




# Humoral Response to the Pfizer BNT162b2 Vaccine in Patients Undergoing Maintenance Hemodialysis

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## Abstract

**Background and objectives** Coronavirus disease 2019 (COVID-19) is associated with higher morbidity and mortality in patients on maintenance hemodialysis. Patients on dialysis tend to have a reduced immune response to infection or vaccination. We aimed to assess, for the first time to the best of our knowledge, the humoral response following vaccination with the BNT162b2 vaccine in patients on maintenance hemodialysis and the factors associated with it.

**Design, setting, participants, & measurements** The study included 56 patients on maintenance hemodialysis (dialysis group) and a control group composed of 95 health care workers. All participants had received two doses of the BNT162b2 (Pfizer-BioNTech) vaccine. The serology testing was done using Quant II IgG anti-Spike severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assay by Abbott a median of 30 days after receipt of the second dose of the vaccine.

**Results** All subjects in the control group developed an antibody response compared with 96% (54 of 56) positive responders in the dialysis group. The IgG levels in the dialysis group (median, 2900; interquartile range, 1128–5651) were significantly lower than in the control group (median, 7401; interquartile range, 3687–15,471). A Mann–Whitney *U* test indicated that this difference was statistically significant ( $U=1238$ ;  $P<0.001$ ). There was a significant inverse correlation of age and IgG levels in both groups. The odds of being in the lower quartile were significantly higher for older individuals (odds ratio, 1.11 per year of age; 95% confidence interval, 1.08 to 1.20;  $P=0.004$ ) and for the dialysis group compared with the control group (odds ratio, 2.7; 95% confidence interval, 1.13 to 7.51;  $P=0.05$ ). Within the dialysis group, older age and lower lymphocyte count were associated with antibody response in the lower quartile (odds ratio, 1.22 per 1-year older; 95% confidence interval, 1.13 to 1.68;  $P=0.03$  and odds ratio, 0.83 per  $10^{-e^3}/\mu\text{l}$ -higher lymphocyte count; 95% confidence interval, 0.58 to 0.97;  $P=0.05$ ).

**Conclusions** Although most patients on maintenance hemodialysis developed a substantial humoral response following the BNT162b2 vaccine, it was significantly lower than controls. Age was an important factor in the humoral response, regardless of chronic medical conditions.

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## Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with higher morbidity and mortality in patients on maintenance hemodialysis (HD) (1,2).

Prioritizing patients on dialysis for vaccination has been at the forefront of SARS-CoV-2 vaccination programs internationally (3). Patients with CKD, but especially those with kidney failure, treated with maintenance HD tend to have a reduced immune response to infection or vaccination, as demonstrated with the hepatitis B virus vaccine. Consequently, there is often a need for higher vaccine dosage or scheduling changes in these patients (4–6).

Several vaccines have been approved for SARS-CoV-2 infection. Live attenuated vaccines generally should be avoided in patients on maintenance HD due to their dysregulated immune system. Both the mRNA vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) and the replication-defective viral-vectored

vaccines, such as ChAdOx1 nCoV-19 (Oxford-AstraZeneca), are considered safe for use in patients treated with maintenance HD (7,8). This study is aimed at establishing one aspect of the immune response, the humoral response to the BNT162b2 (Pfizer-BioNTech) vaccine in patients with kidney failure on maintenance HD. We determined the level of antibodies directed against the spike antigen following vaccination of patients on maintenance HD and compared it with controls with no kidney failure, searching for factors that may be associated with the humoral response.

## Materials and Methods

### Study Design

The study included two cohorts: patients on maintenance HD (dialysis group) and a control group composed of 95 health care workers without kidney failure (control group) from our institution. All participants

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had been previously vaccinated with the BNT162b2 (Pfizer-BioNTech) vaccine, with the recommended dosing interval of 21 days between the first and second doses. All participants received the second vaccine at least 7 days prior to trial entrance. In total, 56 of 83 patients on maintenance HD in our institution had received two doses of the vaccine and, thus, were eligible to participate in our study. Twenty-five received the vaccine in our hospital, and the remaining 31 patients were vaccinated by their health maintenance organizations. Twenty-seven patients (eight women and 19 men) were not vaccinated and, hence, were excluded from the study. Four patients were sick with COVID-19 and were not eligible for vaccine at the time of vaccine administration. One patient was hospitalized for different reasons during this period, and one patient had a history of severe allergic reaction and hence was not vaccinated. The remaining 21 patients refused to receive the vaccine at the time of the study.

Following the approval of the local institutional review board, we obtained informed consent from the participants to draw 5 ml of blood at the beginning of the dialysis session for the dialysis group and venous blood samples for the control group. Immunogenicity assessment was determined using a method previously published by Walsh *et al.* (9) (phase 1 by Pfizer). In brief, we used a chemiluminescent microparticle immunoassay (SARS-CoV-2 IgG II Quant assay on an ARCHITECT analyzer; Abbott) to quantify IgG antibodies from the patient's plasma. The assay detects antibodies against the receptor binding protein of the S1 subunit of the spike protein of SARS-CoV-2. The assay presents a positive predictive agreement of 99.4% (95% confidence interval [95% CI], 96.50% to 99.97%) and a negative predictive agreement of 99.6% (95% CI, 99.15% to 99.37%), and it is in agreement with a neutralization method (positive agreement, 100.0%; 95% CI, 95.72% to 100.00%) (9,10). A value  $\geq 50$  arbitrary units per milliliter (AU/ml) was considered evidence of vaccination response (10).

The dialysis dose was measured by Kt/V, calculated manually using the Daugirdas formula (11).

Body mass index was defined as dry weight in kilograms divided to height in square meters. We used recorded laboratory tests that were routinely taken for each patient on HD at the beginning of the month prior to their first dose of the SARS-CoV-2 vaccine. Control patients self-reported their medical history and any long-term medications. Details about patients on maintenance HD were obtained from their medical charts.

### Statistical Analyses

All data were summarized and displayed as mean (SD) for the continuous variables and as number of patients and the percentage in each group for categorical variables. For all categorical variables, the chi-square statistic was used to assess the statistical significance between groups. Continuous variables were first tested for normal distribution using the Kolmogorov-Smirnov test and quantile-quantile plots; then, parameters were compared by using a *t* test if normally distributed or by Kruskal-Wallis/Mann-Whitney *U* test if not normally distributed.

Correlation between two continuous parameters was calculated by Spearman analysis.

We fitted binary logistic regression models for the risk of being in the lower quartile for all participants and for the study group, adjusted for covariates.

In order to describe the frequencies of antibody levels in both cohorts, we used a histogram, with bin sizes of 3000 AU/ml.

$P=0.05$  was considered statistically significant for all analyses.

IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY) was used for all statistical analyses.

## Results

Ninety-five participants were included in the control group, and 56 were in the dialysis group. Baseline characteristics of both groups are shown in Table 1. Both cohorts included only White participants. Patients in the dialysis group were older and had a higher prevalence of men compared with the control group.

### Patients on Hemodialysis Develop a Lower Level of Antibodies Compared with Control

All subjects in the control group developed a positive antibody response (defined as 50 AU/ml or higher) as compared with 96% (54 of 56) in the dialysis group. The two patients with no serologic response were a 75-year-old man with long-term immunosuppression (low-dose prednisone), diabetes mellitus, and hypertension and a 90-year-old diabetic man.

The mean IgG levels in the dialysis group (median, 2900; interquartile range, 1128–5651) were significantly lower than those in the control group (median, 7401; interquartile range, 3687–15,471) (Figure 1). A Mann-Whitney *U* test indicated that this difference was statistically significant ( $U=1238$ ;  $P<0.001$ ).

### Correlation of Age and Antibody Levels

There was a significant inverse correlation of older age and antibodies levels in both study groups (Spearman correlation =  $-0.29$ ;  $P=0.03$  and  $-0.32$ ;  $P<0.001$  for dialysis and control groups, respectively).

For each age range, there were higher levels of antibodies in the control group compared with the dialysis group, which was significant for ages  $<60$  and 60–70 years old (Figure 2).

### Factors Associated with Lower Antibody Levels

For all participants in the dialysis group, the lower 25th percentile of IgG levels was 2336 AU/ml. The odds of being in the lower quartile were significantly higher for older individuals (odds ratio, 1.11 per 1 year of age; 95% CI, 1.08 to 1.20;  $P=0.004$ ) and for patients on dialysis compared with controls (odds ratio, 2.71; 95% CI, 1.13 to 7.51;  $P=0.05$ ).

In the dialysis group, the lower quartile of IgG was 1128 AU/ml. In a regression model for the lower quartile of antibodies, age was again significantly related to the level of immunization, while a higher lymphocyte count was protective (Table 2).

**Table 1. Characteristics of patients on dialysis and control subjects who received the Pfizer BNT162b2 vaccine**

Factor	Dialysis Group, <i>n</i> =56	Control Group, <i>n</i> =95
Age, yr	74 (11)	57 (9)
Sex, women, <i>n</i> (%)	14 (25)	69 (73)
BMI, kg/m <sup>2</sup>	27.2 (4)	
Diabetes mellitus, <i>n</i> (%)	35 (63)	6 (6)
Immunosuppression medication, <i>n</i> (%)	1 (2)	4 (4)
Kidney failure etiology, diabetes or nephrosclerosis, <i>n</i> (%)	41 (72)	
Transplantation candidate (%)	13 (23)	
Dialysis vintage, mo	38 (37)	
Dialysis access, AVF, <i>n</i> (%)	42 (74)	
Mean Kt/V	1.33 (0.23)	
Days after first dose, median (IQR) <sup>a</sup>	53 (42–56)	52 (41–60)
Days after second dose, median (IQR) <sup>b</sup>	30 (27–34)	30 (26–34)
White blood cell count, 10 <sup>3</sup> /μl	7.9 (3.1)	
Polymorphonuclears count, 10 <sup>3</sup> /μl	5.5 (2.4)	
Lymphocyte count, 10 <sup>3</sup> /μl	1.5 (0.6)	
Serum albumin, g/dL	4.0 (0.35)	

Values are mean (SD), unless otherwise stated. BMI, body mass index; AVF, arteriovenous fistula; IQR, interquartile range.

<sup>a</sup>Range is 34–60 days for the study group and 35–67 days for the control group.

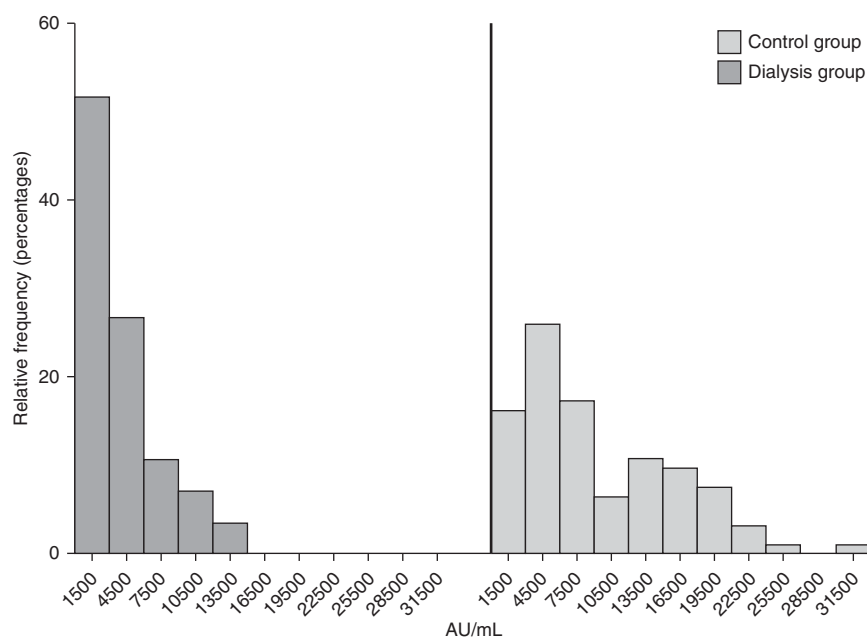
<sup>b</sup>Range is 12–34 days for the study group and 14–34 days for the control group.

## Discussion

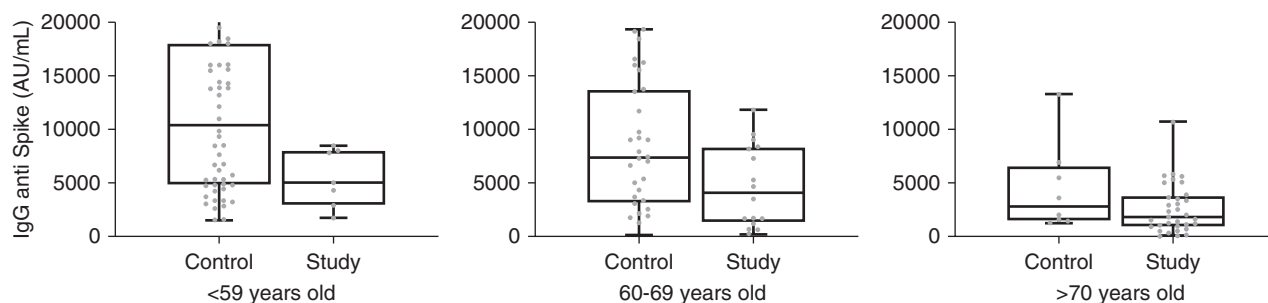
In this study, we describe, for the first time to the best of our knowledge, the IgG antibody response to the spike protein following vaccination with the BNT162b2 (Pfizer-BioNTech) vaccine in patients on maintenance HD compared with a cohort vaccinated health care workers. The pivotal trial that demonstrated 95% protection against COVID-19 infection following a two-dose regimen of the BNT162b2 vaccine did not include patients on maintenance HD (12). It is well known that patients on dialysis may have a reduced response to vaccination. We, therefore, aimed to assess this assumption regarding the BNT162b2 (Pfizer-BioNTech) vaccine.

Our major finding is that the majority of patients on maintenance HD developed a substantial humoral response following the two vaccine doses; however, it was significantly lower than controls.

The cutoff for a positive response in our assay was 50 AU/ml, and >90% of our cohort was well above this threshold. Interestingly, one of the two subjects who did not develop a response reported long-term prednisone use. Other patients were treated with prednisone and responded; therefore, this alone did not explain the lack of response in the one patient. Preliminary reports have shown a lack of humoral response following vaccination with the mRNA-1273 vaccine



**Figure 1. | Patients on dialysis develop a lower IgG antisevere acute respiratory syndrome coronavirus 2 spike antibodies level compared with controls ( $P<0.001$ ).** Two patients from the dialysis group had undetectable antibody levels defined as <50 arbitrary units per milliliter (AU/ml).



**Figure 2. | Mean antibody level in different age ranges was lower in dialysis compared with control group.** Ends of the boxes are the upper and lower quartiles. The medians are marked by horizontal lines inside the boxes. Every dot represents one participant's level of antibodies. Error bars represent the range between minimal and maximal points. The top eight whiskers in plot age <59 (all subjects from the control group) are beyond the range of the y axis (range, 23,177–40,000 AU/ml). It was done in order to provide a greater resolution to the age >70 group ( $P=0.003$  for age <60,  $P=0.007$  for age 60–70, and  $P=0.20$  for age >70 for study group versus control group).

(Moderna) and the BNT162b2 vaccine (Pfizer-BioNTech) in patients with transplants treated with long-term immunosuppression (13). This may warrant further studies on the timing and efficacy of vaccination in patients treated with immunosuppressants.

When comparing the groups and data within groups, age is a substantial factor in determining the level of response.

There was little difference between the antibody response in vaccinees older than 70 years of age in the dialysis group compared with the control group, implying that age is an important factor in humoral response, regardless of chronic medical conditions.

We did find a correlation between the level of antibodies and lymphocyte count. This is not surprising given the lymphocyte role in the immune system in general and the production of antibodies in particular (14). The role of the cellular arm in the immune response following vaccination with the BNT162b2 vaccine remains to be elucidated.

We found no association between the level of IgG response and body mass index, dialysis dose, dialysis vintage, transplantation candidacy status, or albumin levels.

Although our findings are preliminary and warrant further clarifications and verifications, we believe these findings should prompt consideration following further information for changing the dose/schedule of vaccinations in patients on maintenance HD as has been done with different vaccines in the past: for example, with the hepatitis B vaccine Engerix-B, which is given in double doses as a four-series vaccine instead of a three-series vaccine in healthy individuals (15,16).

Further assessments of other parts of the immune response are needed, mainly assessing the cellular response. However, it is difficult to assume that the cellular immune response will fare better (17,18).

Although the level of the humoral response in most patients on maintenance HD in our study is considered positive, the weaker seroresponse (relative to controls and the populations in which the vaccine trials were conducted) may have consequences, including lower vaccine efficacy to the parent strain or to variants that are evolving and/or a shorter period of immunoprotection after vaccination. Studies to assess vaccine efficacy, antibody waning, and efficacy against variants in this population are needed (19).

Our study has several limitations. Size of the study is an inherent limitation in this study. Given the need to perform the assay in close proximity to the vaccine schedule, we were not able to recruit more centers in this study.

In many countries, incidence and/or severity of COVID-19 have varied by ethnicity, which we could not investigate due to the homogenous nature of our maintenance HD population.

Furthermore, there is a considerable age difference between the dialysis group and the control group that stems from the nature of both populations (health care workers versus patients on maintenance HD). We tried to overcome this limitation by adjusting the statistical analysis for age and by dividing into similar age groups in both cohorts.

We did not perform baseline antibody titers, and thus, we cannot exclude the possibility that the seroresponse results may reflect infection versus vaccination in some patients.

**Table 2. Clinical factors associated with low humoral response to the Pfizer BNT162b2 vaccine among patients on hemodialysis**

Variable	Adjusted Odds Ratio (95% Confidence Interval)	P Value
Age per 1 yr	1.2 (1.1 to 1.6)	0.03
Dialysis vintage per 1 yr	1.1 (0.9 to 1.4)	0.19
Kt/V per 1 U	0.9 (0.7 to 1.9)	0.43
Diabetes mellitus	1.4 (0.8 to 2.5)	0.58
Lymphocyte count per $10^3/\mu\text{l}$	0.8 (0.6 to 0.9)	0.04

Low humoral response was defined as below the 25th percentile of antibody concentration. Covariates included in the analysis are age, dialysis vintage, Kt/V, lymphocyte count, and diagnosis of diabetes mellitus.

However, we tested (using PCR) patients presenting with symptoms, contacts of SARS-CoV-2–positive patients, and the entire shift in the case of a positive case from that shift. The last PCR test prior to the vaccine was  $59 \pm 29$  days on average.

Although this is one of our limitations because we cannot rule out asymptomatic infection, we have not detected any asymptomatic infection in our unit during the previous 12 months, and patients on dialysis have been reported to have a low rate of asymptomatic infection (<10%) (20).

The clinical implications of the serology test and the presence of antibodies and their levels remain to be fully clarified. There are several reports regarding the correlation of antibodies to SARS-CoV-2. Lumley *et al.* (21) followed 12,541 health care workers, of whom 1265 were seropositive for antispike IgG following infection with SARS-CoV-2 and demonstrated a substantially reduced risk for reinfection 6 months following infection. Bartsch *et al.* (22) describe a relationship between antibody titers and functional antibody activity to SARS-CoV-2 over time. Regarding protection following vaccine, there is a recent study in press that presents that neutralization level is highly predictive of immune protection.

In conclusion, patients on maintenance HD develop a substantial humoral immune response following the BNT162b2 vaccine. This finding is reassuring and should encourage patients on maintenance HD and their caregivers to receive the vaccine, especially considering the safety profile emerging from real-world data regarding the vaccine.

#### Disclosures

A. Grupper reports employment with Tel Aviv Medical Center. I.F. Schwartz reports employment with Tel Aviv Medical Center. M. Israel reports employment with Laniado Hospital. Y. Rechavi reports employment with Laniado Hospital. D. Schwartz reports employment with Sourasky Medical Center. All remaining authors have nothing to disclose.

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