Supplementary Appendix

Supplement to: Huth L, Schäfer L, Almanzar G, et al. Immunologic effect of bivalent mRNA booster in patients undergoing hemodialysis. N Engl J Med. DOI: 10.1056/NEJMc2216309

This appendix has been provided by the authors to give readers additional information about the work.

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Supplemental Methods

Study population and ethics. Routine serum samples were obtained at the Hemodialysis Centre, University Hospital Wuerzburg, Wuerzburg, Germany. The study included individuals who had their last immunological event, either vaccination or breakthrough infection, at least 3 months before fifth vaccination with Comirnaty Original/Omicron BA.4-5. Only end-stage renal disease patients on hemodialysis with normal total serum IgG levels, normal leukocyte counts and without pharmacologically relevant immunosuppressants were included into analysis. PCR confirmed Omicron breakthrough infections (BTI) have been recognized in 18 patients before fifth vaccination (mean age 72.8±11.5 years; females n=3; 16.7%), whereas 37 had no Omicron BTI in their history (non-BTI) (mean age 68.2±13.9 years, females n=17; 45.9%). The study was performed according to the declaration of Helsinki 2013 and retrospective analysis approved by the local ethics committee (protocol number 20201105_01).

Antibody concentration and avidity testing. SARS-CoV-2-specific anti-spike-IgG antibody concentrations were measured using SERION-ELISA agile SARS-CoV-2 IgG (Institute Virion/Serion GmbH, Wuerzburg, Germany; IgG ELISA: sensitivity 96.2%, specificity 100%). Individuals with IgG concentrations below 31.5 BAU/mL were considered as serological non-responders. IgG-SARS-Cov-2 antibody avidity was determined by adaptation of IgG agile SARS-CoV-2 ELISA (Virion/Serion) using ammonium thiocyanate (NH_4SCN, Roth, Karlsruhe, Germany) as chaotropic agent and expressed as relative avidity.[4]

SARS-CoV-2 neutralization assays. High-titer, replication-competent virus stocks were generated and characterized as reported.[4,5] Stocks of clinical isolates of SARS-CoV-2 variants of concern Omicron BA.4 (GISAID EPI_ISL_13900812) and Omicron BA.5 (GISAID EPI_ISL_13900731) were used. Infection neutralization activities in serum samples were quantified as described.[5]

SARS-CoV-2-spike-specific IFN-gamma (IFN-γ) ELISpot. SARS-CoV-2-specific cellular reactivity was determined in an IFN-γ Enzyme-linked-Immunospot-Assay (ELISpot). Peripheral blood mononuclear cells (PBMC) were obtained by venous puncture and isolated by density-gradient centrifugation and kept in liquid nitrogen until use according to a standardized protocol. Briefly, 2.5 x10^5 PBMCs were seeded in triplicates in 100 µL X-Vivo 15 (Lonza, Verviers, Belgium) in a precoated hydrophobic high protein binding immobilon-P membrane 96-well plates (Merck Millipore Ltd., Carrigtwohill, Ireland) with 5 µg/ml anti-human IFN-γ 1 capture-Antibody (1-D1K, Mabtech, Stockholm, Sweden) in PBS overnight at 4°C. Unspecific binding places were blocked with 5% BSA (Milteny, Bergisch-Gladbach, Germany) in PBS for 1 hour at room temperature. Cells were stimulated either with 10 µg/ml SARS-Cov-2 Spike Ectodomain S1-S2-RBD antigen or 20 ng/mL Staphylococcal enterotoxin B (SEB, Sigma, St. Louis, USA) as positive control for 18h under cell culture conditions. Medium was used as negative control. After incubation, cells were removed followed by washing steps. Detection antibody (2µg/mL, mAb 7-B6-1, Mabtech) was added and incubated for 1 hour at 37°C. Spots were developed after treatment with 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT, Mabtech). Spot forming units (SFU) were quantified using C.T.L. ELISpot reader software (Bonn, Germany) and normalized to 10^6 cells.

Statistics. Distribution of parameters was analyzed using Kolmogorow-Smirnow-test (IBM Statistics, SPSS Version 29.0, Chicago, IL). Non-parametric dependent parameters were statistically analyzed using Wilcoxon-signed-rank and Friedman test, respectively. Non-parametric independent parameters were compared by Mann-Whitney-U and Kruskal-Wallis test, respectively. Bonferroni correction to avoid bias by multiple testing was performed. Correlations between age and anti-spike-IgG concentrations were investigated applying Spearman's rank correlation coefficient R. Stepdown multivariate linear regression analysis was performed on the outcome variables IC_{50} neutralization against BA.4 and BA.5 Omicron subtype, respectively, including age, sex, anti-spike-IgG concentration at six and two weeks before booster vaccination and assignments to the groups BTI or non-BTI. A p-value of less than 0.05 was considered statistically significant.

Supplemental Figure S1

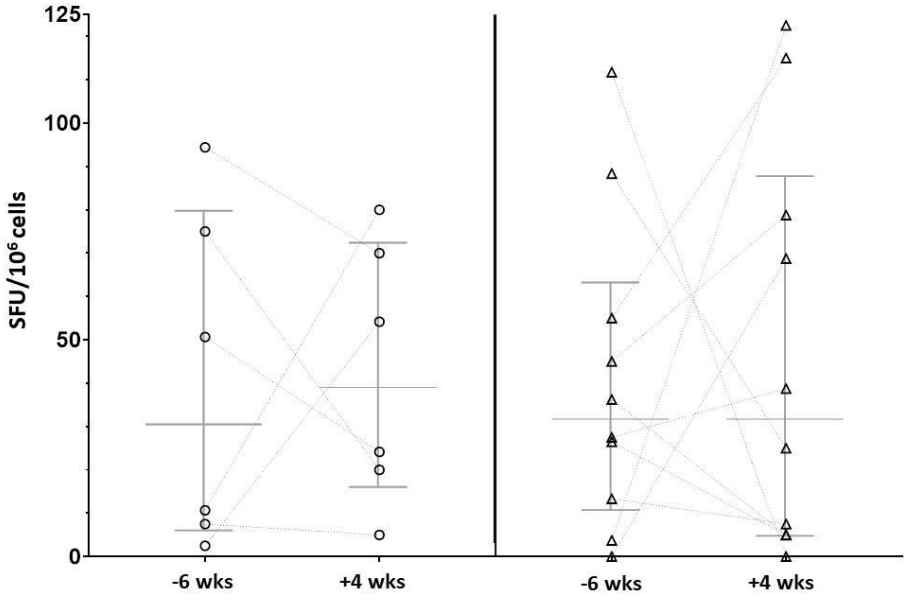

Figure S1. Cell-mediated immunity before and after bivalent BA.4/BA.5 Omicron mRNA booster vaccination in hemodialysis patients. Frozen peripheral mononuclear blood cells were available from 6 BTI (open circles) and 10 non-BTI (triangles) 6 weeks before (-6 wks) and 4 weeks after bivalent BA.4/BA.5 Omicron mRNA booster vaccination (+4 wks). Cellular reactivity was assessed by Interferon-(IFNγ)-gamma-ELISpot assay after stimulation with SARS-CoV-2-spike-ectodomain S1, S2 and recombinant binding domain RBD. IFNγ-producing cell clones were quantified by spot-forming-units (SFU) per $1x10^6$ cells after background subtraction. Median (horizontal line) and interquartiles (whiskers) are shown. SFU were not significantly different between time points and between BTI and non-BTI.

Supplemental Figure S2.

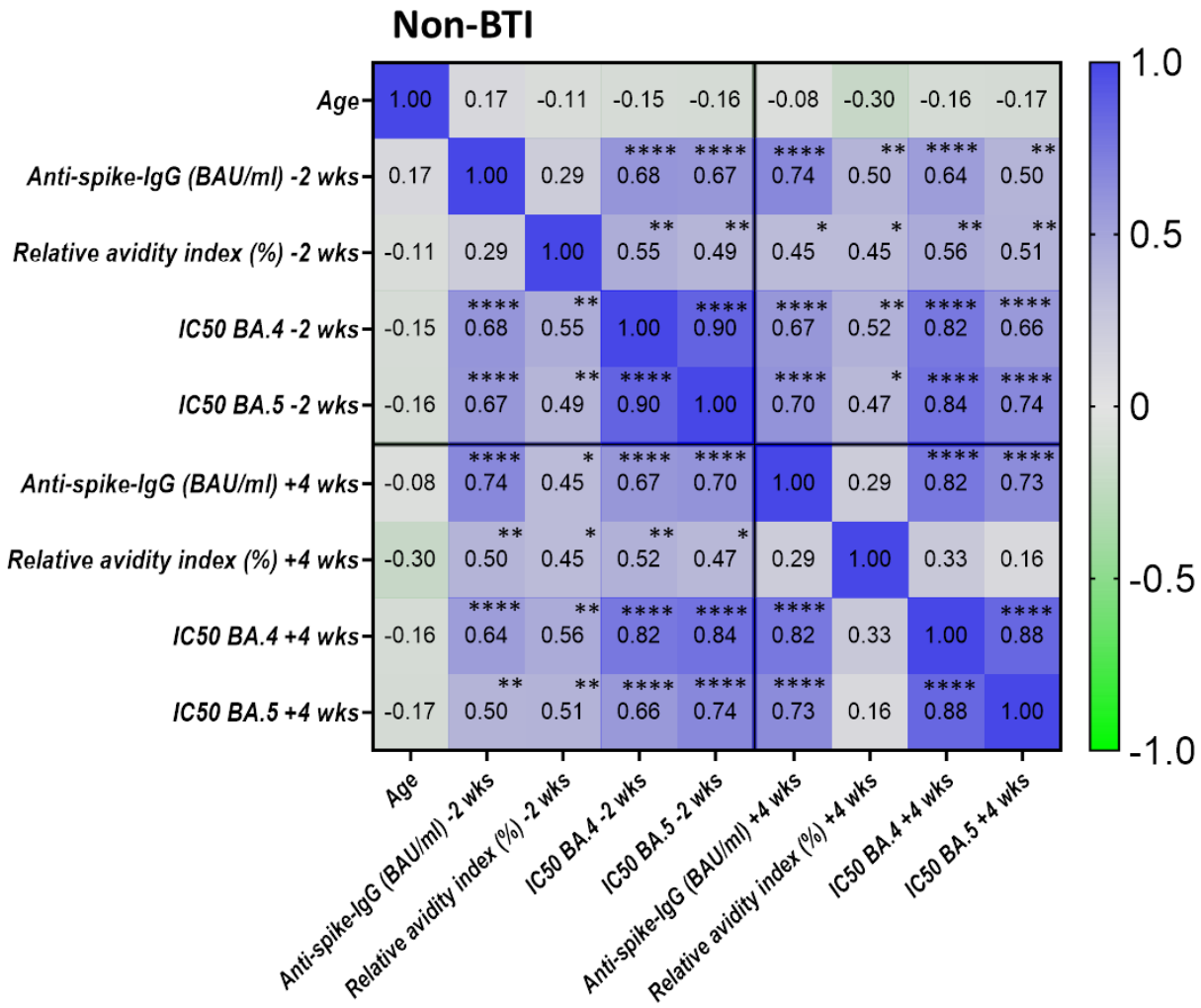

Figure S2. Correlations between serological parameters 2 weeks before (-2 wks) and 4 weeks after bivalent mRNA booster vaccination (+4 wks) in Non-BTI. Numbers indicate Spearman's rank correlation coefficient R. Significant correlations are indicated by ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05.

Supplemental Figure S3.

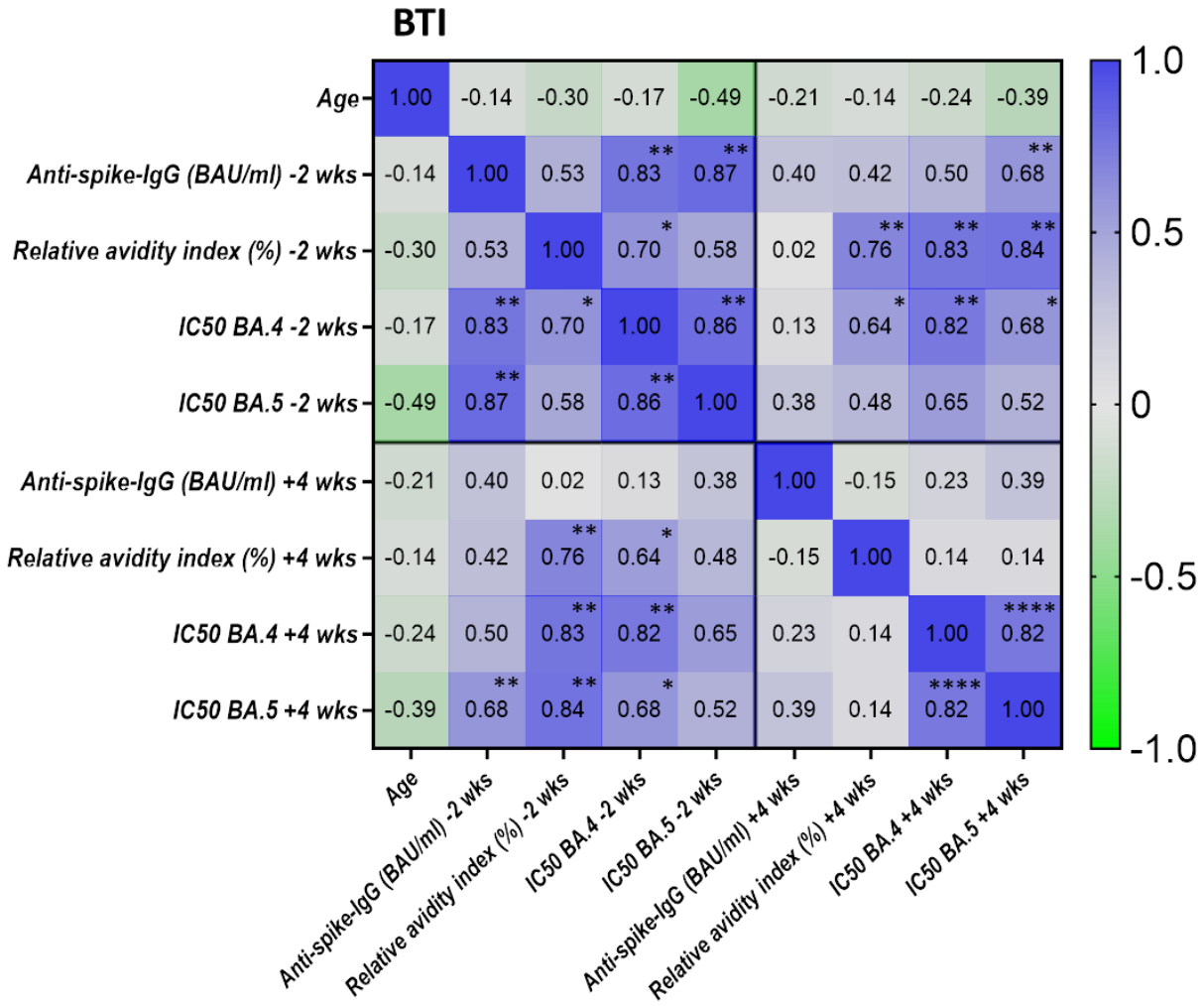

Figure S3. Correlations between serological parameters 2 weeks before (-2 wks) and 4 weeks after bivalent mRNA booster vaccination (+4 wks) in BTI. Numbers indicate Spearman's rank correlation coefficient R. Significant correlations are indicated by ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05.

References

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