct impact of BMI on serological response has not been constantly reported [32,33].

We believe that this analysis has several important implications. First, the observation that patients with CNI and MPA based maintenance immunosuppression are likely to develop a serological response after four or five SARS-CoV-2 vaccines suggests that repeated vaccination is an alternative immunization strategy to the administration of monoclonal antibodies in non-responders after 3 vaccinations, with the latter being performed by several transplant centers. Second, serological response can be improved by pausing MPA and adding 5 mg prednisolone equivalent 1 week before until 4 weeks after vaccination without increased short-term risk of rejection.

Still, anti-HLA antibody development may occur with time delay in a low percentage and has to be weighed against the benefit of protection from a potentially life-threatening disease. As a consequence, we recommend close monitoring after temporary change of immunosuppression.

Third, patients receiving belatacept are likely to be sustained non-responders even after five vaccinations. For these patients, the optimal strategy to prevent severe COVID-19 has to be defined. We advocate for pre-exposure prophylaxis with monoclonal anti-S-protein antibodies for non-responders after three vaccines who receive belatacept treatment. Switching from belatacept to a CNI-based regimen is another option, but the effect of belatacept lasts two to three months after cessation. Since some patients receive belatacept as a rescue therapy in case of poor graft function (e.g., due to CNI toxicity or thrombotic microangiopathy), the optimal strategy for belatacept-treated patients remains a challenge. Other concepts might include a pill-in-the-pocket concept using nirmatrelvir/ritonavir

for early treatment after exposure. This needs to be performed under dose adaption or pause of CNI and close monitoring of CNI levels. Alternatively, early treatment with remdesevir or post-exposure prophylaxis with monoclonal antibodies are more widely available alternatives.

Limitations arise from the study's retrospective design, which in combination with the large cohort size, did not enable complete follow-up for every patient. Hence, patients with incomplete medication data were excluded from all analyses, since these patients are not regularly followed up at our transplant center, and their records are prone to incomplete and erroneous data, including that about vaccination and serological outcome. While serological measurements were routinely performed at our institution, there was a considerable number of vaccinations that were not preceded or followed by serological measurements (Figure 1). Consequently, a patient-based approach was discarded in favor of a vaccination-based evaluation, introducing a risk of selection bias. However, our approach allows us to account for the incompleteness of the data and to optimize the number of examinable vaccinations. Intraindividual changes of titers between or after vaccinations were not represented with this approach, whereas protection after vaccination will evolve and might effectively decrease over time.

With regard to the effects of MPA dose adjustment around the fourth vaccination, the retrospective assignment to treatment groups limits the validity of the results because the groups were not fully matched and confounding factors could have influenced the group assignment. Nevertheless, comparison of the major potential influencing factors was provided to account for these risks.

Finally, while this study focuses on vaccine-induced humoral response as correlate of protection from disease, there are other contributors to immunity, such as T cell response, that may influence the degree of protection.

In conclusion, repeated vaccination against SARS-CoV-2 of up to five times effectively induces humoral serological response in kidney transplant recipients. Serological response can be enhanced by pausing MPA at the time of vaccination without increased short-term risk of acute rejection. Patients with belatacept immunosuppression and full-dose MPA are unlikely to achieve a sufficient serological response, thus requiring a different approach to ensure protection for this population at-risk.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11092565/s1. Item S1: Detailed description of institutional SARS-CoV-2 vaccination protocol [34–36]. Figure S1: Graphical representation of the institutional SARS-CoV-2 vaccination protocol. Table S1: Variable definition for descriptive statistics and multivariable analysis. Table S2: Predictors of serological response after three doses of SARS-CoV-2 vaccines for patients with CNI-based immunosuppression identified in multivariable analysis (n = 529). CNI through levels were assessed as deviation from target through level of 6 ng/mL for tacrolimus and 80 ng/mL for cyclosporine A. Table S3: Predictors of serological response after four doses of SARS-CoV-2 vaccines for patients with CNI-based immunosuppression identified in multivariable analysis. (n = 218). CNI through levels were assessed as deviation from target through level of 6 ng/mL for tacrolimus and 80 ng/mL for cyclosporine A. Table S4: Detailed analysis of all belatacept patients with sufficient serological response showing special immunological circumstances or reduced immunosuppressive medication in 8 out of 9 patients.

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Abbreviations

CNI	calcineurin inhibitor
COVID-19	coronavirus disease 2019
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assays
KTR	kidney transplant recipients
MPA	mycophenolic acid
N	nucleocapsid protein
RBD	receptor binding domain
S	spike protein
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SOT	solid organ transplantation
TCMR	T cell mediated rejection

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