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Original Article

ORIGINAL ARTICLE

Diminished and waning immunity to COVID-19 vaccination among hemodialysis patients in Israel: the case for a third vaccine dose

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ABSTRACT

Background. Humoral responses to coronavirus disease 2019 (COVID-19) vaccines in hemodialysis (HD) patients can direct vaccination policy.

Methods. We compared 409 COVID-19-naïve HD patients from 13 HD units in Israel to 148 non-dialysis-dependent COVID-19-naïve controls. Twenty-four previously infected (antinucleocapsid positive) HD patients were analysed separately. Blood samples were obtained ≥14 days post-vaccination (BNT162b2, Pfizer/BioNTech) to assess seroconversion rates and titers of anti-spike (anti-S) and neutralizing antibodies.

Results. The median time from vaccination to blood sample collection was 82 days [interquartile range (IAR) 64–87] and 89 days (IQR 68–96) for HD patients and controls, respectively. Seroconversion rates were lower in HD patients compared with controls for both anti-S and neutralizing antibodies (89% and 77% versus 99.3%, respectively; $P < 0.0001$). Antibody titers were also significantly lower in HD patients compared with controls {median 69.6 [IQR 33.2–120] versus 196.5 [IQR 118.5–246], $P < 0.0001$; geometric mean titer [GMT] 23.3 [95% confidence interval (CI) 18.7–29.1] versus 222.7 [95% CI 174–284], $P < 0.0001$, for anti-S and neutralizing antibodies, respectively}. Multivariate analysis demonstrated dialysis dependence to be strongly associated with lower antibody responses and antibody titers waning with time. Age, low serum albumin and low lymphocyte count were also associated with lower seroconversion rates and antibody titers. HD patients previously infected with sudden acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had no difference in their seroconversion rates or antibody titers compared with COVID-19-naïve patients.

Conclusion. This study demonstrates diminished and waning humoral responses following COVID-19 vaccination in a large and diverse cohort of HD patients, including those previously infected with SARS-CoV-2. Considering these results

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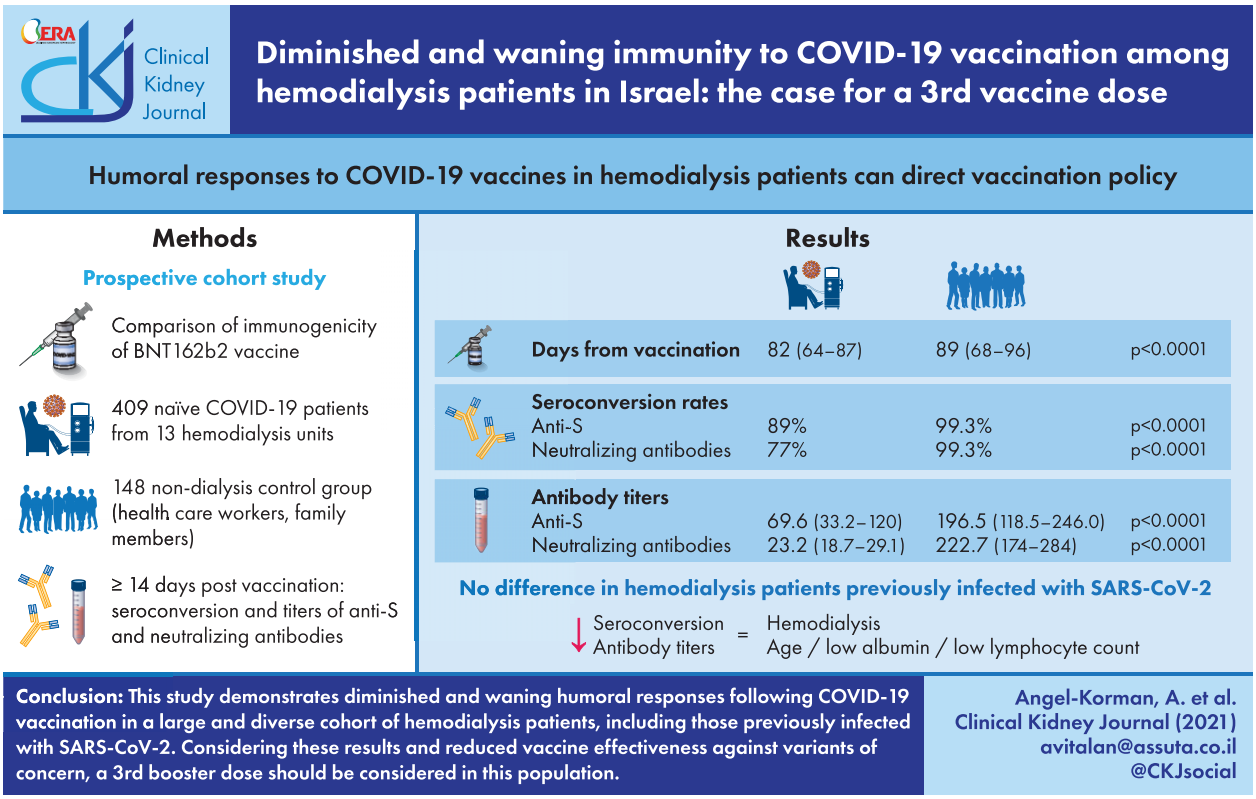
and reduced vaccine effectiveness against variants of concern, in addition to continued social distancing precautions, a third booster dose should be considered in this population.

GRAPHICAL ABSTRACT



Keywords: anti-S antibodies, BNT162b2 vaccine, COVID-19, hemodialysis, humoral response, neutralizing antibodies, seroconversion, waning immunity

INTRODUCTION

The messenger RNA (mRNA)-based coronavirus disease 2019 (COVID-19) vaccine BNT162b2 (Pfizer/BioNTech) received emergency authorization by the US Food and Drug Administration in November 2020, following a phase III study that included more than 43 000 subjects. Over a follow-up period of 3 months the vaccine showed 95% efficacy against symptomatic SARS-CoV-2 [1].

Various studies have shown high real-world vaccine effectiveness in the prevention of severe disease, hospitalization and death related to COVID-19 [1–5]. Nevertheless, diminished effectiveness was shown in patients with multiple comorbidities [6]. Moreover, patients with severe breakthrough sudden acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections leading to hospitalizations have been found to have a high rate of comorbidities and immunosuppression [7]. Diminished immunogenicity has been previously reported in immunocompromised patients, including patients with haematological malignancies, transplant recipients and patients receiving immunosuppressive therapy [8–13].

Patients with end-stage renal disease (ESRD) are known to have reduced immune responses [14], as evidenced by their diminished response to several types of vaccines, including the hepatitis B vaccine [15, 16], as well as by a relatively rapid antibody titer waning following pneumococcal vaccination [17].

Limited data are available regarding the humoral response following BNT162b2 vaccination in ESRD patients requiring dialysis, all studied in relatively small cohorts. Antibody titers were measured only for a short time following the second dose of the vaccine and most studies have not looked at neutralizing antibody levels, which are correlated with protection against SARS-CoV-2 infection [18, 19]. Nevertheless, lower seroconversion rates and lower anti-spike (anti-S) binding antibody titers were demonstrated in this patient population [20–23].

In the current study we investigated humoral responses including anti-S antibody levels, neutralizing antibody levels and factors associated with it, 2–3 months after the second BNT162b2 vaccine dose in a large and diverse maintenance hemodialysis (MHD) cohort.

MATERIALS AND METHODS

Study design and setting

A prospective cohort study comparing the immunogenicity of BNT162b2 vaccine in adult patients on chronic MHD with control group participants not on dialysis. The dialysis cohort consisted of patients recruited from 13 HD units, 11 of which were community units operated by the A.P.C Health Specialists Clinics HD chain and 2 were hospital-based HD units at the Assuta University Medical Center in Ashdod and the Barzilai

University Medical Center in Ashkelon. The control group consisted of healthcare workers of the participating HD units as well as adult family members of the dialysis subjects enrolled in the study.

Venous blood was drawn from patients and controls at least 14 days following the second dose of BNT162b2 vaccination and assayed for SARS-CoV-2 anti-S, antinucleocapsid (anti-N) and SARS-CoV-2 neutralizing antibody levels.

Humoral response assessment

Anti-S antibody levels were tested with the LIAISON SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Saluggia, Italy), which targets the S1 and S2 subunits of the spike protein. The assay is considered highly sensitive and specific (97.4% sensitivity and 98.9% specificity), correlating well with neutralizing antibody titers [24]. Antibody titers are presented as AU/mL. The cut-off value for a positive result being ≥15 AU/mL.

Anti-N antibody presence, which is indicative of previous SARS-CoV-2 infection, was tested using the qualitative Elecsys anti-SARS-CoV-2 assay (Cobas, Roche Diagnostics, Basel, Switzerland). The test has a sensitivity of 89% and a specificity of 100%.

Neutralizing antibody levels were measured by a pseudovirus microneutralization assay as previously described [25] using a green fluorescent protein reporter–based pseudotyped virus with a vesicular stomatitis virus backbone coated with SARS-CoV-2 spike protein, which was obtained from Gert Zimmer (Institute of Virology and Immunology, Mittelhäusern, Switzerland). Sera not capable of reducing viral replication by 50% at 1: 8 were considered non-neutralizing. Levels are provided as geometric mean titer (GMT) and 95% confidence interval (CI).

Other variables

All participants filled out a questionnaire with demographic details. For all dialysis patients, relevant medical history including comorbidities, ESRD etiology, use of immunosuppressive medications, dialysis treatment details (Kt/V_{urea}, number of treatments per week, etc.) and most recent serum albumin level, hemoglobin level, white blood cell and lymphocyte counts as well as hepatitis B surface antibody (anti-HBsAb) levels were retrieved from the dialysis units' medical records.

Outcomes

We analysed four serological outcomes: the post-vaccination seropositivity rate with the anti-S and neutralization assays and the antibody titers achieved by vaccination in these two assays.

Statistical methods

Categorical variables were summarized as frequency and percentages. Continuous variables were evaluated for normal distribution using histograms and the Kolmogorov–Smirnov test. Non-normally distributed variables are reported as median and interquartile range (IQR). Chi-square and Fisher's exact tests were used to study the association between categorical variables. Spearman's correlation coefficient was used to evaluate the association between continuous variables. The Kruskal–Wallis and Mann–Whitney tests were applied to compare continuous variables between categories. All statistical tests were two-sided and P-values <0.05 were considered statistically significant. For multivariate analysis we used logistic regression models including variables with a significant effect on the outcome in the univariate analysis. We used NCSS 2021 version 21.0.2 software (NCSS, Kaysville, UT, USA) for all statistical analyses.

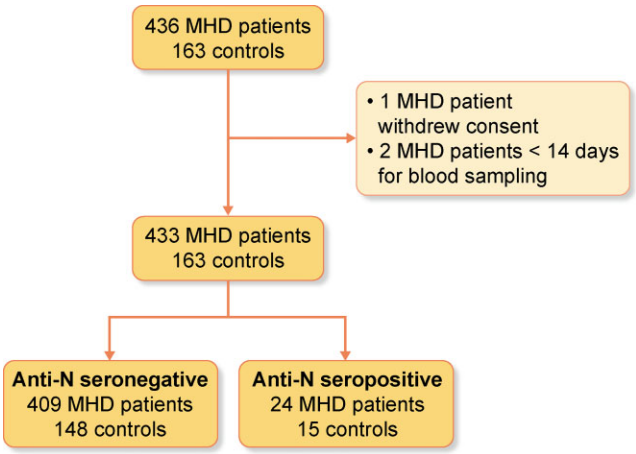


FIGURE 1: Study cohort.

Ethical considerations

All participants signed an informed consent and the study was approved by the Samson Assuta Ashdod University Hospital Institutional Review Board (IRB) and by the Barzilai University Medical Center IRB.

RESULTS

Study cohort

The original study cohort included 436 MHD patients and 163 controls. One MHD patient withdrew consent prior to obtaining blood samples. Two more MHD patients were excluded, as <14 days had passed from the date of the second vaccine dose to blood sampling. Twenty-four MHD and 15 control samples were found to have anti-N-positive tests, signifying previous SARS-CoV-2 infection, and were therefore analysed separately. The final analysis included a cohort of COVID-19-naïve subjects (409 MHD patients and 148 controls) and a cohort of previously infected subjects (24 MHD patients and 15 controls) (Figure 1).

Comparison of COVID-19-naïve MHD patients and controls

The time from vaccination to sampling ranged from 15 to 120 days, but the medians were >80 days (IQR 65–90), satisfying the goal for a late (2–3 months) assessment of immunogenicity.

The subjects' demographic characteristics, seroconversion rates and antibody titers are presented in Table 1. MHD patients were older than controls [median 71.9 years (IQR 63–80) versus 48.5 years (IQR 38–58); P < 0.0001, respectively] and had a higher percentage of males. The median time from the second dose of BNT162b2 to blood sampling for serology was shorter in MHD

Table 1. Characteristics and humoral responses in naïve MHD patients and controls

Characteristics	MHD group ($n = 409$)	Control group ($n = 148$)	P-value
Age (years) median (IQR)	71.9 (63–80)	48.5 (38–58)	**<0.0001**
Sex (male), *n* (%)	269 (65.7)	51 (34.4)	**<0.0001**
Time to serologic sampling (days), median (IQR)	82 (64–87)	89 (68–96)	**<0.0001**
Anti-S seropositive, *n* (%)	364 (89)	147 (99.3)	**<0.0001**
Anti-S titer, median (IQR)	69.6 (33.2–120)	196.5 (118.5–246)	**<0.0001**
Neutralizing Ab seropositive, *n* (%)	315 (77)	147 (99.3)	**<0.0001**
Neutralizing Ab, GMT (95% CI)	23.3 (18.7–29.1)	222.7 (174–284)	**<0.0001**

Ab, antibody.

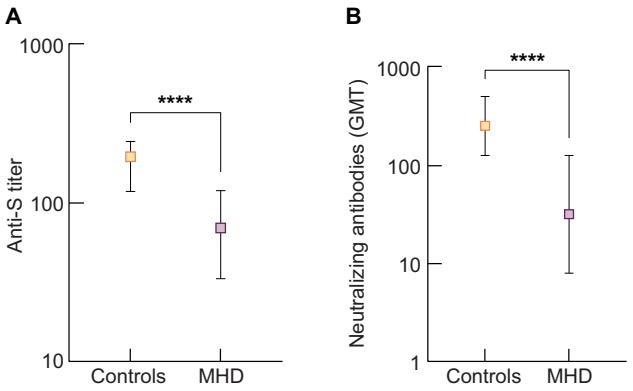


FIGURE 2: Antibody titers of naïve MHD patients and controls, including (**A**) anti-S and (**B**) neutralizing antibodies. Boxes represent medians and whiskers represent the IQR. Neutralizing antibody levels are displayed as GMT.

patients than in controls [82 days (IQR 64–87) versus 89 days (IQR 68–96), respectively ($P < 0.0001$)].

A positive anti-S antibody titer developed in 364 of 409 (89%) naïve MHD patients compared with 147 of 148 (99.3%) controls ($P < 0.0001$). The median anti-S titer was significantly lower in MHD patients compared with controls [median 69.6 (IQR 33.2–120) versus 196.5 (IQR 118.5–246); $P < 0.0001$] (Table 1, Figure 2A).

Neutralizing antibodies developed in 315 of 409 (77%) naïve MHD patients compared with 147 of 148 (99.3%) controls ($P < 0.0001$). Neutralizing titers were lower in MHD patients than controls, with a GMT of 23.3 (95% CI 18.7–29.1) versus 222.7 (95% CI 174–284) ($P < 0.0001$), respectively (Table 1, Figure 2B).

Importantly, anti-S and neutralizing antibodies in both controls and study group participants were strongly correlated [Spearman correlation coefficient (r_s) = 0.84].

On multivariate analysis including age and sex, dialysis dependence was strongly associated with reduced seroconversion rates and antibody titers (Figure 3 and Supplementary Tables S1–S4). MHD patients had 54.2% and 76.5% reduced anti-S and neutralizing antibody titers, respectively, compared with controls, whereas age had a more modest contribution to titer decline, with 18% and 36.8% decreases per decade, respectively.

Analysis of COVID-19-naïve MHD patients

Clinical and laboratory characteristics including comorbidities, dialysis-related details and etiologies for ESRD as well as humoral responses of COVID-19-naïve MHD patients are presented in Table 2.

Of the 364 naïve MHD patients who had positive anti-S antibodies, 57 (15.7%) did not develop neutralizing antibodies. Anti-S seropositivity (seroconversion) in MHD patients was significantly associated with younger age, higher albumin levels, higher absolute lymphocyte count, higher hemoglobin levels and higher serum iron levels and was negatively associated with use of immunosuppressive medications including the use of steroids, calcineurin inhibitors (CNIs), rituximab and chemotherapy (all at the time of blood sampling). Other comorbidities, including dialysis vintage, Kt/V_{urea} etiology of ESRD, residual urine output, previous kidney transplantation and the time from vaccination to blood sampling, were not significantly associated with anti-S positivity (Table 2).

For neutralizing antibodies, seropositivity was significantly associated with younger age, higher albumin levels, higher absolute lymphocyte count and serum iron level and negatively associated with immunosuppressive therapy as well as the time from vaccination to blood sampling (Table 2).

On univariate analysis, higher anti-S and neutralizing antibody titers were associated with younger age, higher albumin levels and higher absolute lymphocyte count and negatively associated with longer time from vaccination to blood sampling and immunosuppressive medications (Supplementary data, Table S5).

Multivariable logistic regression analysis, for variables influencing anti-S and neutralizing antibodies, verified that younger age remained significantly associated with neutralizing antibodies for seroconversion and with titer levels of both antibody types. Higher albumin levels remained significantly associated with anti-S seropositivity and with titer levels of both anti-S and neutralizing antibodies. Higher absolute lymphocyte count remained significantly associated with seropositivity and titer levels of neutralizing antibodies. Immunosuppression remained negatively associated with seroconversion and titer levels for both anti-S and neutralizing antibodies. Longer time from vaccination to blood sampling remained associated with seronegativity of neutralizing antibodies (Table 3) and with titer levels for both antibody types (Figure 4; Supplementary data, Table S6).

Association of SARS-CoV-2 antibodies with hepatitis B antibodies in MHD patients

Considering that MHD patients are routinely vaccinated against hepatitis B, we compared their anti-HBsAb seropositivity and titer levels with the SARS-CoV-2 antibodies seroconversion rates and titer levels following the second dose of BNT162b2 vaccine. Anti-S seroconversion rates were not significantly associated with anti-HBsAb titers ($P = 0.14$); however, the association was

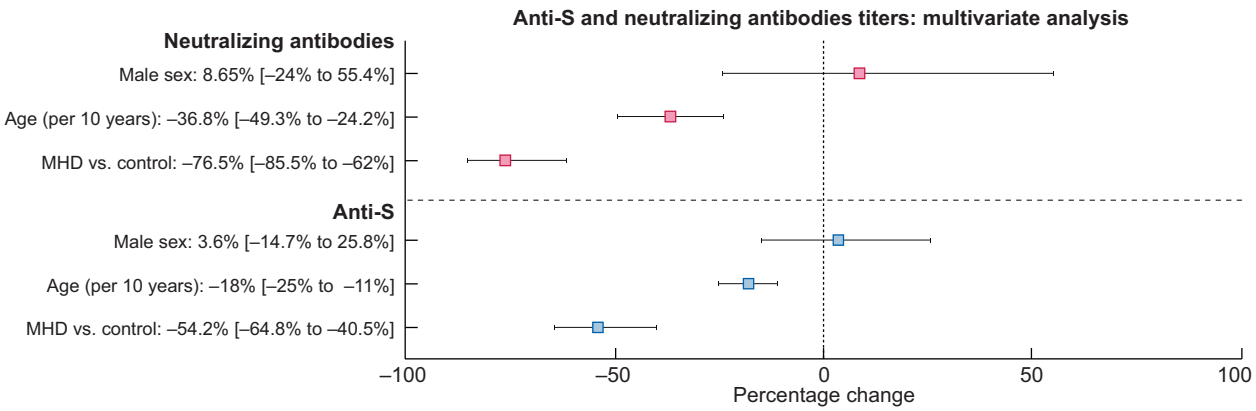


FIGURE 3: Multivariate regression analysis of factors associated with anti-S (blue) and neutralizing antibody (red) titers in MHD patients and controls. Associations are presented as the percent change (boxes) and 95% CI (whiskers).

significant for neutralizing antibodies seropositivity (P = 0.04) (Table 2, Figure 4).

In a univariate analysis, anti-S and neutralizing antibody titers were only weakly associated with anti-HBsAb titers ($r_s = 0.18$ and $r_s = 0.16$, $P < 0.01$, respectively; Table 2). Accordingly, multivariate analysis demonstrated a very weak effect of anti-HBsAb titers on both anti-S and neutralizing antibody titers: 1% per 100 IU/mL (Figure 4).

Humoral response in MHD patients previously infected with SARS-CoV-2 (positive anti-N antibodies)

Twenty-four MHD patients and 15 controls were found to have positive anti-N antibodies, indicating previous infection with SARS-CoV-2. The median anti-S titer was higher in controls versus MHD patients (174 versus 101.8 AU/L; P = 0.0001). Notably, four of the anti-N-positive MHD patients (17%) did not seroconvert for anti-S antibodies following both infection and vaccination. For neutralizing antibodies, six MHD patients (25%) did not seroconvert—four of whom had no anti-S antibodies and two additional patients who had an anti-S titer of 57 AU/L and 3130 AU/L. Notably, all were elderly (69–91 years old) and two of them were immunosuppressed. Conversely, in the control group, all anti-N-positive subjects (100%) had seroconversion for both antibody types. Importantly, there was no statistically significant difference in the seroconversion rate as well as titer levels for both anti-S and neutralizing antibodies between MHD patients who were COVID-19 naïve (anti-N negative) and those who were previously infected with SARS-CoV-2 (anti-N positive) (Table 4).

DISCUSSION

Similar to the findings in previous studies, this study confirms that when MHD patients are compared with healthy controls, older age and being on MHD conferred a higher risk for lack of seroconversion as well as for lower antibody titers [21, 22]. Notably, the effect of being dialysis dependent was much more pronounced than advancing age on both seroconversion and antibody titer levels.

Unlike other studies, we demonstrated waning of immune response over time and reduced neutralizing antibody response, hinting toward suboptimal protection in MHD patients, as well as a reduced response among vaccinated convalescent MHD patients, whom we previously considered as relatively protected.

In our study, the timing for blood sampling was 2–3 months after the second vaccine for most subjects: 82 days (IQR 64–87) for MHD patients and 89 days (IQR 68–96) for controls. This time period was similar to the one used in the original study that led to vaccine authorization [1] and significantly longer than the median time in other published studies (30–58 days) [20–22].

Waning immunity following BNT162b2 vaccination was a significant unknown at the time of emergency authorization. In the current study, multivariate analysis demonstrated a gradual antibody waning in MHD patients, with anti-S titers decreasing by 1.36%/day (95% CI 0.74–1.38) and neutralizing antibodies by 2.37%/day (1.29–3.63, as well as loss of neutralizing antibodies with time. Interestingly, the anti-S titers published by Yanay *et al.* [21], who used the same laboratory assay, measured 21–35 days post-vaccination, were 116 AU/mL in MHD patients and 176 AU/mL in controls, whereas our study showed a lower median titer of 69.6 AU/mL in MHD patients after a median of 82 days and a similar median titer of 196.5 AU/mL in controls. These findings suggest a significant decline over time in anti-S titers in dialysis patients as opposed to younger and healthier controls. Waning immunity might be more important with emerging vaccine-escape variants and was reported in elderly individuals infected with the Delta variant in Israel [26].

Neutralizing antibodies are considered to be associated with protection from SARS-CoV-2 infection and development of severe disease [18] and therefore serve as a better indication for the level of protection for MHD patients. Currently only limited data are available regarding the neutralizing antibody response in dialysis patients, with one study measuring antibody titers after a single dose [27] and the other measuring neutralizing antibody response in 22 MHD patients only, shortly after vaccination [28] (Table 5).

Our study was the first one to demonstrate in a large cohort and in a relatively long interval after full vaccination reduced immune responses, with only 77% of MHD patients achieving any titer of neutralizing antibodies.

Older age, poor nutritional status (lower albumin levels, lower absolute lymphocyte count) and use of immunosuppressive therapy (steroids, CNIs, rituximab and chemotherapy) were significantly associated with a lack of seroconversion and lower titers for both anti-S and neutralizing antibodies.

Table 2. Clinical characteristics and humoral responses of naïve MHD patients: univariate analysis of anti-S and neutralizing antibody seropositivity

Characteristics	Total	Anti-S seropositive ($n = 364$)	Anti-S seronegative ($n = 45$)	P-value	Neutralizing antibodies seropositive ($n = 315$)	Neutralizing antibodies seronegative ($n = 94$)	P-value
Sex (male) *n/N* (%)	269/409 (66)	241/364 (66.2)	28/45 (62.2)	0.59	209/315 (66.4)	60/94 (63.8)	0.65
Comorbidities, *n/N* (%)							
HTN	347/399 (87)	310/356 (87.1)	37/43 (86.1)	0.85	266/308 (86.4)	81/91 (89)	0.5
DM	235/399 (58)	211/356 (58.3)	24/43 (55.8)	0.76	180/312 (57.7)	55/93 (59.1)	0.8
CHF	68/399 (17)	59/356 (16.6)	9/43 (20.9)	0.47	50/308 (16.2)	18/91 (19.8)	0.43
Cancer	26/399 (7)	22/356 (6.2)	4/45 (9.3)	0.51	17/308 (5.5)	9/91 (9.9)	0.14
Kidney transplant	21/399 (5)	16/364 (4.4)	5/45 (11.1)	0.07	15/315 (4.8)	6/94 (6.4)	0.59
Immunosuppression, *n/N* (%)							
Any type	23/409 (6)	15/364 (4.1)	8/45 (17.8)	**<0.01**	11/308 (3.6)	12/91 (13.2)	**<0.0001**
Prednisone	18/409 (4)	12/356 (3.4)	6/43 (14)	**0.01**	9/308 (2.9)	9/91 (9.9)	**<0.01**
Rituximab	1/409 (0.002)	1/356 (0.3)	0/43 (0)	1.0	1/308 (0.3)	0/91 (0)	1.0
Chemotherapy	4/409 (1)	2/356 (0.56)	2/43 (4.65)	0.06	1/308 (0.3)	3/91 (3.3)	**0.04**
CNI	7/409 (2)	3/356 (1.8)	4/43 (9.3)	**<0.01**	2/308 (0.7)	5/91 (5.5)	**<0.01**
ESRD etiology, *n/N* (%)							
Unknown	57/409 (14)	47/364 (12.9)	10/45 (22.2)	0.41	43/315(13.7)	14/94 (14.9)	0.72
DM	177/409 (43)	159/364 (43.7)	18/45 (40)		132/315 (41.9)	45/94 (47.9)	
HTN	62/409 (15)	54/364 (14.8)	8/45 (17.8)		47/315 (14.9)	15/94 (16)	
ADPKD	21/409 (5)	21/364 (5.8)	0/45 (0)		18/315 (5.7)	3/94 (3.2)	
Ischaemic	6/409 (1.5)	6/364 (1.65)	0/45 (0)		6/315 (1.9)	0/94 (0)	
Glomerulonephritis	38/409 (9)	33/38 (9.1)	5/45 (11.1)		29/315 (9.2)	9/94 (9.6)	
Urologic	42/409 (10)	38/364 (10.4)	4/45 (8.9)		35/315 (11.1)	7/94 (7.5)	
Other	6/409 (1.5)	6/364 (1.5)	0/45 (0)		5/315 (1.6)	1/94 (1.1)	
Residual urine output, *n/N* (%)	215/342 (63)	194/306 (63.4)	21/36 (58.3)	0.55	170/260 (65.4)	45/82 (54.9)	0.09
Age (years), median (IQR)	72 (63–80)	71.4 (62–79.9)	76.6 (68.3–82.5)	**0.02**	70.6 (61.1–79.3)	75.2 (68.3–82.3)	**<0.001**
Dialysis vintage (years), median (IQR)	2.7 (1.2–5.2)	2.6 (1.2–5)	3.1 (1.4–6.3)	0.59	2.5 (1.2–4.8)	3.2 (0.96–5.7)	0.81
Kt/V_{urea}, median (IQR)	1.4 (1.2–1.6)	1.4 (1.2–1.6)	1.4 (1.2–1.6)	1.0	1.4 (1.2–1.6)	1.4 (0.96–5.7)	0.94
Anti-HBsAb titer (AU/mL), median (IQR)	6 (0–99)	7.6 (0–110)	2 (0–42)	0.14	11 (0–112)	2(0–36)	**0.04**
Albumin (mg/L), median (IQR)	3.9 (3.7–4.1)	3.9 (3.7–4.1)	3.7 (3.3–3.9)	**<0.001**	3.9 (3.7–4.1)	3.8 (3.5–4.1)	**0.04**
Ferritin (μg/L), median (IQR)	592 (386–885)	605 (399–900)	478 (321–746)	0.12	614 (403–876)	480 (321–966)	0.1
Haemoglobin (g/dL), median (IQR)	11.2(10.4–11.9)	11.2 (10.5–11.9)	10.7 (9.8–11.8)	**0.02**	11.2 (10.5–11.9)	11.1 (10–12)	0.54
WBC ($10^3/\mu$L), median (IQR)	6.7 (5.6–8.1)	6.7 (5.6–8.1)	6.5 (5.2–8.7)	0.96	6.7 (5.7–8.2)	6.7 (5.1–8)	0.26
Absolute lymphocyte count ($10^3/\mu$L), median (IQR)	1.3 (9.3–1.8)	1.3 (1–1.8)	1.0 (0.7–1.4)	**<0.01**	1.3 (1–1.8)	1.1 (0.7–1.5)	**<0.001**
Time to sample (days), median (IQR)	82 (15–120)	81 (64–87)	84 (68.5–90)	0.06	81 (62–87)	83 (71.8–90)	**0.01**

HTN, hypertension; DM, diabetes mellitus; CHF, congestive heart failure; ADPKD, autosomal dominant polycystic renal disease; Ab, antibody.
Values in bold are statisticall significant.

Table 3. Multivariate regression analysis for seronegativity of anti-S and neutralizing antibodies among naïve MHD patients: results of regression analysis displaying odds ratios (ORs) for being seronegative

	Anti-S seronegativity		Neutralizing Ab seronegativity	
Variable	OR (95% CI)	P-value	OR (95% CI)	P-value
Immunosuppression	7.8 (2.4–25.4)	**0.0001**	6.6 (2.1–21.1)	**0.001**
Age (years)	1.03 (1–1.07)	0.06	1.04 (1.01–1.07)	**0.004**
Albumin (mg/L)	0.24 (0.09–0.63)	**0.003**	0.55 (0.26–1.16)	0.11
Hemoglobin level (mg/dL)	0.91 (0.68–1.21)	0.5	Not included	
Absolute lymphocyte count ($10^3/\mu$L)	0.57 (0.3–1.06)	0.08	0.58 (0.36–0.92)	**0.02**
Time to sample (days)	1.2 (0.99–1.43)	0.06	1.2 (1.02–1.32)	**0.02**
Anti-HBsAb (AU/mL)	Not included		0.86 (0.75–0.99)	**0.03**

Values in bold are statistically significant.

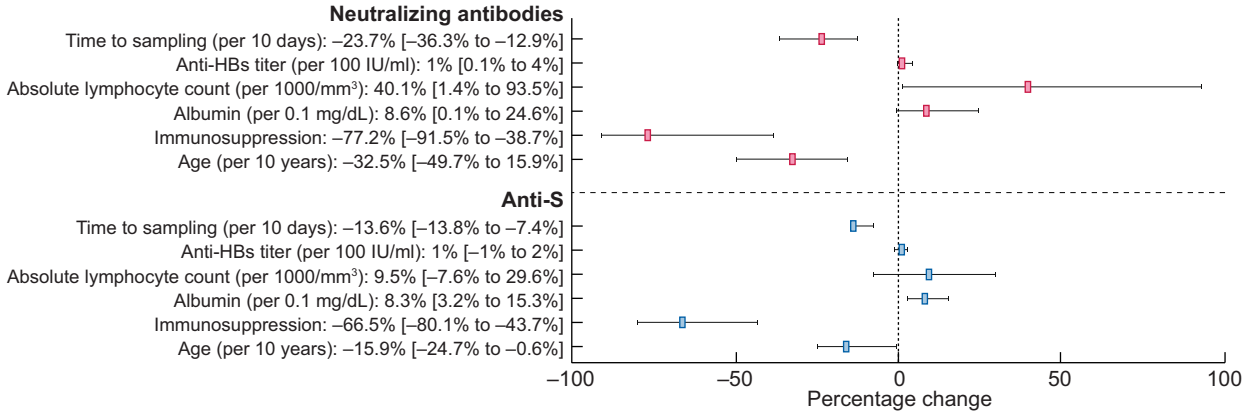


FIGURE 4: Multivariate regression analysis of various factors associated with anti-S (blue) and neutralizing antibody (red) titers in MHD patients. Associations are presented as the percent change (boxes) and 95% CI (whiskers).

HD patients are routinely vaccinated against Hepatitis B, but some of them do not develop antibodies or their titers decrease over time [29, 30]. Hypothesizing that in a given patient the response to one vaccine would be similar to their response to other viral vaccines, we compared between the humoral response to hepatitis B vaccine and the response to the mRNA-based vaccine BNT162b2. In univariate analysis, anti-HBsAb titers were only weakly associated with SARS-CoV-2 serologic response ($r_s = 0.18$ and $r_s = 0.16$ for anti-S and neutralizing antibodies, respectively). A multivariate analysis demonstrated an extremely modest effect (1% per 100 IU/mL) of anti HBsAb titers on both anti-S and neutralizing antibodies titers. These results are contradictory to the study published by Danthu *et al.* [31], which demonstrated that non-responders to hepatitis B vaccine had the lowest anti-SARS-CoV-2 antibody titers. Nonetheless, the cohort in that study was much smaller, composed of 78 MHD patients and 74 kidney transplant recipients. The missing information regarding the timing of hepatitis B vaccination in most of our patients could possibly explain the weak correlation, although it was also not documented in the aforementioned study [31]. The association between anti-HBsAb and SARS-CoV-2 post-vaccination antibodies in HD patients deserves further investigation.

MHD patients who were previously infected with SARS-CoV-2 as indicated by positive anti-N had no advantage in mounting humoral responses for both anti-S and neutralizing antibodies. These results are contradictory to a study by Saadat *et al.* [32] that demonstrated a much higher neutralizing antibody titer in subjects previously infected with SARS-CoV-2 compared with naïve subjects. Nevertheless, the subjects who were studied were young healthcare workers, who are considered to be a healthy population. Lacson *et al.* [33] demonstrated a 100% seropositivity of anti-S antibodies post-vaccination in 38 previously infected MHD patients, although the timing of the serologic test was not indicated. Chan *et al.* [34] demonstrated an earlier increase in antibodies as well as higher antibody titers post-vaccination with mRNA-1273 vaccine (Moderna) in previously SARS-CoV-2-infected MHD patients compared with infection-naïve patients, although the last serologic test was obtained only 2 weeks after the second vaccine dose.

The strengths of this study are the size and diversity of our study group, with >400 MHD patients from different locations all over the country treated in both community and hospital-based dialysis units, the comprehensive clinical and laboratory data collected and the longer time frame between a second vaccine dose and the timing of serology testing. Furthermore, the serologic assessment included not only the "classic" anti-S antibodies, but also neutralizing antibody titer levels, which are a better indicator for protection and have not been well studied in dialysis patients thus far. Another important strength is the measurement of anti-N antibodies, which enabled us to identify patients with previous COVID-19, which could potentially bias the results. A separate analysis of previously infected anti-N-positive patients gave us further insights into the reduced humoral immune response in MHD patients.

Some limitations also exist in this study. First, although the Israeli population is composed of varied immigrant origins, most participants were Caucasian Jews and we therefore may not be able to generalize our findings to different races and ethnic groups. Second, the median age was significantly lower in the control group since most of it was composed of healthcare workers. However, given our large cohort size, we were able to adjust for the effect of age differences between groups. Lastly, ideally, in order to obtain a more comprehensive image of immune response to SARS-CoV-2, the T-cell response component should also have been evaluated. Cellular immunity has been shown to play an important role in protecting against SARS-CoV-2 infection, regardless of antibody titer levels [35]. Only limited data are available regarding early cellular response [36], showing diminished cellular response in MHD patients 10–14 days following the second BNT162b2 vaccine. Unfortunately we were unable to evaluate this component of the immune response given the size of our cohort and the complexity and cost of cellular immunity testing.

In conclusion, our study demonstrates that MHD dependence confers a significant risk for decreased humoral response following vaccination for SARS-CoV-2. This diminished response is most evident in those MHD patients who are older, suffer from poor nutritional status and are treated with immunosuppressive medications. Furthermore, humoral responses show significant waning 2–3 months after vaccination in MHD patients.

Given these results, and in light of decreased vaccine effectiveness against emerging variants of concern [37, 38], MHD patients should be considered less protected by COVID-19 vaccines. This should indicate the need for continued social distancing precautions. MHD patients are likely to benefit from

Table 4. Comparison of humoral response in MHD patients according to anti-N status

Status	Anti-S positive, *n*/*N* (%)	P-value	Neutralizing Ab negative, *n*/*N* (%)	P-value	Anti-S titer level, median (IQR)	P-value	Neutralizing Ab titer level, GMT (95% CI)	P-value
Anti-N positive ($n = 24$)	20/24 (83)	0.33	19/24 (78)	>0.99	101.8 (35.6–193.8)	0.2	57.5 (16–206.1)	0.09
Anti-N negative ($n = 409$)	364/409 (89)		315/409 (77)		69.6 (33.2–120)		23.3 (18.7–29.1)	

Ab, antibody.

Table 5. Summary of published data on humoral response after BNT162b2 vaccination in MHD patients

Study	Number of participants	MHD patients' age (years) median (range) or mean ± SD	Time from vaccination to sampling (days), median (range)	Anti-S seroconversion, %	Neutralizing antibody seroconversion, %
Grouper *et al.* [22]	HD 56 Controls 95	74 ± 11	30 (27–34)	96	n/a
Yanay *et al.* [21]	HD 127 Controls 132	69 (62–78)	21–35	90	n/a
Agur *et al.* [20]	HD 122	72 ± 12	36 (32–40)	93	n/a
Speer *et al.* [28]	HD 22 Controls 48	74	18–22	77	82
Simon *et al.*	HD 81 Controls 80	67 (34–86)	21	80	n/a
Frantzen *et al.*	HD 244	76 ± 13	30	91	n/a
Lacson *et al.*	HD 186	68 ± 12	23 (15–31)	89	n/a
Attias *et al.*	HD 64	70 ± 12	21	86	n/a
Our cohort Angel-Korman *et al.*	HD 409 Controls 148	72 (26–97)	82 (18–99)	89	77

n/a, not applicable.

a third vaccine dose. This strategy for individuals >60 years of age was implemented in Israel starting 1 August 2021.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

A.A.K., A.L. and T.B.S. conceptualized the study and wrote the manuscript. A.A.K., E.P. and V.R. curated the data. A.L., Y.Y., N.L.I., Z.K. and T.B.S. supervised the study. G.B., Y.L., V.I. and S.A. contributed the resources. T.B.S. was in charge of data analysis. All authors edited and reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The results presented in this article have not been published previously in whole or part, except in abstract format.

REFERENCES

1. Polack FP, Thomas SJ, Kitchin N *et al.* Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. *N Engl J Med* 2020; 383: 2603–2615
2. Angel Y, Spitzer A, Henig O *et al.* Association between vaccination with BNT162b2 and incidence of symptomatic and asymptomatic SARS-CoV-2 infections among health care workers. *JAMA* 2021; 325: 2457–2465
3. Dagan N, Barda N, Kepten E *et al.* BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. *N Engl J Med* 2021; 384: 1412–1423
4. Amit S, Regev-Yochay G, Afek A *et al.* Early rate reductions of SARS-CoV-2 infection and COVID-19 in BNT162b2 vaccine recipients. *Lancet* 2021; 397: 875–877
5. Haas EJ, Angulo FJ, McLaughlin JM *et al.* Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths

following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet* 2021; 397: 1819–1829
6. Barda N, Dagan N, Balicer RD. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. Reply. *N Engl J Med* 2021; 384:1970
7. Brosh-Nissimov T, Orenbuch-Harroch E, Chowers M *et al*. BNT162b2 vaccine breakthrough: clinical characteristics of 152 fully-vaccinated hospitalized COVID-19 patients in Israel. *Clin Microbiol Infect* 2021; doi: 10.1016/j.cmi.2021.06.036
8. Grupper A, Katchman H. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus: not alarming, but should be taken gravely. *Am J Transplant* 2021; 21: 2909
9. Peled Y, Ram E, Lavee J *et al*. BNT162b2 vaccination in heart transplant recipients: clinical experience and antibody response. *J Heart Lung Transplant* 2021; 40: 759–762
10. Rabinowich L, Grupper A, Baruch R *et al*. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol* 2021; 75: 435–438
11. Herishanu Y, Avivi I, Aharon A *et al*. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* 2021; 137: 3165–3173
12. Boyarsky BJ, Ruddy JA, Connolly CM *et al*. Antibody response to a single dose of SARS-CoV-2 mRNA vaccine in patients with rheumatic and musculoskeletal diseases. *Ann Rheum Dis* 2021; 80:1098–1099
13. Ray K. Antibody responses to SARS-CoV-2 infection are attenuated in infliximab-treated patients with IBD. *Nat Rev Gastroenterol Hepatol* 2021; 18: 286
14. Kato S, Chmielewski M, Honda H *et al*. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol* 2008; 3: 1526–1533
15. Krueger KM, Ison MG, Ghossein C. Practical guide to vaccination in all stages of CKD, including patients treated by dialysis or kidney transplantation. *Am J Kidney Dis* 2020; 75: 417–425
16. Crosnier J, Jungers P, Courouce AM *et al*. Randomised placebo-controlled trial of hepatitis b surface antigen vaccine in French haemodialysis units: II, haemodialysis patients. *Lancet* 1981; 317: 797–800
17. Gilbertson DT, Guo H, Arneson TJ *et al*. The association of pneumococcal vaccination with hospitalization and mortality in hemodialysis patients. *Nephrol Dial Transplant* 2011; 26: 2934–2939
18. Khoury DS, Cromer D, Reynaldi A *et al*. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; 27: 1205–1211
19. Bergwerk M, Gonen T, Lustig Y *et al*. Covid-19 breakthrough infections in vaccinated health care workers. *N Engl J Med* 2021; 385: 1474–1484
20. Agur T, Ben-Dor N, Goldman S *et al*. Antibody response to mRNA SARS-CoV-2 vaccine among dialysis patients—a prospective cohort study. *Nephrol Dial Transplant* 2021; 36: 1347–1349
21. Yanay NB, Freiman S, Shapira M *et al*. Experience with SARS-CoV-2 BNT162b2 mRNA vaccine in dialysis patients. *Kidney Int* 2021; 99: 1496–1498
22. Grupper A, Sharon N, Finn T *et al*. Humoral response to the Pfizer BNT162b2 vaccine in patients undergoing maintenance hemodialysis. *Clin J Am Soc Nephrol* 2021; 16: 1037–1042
23. Broseta JJ, Rodriguez-Espinosa D, Rodriguez N *et al*. Humoral and cellular responses to mRNA-1273 and BNT162b2 SARS-CoV-2 vaccines administered to hemodialysis patients. *Am J Kidney Dis* 2021; 78: 571–581
24. Valdivia A, Torres I, Latorre V *et al*. Inference of SARS-CoV-2 spike-binding neutralizing antibody titers in sera from hospitalized COVID-19 patients by using commercial enzyme and chemiluminescent immunoassays. *Eur J Clin Microbiol Infect Dis* 2021; 40: 485–494
25. Lustig Y, Sapir E, Regev-Yochay G *et al*. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir Med* 2021; 9: 999–1009
26. Mizrahi B LR, Kalkstein N, Peretz A *et al*. Correlation of SARS-CoV-2 breakthrough infections to time-from-vaccine; preliminary study. *medRxiv* 2021; doi: 10.1101/2021.07.29.21261317
27. Torreggiani M, Blanchi S, Fois A *et al*. Neutralizing SARS-CoV-2 antibody response in dialysis patients after the first dose of the BNT162b2 mRNA COVID-19 vaccine: the war is far from being won. *Kidney Int* 2021; 99: 1494–1496
28. Speer C, Goth D, Benning L *et al*. Early humoral responses of hemodialysis patients after COVID-19 vaccination with BNT162b2. *Clin J Am Soc Nephrol* 2021; 16: 1073–1082
29. DaRoza G, Loewen A, Djurdjev O *et al*. Stage of chronic kidney disease predicts seroconversion after hepatitis B immunization: earlier is better. *Am J Kidney Dis* 2003; 42: 1184–1192
30. Burdick RA, Bragg-Gresham JL, Woods JD *et al*. Patterns of hepatitis B prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2003; 63: 2222–2229
31. Danthu C, Hantz S, Dahlem A *et al*. Humoral response after SARS-Cov-2 mRNA vaccine in a cohort of hemodialysis patients and kidney transplant recipients. *J Am Soc Nephrol* 2021; 32: 2153–2158
32. Saadat S, Rikhtegaran Tehrani Z, Logue J *et al*. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. *JAMA* 2021; 325: 1467–1469
33. Lacson E, Argyropoulos CP, Manley HJ *et al*. Immunogenicity of SARS-CoV-2 vaccine in dialysis. *J Am Soc Nephrol* 2021; 32: 2735–2742
34. Chan L, Fuca N, Zeldis E *et al*. Antibody response to mRNA-1273 SARS-CoV-2 vaccine in hemodialysis patients with and without prior COVID-19. *Clin J Am Soc Nephrol* 2021; 16: 1258–1260
35. David Wyllie RM, Jones HE, Taylor-Phillips S *et al*. SARS-CoV-2 responsive t cell numbers are associated with protection from COVID-19: a prospective cohort study in keyworkers. *medRxiv*. 2020; doi: 10.1101/2020.11.02.20222778
36. Espi M, Charmetant X, Barba T *et al*. The ROMANOV study found impaired humoral and cellular immune responses to SARSCov-2 mRNA vaccine in virus unexposed patients receiving maintenance hemodialysis. *Kidney Int* 2021; 100: 928–936
37. Wall EC, Wu M, Harvey R *et al*. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. *Lancet* 2021; 397: 2331–2333
38. Lopez Bernal J, Andrews N, Gower C *et al*. Effectiveness of Covid-19 vaccines against the B.1.617.2 (Delta) variant. *N Engl J Med* 2021; 385: 585–594