Humoral Response and SARS-CoV-2 Infection Risk following the Third and Fourth Doses of the BNT162b2 Vaccine in Dialysis Patients

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Abstract
The optimal SARS-CoV-2 vaccination schedule in dialysis patients and the potential need for a fourth vaccine dose are debatable. We prospectively assessed the humoral responses to three and four doses of BNT162b2 among dialysis patients. The study included 106 dialysis patients; 60 (56.6%) and 46 (43.4%) received 3 and 4 vaccine doses, respectively. Anti-spike (anti-S) antibody titer significantly increased after the third vaccine dose, followed by a decline, yet still remained higher than all previous measurements. The fourth vaccine dose led to another profound rise in anti-S titers. The absolute increase following the fourth dose correlated with response to the third dose. Infection risk however was similar between patients vaccinated with three or four doses.

Introduction
Vaccination has been shown to be a highly successful strategy in reducing SARS-CoV-2 transmission and COVID-19 illness severity [1, 2], even in the high-risk dialysis population. A third “booster” dose of the BNT162b2 (Pfizer-BioNTech) vaccine effectively overcame waning immunity over time and the emergence of the highly transmissible B.1.617.2 (Delta) variant and significantly reduced the rate of SARS-CoV-2 infections and illness severity in the general population [2]. The third dose also led to a significant humoral response in hemodialysis (HD) patients [3, 4].

Based on the beneficial effect of the third dose and the rising predominance of the B.1.1529 (Omicron) variant, the Israeli Ministry of Health recommended a fourth vaccine dose to high-risk populations (including dialysis pa-
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Patients, at least 4 months after the third dose. Our primary objective was to report the humoral responses of the dialysis population to three and four doses of BNT162b2 and to preliminarily assess their protective effect against SARS-CoV-2 infection.

Materials and Methods

This prospective observational study was conducted at two dialysis centers in Israel. The study population included adult maintenance dialysis patients receiving treatment for ≥3 months who were able to sign informed consent. All participants received at least 3 doses of the BNT162b2 vaccine. Subjects with evidence of SARS-CoV-2 infection before the third vaccine dose, either by RT-PCR positivity or by anti-nucleocapsid antibody (anti-N Ab) levels, were excluded.

Patients received the first vaccine doses in December 2020 and the second 21 days later. The third dose was recommended to dialysis patients since July 13, 2021, and the fourth since January 2, 2022, for those who had received the third dose more than 4 months earlier. Patients self-reported adverse events following the fourth dose.

Levels of antibodies targeting SARS-CoV-2 spike protein (IgG anti-spike [anti-S]) were measured using the Abbott AdviseDx SARS-CoV-2 IgG II Quant assay. Simultaneously, levels of anti-N Ab were measured using the Abbott SARS-CoV-2 IgG nucleocapsid protein assay, both on an Alinity analyzer. As previously suggested, anti-N levels above 0.8 S/C were considered positive for prior infection [5].

Antibody measurements were taken at up to 5 predefined time points (online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000525309): 6 months following the first vaccine dose (T1), 2–3 weeks after third and ~8 months following first vaccine dose (T2), 12 months following the first dose (T3), within a week after the fourth dose (T4), 2–3 weeks after the fourth dose (T5). Measurements T4 and T5 were taken only for subjects who received four vaccine doses. SARS-CoV-2 infection was diagnosed by a positive RT-PCR from nasopharyngeal swabs, new anti-N Ab (with previous negative anti-N), or both. Results are reported according to STROBE guidelines.

Statistical Analysis

Descriptive statistics are presented as means, with standard deviation, median and range, or percentages. Continuous variables were examined for normality (Shapiro-Wilk test), and data were analyzed accordingly. t test or one-way ANOVA was used for normally distributed variables and the Mann-Whitney or Kruskal-Wallis test for nonparametric variables. Variables between two study groups were compared using t-test, Mann-Whitney test, Fisher’s exact test, or χ² test, according to the scale of measured variables. p values <0.05 were considered statistically significant.

Table 1. Baseline clinical and laboratory characteristics of study participants

| Characteristic                        | 3 doses of vaccine, N = 60 (57%) | 4 doses of vaccine, N = 46 (43%) | p value |
|---------------------------------------|----------------------------------|-----------------------------------|---------|
| Age, years                            | 67.9±15.7                        | 73.9±11.3                         | 0.03    |
| Male                                  | 43 (61)                          | 28 (39)                           | 0.2     |
| Dialysis vintage, months              | 31.2±25.8                        | 30.5±20.9                         | 0.9     |
| Dry weight, kg                        | 77.6±17.2                        | 78.0±19.3                         | 0.9     |
| HD/PD                                 | 31/29 (52/48)                    | 44/2 (96/4)                       | <0.001  |
| Comorbidities                         |                                  |                                   |         |
| Diabetes mellitus                     | 32 (53)                          | 30 (65)                           | 0.2     |
| Hypertension                          | 48 (80)                          | 40 (76)                           | 0.3     |
| Ischemic heart disease                | 16 (27)                          | 17 (37)                           | 0.2     |
| Congestive Heart failure              | 13 (22)                          | 13 (28)                           | 0.4     |
| Peripheral vascular disease           | 2 (3)                            | 6 (13)                            | 0.06    |
| Chronic obstructive pulmonary disease | 6 (10)                           | 7 (15)                            | 0.5     |
| Malignancy                            | 1 (2)                            | 3 (7)                             | 0.2     |
| Immunosuppressive therapy             | 3 (5)                            | 3 (6.5)                           | 0.7     |
| Baseline laboratory data              |                                  |                                   |         |
| Urea, mg/dL                           | 125.8±36.7                       | 121.4±30.5                        | 0.5     |
| Phosphate, mg/dL                      | 5.2±1.1                          | 5.3±1.3                           | 0.4     |
| Albumin, gr/dL                        | 3.6±0.4                          | 3.7±0.3                           | 0.4     |
| CRP, mg/dL                            | 1.4±2.4                          | 1.6±1.8                           | 0.6     |
| PTH, pg/mL                            | 289.8±181.9                      | 282.7±157.7                       | 0.8     |
| WBC, K/mL                             | 7.2±2.6                          | 6.5±1.7                           | 0.1     |
| Hemoglobin, g/dL                      | 10.8±1.3                         | 10.6±1.0                          | 0.5     |

Values are presented as absolute n (%) or as mean ± SD. Laboratory tests were taken at dialysis initiation or as part of routine follow-up of PD patients. CRP, C-reactive protein; HD, hemodialysis; PD, peritoneal dialysis; PTH, parathyroid hormone; WBC, white blood cells.
Estimated marginal means of IgG anti-S titers were analyzed using the general linear model (repeated measures ANOVA), yielded means, and error bars that represent 95% confidence interval from the repeated anti-S measurements at different time points. A multivariable Cox proportional hazards model was constructed to obtain covariate-adjusted measures of the association of number of vaccine doses with the rate of SARS-CoV-2 infection. Data were analyzed using SPSS, Version 27 (IBM Corp., Armonk, NY, USA).

**Results**

The cohort included 106 patients, mean age was 70.5 ± 14.2 years; 75 patients were HD patients, and 31 peritoneal dialysis patients. Sixty patients (56.6%) received only three doses, and 46 (43.4%) received four doses of vaccine. Subjects who received 4 vaccine doses were on average older and more often HD patients than those who received 3 doses, as summarized in Table 1. The third vaccine dose led to a significant increase in IgG anti-S titers among all participants, from a mean of 412 ± 537 AU/mL to 16,353 ± 15,656 AU/mL (T1 to T2; \( p < 0.001 \)).

This was followed by a decline to 7,029 ± 8,181 AU/mL at 1 year (T3; \( p < 0.001 \)). The fourth vaccine dose led to another significant rise in mean IgG anti-S titers 2–3 weeks after administration (T5), mean 29,710 ± 13,139 AU/mL (\( p < 0.001 \), compared with T3). An estimated marginal means model of anti-S Ab titers is presented in Figure 1.

Anti-S Ab titers at T1, T2, and T3 were similar between subjects who received three or four vaccine doses. The absolute increase of IgG anti-S titers following the fourth vaccine dose correlated with the previous response to the third dose (Spearman’s rho = 0.392, \( p = 0.02 \)) as well as with titers in measurements T2 and T3 (Spearman’s rho = 0.376, \( p = 0.02; 0.396, 0.01 \), respectively).

Age inversely correlated with IgG anti-S titers at T1 (Spearman’s rho = −0.23, \( p = 0.02 \)); however, this correlation was not observed in other measurements (T2–T5). Antibody levels were similar between peritoneal dialysis and HD patients across all measurements (\( p > 0.65 \)).

No adverse events were reported by participants after the fourth vaccine dose. Twenty-two subjects from our cohort were diagnosed with SARS-CoV-2 infection. All cases occurred during January and February 2022, after the fourth vaccine dose became available, and all resulted in asymptomatic or mild COVID-19, with neither hospital admissions nor related mortality. The proportions of infections cases were comparable between subjects who received three or four vaccine doses (21.6% and 19.6%, respectively, online suppl. Fig. S2). A similar risk for infection between groups was also demonstrated by a Cox proportional hazard model which adjusted for age and dialysis modality (HR for infection 0.73, 95% CI: 0.25–2.11, \( p = 0.56 \), for patients who were vaccinated with four vs. three vaccine doses).

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**Fig. 1:** Estimated marginal means of anti-S for subjects who received 3 vaccine doses (a) and 4 vaccine doses (b). Kinetics of response to vaccine doses at different time points were analyzed using a general linear model demonstrating the estimated marginal means of anti-S Ab. Arrows represent four vaccine doses. Antibody measurements were taken at 5 different time points: T1: 6 months following the first dose of vaccine; T2: 2–3 weeks after third dose of vaccine (~8 months following first vaccine dose); T3: 12 months following the first dose (4–5 months following the third dose); T4: within a week after the 4th dose was administrated, mostly 5 months after third vaccine dose; T5: 2–3 weeks after the fourth dose (among patients who received a fourth dose). * \( p \leq 0.001 \).
Discussion

Dialysis patients elicit weaker early humoral responses to vaccinations, when compared to healthy controls, which also decline over time [6]. Similar to previous reports, the third vaccine dose led to a profound increase in antibody titers [3, 7]. Nevertheless, the durability and efficacy of this immune response are still undefined in general, let alone in dialysis patients. Indeed, according to our results, although antibody levels decline over 4–5 months following the third dose, they remain higher than all prior measurements. The fourth dose, administered a year after the initial doses, again led to a marked humoral response [8]. As could be expected, older patients and the HD population chose to get vaccinated by a fourth dose more often. Antibody titers in the shared time points between 3 and 4 vaccine doses (T1–T3) and the degree of increase in anti-S Ab after the third vaccine dose were not different between groups. The degree of response was in line with previous response to the third dose. Correlation between the humoral response to the third dose and the response to previous doses, as well as an inverse correlation with age, has been previously reported by our group and others [3, 4].

Our study was not powered to detect infection risk which was an exploratory outcome and should be interpreted as such. All infection cases occurred after January 2022, when the Omicron variant became overwhelmingly dominant in Israel, causing over 99% of COVID-19 cases [9]. Thus, we can safely assume that Omicron was responsible to SARS-CoV-2 cases in our cohort. All infected cases resulted in asymptomatic or mild COVID-19 which did not require hospital admission. In our small cohort, the fourth dose did not confer more protection against Omicron variant when compared to third dose. However, since our study was observational with a bias for higher risk population to receive the fourth dose, such an advantage could not be accurately assessed. It was recently reported that the fourth vaccine dose confers only marginal and short-lived protection from infection by Omicron, yet protection from severe disease is more pronounced [10]. Our results regarding infection risk in the dialysis population, although preliminary, are in-line with those reports.

The main limitations of the study are its modest sample size and observational design. We did not measure neutralizing antibody activity or cellular response to the vaccine which could expand our understanding of the immunity achieved by repeated vaccine doses. Finally, adverse events following vaccination were based on voluntary self-reports only.

In summary, a fourth vaccine dose administered 5–6 months after the third dose has led to a marked increase in anti-S titers among dialysis patients. The clinical effectiveness and ideal vaccine schedules required larger studies with longer follow-up and continuous reevaluation in the context of emerging variants.

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Statement of Ethics

The study was approved by the Ethics Committee and Institutional Review Board of the Meir Medical Center and Shaare Zedek Medical Center and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all participants prior to study entry. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Conflict of Interest Statement

The authors have no financial or other conflicts of interest to declare.

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Author Contributions

Research area and study design: Y.E., N.N., S.B., O.W., and K.C.H.; data acquisition: Y.E., A.B., L.S., N.N., D.E., and K.C.H.; data analysis and interpretation: L.S., O.W., S.B., M.S., A.G., T.H., and K.C.H.; statistical analysis: Y.E., O.W., and K.C.H.; supervision or mentorship: J.P., S.B., and K.C.H. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual’s own contributions.

Data Availability Statement

All data analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.
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