ORIGINAL ARTICLE

Neutralizing antibody response against the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants after a third mRNA SARS-CoV-2 vaccine dose in kidney transplant recipients

Louise Benning[1] | Christian Morath[1] | Marie Bartenschlager[2] | Heeyoung Kim[2] | Marvin Reineke[1] | Jörg Beimler[1] | Mirabel Buylaert[1] | Christian Nusshag[1] | Florian Kälble[1] | Paula Reichel[1] | Maximilian Töllner[1] | Matthias Schaier[1] | Katrin Klein[1] | Vladimir Benes[3] | Tobias Rausch[3] | Susanne Rieger[4] | Maximilian Stich[4] | Burkhard Tönshoff[4] | Niklas Weidner[5] | Paul Schnitzler[5] | Martin Zeier[1] | Caner Süsal[6,7] | Thuong Hien Tran[6] | Ralf Bartenschlager[2,8,9] | Claudius Speer[1,10]

[1]Department of Nephrology, University of Heidelberg, Heidelberg, Germany

[2]Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Heidelberg, Germany

[3]Genomics Core Facility, European Molecular Biology Laboratory, Heidelberg, Germany

[4]Department of Pediatrics I, University Children's Hospital Heidelberg, Heidelberg, Germany

[5]Department of Virology, University of Heidelberg, Heidelberg, Germany

[6]Institute of Immunology, University of Heidelberg, Heidelberg, Germany

[7]Transplant Immunology Research Center of Excellence, Koç University Hospital, Istanbul, Turkey

[8]German Center for Infection Research, Heidelberg, Germany

[9]Division Virus-Associated Carcinogenesis, German Cancer Research Center, Heidelberg, Germany

[10]Department of Molecular Medicine Partnership Unit Heidelberg, European Molecular Biology Laboratory, Heidelberg, Germany

Correspondence
Louise Benning, Department of Nephrology, University of Heidelberg, INF162, 69120 Heidelberg, Germany.
Email: louise.benning@med.uni-heidelberg.de

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Seroconversion after COVID-19 vaccination is impaired in kidney transplant recipients. Emerging variants of concern such as the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants pose an increasing threat to these patients. In this observational cohort study, we measured anti-S1 IgG, surrogate neutralizing, and anti-receptor-binding domain antibodies three weeks after a third mRNA vaccine dose in 49 kidney transplant recipients and compared results to 25 age-matched healthy controls. In addition, vaccine-induced neutralization of SARS-CoV-2 wild-type, the B.1.617.2 (delta), and the B.1.1.529 (omicron) variants was assessed using a live-virus assay. After a third vaccine dose, anti-S1 IgG, surrogate neutralizing, and anti-receptor-binding domain antibodies were significantly lower in kidney transplant recipients compared

Abbreviations: ID_{50}, inhibitory dilution 50; IQR, interquartile range; MFI, mean fluorescence intensity; RBD, receptor-binding domain; snABs, surrogate neutralizing antibodies; TCID, tissue culture infectious dose; VEP, variant effect predictor; VoCs, variants of concern.

to healthy controls. Only 29/49 (59%) sera of kidney transplant recipients contained neutralizing antibodies against the SARS-CoV-2 wild-type or the B.1.617.2 (delta) variant and neutralization titers were significantly reduced compared to healthy controls ($p < 0.001$). Vaccine-induced cross-neutralization of the B.1.1.529 (omicron) variants was detectable in 15/35 (43%) kidney transplant recipients with seropositivity for anti-S1 IgG, surrogate neutralizing, and/or anti-RBD antibodies. Neutralization of the B.1.1.529 (omicron) variants was significantly reduced compared to neutralization of SARS-CoV-2 wild-type or the B.1.617.2 (delta) variant for both, kidney transplant recipients and healthy controls ($p < .001$ for all).

KEYWORDS
clinical decision-making, clinical research, immune modulation, immunosuppression, kidney transplantation, nephrology, practice, solid organ transplantation, vaccine

1 | INTRODUCTION

A coordinated innate and adaptive immune response is crucial for successfully combating SARS-CoV-2 infection.[1] The innate immune response slows down viral replication and spread by impeding viral replication within infected cells and creating an antiviral microenvironment in infected tissue. An adaptive immune response is then triggered by the early responses of the innate immune system resulting in highly antigen-specific effector T and B cells. However, naïve T and naïve B cells need time to proliferate and differentiate into effector cell subsets. Severe COVID-19 cases have been shown to be associated with failure of a timely coordinated immune response during natural infection, whereas rapid induction of SARS-CoV-2 specific CD4+ T cells has been shown to play a key role in milder disease and enhanced viral clearance.[2,3] Successful vaccination against COVID-19 counteracts a delayed immune response by activating the immune system before exposure to SARS-CoV-2 and is essential to prevent severe COVID courses, especially in immunocompromised individuals.

However, immune response to two-dose COVID-19 vaccination is impaired in solid organ transplant recipients compared to the general population and recent data have shown inferior real-world effectiveness of different vaccination schemes against COVID-19 disease.[4-7] Qin et al. recently demonstrated an 82-fold higher risk of a COVID-19 breakthrough infection and a 485-fold higher risk of associated hospitalization and death for solid organ transplant recipients compared to fully vaccinated adults in the United States through April 2021.[8] Due to waning humoral immunity and a rapid increase in breakthrough infections, a third vaccine dose was recommended for the general population, including immunocompromised individuals with impaired vaccination response.[9-11] First results describe an increased immune response in transplant recipients to a third vaccine dose with an induction of serologic response in 25%–49% of previous non-responders and a significant increase in antibody titers for patients who were seropositive already before the third dose.[12-15]

Emerging variants of concern (VoCs), such as the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants with partial immune escape are rapidly displacing other circulating strains and increasingly lead to breakthrough infections. In particular, the omicron variant escapes antibody neutralization and early real-world data indicate reduced effectiveness and reduced protection from hospitalization after two-dose vaccination or prior infection in the general South African population where omicron was first described.[16,17] Schmidt et al. and Nemet et al. recently independently demonstrated a substantial gain in neutralizing antibody activity against the omicron variant in healthy persons who were vaccinated after COVID-19 infection or received a third mRNA vaccine dose compared to individuals with standard two-dose vaccination.[18,19]

We recently first demonstrated impaired neutralization of the B.1.1.7 (alpha), B.1.351 (beta), and B.1.617.2 (delta) variant in seroconverted kidney transplant recipients compared to healthy controls after two-dose vaccination.[20] However, little is known about neutralization of the currently predominant B.1.617.2 (delta) and B.1.1.529 (omicron) variants in kidney transplant recipients after a third mRNA vaccine dose. Data regarding neutralization against both VoCs are urgently needed to guide further vaccination strategies for non- and low-responders and ultimately help protect highly vulnerable kidney transplant recipients from severe COVID-19.

2 | METHODS

2.1 | Study design

In this ongoing observational cohort study to assess immunogenicity after COVID-19 vaccination in kidney transplant recipients and healthy controls, we enrolled 49 kidney transplant recipients and 25 healthy controls who received a third mRNA vaccine dose between August and October 2021 at the Department of Nephrology and at the Department of Pediatrics I at Heidelberg University Hospital. Serum was collected after a median (IQR) of 21 (20–32) and

21 (18–22) days after a third vaccine dose in kidney transplant recipients and healthy controls, respectively. Vaccine interval between a second and third dose was a median (IQR) of 138 (117–162) and 228 (218–236) days in kidney transplant recipients and healthy controls, respectively. All 25/25 (100%) healthy controls received three vaccinations with BNT162b2, whereas 40/49 (82%) kidney transplant recipients received three doses of an mRNA vaccine, 7/49 (14%) a priming dose with the replication-deficient adenoviral vector vaccine ChAdOx1 followed by two doses of an mRNA vaccine, and 2/49 (4%) two doses of ChAdOx1 followed by a third dose of an mRNA vaccine. Study participants with antibodies against the nucleocapsid protein (indicative of previous SARS-CoV-2 infection) or a medical history of SARS-CoV-2 infection were excluded from the analysis.

We determined IgG antibodies against the SARS-CoV-2 spike S1 subunit, surrogate neutralizing antibodies (snABs), and IgG antibodies against different target epitopes of the SARS-CoV-2 in all 49 kidney transplant recipients and 25 healthy controls after a third mRNA vaccine dose. In addition, IgG antibodies against the spike S1 of 4 common cold coronaviruses, namely HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43, were assessed.

Neutralizing antibodies against the SARS-CoV-2 wild-type, the B.1.617.2 (delta), and the B.1.1.529 (omicron) variants were quantified by using a live-virus neutralization assay. Neutralization against the B.1.1.529 (omicron) variants was tested in 35 kidney transplant recipients with seropositivity for anti-S1 IgG, snABs, and/or anti-receptor-binding domain (anti-RBD) antibodies. Results for neutralization against the B.1.1.529 (omicron) variants in kidney transplant recipients were compared to 10 age- and sex-matched healthy controls.

In 33/49 (67%) kidney transplant recipients with sera available before third vaccine dose, the same assays including live-virus neutralization against wild-type and the B.1.617.2 (delta) variant were performed using to analyze interindividual courses of humoral responses.

The study was approved by the Ethics Committee of the University of Heidelberg and conducted in accordance with the Declaration of Helsinki. All study participants provided written informed consent. The study is registered at the German Clinical Trial Register (DRKS00024668).

2.2 | Assessment of humoral responses after COVID-19 vaccination using commercially available tests

We used the SARS-CoV-2 Total Assay (Siemens) and the Elecsys anti-SARS-CoV-2 assay (Roche) to detect anti-S1 IgG and anti-nucleocapsid antibodies, respectively. A semi-quantitative index of ≥1 defines positivity for both assays.

Surrogate neutralizing antibodies were detected using a surrogate virus neutralization test (Medac) that mimics the virus interaction with the host cell by direct protein-protein interaction using purified RBD protein from the viral spike and the ACE-2 host cell receptor.[21] An inhibition ≥30% of RBD:ACE-2 binding defines positivity for this assay.

A bead-based multiplex assay for the Luminex platform (LabScreen Covid Plus, One Lambda Inc.) was used to detect IgG antibodies against four different SARS-CoV-2 target epitopes and IgG antibodies against the spike S1 of four common cold coronaviruses.[22] The mean fluorescence intensity (MFI) was analyzed using a Luminex 200 device (Luminex Corporation).

All assays were performed according to the manufacturer's instructions and as described previously.[20,23-29]

2.3 | Live-virus neutralization against wild-type, the B.1.617.2 (delta), and the B.1.1.529 (omicron) variants

All experiments have been described in detail previously.[20,26-28,30] In brief, we determined neutralization titers in titration experiments using VeroE6 cells. Virus stocks were produced by either amplification of the BavPat1/2020 strain (European Virus Archive) or isolation and amplification of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants from nasopharyngeal and oropharyngeal swabs of PCR-confirmed SARS-CoV-2 positive patients.[20,26-28,31] BavPat1/2020 and B.1.617.2 (delta) variant were amplified in VeroE6 cells and virus titers of stocks were determined by plaque assay and Tissue Culture Infectious Dose (TCID) 50 assay in VeroE6 cells. To avoid rapid cell culture adaptation, stocks of B.1.1.5291 (omicron) were produced in Calu-3 cells and titers were determined in VeroE6 cells using TCID 50 assay. Virus stocks were validated by genome sequencing. For neutralization assays, two-fold serial dilutions of vaccine sera were incubated with 6×10^4 TCID 50 of wild-type, the B.1.617.2 (delta), and the B.1.1.529 (omicron) variants. Virus replication was determined by immunostaining for the viral nucleocapsid protein using an in-cell ELISA. Data were normalized to a mock-infected (0%) and a no-serum control (100%). The inhibitory dilution 50 (ID_{50}) is defined as the serum dilution that results in 50% reduction of normalized signal.

2.4 | SARS-CoV-2 genome sequencing

SARS-CoV-2 genomes were sequenced with the ARTIC protocol using the NEBNext ARTIC FS kit to prepare sequencing libraries. To increase sample throughput the I.DOT liquid dispenser (Dispendix) was employed, and 4×96 libraries were pooled for sequencing in paired-end mode (2×75 bp) on a NextSeq instrument. Sequencing adapters were trimmed using trim_galore and host-read contamination was assessed and filtered using kraken2, as described previously.[31,32] Reads were aligned to the SARS-CoV-2 reference genome using bwa and alignments were sorted and indexed using samtools and quality-controlled using alfred.[33-35] Priming regions were masked with iVar, followed by variant calling with FreeBayes, normalization of variants with bcftools, and annotation of variants with

the Ensembl Variant Effect Predictor (VEP).[36-39] iVar was employed to compute a viral consensus sequence that was then classified by Pangolin and Nextclade to determine the viral lineage and clade, respectively.[40,41]

2.5 | Statistics

The statistical analysis was performed using GraphPad Prism version 9.0.0 (GraphPad Software). Data are given as median and interquartile range (IQR) or number (N) and percent (%). Continuous variables were compared using the Mann-Whitney U test for unpaired or the Wilcoxon matched-pairs rank test for paired variables. When comparing more than three paired continuous variables, we applied the Friedman's test with Dunn's post-test. Categorial data were compared using the Fisher's exact test. Spearman's rho was calculated to describe the correlation of different commercially available assays to the current gold standard to assess humoral immunity using a live virus assay. Statistical significance was assumed at a $p < .05$.

2.6 | Role of the funding source

The funding source did not affect in the trial design, conduct, or reporting of this study.

3 | RESULTS

3.1 | Study population

Humoral immune response was assessed in 49 kidney transplant recipients and 25 age-matched healthy controls. Median (IQR) age was 55 (46–65) for kidney transplant recipients and 53 (39–59) years for healthy controls. With 20/49 (41%) kidney transplant recipients and 17/25 (68%) healthy female controls, the healthy controls cohort consisted of more females ($p = .048$). Baseline characteristics of all 49 kidney transplant recipients stratified for seropositivity in at least one of three commercially available assays after a third vaccine dose are shown in Table 1.

3.2 | Humoral immune responses in kidney transplant recipients compared to healthy controls

Kidney transplant recipients showed significantly impaired humoral response to a third mRNA vaccine dose in all commercially available assays with a median (IQR) anti-spike S1 IgG index of 4.79 (0.1–31.4), a median (IQR) % inhibition for surrogate neutralizing antibodies of 65 (27.4–97.2), and a median (IQR) MFI for anti-RBD antibodies of 12,322 (0–21,413) compared to 482.1 (253.6–811.9), 97.9 (97.8–98), and 23,214 (22,603–23,429),

respectively, in healthy controls ($p < .001$ for all; Figure 1A). IgG antibodies against the full spike, the spike S1, and the spike S2 subunits as determined by a bead-based multiplex assay were also significantly lower in kidney transplant recipients with a median (IQR) MFI of 19,084 (0–22,419) for the full spike, 9482 (0–15,117) for the spike S1, and 1716 (0–6,042) for the spike S2, respectively, when compared to healthy controls (24,657 [23,619–24,913]; 21,237 [20,572–22,084]; 15,953 [13,159–19,137]; $p < .001$ for all; Figure 1B). Healthy controls also exhibited significantly higher IgG antibodies against the four tested common cold coronaviruses, but the difference of antibody response between healthy controls and kidney transplant recipients was less pronounced than for SARS-CoV-2 ($p = .003$ for HCoV-229E, $p = .004$ for HCoV-HKU1, $p = .003$ for HCoV-NL63, and $p < .001$ for HCoV-OC43; Figure S1).

All 25/25 (100%) healthy controls were seropositive for anti-spike S1 IgG, surrogate neutralizing antibodies, and anti-RBD antibodies after a third vaccine dose whereas only 26/49 (53%) kidney transplant recipients showed concurrent seropositivity in all three commercially available tests (Figure 1C).

3.3 | Neutralizing antibody response against SARS-CoV-2 wild-type and the B.1.617.2 (delta) variant

After a third mRNA vaccine dose, kidney transplant recipients showed significantly impaired neutralization against SARS-CoV-2 wild-type and the B.1.617.2 (delta) variant when compared to healthy controls ($p < .001$ for both; Figure 2A). The median (IQR) ID_{50} was 1:20 (0–1:160) versus 1:640 (1:640–1:1280) for wild-type neutralization, and 1:20 (0–1:160) versus 1:1280 (1:640–1:1280) for neutralization of the B.1.617.2 (delta) variant in kidney transplant recipients compared to healthy controls, respectively. All 25/25 (100%) healthy controls were above the cut-off for detection of 1:10 for neutralization against wild-type and the B.1.617.2 (delta) variant, whereas 20/49 (41%) kidney transplant recipients remained below the threshold for wild-type and B.1.617.2 (delta) neutralization.

When assessing interindividual changes in neutralization against the SARS-CoV-2 wild-type strain and the B.1.617.2 (delta) variant in the 33 kidney transplant recipients with sera available before and after a third vaccine dose, neutralizing activity against wild-type and the B.1.617.2 (delta) variant increased significantly with a third mRNA vaccine dose ($p < .001$ for both; Figure 2B). Corresponding interindividual changes in anti-S1 IgG, surrogate neutralizing, and anti-RBD antibodies are displayed in Figure S2.

Commercially available assays suitable for clinical routine use showed a strong correlation to the ID_{50} as determined by live-virus neutralization assay. Correlation for anti-spike S1 IgG index, surrogate neutralizing antibodies, and anti-RBD antibodies to wild-type neutralization was slightly better compared to neutralization of the B.1.617.2 (delta) variant with a Spearman's rho of 0.94 and 0.88 for anti-spike S1 IgG, 0.89 and 0.85 for surrogate neutralizing

TABLE 1 Clinical characteristics of kidney transplant recipients according to humoral response 3 weeks after a third vaccine dose

Characteristic	All patients (N = 49)	Seropositive after third dose (N = 35)	Seronegative after third dose (N = 14)	p value
Female, N (%)	20 (41)	16 (46)	4 (29)	.27
Age, median (IQR)	55 (46–65)	57 (49–65)	54 (45–67)	.67
Vaccine type				
3 × mRNA[a]	40 (82)	28 (80)	12 (86)	>.99
ChAdOx1 + 2 × mRNA[b]	7 (14)	6 (17)	1 (7)	.66
2 × ChAdOx1 + mRNA[c]	2 (4)	1 (3)	1 (7)	.49
Time between vaccine and transplant, years (IQR)	8.1 (2.4–13.6)	8.5 (4.4–13.7)	3.7 (0.8–12.7)	.12
First transplant, N (%)	46 (94)	33 (94)	13 (93)	>.99
Type of immunosuppressive regimen, N (%)				
Calcineurin inhibitors	44 (90)	32 (91)	12 (86)	.62
Tacrolimus	32 (65)	20 (57)	12 (86)	.10
Cyclosporin A	12 (24)	12 (34)	0 (0)	.01
Mycophenolic acid	39 (80)	27 (77)	12 (86)	.70
mTOR inhibitors	5 (10)	5 (14)	0 (0)	.30
Belatacept	2 (4)	1 (3)	1 (7)	.49
Steroids	47 (96)	35 (100)	12 (86)	.08
Comorbidities, N (%)				
Hypertension	35 (71)	26 (74)	9 (64)	.50
Diabetes	6 (12)	3 (9)	3 (21)	.33
Chronic artery disease	12 (24)	9 (26)	3 (21)	>.99
Chronic lung disease	5 (10)	5 (14)	0 (0)	.30
Chronic liver disease	2 (4)	2 (6)	0 (0)	>.99
Malignancy	8 (16)	5 (14)	3 (21)	.67

Abbreviations: mTOR, mammalian target of rapamycin; N, Number.

[a]36 received three doses of BNT162b2; 4 received 2 doses of BNT162b2 followed by a third dose of mRNA-1273.

[b]6 received a priming dose with ChAdOx1 followed by two doses of BNT162b2; 1 received a priming dose with ChAdOx1 followed by two doses of mRNA-1273.

[c]2 received two doses of ChAdOx1 followed by a third dose of BNT162b2.

antibodies, and 0.95 and 0.91 for anti-RBD antibodies, respectively (Figure 2C).

3.4 | Neutralizing antibody response against the B.1.1.529 (omicron) variants

Neutralization against the B.1.1.529 (omicron) variants was assessed in 35 kidney transplant recipients that showed seroconversion for anti-S1 IgG, surrogate neutralizing, and/or anti-RBD antibodies and was compared to 10 age- and sex-matched healthy controls (Figure 3A). Neutralization against the B.1.1.529 (omicron) variants in seroconverted kidney transplant recipients was with a median (IQR) ID_{50} of 0 (0–1:20) significantly lower compared to healthy controls with a median (IQR) ID_{50} of 1:80 (1:40–1:160) ($p < .001$; Figure 3B). For both, kidney transplant recipients and healthy controls, neutralization of the B.1.1.529 (omicron) variants

was significantly reduced compared to neutralization of the wild-type or the B.1.617.2 (delta) variant ($p < .001$ for both; Figure 3B). Anti-S1 IgG showed the strongest correlation to neutralization of the B.1.1.529 (omicron) variants with a Spearman's rho of 0.85 (Figure 3C).

3.5 | Breakthrough infections

All study participants were inquired about the occurrence of breakthrough infection at a median (IQR) of 5.9 (5.4–6.5) months after receiving a third mRNA vaccine dose. PCR-confirmed infection occurred in 12/49 (25%) kidney transplant recipients a median (IQR) of 5.2 (4.1–5.8) months after receiving their third vaccine dose. One infection occurred in December 2021, when the B.1.617.2 (delta) variant was the predominant SARS-CoV-2 variant in Germany, whereas the remaining 11/12 infections
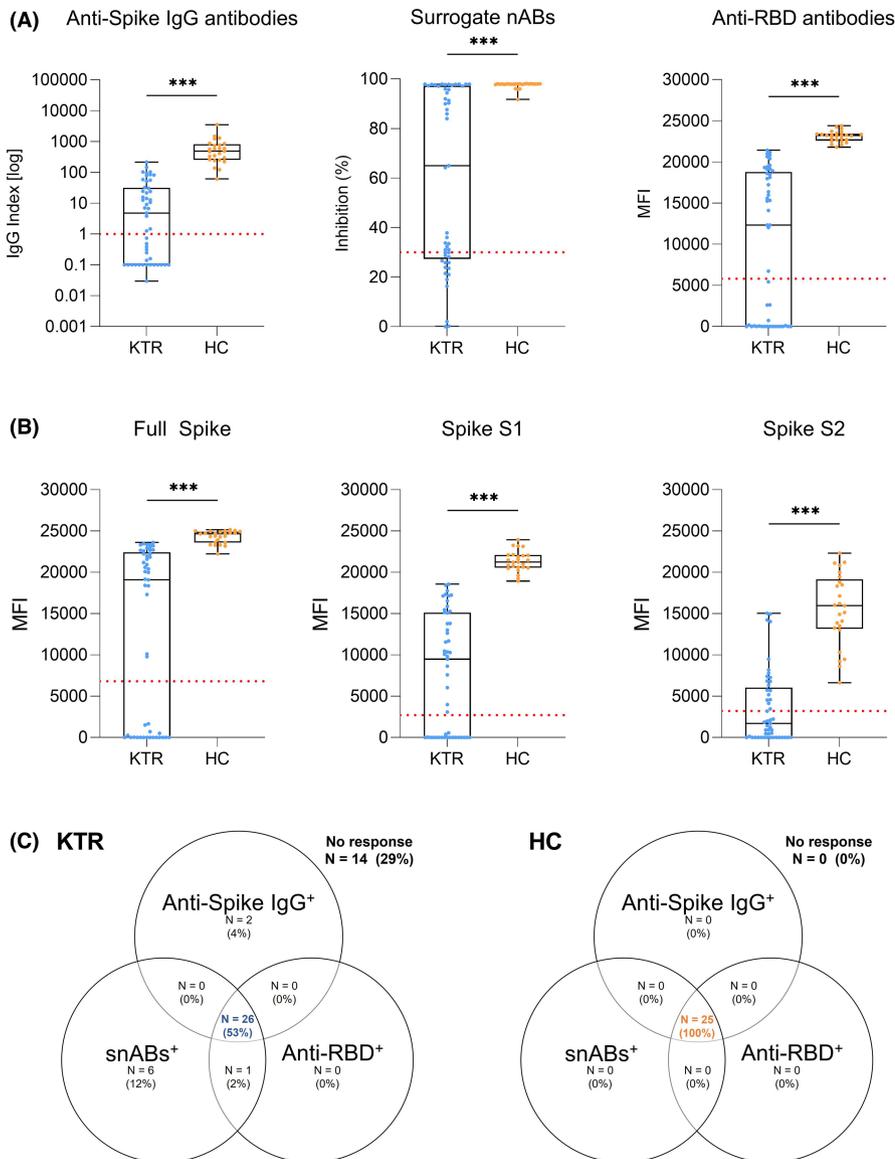
FIGURE 1 Humoral immune response after a third mRNA vaccine dose in kidney transplant recipients and healthy controls as determined by commercially available assays. (A) Anti-spike IgG (left panel), surrogate neutralizing (middle panel), and anti-receptor-binding domain (right panel) antibodies in 49 kidney transplant recipients and 25 age-matched healthy controls after a third mRNA vaccine dose. The dashed red line indicates the respective cut-off for each assay. (B) IgG antibodies against the SARS-CoV-2 full spike, spike S1, and spike S2 subunits in 49 kidney transplant recipients and 25 age-matched healthy controls. The y-axis represents the mean fluorescence intensity (MFI) where the dashed red line indicates the cut-off for each target. (C) Seropositivity for anti-spike IgG, surrogate neutralizing, and anti-receptor-binding domain antibodies in 49 kidney transplant recipients and 25 age-matched healthy controls after a third mRNA vaccine dose shown in a Venn diagram. HC, healthy controls; KTR, kidney transplant recipients; MFI, mean fluorescence intensity; N, number; RBD, receptor-binding domain; snABs, surrogate neutralizing antibodies. ***p < .001 [Color figure can be viewed at wileyonlinelibrary.com]

occurred in 2022 in parallel with the surge of the B.1.1.529 (omicron) variants. In 6/12 (50%) kidney transplant recipients with breakthrough infections, seroconversion was detectable in all three commercially available assays before SARS-CoV-2 infection. Notably, all patients were oligosymptomatic with no patient requiring hospitalization due to COVID-19.

4 | DISCUSSION

This is the first study to describe live-virus neutralization of the SARS-CoV-2 wild-type, the B.1.617.2 (delta) variant, and the B.1.1.529 (omicron) variants by sera of kidney transplant recipients before and after a third vaccine dose in comparison to healthy controls.

In commercially available assays, we detected seropositivity for 26/49 (53%) kidney transplant recipients after a third vaccine dose. This is in line with other studies that found a 36%–68% seroresponse rate for kidney transplant recipients after a third COVID-19 vaccine dose and a 25%–49% seroconversion rate of previous non-responders.[12-15] However, the authors of these studies only used commercially available tests that may not fully reflect the actual protection against variants of concern as they test for antibodies against the SARS-CoV-2 wild-type strain.

Only recently, Kumar et al. published results on neutralization of SARS-CoV-2 variants by sera of transplant recipients after two and three vaccine doses of mRNA-1273 (Moderna) vaccine using a SARS-CoV-2 spike-pseudotyped lentivirus-based neutralization assay.[42] The authors showed that neutralizing antibody positivity after two doses of mRNA-1273 vaccine is low for B.1.1.7 (alpha), B.1.351 (beta), and B.1.617.2 (delta) but subsequently increases with the administration of a third vaccine dose.[42] In concordance with our results, the authors were not able to detect sufficient neutralization response in 25/60 (42%) and 27/60 (45%) patients against the SARS-CoV-2 wild-type or B.1.617.2 (delta) variant, respectively.[42] Although lentivirus-based assays show a good correlation to results obtained
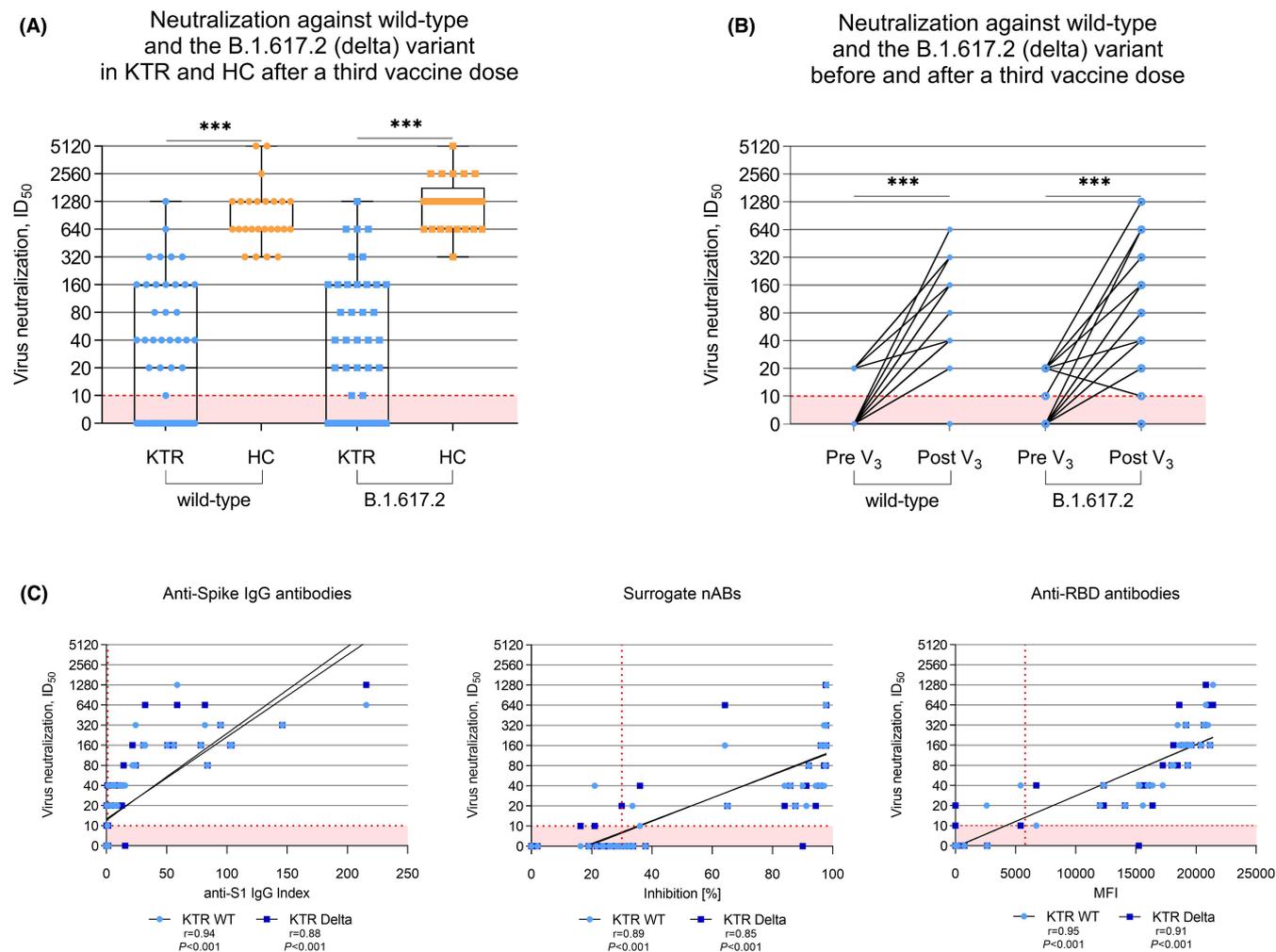
FIGURE 2 Neutralization of SARS-CoV-2 wild-type and the B.1.617.2 (delta) variant by antibodies in sera of kidney transplant recipients and healthy controls taken after a third mRNA vaccine dose. (A) Vaccine-induced neutralization of the SARS-CoV-2 wild-type and cross-neutralization of the B.1.617.2 (delta) variant by antibodies in sera of 49 kidney transplant recipients and 25 age-matched healthy controls taken after a third mRNA vaccine dose as determined using a live-virus assay. The dashed red line indicates the cut-off for detection which is the 1:10 dilution in this assay. (B) Interindividual course of neutralization against SARS-CoV-2 wild-type and cross-neutralization against the B.1.617.2 (delta) variant in 33 kidney transplant recipients with sera available before and after a third mRNA vaccine dose. (C) Correlation analyses of three commercially available assays for anti-spike IgG, surrogate neutralizing, and anti-receptor-binding domain antibodies with neutralization titers of SARS-CoV-2 wild-type and cross-neutralization titers of the B.1.617.2 (delta) variant by sera of kidney transplant recipients taken after a third mRNA vaccine dose. The dashed red line indicates the respective cut-off for each assay. HC, healthy controls; ID_{50}; inhibitory dilution 50; KTR, kidney transplant recipients; MFI, mean fluorescence intensity; RBD, receptor-binding domain; r; Spearman's rho; snABs, surrogate neutralizing antibodies; V, vaccination; WT, wild-type. ***$p < .001$ [Color figure can be viewed at wileyonlinelibrary.com]

by live-virus neutralization, the testing performed in our study is generally considered the current gold standard to assess actual neutralization titers.[43]

In our results including neutralizing antibody activity detected by a live-virus assay, kidney transplant recipients with detectable seroconversion before the administration of a third vaccine dose showed significantly stronger neutralization of the SARS-CoV-2 wild-type and the B.1.617.2 (delta) variant after a third vaccine dose. However, sera of 20/49 (41%) kidney transplant recipients did not contain neutralizing antibodies above the threshold for detection of neutralization of SARS-CoV-2 wild-type or the B.1.617.2 (delta) variant even after the administration of a third mRNA vaccine dose. Notably, sera of only 15/35 (43%) kidney transplant recipients with seropositivity in any

of the three used commercially available assays showed neutralizing antibody activity against the emerging B.1.1.529 (omicron) variants. Impaired neutralization of the B.1.1.529 (omicron) variants has been described previously in healthy cohorts and is explained by the various mutations in the spike region of the B.1.1.529 (omicron) variants that facilitate immune escape.[16-19,44] Therefore, even seroconverted kidney transplant recipients may not be adequately protected against infection with the B.1.1.529 (omicron) variants. These results are in line with only recently published data by Kumar et al. analyzing 60 solid organ transplant recipients (kidney, kidney-pancreas, lung, heart, and liver) one and three months after completion of 3 doses of the mRNA-1273 vaccine.[45] Kumar et al. showed a significantly lower proportion of patients with detectable neutralizing antibody responses to
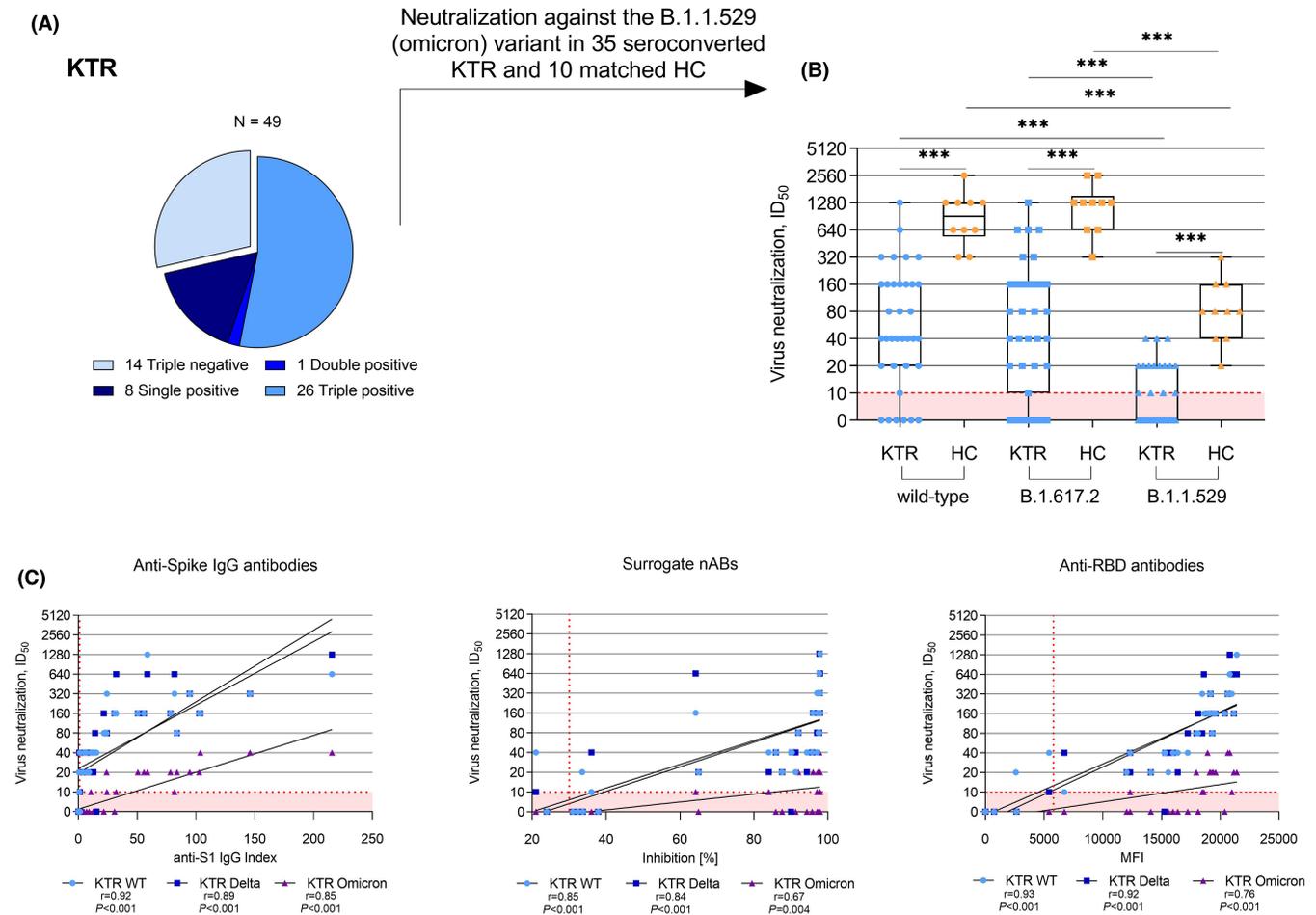
FIGURE 3 Neutralization of the B.1.1.529 (omicron) variants in kidney transplant recipients showing seroconversion in at least one commercially available assay and healthy controls after a third mRNA vaccine dose. (A) Neutralization of the B.1.1.529 (omicron) variants was assessed in 35 kidney transplant recipients with seropositivity for anti-S1 IgG, surrogate neutralizing, and/or anti-RBD antibodies. (B) Vaccine-induced neutralization of the SARS-CoV-2 wild-type and cross-neutralization of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants by sera of 35 seroconverted kidney transplant recipients and 10 age- and sex-matched healthy controls after a third mRNA vaccine dose as determined with a live-virus assay. (C) Correlation analyses of anti-spike IgG, surrogate neutralizing, and anti-RBD antibodies with neutralization titers of SARS-CoV-2 wild-type and cross-neutralization of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants in sera of seroconverted kidney transplant recipients after a third mRNA vaccine dose. The dashed red line indicates the respective cut-off for each assay. HC, healthy controls; ID_{50}, inhibitory dilution 50; KTR, kidney transplant recipients; MFI, mean fluorescence intensity; N, number; RBD, receptor-binding domain; r; Spearman's rho; snABs, surrogate neutralizing antibodies; WT, wild-type. ***$p < .001$ [Color figure can be viewed at wileyonlinelibrary.com]

omicron compared to neutralization against wild-type or delta using a SARS-CoV-2 spike pseudotyped lentivirus assay.[45] Our data with an increasing number of breakthrough infections about 6 months after reception of a third vaccine dose, even in seroconverted kidney transplant recipients, illustrate the insufficient neutralization against the B.1.1.529 (omicron) variants. As kidney transplant recipients have a higher risk for breakthrough infections, hospitalization, and death due to COVID-19 than the general population, the high proportion of non-responders even after a third vaccine dose and the significantly lower neutralization against the B.1.1.529 (omicron) variants is highly distressing and poses an urgency to optimize vaccine responsiveness in kidney transplant recipients.

One approach to optimize vaccination response in kidney transplant recipients is to combine different vaccines to a heterologous vaccination regimen. However, heterologous vaccination regimens

have not yet shown any superior outcome regarding seroconversion rates in kidney transplant recipients as they are all still based on the original SARS-CoV-2 strain.[46,47] In our study cohort, 9/49 (18%) received either one or two doses of ChAdOx1 before a third mRNA vaccine dose. Among kidney transplant recipients who received heterologous vaccination, 4/9 (44%) were seropositive in all three assays, compared to 22/40 (55%) who received three doses of an mRNA vaccine. Although limited by the small number of patients vaccinated with a heterologous vaccination regimen, we did not detect a trend toward better serologic response after heterologous vaccination.

Another approach to improve the vaccine-induced immune response in kidney transplant recipients is by modulation of immunosuppression. Recent data suggest that number and type of immunosuppressive agents, especially treatment with mycophenolate mofetil and belatacept, act as major determinants of seroconversion

failure in kidney transplant recipients after standard two-dose vaccination.[6,48] Both the magnitude of humoral responses and spike-specific T cells have been shown to depend on immunosuppressive treatment during vaccine administration for patients with systemic rheumatic diseases, rheumatoid arthritis, or multiple sclerosis receiving disease-modifying therapies.[49-51] We did not see any significant differences between responders and non-responders after a third mRNA vaccine dose for a particular type of immunosuppressive regimen, which may be due to the size of our study population. However, we found that patients who were transplanted more recently tended to remain seronegative even after a third vaccine dose, which may indicate a better vaccination response with reduced immunosuppressive maintenance therapy, as is common in long-term kidney transplant recipients. D'Offizi et al. recently reported similar findings with a higher immunologic response after two-dose vaccination in liver transplant recipients with ≥6 years since transplantation, which they also attribute to progressive dose reduction.[52] The two patients of our cohort with Belatacept maintenance therapy showed low antibody levels or no seroconversion which has been described previously, even after a third mRNA vaccine dose.[53]

The administration of a fourth vaccine dose is another attempt to optimize vaccination response in kidney transplant recipients, ideally using a vaccine formulation that is based on more recently circulating variants such as B.1.1.529 (omicron). First results indicate an improved humoral response after a fourth vaccine dose among those with a weak response after three doses but little to no improvement among those with no response after three doses.[54,55] Although this may suggest immunogenic potential for poor responders after a third vaccine dose, additional actions seem necessary to reach vaccine-induced immunity and protection from severe disease courses.[55] Passive immunization of those patients that do not mount immune response at all with therapeutic antibodies that have shown to inhibit SARS-CoV-2 and variants of concern including the B.1.1.529 (omicron) variants is another attempt to protect these patients from severe COVID-19 disease.[56,57] Our data show that anti-spike S1 IgG and other commercially available tests may aid in clinical decision-making for additional booster vaccination(s) as these tests show a strong correlation to live-virus neutralization which is unfortunately not yet feasible in clinical routine. A strong correlation between commercially available tests and live-virus neutralization has been described previously by us and others in different cohorts.[20,26,27,58-60]

A limitation of our study is the lack of data on B and T cell responses after vaccination. Recent studies provided evidence on highly reproducible whole-blood assays to detect SARS-CoV-2 spike specific T cell response, using a similar platform to assays measuring T cell specific responses against *Mycobacterium tuberculosis*.[49,50,61] A strong correlation between anti-RBD antibodies and SARS-CoV-2 specific IFN-*γ* T cell response was shown for healthy cohorts, immunosuppressed patients with rheumatoid arthritis, and multiple sclerosis patients on various disease-modifying therapies.[49,50,62] Similarly, in kidney and liver transplant recipients, a strong correlation between quantitative and functional CD4+ T-cell

responses and anti-S1 IgG antibodies was demonstrated after two vaccine doses.[6,52] These results suggest that anti-spike titers may be used as a surrogate parameter to assess immunologic response after COVID-19 vaccination as T cell studies are more resource intensive and less standardized between different laboratories.

In healthy individuals, a substantial increase in neutralizing antibody activity against omicron was observed after a third vaccine dose, possibly due to the presence of memory B cells recognizing the omicron RBD.[44,63,64] Tarke et al. recently demonstrated preserved T cell responses in healthy individuals against variants of concern, including the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants, up to 6 months after second vaccination.[63] These data provide cause for optimism at a time of rising incidence and waning humoral immunity at least for the general population.[58,63] Regarding kidney transplant recipients, Schrezenmeier et al. found significantly increased spike-reactive CD4$^+$ T helper cells with higher portions of IL-2 and IL-4 secreting and polyfunctional (IFNγ$^+$TNFα$^+$IL-2$^+$) T cells in seroconverted kidney transplant recipients after a third vaccine dose, however, non-responders showed only marginal improvements in antigen-specific B and T cells.[65]

In conclusion, a third mRNA vaccine dose increases vaccine-induced immunity in most kidney transplant recipients. However, neutralizing antibody activity against immune-escape variants such as the B.1.1.529 (omicron) variants is barely detectable even in seroconverted individuals after a third vaccine dose and poses the urgent need to optimize vaccination strategies for highly vulnerable kidney transplant recipients.

ORCID

Louise Benning https://orcid.org/0000-0002-8366-2100
Christian Morath https://orcid.org/0000-0003-2218-7817
Christian Nusshag https://orcid.org/0000-0001-7776-3784
Matthias Schaier https://orcid.org/0000-0003-1040-1150
Burkhard Tönshoff https://orcid.org/0000-0002-6598-6910
Claudius Speer https://orcid.org/0000-0002-3668-5916

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