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# Potent induction of humoral and cellular immunity after bivalent BA.4/5 mRNA vaccination in dialysis patients

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Knowledge on immunogenicity of the bivalent Omicron BA.4/5 vaccine in dialysis patients and the effect of a previous infection is limited. Therefore, vaccine-induced humoral and cellular immunity was analyzed in dialysis patients and immunocompetent controls with and without prior infection. In an observational study, 33 dialysis patients and 58 controls matched for age, sex and prior infection status were recruited. Specific IgG, neutralizing antibody activity and cellular immunity towards the spike-antigen from parental SARS-CoV-2 and Omicron-subvariants BA.1, BA.2 and BA.4/5 were analyzed before and 13–18 days after vaccination. The bivalent vaccine led to a significant induction of IgG, neutralizing titers, and specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell levels. Neutralizing activity towards the parental strain was higher than towards the Omicron-subvariants, whereas specific T-cell levels towards parental spike and Omicron-subvariants did not differ indicating substantial cross-reactivity. Dialysis patients with prior infection had significantly higher spike-specific CD4<sup>+</sup> T-cell levels with lower CTLA-4 expression compared to infection-naïve patients. When compared to controls, no differences were observed between infection-naïve individuals. Among convalescent individuals, CD4<sup>+</sup> T-cell levels were higher in patients and neutralizing antibodies were higher in controls. Vaccination was overall well tolerated in both dialysis patients and controls with significantly less adverse events among patients. In conclusion, our study did not provide any evidence for impaired immunogenicity of the bivalent Omicron BA.4/5 vaccine in dialysis patients. Unlike in controls, previous infection of patients was even associated with higher levels of spike-specific CD4<sup>+</sup> T cells, which may reflect prolonged encounter with antigen during infection.

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## INTRODUCTION

End-stage chronic kidney disease (CKD) and dialysis are risk factors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the development of severe COVID-19 disease with fatal outcome which was particularly evident at the beginning of the pandemic<sup>1</sup>. This resulted from the relative inability to perform stringent physical distancing due to regular hemodialysis treatment and associated travel to and from the dialysis centers<sup>2</sup> as well as from general immunological impairments due to uremic immunodeficiency, advanced age, and multiple comorbidities<sup>3</sup>. To protect this vulnerable group from severe COVID-19 disease, hospitalizations, and death, patients were prioritized for COVID-19 vaccination once licensed. In line with a decreased response rate to vaccinations such as hepatitis B, tetanus<sup>3,4</sup>, or influenza<sup>5</sup>, patients were shown to frequently mount an inadequate humoral and cellular immune response after COVID-19 vaccination<sup>6–10</sup> which waned rapidly after administration of prime and booster doses<sup>11,12</sup>. This necessitated more frequent booster vaccinations in this vulnerable patient group to achieve a similar level of immunity as in immunocompetent individuals. In 2022, the immune-escaping omicron subvariants of concern dominated the pandemic, which led to an increased incidence of breakthrough infections in both patients and immunocompetent individuals. This is illustrated by the fact that four doses of the monovalent mRNA vaccine resulted in a high neutralizing activity against the ancestral strain, but a much lower rate against the omicron subvariant BA.1<sup>13</sup>, BA.4 and BA.5<sup>14</sup>. Therefore, bivalent

mRNA vaccines targeting both the parental strain and either BA.1 or BA.4/5 were developed to more specifically induce variant-adapted humoral and cellular immunity. First studies among immunocompetent controls showed a more pronounced induction of neutralizing antibodies after bivalent booster vaccination compared to the monovalent vaccination<sup>15–18</sup>. The bivalent BA.4/5 vaccine effectiveness was 72% for preventing COVID-19 hospitalization, and 68% for preventing COVID-19-related deaths compared to individuals who did not receive a booster vaccination<sup>19</sup>. With increasing incidence of breakthrough infection, knowledge on the effect of hybrid immunity on subsequent booster vaccinations becomes increasingly important. We and others have previously shown that the induction of humoral immunity after bivalent vaccination in immunocompetent individuals is more pronounced in previously non-infected individuals<sup>15,16,20</sup>, whereas vaccine-induced T-cell levels were similar in individuals with and without prior infection<sup>20</sup>. Similar data on bivalent vaccines in dialysis patients are limited. First immunogenicity data are available that were either restricted to the analysis of humoral immunity<sup>21</sup> or did not differentiate between individuals with and without prior infection<sup>22</sup>. Moreover, no head-to-head analyses with immunocompetent controls are available.

We therefore prospectively characterized induction of humoral and cellular immunity after bivalent BA.4/5 vaccination in dialysis patients. To analyse the effect of prior infections on vaccine-responses, convalescent patients with a history of prior infection and infection-naïve patients were compared regarding spike-specific IgG, neutralizing titers as well as CD4<sup>+</sup> and CD8<sup>+</sup> T-cell

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levels against parental SARS-CoV-2 and variants of concern. Moreover, immunogenicity and reactogenicity of the bivalent BA.4/5 booster vaccination were compared between dialysis patients and age-matched non-dialysing immunocompetent controls.

## RESULTS

### Study population

Thirty-three Caucasian patients undergoing hemodialysis ( $n = 32$ ) or continuous ambulatory peritoneal dialysis ( $n = 1$ ) were recruited, of which 17 had a history of a prior SARS-CoV-2 infection ("convalescent group"). In addition, despite no reported history of SARS-CoV-2 infection, two additional patients were assigned to the convalescent group due to a positive nucleocapsid (NCAP)-specific IgG. These individuals were distinguished from infection-naïve patients without a history of infection and negative NCAP-specific IgG. All patients were tested prior to and at a median of 16 (IQR 2) days after vaccination with the bivalent BA.4/5 vaccine. In both groups, most patients had a history of homologous mRNA vaccination with at least two and up to five prior immunization events (including vaccinations and infections, Fig. 1a). Convalescent patients with prior infection were younger and had a longer time on dialysis than the infection-naïve patients (Table 1). Demographic characteristics including primary disease that led to renal failure resulting in dialysis treatment, comorbidities, and differential blood counts are shown in Table 1. The number of leukocytes and granulocytes did not differ between the groups, whereas infection-naïve patients showed a significantly higher number of monocytes ( $p = 0.011$ , Mann–Whitney test) and lymphocytes ( $p = 0.023$ , Mann–Whitney test). The percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T cells did not differ between the two groups (Table 1).

### SARS-CoV-2-specific antibodies after bivalent vaccination in patients with and without prior infection

Blood samples were drawn prior to and a median of 16 (IQR 2) days after vaccination (Fig. 1a). IgG towards the parental spike were detectable in 32/33 patients prior to vaccination with no difference between individuals with and without prior infection ( $p = 0.483$ , Mann–Whitney test). Both groups showed a significant induction of IgG after vaccination with no difference in the relative increases (Fig. 1b,  $p = 0.0001$ , 3.17-fold and  $p < 0.0001$ , 4.9-fold increase in infection-naïve and convalescent individuals, respectively, both Wilcoxon signed rank test). Despite similar baseline IgG-levels, vaccine-induced median IgG-levels in convalescent patients were slightly higher (7689 (IQR 10217) BAU/ml) than in patients without prior infection (2604 (IQR 7011) BAU/ml), although the difference did not reach statistical significance ( $p = 0.055$ , Mann–Whitney test, Fig. 1b). Antibodies were further characterized for their neutralizing ability towards the spike proteins targeted by the vaccine (parental strain (FFM7) and omicron BA.5) as well as Omicron BA.1 and BA.2 (Fig. 1c). Baseline neutralizing titers against the parental strain were considerably high in both convalescent and infection-naïve patients, and only slightly increased after vaccination. In contrast, baseline neutralizing titers against the Omicron variants BA.5, BA.1 and BA.2 were lower, and significantly increased in both patient groups. Both the percentage of individuals with detectable neutralizing antibodies as well as median titers reached after vaccination were slightly higher in convalescent than in infection-naïve patients. Overall, IgG levels and neutralizing antibody activity towards the parental strain and all omicron subvariants showed a significant correlation in both patient groups (Fig. 1d).

### SARS-CoV-2-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell levels after bivalent vaccination in patients with and without prior infection

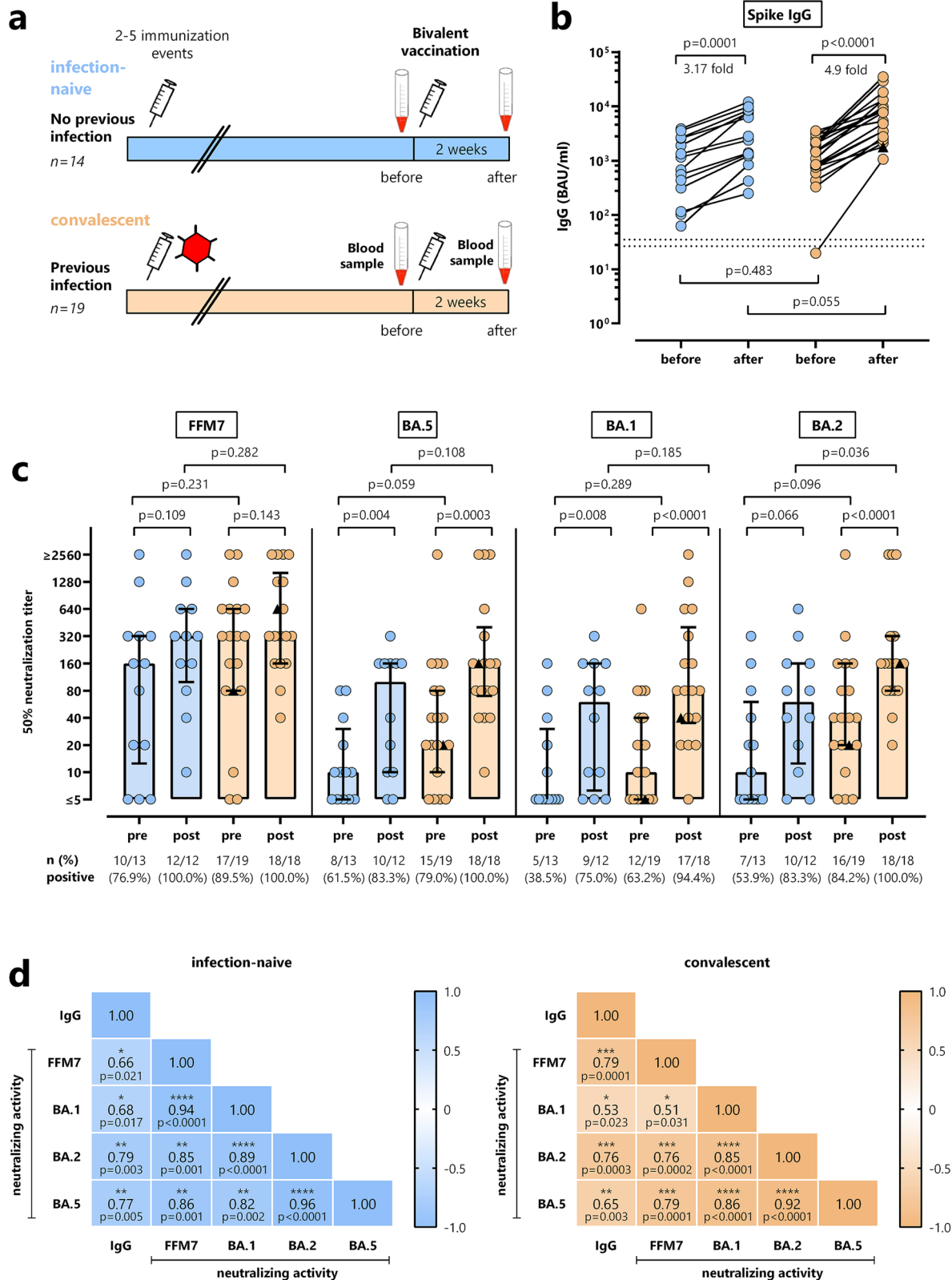
Characterization of the spike-specific cellular immune response before and after vaccination was performed after stimulation with overlapping peptide pools derived from the parental spike protein followed by intracellular cytokine staining. In addition, SEB-stimulation was used to analyze polyclonal T-cell responses, and to control for general T-cell reactivity. Spike-specific T cells were identified by co-expression of CD69 and IFN $\gamma$ , and both vaccine-induced CD4<sup>+</sup> and CD8<sup>+</sup> T-cell levels towards parental spike exceeded the detection limit in the majority of cases (Fig. 2a). The vaccine induced a significant increase in spike-specific CD4<sup>+</sup> T-cell levels in both infection-naïve (1.76-fold,  $p = 0.014$ , Wilcoxon signed rank test) and convalescent patients (1.86-fold,  $p = 0.006$ , Wilcoxon signed rank test). Likewise, median percentages of spike-specific CD8<sup>+</sup> T cells showed a significant increase (2.90-fold in infection-naïve ( $p = 0.004$ , Wilcoxon signed rank test) and 2.64-fold in convalescent patients ( $p = 0.008$ , Wilcoxon signed rank test)). Except from a significant increase in SEB-reactive CD4<sup>+</sup> T cells among infection-naïve patients, polyclonal CD4<sup>+</sup> and CD8<sup>+</sup> T-cell levels did show any pronounced vaccine-induced dynamics (Fig. 2b).

When comparing the specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards spike from the parental strain and Omicron subvariants between infection-naïve and convalescent patients after vaccination, the median percentage of parental spike-specific CD4<sup>+</sup> T cells was lower in infection-naïve patients (Fig. 2c), whereas spike-specific CD8<sup>+</sup> T-cell levels did not differ between the two groups (Fig. 2d). Likewise, there was no significant difference in median percentages of SEB-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells between the two groups (Fig. 2c, d). Interestingly, the bivalent vaccine also induced specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards all Omicron subvariants, which not only included Omicron BA.4/5 as part of the vaccine, but also Omicron BA.1 and BA.2. As with specific CD4<sup>+</sup> T cells towards parental spike, BA.1, BA.2 and BA.4/5-specific CD4<sup>+</sup> T-cell levels were significantly higher among convalescent than in infection-naïve patients (Fig. 2c). In addition, among both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, there was a significant correlation between spike-specific T-cell levels towards the parental spike and all tested Omicron subvariants BA.1, BA.2 and BA.4/5 (Fig. 2e). Interestingly, as shown in the lower left part of the correlation matrices, significant correlations between specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell populations were only found among individuals with prior infection (Fig. 2e).

Although patients differed in the number of prior immunization events and convalescent patients were younger than infection-naïve patients (Table 1), age and number of prior immunization events had no confounding effects on spike-specific IgG-levels or neutralizing activity or on spike-specific CD4<sup>+</sup> or CD8<sup>+</sup> T cells (Supplementary Table 1).

### Functional and phenotypical characteristics of bivalent BA.4/5 vaccine-induced T cells of patients with and without prior infection

Apart from quantitative analyses of spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells after vaccination, which was based on the induction of IFN $\gamma$ , the phenotypical and functional characteristics of specific T cells were further evaluated by cytokine profiling. Individual or combined expression of IFN $\gamma$ , TNF $\alpha$  and IL-2 was characterized by Boolean gating, allowing the distinction of seven subpopulations which included polyfunctional cells simultaneously expressing all three cytokines, two cytokines, or one cytokine only. As shown in Fig. 3a, b, the cytokine profile of spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells was clearly distinct from SEB-reactive T cells. The majority of spike-specific CD4<sup>+</sup> T cells were polyfunctional, followed by dual-positive cells expressing TNF $\alpha$  in combination



with either IFN $\gamma$  or IL-2. The percentage of triple positive polyfunctional CD4 $^{+}$  T cells towards parental spike was slightly higher among patients with prior infection, whereas non-infected patients had slightly higher levels of TNF $\alpha$  $^{+}$ IL-2 $^{+}$  dual positive CD4 $^{+}$  T cells towards spike from BA.4/5 (Fig. 3a). Spike-specific CD8 $^{+}$  T cells produced less IL-2 and were predominantly

IFN $\gamma$  $^{+}$ TNF $\alpha$  $^{+}$ . In addition, a similar cytokine profile was observed for specific CD4 $^{+}$  or CD8 $^{+}$  T cells towards the Omicron subvariants BA.1 and BA.2 (Supplementary Fig. 1).

As evidence for recent encounter with antigen, spike-specific and SEB-reactive CD4 $^{+}$  and CD8 $^{+}$  T cells from convalescent and infection-naive patients were compared regarding their CTLA-4

**Fig. 1 Bivalent BA.4/5 vaccine-induced antibodies levels and neutralizing activity in dialysis patients with and without prior infection.** **a** Schematic outline of the study design, where blood was drawn from infection-naïve dialysis patients without ( $n = 14$ , blue) and convalescent patients with prior infection ( $n = 19$ ; orange) before and after bivalent BA.4/5 vaccination. **b** Levels of spike-specific IgG antibodies towards parental spike protein (expressed as BAU/ml) were analyzed at baseline and after vaccination. Lines represent medians with interquartile ranges. **c** Antibody-mediated neutralization of parental SARS-CoV-2 (FFM7) and Omicron BA.1, BA.2 and BA.5 variants of concern (expressed as 50% neutralization titers) was tested among dialysis patients with and without prior infection as well as before and after vaccination with the bivalent BA.4/5 vaccine. Bars represent median titers with interquartile ranges. Differences were calculated using Wilcoxon signed rank test (before/after) or Mann–Whitney test for group comparisons at baseline and after vaccination. **d** Correlation between IgG levels towards parental spike and neutralizing activity towards parental SARS-CoV-2 (FFM7) and Omicron BA.1, BA.2 and BA.5 variants of concern are displayed in a correlation matrix. Correlations coefficients were calculated according to two-tailed Spearman and displayed using a color code. One patient, marked by a black triangle, was infected with SARS-CoV-2 two days after bivalent vaccination.

expression (Fig. 3c). CTLA-4 expression levels on spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in infection-naïve patients were numerically higher than in convalescent patients with statistically significant differences observed for spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards Omicron BA.4/5. These differences in CTLA-4 expression were spike-specific, as CTLA-4 expression on SEB-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells were similarly low in both groups.

### Comparison of vaccine-induced humoral and cellular immunity in dialysis patients and immunocompetent controls

To evaluate potential differences in vaccine-induced immunity in patients and individuals without immunodeficiency, patient data were compared with those of 58 immunocompetent non-dialysing individuals who were matched for age, sex and prior infection status. Convalescent patients and controls showed some differences in the distribution of the infecting strains, with the parental strain dominating among patients, and the omicron BA.2 variant dominating among controls. Demographic characteristics and differential blood counts are shown in supplementary Table 2. While the number of granulocytes and monocytes did not differ between patients and controls, leukocyte counts among individuals with prior infection were significantly lower in patients than in controls. Moreover, irrespective of prior infection, lymphocyte counts were significantly lower in patients than in controls.

Irrespective of prior infection, spike-specific IgG levels after vaccination were induced to a similar extent in both patients and controls (Fig. 4a). The neutralizing activity among infection-naïve individuals was also similar in patients and controls. Interestingly, among convalescent individuals with prior infection, median neutralizing titers were numerically higher in immunocompetent controls, with significant differences for titers towards the parental strain and towards BA.2 (Fig. 4b). Comparison of vaccine-induced parental and Omicron subvariant-specific T cells showed no significant difference in spike-specific CD4<sup>+</sup> T cells between infection-naïve patients and controls (Fig. 4c). Interestingly, however, while spike-specific CD4<sup>+</sup> T-cell levels among controls was similar irrespective of prior infection, dialysis patients with prior infection showed a significantly higher percentage of spike-specific CD4<sup>+</sup> T cells than controls (Fig. 4c). This was different from CD8<sup>+</sup> T cells, where there was no difference between patients and controls, which held true for both individuals with and without prior infection (Fig. 4d). Although SEB-reactive CD4<sup>+</sup> T-cell responses among convalescent individuals were also slightly higher in patients than in controls ( $p = 0.001$ , Mann–Whitney test), polyclonal T-cell responses were generally higher and rather of similar magnitude in all groups (Fig. 4c, d). Pre-vaccine levels and comparative analyses of vaccine-induced changes in specific immunity are shown in Supplementary Figure 2. Disaggregated data for females and males are shown in supplementary Figures 3 and 4, respectively.

### Reactogenicity after bivalent vaccination in patients and controls

Finally, self-reported local and systemic adverse events within the first week after vaccination were compared between patients and controls using a questionnaire. Irrespective of prior history of infection, the vaccine was well tolerated among dialysis patients with most reporting either no (> 50%) or only local adverse events at the injection site (Fig. 5a). Systemic adverse events included chills and gastrointestinal symptoms but were only rarely reported among patients. In contrast, irrespective of prior infection status, local (mainly pain at the injection site) and systemic adverse events (mainly fatigue) were significantly more frequently reported among controls (Fig. 5a, b). Considering these rather minor adverse events of the bivalent vaccination, it is notable that all previous COVID-19 vaccinations were also very well tolerated in patients, as adverse events were all perceived as of similarly low severity. This was significantly different from controls, where a larger fraction felt most affected by the bivalent vaccination (Fig. 5c).

### DISCUSSION

Bivalent COVID-19 vaccines have been recommended for use as booster doses in both immunocompetent and immunocompromised individuals, although knowledge on the reactogenicity and immunogenicity and the impact of prior infection on cellular and humoral immunity in dialysis patients is limited. We now show that dialysis patients mount a robust antibody and T-cell response against the parental SARS-CoV-2 and Omicron BA.4/5 strains targeted by the vaccine, as well as the Omicron BA.1 and BA.2 variants. In line with results from immunocompetent controls, neutralizing activity towards the parental strain was higher compared to Omicron subvariants, whereas T-cell levels towards parental strain and Omicron subvariants were of similarly high magnitude. While IgG-levels, neutralizing antibody activity, and CD8<sup>+</sup> T-cell levels after vaccination did not differ in patients with and without prior infection, vaccine-induced CD4<sup>+</sup> T-cell levels were significantly higher in convalescent patients. Finally, immunogenicity was largely comparable in infection-naïve patients and immunocompetent controls. In contrast, among convalescent individuals, controls had higher neutralizing antibody activity than patients, and dialysis patients had higher CD4<sup>+</sup> T-cell levels than respective controls.

Our observation that both patients and controls showed lower neutralizing antibody activity towards the Omicron subvariants than towards the parental strain is in line with previous reports on neutralizing antibodies after bivalent vaccination in immunocompetent controls<sup>15–17,23,24</sup>, and with first series of hemodialysis patients<sup>21,22</sup>, of which one study also compared antibody responses in patients with and without infection<sup>21</sup>. Overall, the dominance of neutralizing activity towards the parental strain may result from immune imprinting by previous exposures to the monovalent vaccines and/or to the parental SARS-CoV-2 virus<sup>25,26</sup>, which was the dominant strain in our cohort of convalescent

**Table 1.** Demographic and clinical characteristics of the study population of dialysis patients.

	Infection-naive <i>n</i> = 14	convalescent <i>n</i> = 19	<i>p</i> value
Years of age, mean (SD)	71.8 (11.3)	58.7 (15.5)	0.012 <sup>a</sup>
Sex, <i>n</i> (%) <sup>d</sup>			
Female	7 (50.0%)	5 (26.3%)	0.273 <sup>b</sup>
Male	7 (50.0%)	14 (73.7%)	
Vaccine regimen, <i>n</i> (%) <sup>e</sup>			
mRNA	13 (92.9%)	17 (89.5%)	
Vector/mRNA combination	1 (7.1%)	2 (10.5%)	
Total number of prior immunization events, <i>n</i> (%)			
2	1 (7.1%)	0 (0%)	
3	8 (57.1%)	12 (63.2%)	
4	5 (35.7%)	5 (26.3%)	
5	0 (0%)	2 (10.5%)	
Type of latest immunization prior to vaccination with Comirnaty BA.4/5, <i>n</i> (%)			
Infection	n.a.	4 (21.1%)	
Vaccination	14 (100.0%)	13 (68.4%)	
Unknown <sup>f</sup>		2 (10.5%) <sup>f</sup>	
Weeks between last immunization event and bivalent vaccination, median (IQR)	43.2 (23.2)	40.0 (12.9)	0.559 <sup>a</sup>
Analysis time (days after vaccination), median (IQR)	16 (2)	16 (2)	
Type of dialysis, <i>n</i> (%)			
Peritoneal dialysis	1 (7.1%)	–	
Hemodialysis	13 (92.9%)	19 (100.0%)	
Time on dialysis (years), mean (SD)	1.48 (1.61)	5.25 (4.09)	0.003 <sup>a</sup>
Cause of kidney failure, <i>n</i> (%)			
Autoimmune-mediated nephropathy	2 (15.4%)	1 (5.3%)	
Chronic glomerulonephritis	1 (7.1%)	3 (15.8%)	
Secondary chronic renal disease	10 (71.4%)	9 (47.4%)	
Innate	1 (7.1%)	5 (26.3%)	
Unknown		1 (5.3%)	
Comorbidities ( <i>n</i> )			
Diabetes mellitus type 2	7	10	
Insulin-dependent diabetes mellitus type 1	3	5	
Arterial hypertension	11	18	
Coronary heart disease	4	6	
Lung disease		3	
Liver cirrhosis		2	
Active tumor	1	1	
ANCA-associated vasculitis		1	
	Infection-naive <i>n</i> = 13	convalescent <i>n</i> = 19	<i>p</i> value
Differential blood counts median (IQR) cells/ $\mu$ l			
Leukocytes	6600 (4550)	5800 (1900)	0.420 <sup>c</sup>
Granulocytes	4891 (2536)	4437 (1764)	0.821 <sup>c</sup>
Monocytes	704 (266)	536 (130)	0.011 <sup>c</sup>
Lymphocytes	1280 (663)	1051 (480)	0.023 <sup>c</sup>
Percentage of T cells median (IQR) % among lymphocytes			
CD4 <sup>+</sup> T cells	36.0 (23.0)	34.9 (11.6)	0.957 <sup>c</sup>
CD8 <sup>+</sup> T cells	18.4 (18.9)	18.9 (9.8)	0.706 <sup>c</sup>

*IQR* interquartile range.

<sup>a</sup>unpaired t-test.

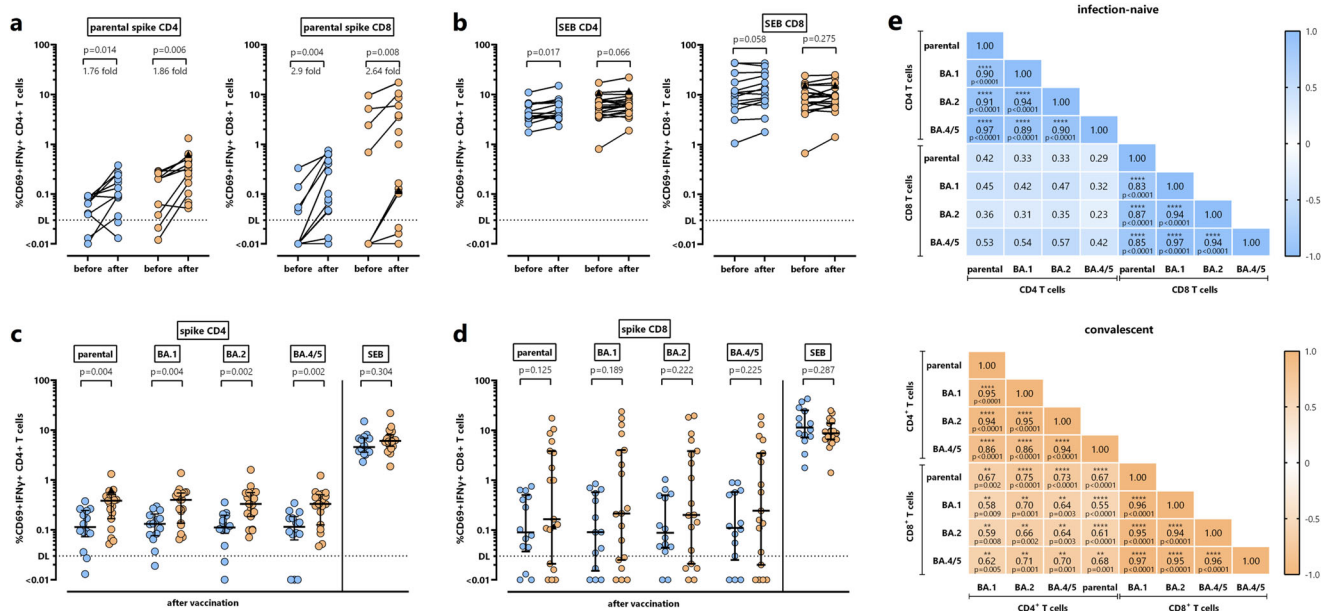
<sup>b</sup>Fisher's exact test.

<sup>c</sup>Mann–Whitney test.

<sup>d</sup>Information on sex was based on individual self-declaration.

<sup>e</sup>Among the 30 patients with mRNA vaccines, 16 patients had received the vaccine by Biontech/Pfizer only (7 infection-naive and 9 convalescent), whereas the previous vaccine regimen contained one dose of the Moderna in 13 patients (5 infection-naive and 8 convalescent), and 2 doses of Moderna in 1 infection-naive patient.

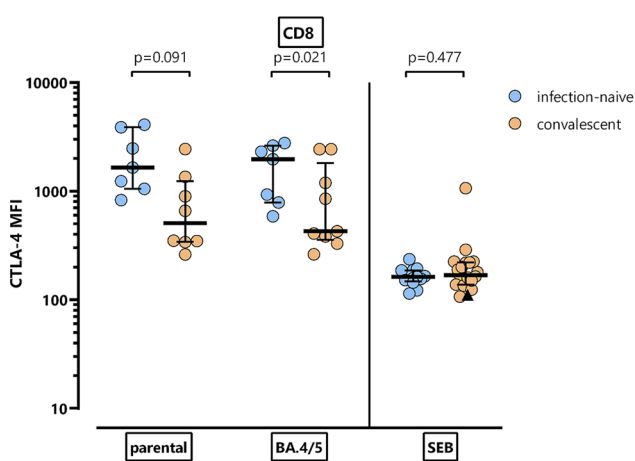
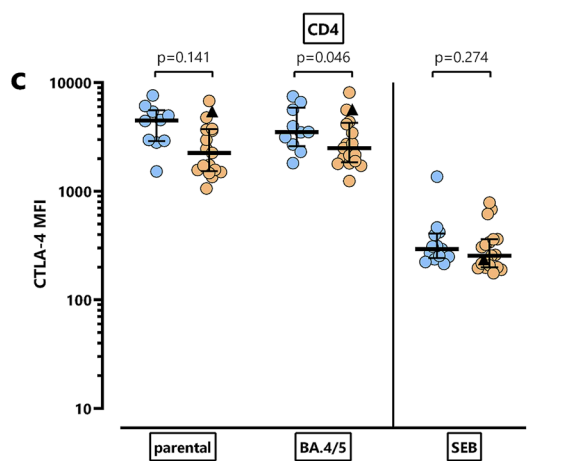
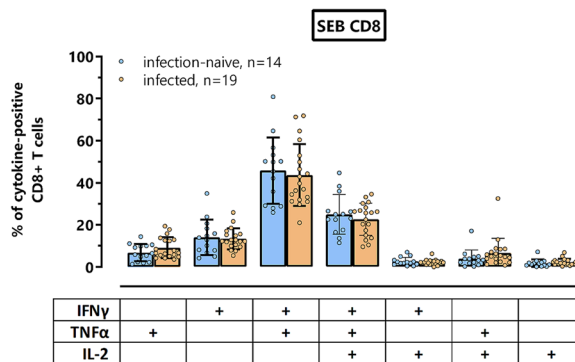
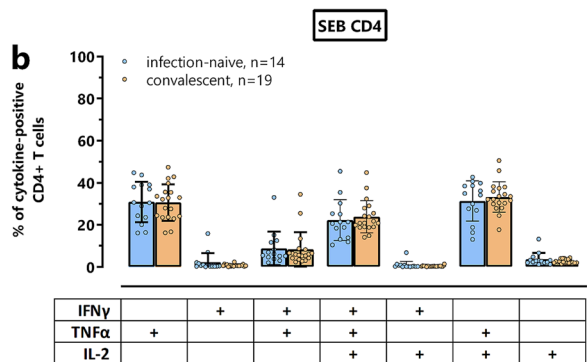
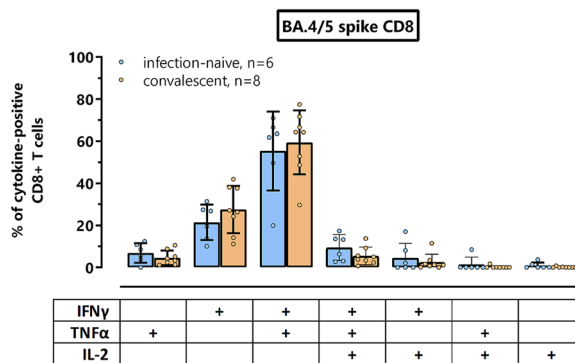
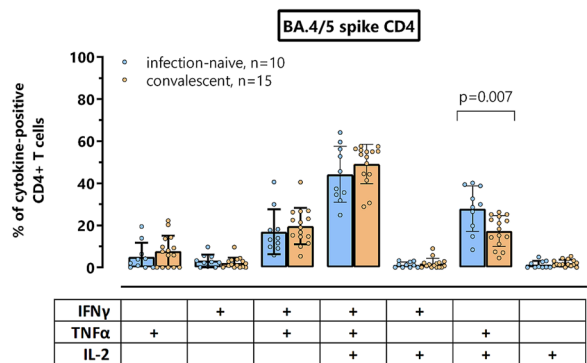
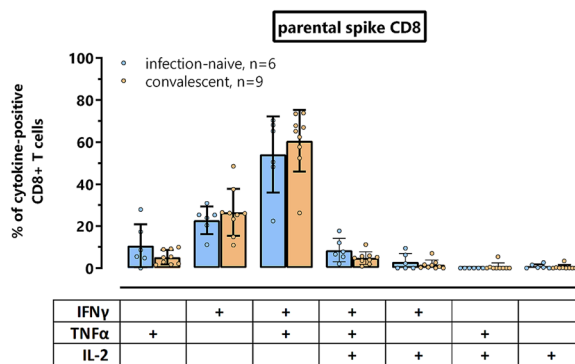
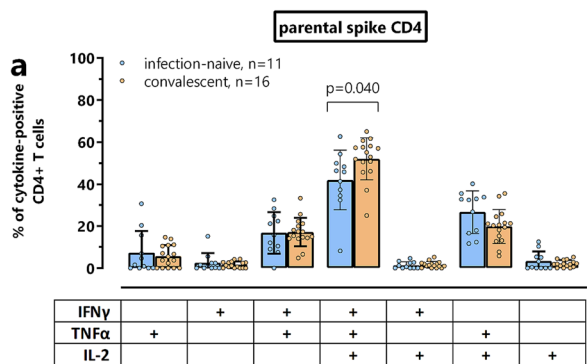
<sup>f</sup>2 patients were assigned as previously infected based on NCAP-IgG positivity despite no known history of infection.



**Fig. 2** Bivalent BA.4/5 vaccine-induced CD4<sup>+</sup> and CD8<sup>+</sup> T-cell immunity in dialysis patients with and without prior infection. **a** CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards parental spike were determined at baseline ( $n = 10$  in patients with and without prior infection) and after vaccination in infection-naïve dialysis patients ( $n = 14$ , blue symbols) and in convalescent patients ( $n = 19$ ; orange symbols). Fold changes after vaccination are indicated above the graphs and were calculated by dividing the individual levels after vaccination and levels prior to vaccination (with 0.03% added to each value prior to division to avoid division by 0). **b** *Staphylococcus aureus* enterotoxin B (SEB)-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells were determined at baseline and after vaccination in infection-naïve dialysis patients ( $n = 14$ ) and in convalescent patients with prior infection ( $n = 19$ ). **c** CD4<sup>+</sup> and **d** CD8<sup>+</sup> T cells towards parental spike and towards spike from Omicron subvariants BA.1, BA.2, BA.4/5, and SEB-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells were compared after vaccination of patients without ( $n = 14$ ) and with prior infection ( $n = 19$ ). One patient, marked by a black triangle, was infected with SARS-CoV-2 two days after vaccination. Bars represent medians with interquartile ranges. Differences between the time points among the 10 paired datasets were calculated by Wilcoxon signed rank test (**a**) and between the groups using Mann–Whitney test (**b**, **c**). Dotted lines indicate detection limits (DL) for spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells. **e** Correlation matrix of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell levels towards parental spike and Omicron subvariants BA.1, BA.2 and BA.4/5 ( $n = 33$  dialysis patients). Correlation coefficients were calculated according to two-tailed Spearman and displayed using a color code.

patients. In addition, specific neutralizing activity may further be shaped by infection with SARS-CoV-2 subvariants. While neutralizing activity among infection-naïve patients and controls did not differ, convalescent controls who were predominantly infected with BA.2 reached significantly higher neutralizing activity towards parental and Omicron BA.2 than convalescent patients. Given the similarity in neutralizing antibody response between infection-naïve patients and controls, it is tempting to speculate whether the higher neutralizing activity among convalescent controls is mainly driven by the infecting strain, and to a lesser extent by better immunocompetence. In general, the relative increase in antibody levels and neutralizing activity towards Omicron subvariants in infection-naïve individuals is more pronounced than in convalescent individuals, which may suggest that infection-naïve patients derive greater benefit from bivalent vaccination than individuals with a history of infection. Among convalescents, the benefit may be stronger in individuals with parental SARS-CoV-2 infection and/or longer distance from infection. When comparing baseline neutralizing activity towards the Omicron subvariants in our cohort of predominantly wildtype-SARS-CoV-2 infected patients with those of a recently published cohort of dialysis patients with a history of Omicron infection<sup>21</sup>, it seems that our cohort had clearly lower baseline levels and a more pronounced relative increase in neutralizing activity towards Omicron subvariants. Together this may explain why the differences in antibody responses between convalescent and infection-naïve patients in our study were less pronounced, and may suggest that the particular benefit of bivalent vaccination for infection-naïve patients may also extend to convalescent patients with a history of wild-type infection.

As with antibody levels, percentages of spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells after bivalent BA.4/5 vaccination increased in both patients with and without prior infection. However, unlike antibodies, specific T-cell levels against the parental spike protein showed a strong correlation and striking similarity with the vaccine-induced T-cell levels of all tested Omicron VOCs indicating substantial cross-reactivity between the strains. In line with vaccine-induced T-cell responses from immunocompetent controls<sup>27</sup>, spike-specific T cells were largely polyfunctional, and showed high expression of the immune checkpoint molecule CTLA-4, a marker indicative of recent antigen contact. The higher expression of CTLA-4 in infection-naïve patients may indicate some phenotypical evidence of de-novo priming and expansion of a new population of T cells after first contact with BA.4/5 antigen. Consistent with some extent of primary induction<sup>28,29</sup>, specific T cells among infection-naïve patients showed a restricted cytokine pattern with a lower percentage of multifunctional cells and a relative dominance of dual cytokine-producing cells expressing IL-2 and TNF $\alpha$ , which is different from reactivations, where less multifunctional cells are associated with an increase in cells expressing IFN $\gamma$ <sup>28,29</sup>. As the most striking finding, dialysis convalescent patients mounted significantly higher levels of spike-specific CD4<sup>+</sup> T cells than convalescent controls or infection-naïve individuals, which was not confounded by the number of prior immunization events or differences in age. This finding is compatible with a generally higher disease severity, prolonged disease courses and longer periods of PCR-positivity in immunocompromised patients<sup>30–33</sup>. As spike-specific T-cell levels in patients with COVID-19 were found to correlate with disease severity<sup>34</sup>, higher CD4<sup>+</sup> T-cell levels in dialysis patients may result from more pronounced exposure with viral antigens at the time of



infection. Convalescent patients were also distinct in that their spike-specific CD4<sup>+</sup> T-cell levels correlated with those of CD8<sup>+</sup> T cells, which was not the case among infection-naive patients. It therefore seems that induction of T cells by natural infection<sup>34</sup> will ensue a more uniform expansion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells after subsequent vaccination.

In our study, adverse events of the bivalent BA.4/5 vaccine were collected on a standardized questionnaire, which also inquired which vaccine dose received so far was perceived to be the worst in terms of side effects. It was remarkable that the bivalent vaccine was very well tolerated in both convalescent and infection-naive dialysis patients. As with previous vaccinations, patients either

**Fig. 3 Functional characterization of parental and Omicron BA.4/5-spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells after bivalent vaccination.** Cytokine expression profiles of CD4<sup>+</sup> and CD8<sup>+</sup> T cells after stimulation with (a) parental and BA.4/5-spike-peptides or (b) *Staphylococcus aureus* enterotoxin B (SEB) were compared between infection-naïve (blue bars) and convalescent patients (orange bars). At the single-cell level, the cytokine-expressing T cells were differentiated into 7 subpopulations according to their expression of IFN $\gamma$ , TNF $\alpha$  and IL-2 (single, double or triple cytokine-expressing cells). Only samples of the patients with at least 30 cytokine-expressing CD4<sup>+</sup> and CD8<sup>+</sup> T cells were included, respectively, to allow for robust statistical analysis. Bars represent means and standard deviations of subpopulations. Differences among subpopulations were determined using unpaired t-test. c Median fluorescence intensity (MFI) of CTLA-4 expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells towards spike from the parental strain or BA.4/5, and on SEB-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells from patients with and without prior infection was determined. To allow robust statistical analysis, only samples with at least 20 cytokine-positive CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively, were included. Differences between groups were analyzed using Mann–Whitney test.

reported no adverse events or primarily local pain at the injection site. This is in line with previous reports of COVID-19 vaccine tolerability and also extends to other vaccine types<sup>35</sup>. Reactogenicity was significantly different from immunocompetent controls where more individuals reported local and/or systemic reactions that were most frequently pain at the injection site followed by fatigue. The fact that patients rather reported pain at the injection site as compared to systemic adverse events may be due to the fact that polypharmaceutical treatment of patients may have contributed to amelioration of some systemic adverse events. On the other hand, the dialysis procedure itself may be associated with headache and fatigue<sup>36,37</sup>; hence some systemic adverse events may not have been perceived as vaccine-related. In any case, in light of our data on the strong immunogenicity, the low rate of adverse events does not seem to correlate with poorer immune responses.

A strength of our study is a detailed analysis of bivalent BA.4-5 vaccine-induced humoral and cellular immunity of dialysis patients, which also assessed the impact of previous infections and a comparison to healthy individuals. Convenience sampling of dialysis patients limits the study to the extent that convalescent patients were significantly younger than the infection-naïve group. Although the in part more pronounced immune responses may be age-dependent<sup>38</sup>, we did not find any confounding effect of age on our results. In addition, the control group is matched for age and sex and therefore allows for a direct comparison of the immunogenicity of immunocompetent individuals. We have also not performed any follow-up analyses to assess stability of the bivalent booster response. Considering the more rapid waning of vaccine-induced immunity in dialysis patients<sup>39</sup>, knowledge on stability will continue to be important to inform future vaccine policies in vulnerable patient groups.

In conclusion, despite insufficient humoral<sup>6,7</sup> and cellular<sup>8,9</sup> immune response compared to healthy controls after the primary COVID-19 vaccine doses, dialysis patients showed a pronounced induction of humoral and cellular immune responses after bivalent booster vaccination. Together with the excellent tolerability, these data are reassuring considering current recommendations towards yearly vaccinations in immunocompromised patients at high risk for severe disease and more rapid loss of specific immunity after vaccination.

## METHODS

### Study design and subjects

In this observational study, continuous ambulatory peritoneal dialysis and hemodialysis patients were enrolled prior to their vaccination with the bivalent BA.4/5 vaccine (Comirnaty® Original/Omicron BA.4/5, BioNTech/Pfizer). Vaccinations were performed after a dialysis procedure. A subgroup of healthy, immunocompetent, non-dialysing volunteers (mainly employees at Saarland University Medical campus) were included as controls. Study participants received a questionnaire for self-reporting their history of vaccination and infection, and of local and systemic adverse events within the first week after vaccination. In addition,

patients were assigned as previously infected based on NCAP-IgG positivity. Heparinized blood samples were collected before and 13-18 days after vaccination to determine specific humoral and cellular immunity toward the spike protein derived from the parental SARS-CoV-2 strain as well as from the Omicron variants BA.1, BA.2 and BA.4/5. Blood sampling was performed prior to dialysis to minimize the effect of temporal dialysis-related leukocyte depletion<sup>40</sup>. The study was performed in adherence to the declaration of Helsinki and approved by the ethics committee of the Ärztekammer des Saarlandes (reference 76/20 including amendment), and written informed consent was obtained from all individuals.

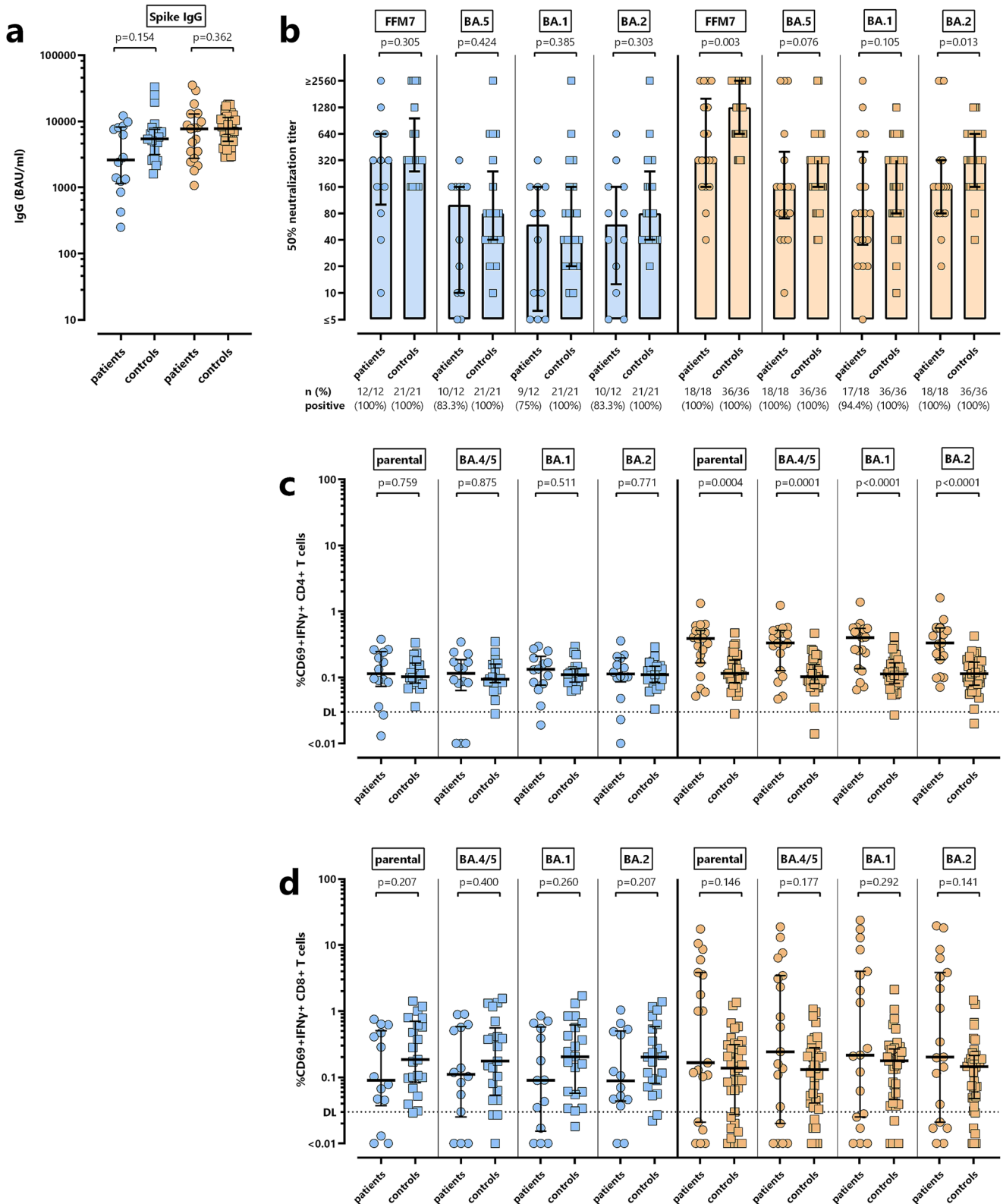
### Quantification of vaccine-induced spike-specific T cells

To determine spike-specific T cells, heparinized whole blood was stimulated for 6 h as described before<sup>27,34</sup> with overlapping peptides (each peptide 2  $\mu$ g/ml) spanning the parental spike or Omicron variants BA.1-, BA.2-, BA.4/5-spike protein (N-terminal receptor binding domain and C-terminal portion including the transmembrane domain, jpt Berlin, Germany) in the presence of co-stimulatory antibodies against CD28 and CD49d (clone L293 and clone 9F10, 1  $\mu$ g/ml each). In addition, stimulation with 0.64% DMSO and 2.5  $\mu$ g/ml of *Staphylococcus aureus* enterotoxin B (SEB; Sigma) served as a negative and positive control, respectively. Moreover, SEB-reactive T cells were analyzed as polyclonal effector/memory T cells which allows to distinguish characteristics of SARS-CoV-2 specific T cells from T cells with specificities largely different from SARS-CoV-2. After stimulation, cells were immunostained using anti-CD4 (clone SK3, 1:33.3), anti-CD8 (clone SK1, 1:12.5), anti-CD69 (clone L78, 1:33.3), anti-IFN $\gamma$  (clone 4S.B3, 1:100), anti-IL-2 (clone MQ1-17H12, 1:12.5), anti-TNF $\alpha$  (clone MAb11, 1:20), and anti-CTLA-4 (clone BNI3, 1:50) and analyzed using flow cytometry (BD FACS Canto II and FACSDiva software 6.1.3.). Activated CD69-positive T cells producing IFN $\gamma$  identified spike (WT, BA.1, BA.2, BA.4/5)-reactive CD4<sup>+</sup> or CD8<sup>+</sup> T cells. Levels of reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells after control stimulations were subtracted from those obtained after spike-specific stimulation, and 0.03% of reactive T cells was set as detection limit as described before<sup>27</sup>. To characterize T-cell functionality, co-expression of IL-2 and TNF $\alpha$  was analyzed as well as the cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4). The gating strategy is shown in supplementary Figure 5.

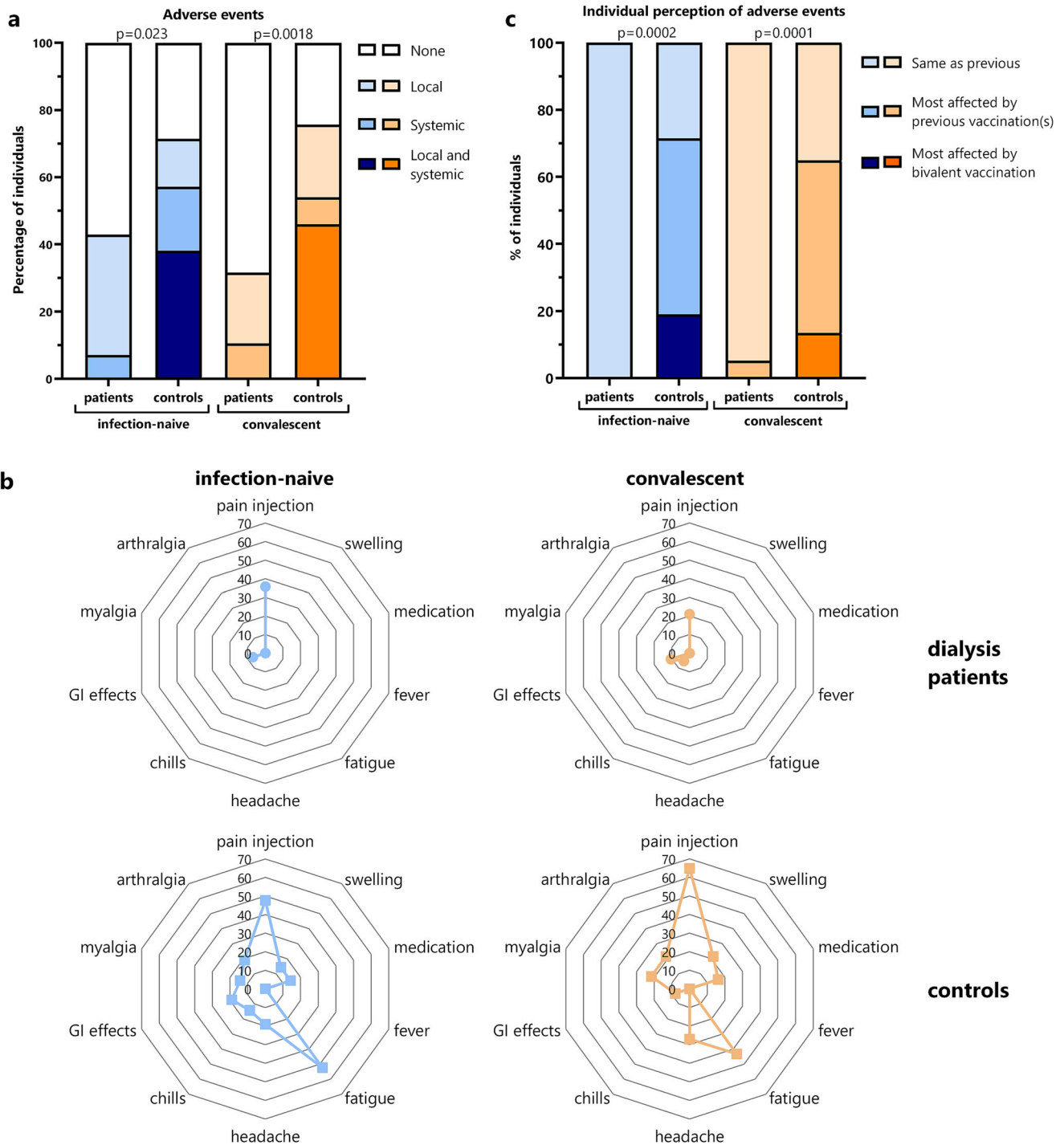
### Viruses used for the quantitation of SARS-CoV-2 neutralizing antibodies

In this study, the following SARS-CoV-2 isolates were used: Parental strain (SARS-CoV-2 B.1 FFM7/2020, GenBank ID MT358643), BA.1 (SARS-CoV-2 B.1.1.529 FFM-SIM0550/2021 (EPI\_ISL\_6959871), GenBank ID OL800702), BA.2 (SARS-CoV-2 BA.2 FFM-BA.2-3833/2022, GenBank ID OM617939), BA.5 (SARS-CoV-2 BA.5 FFM-BA.5-501/2022, GenBank ID OP062267)<sup>41–44</sup>.





**Fig. 4** Comparison of bivalent BA.4/5 vaccine-induced humoral and cellular immunity between dialysis patients and healthy controls stratified according to their history of infection. **a** Spike-specific IgG levels after bivalent vaccination were compared between infection-naïve dialysis patients ( $n = 14$ , blue circle) and controls ( $n = 21$ , blue square) as well as between convalescent dialysis patients ( $n = 19$ , orange dots) and controls ( $n = 37$ ; orange squares). **b** Antibody-mediated neutralization of parental SARS-CoV-2 strain (FFM7) and Omicron BA.1, BA.2 and BA.5 variants of concern after vaccination with the bivalent vaccine (expressed as 50% neutralization titers) was compared between dialysis patients and controls with and without prior infection. The number of tested individuals and the percentage of individuals with detectable neutralizing antibodies are indicated. Levels of **(c)** CD4 and **(d)** CD8 T cells towards spike from the parental strain and Omicron subvariants BA.4/5, BA.1, BA.2 after vaccination were compared in patients and controls with and without prior infection. Dotted lines indicate detection limits (DL) for spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Bars represent median titers with interquartile ranges. Differences between the groups were determined using Mann–Whitney test.



**Fig. 5 Reactogenicity after bivalent BA.4/5 vaccination in dialysis patients and controls.** Reactogenicity within first week after bivalent BA.4/5 vaccination of dialysis patients and controls with and without prior infection was self-reported based on a standardized questionnaire. **a** The percentage of individuals with no adverse events, only local or only systemic adverse events or both are shown. **b** The distribution of the individual local and systemic adverse events in convalescent and infection-naïve patients and controls is shown. **c** Individual perception of adverse events was classified as to whether individuals felt most affected by the bivalent BA.4/5 vaccination, by any of the previous vaccinations, or whether adverse events were similar for all vaccinations. Comparisons between groups were analyzed using the  $\chi^2$  test.

#### Determination of SARS-CoV-2-specific antibodies

All antibody tests were performed according to the manufacturer's instructions (Euroimmun, Lübeck, Germany) as described before<sup>27</sup>. The enzyme-linked immunosorbent assay (ELISA, SARS-CoV-2-Quantivac) was used to quantify the SARS-CoV-2-specific IgG antibodies towards the receptor binding domain of the

parental SARS-CoV-2-spike protein. Antibody binding units (BAU/ml) <25.6 were scored negative,  $\geq 25.6$  and <35.2 were scored intermediate, and  $\geq 35.2$  were scored positive. SARS-CoV-2-specific IgG towards the nucleocapsid (NCAP) protein were determined using the anti-SARS-CoV-2-NCP-ELISA (Euroimmun, Lübeck, Germany). NCAP-ELISA positivity was used as

independent evidence for infection in individuals without history of infection. A micro neutralization assay with A549-AT cells and authentic parental SARS-CoV-2 (FFM7, D614G) and the Omicron variants BA.1, BA.2, and BA.5 was used to determine the in vitro neutralizing activity of the antibodies, as described before<sup>45</sup>.

### Statistical analysis

All statistical analyses were performed using GraphPad Prism 10.0.3 software (GraphPad, San Diego, CA, USA) using two-tailed tests. Categorical analyses on sex and adverse events were performed using Fisher's exact test. Data with normal distribution were analyzed using unpaired *t* test. To compare unpaired nonparametric data between groups, Mann–Whitney and Kruskal–Wallis test followed by Dunn's multiple comparisons test were performed. Wilcoxon matched pairs test was used to compare paired data between two groups. Correlations were analyzed using a correlation matrix according to Spearman. A *p* value less than 0.05 was considered statistically significant. Linear regression analysis was performed from log(10) transformed values to test the effects of prior infection, age and number of immunization events on vaccine-induced immunity.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

### DATA AVAILABILITY

All figures and tables have associated raw data. The data that support the findings of this study are available from the corresponding author upon request.

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## AUTHOR CONTRIBUTIONS

S.B., T.S., J.M. U.S., and M.S. designed the study and the experiments. S.B., R.U., V.K., S.M., C.G., A.A.-O., F.H., M.W. performed experiments. S.B., J.M., U.S., A.A.-O., F.H. and M.S. contributed to study design, patient recruitment, and clinical data acquisition. S.B. and M.S. performed statistical analysis. T.S., J.M., U.S. and M.S. supervised all parts of the study. S.B. and M.S. wrote the manuscript, and J.M., T.S. R.U. V.K. S.M. C.G. A.A.-O., F.H., M.W. provided revisions for important intellectual content. All authors agree on accountability for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors approved the final version of the manuscript.

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## COMPETING INTERESTS

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## ADDITIONAL INFORMATION

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