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ORIGINAL RESEARCH

Anti-SARS-CoV-2 Spike Antibody Response to the Fourth Dose of BNT162b2 mRNA COVID-19 Vaccine and Associated Factors in Japanese Hemodialysis Patients

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Background: We assessed the anti-SARS-CoV-2 spike antibody response to four doses of BNT162b2 mRNA COVID-19 vaccine in Japanese hemodialysis patients and determined factors associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose.
Methods: Fifty-one patients were enrolled in this single-center, prospective, longitudinal study. Change in anti-SARS-CoV-2 spike antibody titers between after the second and fourth doses were evaluated. Multiple linear regression analysis was used to identify factors associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose.
Results: The anti-SARS-CoV-2 spike antibody titer was higher 4 weeks after the fourth dose compared with 4 weeks after the third dose (30,000 [interquartile range (IQR), 14,000–56,000] vs 18,000 [IQR, 11,000–32,500] AU/mL, $p<0.001$) and 4 weeks after the second dose (vs 2896 [IQR, 1110–4358] AU/mL, $p<0.001$). Hypoxia-inducible factor prolyl hydroxylase inhibitor use (standard coefficient [β]=0.217, p=0.011), and the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (β=0.810, $p<0.001$) were correlated with the log-anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose, whereas only the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (β=0.677, $p<0.001$) was correlated with the log-anti-SARS-CoV-2 spike antibody titer 12 weeks after the fourth dose.
Conclusion: Hypoxia-inducible factor prolyl hydroxylase inhibitor use and the anti-SARS-CoV-2 spike antibody titer before the fourth dose were associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose in Japanese hemodialysis patients.
Keywords: coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2, anti-severe acute respiratory syndrome coronavirus 2 spike antibody, BNT162b2 messenger RNA vaccine, hemodialysis

Introduction

Patients undergoing hemodialysis are one of the most vulnerable populations at risk for severe and fatal coronavirus disease 2019 (COVID-19).[1] COVID-19 vaccination was reported to reduce the risk of hospitalization and mortality associated with COVID-19 in hemodialysis patients,[2] and its preventable effects were associated with the anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antibody titer after COVID-19 vaccination.[3] Lower anti-SARS-CoV-2 spike antibody titer was shown to be associated with higher risk of hospital admission due to COVID-19 in hemodialysis patients.[4] Therefore, maintaining an adequate anti-SARS-CoV-2 spike antibody titer is important to prevent severe COVID-19 and COVID-19-related mortality in patients undergoing hemodialysis.

Several dialysis-specific factors including uremic toxins, oxidative stress, and mineral bone disorders are considered as causes of impaired humoral immune response to vaccination in hemodialysis patients.[5] Hemoglobin level, blood urea nitrogen concentration, uric acid concentration, and transferrin saturation were reported to be associated with the anti-SARS-CoV-2 spike antibody response after COVID-19 vaccination in patients undergoing hemodialysis.[6,7] Recently,

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several studies have investigated the anti-SARS-CoV-2 spike antibody response to the fourth dose of BNT162b2 messenger RNA (mRNA) COVID-19 vaccine in patients undergoing hemodialysis.[8–12] One of these studies revealed that immunosuppressive medication use for organ transplantation, cancer, and rheumatologic disease was associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose of COVID-19 vaccine in individuals undergoing hemodialysis.[8] However, factors associated with the anti-SARS-CoV-2 spike antibody response to the fourth dose have not yet been investigated in Asian patients undergoing hemodialysis. Therefore, in the present study, we determined which clinical factors were associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose of COVID-19 vaccine in Japanese patients undergoing hemodialysis. We also assessed change in the anti-SARS-CoV-2 spike antibody titers between after the second and fourth doses of COVID-19 vaccine in these patients.

Patients and Methods

Ethical Approval

The Ethical Committee of Mizue Yuai Clinic approved the study (MYC 2021–01), which was performed in accordance with the Declaration of Helsinki. Written informed consent was gained from all study participants.

Patients

The study's inclusion criteria were: (i) age ≥20 years, (ii) chronic kidney disease stage 5D, (iii) currently receiving hemodialysis, and (iv) vaccinated with three doses of BNT162b2 mRNA COVID-19 vaccine (Pfizer Inc., and BioNTech) with an interval of 3 weeks between the first and second doses and 24 weeks between the second and third doses; and scheduled to receive the fourth dose with an interval of 24 weeks between the third and fourth doses. The following exclusion criteria were applied: (i) unable or unwilling to give consent and (ii) any history of COVID-19 infection.

Study Design

This study was a single-center, prospective, longitudinal study conducted between April 1, 2021 and December 31, 2022 at the Mizue Yuai Clinic in Tokyo. Figure 1 illustrates the Study flow chart. Each participant's anti-SARS-CoV-2 spike antibody titer was measured 4 weeks after the second dose, 1 week before the third dose, 4 weeks after the third dose, 12 weeks after the third dose, 1 week before the fourth dose, 4 weeks after the fourth dose, and 12 weeks after the fourth dose of the BNT162b2 mRNA COVID-19 vaccine. Clinical and demographic parameters were collected during the week when the fourth dose of vaccine was administered. We assessed the change in anti-SARS-CoV-2 spike antibody titers between 4 weeks after the second dose and 12 weeks after the fourth dose of vaccine in hemodialysis patients. Multiple linear regression analysis was used to identify factors associated with the anti-SARS-CoV-2 spike antibody titers 4 and 12 weeks after the fourth dose in these patients.

Laboratory Methods

Patients' blood samples were collected from an arteriovenous fistula immediately before the start of their first hemodialysis session of the week. A commercial laboratory (BML, Tokyo, Japan) measured the patients' anti-SARS-CoV-2 spike antibody titers and blood parameters. The SARS-CoV-2 IgG II Quant immunoassay (Abbott, Sligo, Ireland) was used to determine anti-SARS-CoV-2 spike antibody titers. Shinzato's formula[13] was used to calculate the single-pool urea clearance (Kt/V) and normalized protein catabolism rate.

Statistical Analyses

Data of continuous variables are shown as the mean ± standard deviation when they were normally distributed. Data of not normally distributed continuous variables are shown as the median [interquartile range (IQR)]. Data of categorical variables are presented as numbers and percentages. The hemodialysis vintage, C-reactive protein, and anti-SARS-CoV-2 spike antibody titer did not show normal distributions; therefore, these variables were transformed using the natural logarithm. Friedman test accompanied by Bonferroni test was conducted to compare the anti-SARS-CoV-2 spike antibody titers. To identify variables that were independently correlated with the anti-SARS-CoV-2 spike antibody titers 4 and 12 weeks after

the fourth dose, multiple linear regression analysis included parameters that were significantly correlated with the anti-SARS-CoV-2 spike antibody titers 4 and 12 weeks after the fourth dose in simple linear regression analyses. Probability (p)-values < 0.05 were accepted as significant. All statistical analyses were conducted using JMP ver. 11 (SAS, Cary, NC, USA).

Results

Patient Characteristics

Seventy-five hemodialysis patients were enrolled in this study. During the study period 16 patients developed COVID-19 infection and 2 patients changed to another hospital. Blood samples were not available for 6 patients. Therefore, 51 hemodialysis patients were included in the analysis (Figure 1). Table 1 summarizes patient characteristics and medications. There were 31 males and 20 females with a mean age of 70.7 ± 12.5 years and mean hemodialysis vintage of 4.0 [IQR, 2.0–8.0] years. Ten patients (19.6%) had a history of past or current smoking, and 11 patients (21.6%) had a habit of alcohol consumption. The proportions of patients with hypertension, diabetes mellitus, autoimmune diseases, and allergic diseases were 62.7%, 43.1%, 11.8%, and 29.4%, respectively. The percentages of patients with a history of infection were as follows: hepatitis B virus, 15.7%; hepatitis C virus, 3.9%; and syphilis, 5.9%. Medication use was as follows: renin-angiotensin system inhibitors, 33.3%; statins, 31.4%; erythropoiesis-stimulating agents, 86.3%; hypoxia-inducible factor prolyl hydroxylase inhibitors, 7.8%; iron supplements, 56.9%; zinc supplements, 7.8%; phosphate binders, 80.4%; vitamin D analogs, 78.4%; calcimimetics, 37.3%; and corticosteroids, 5.9%.

Change in the Anti-SARS-CoV-2 Spike Antibody Titers in Hemodialysis Patients

As shown in Figure 2, the anti-SARS-CoV-2 spike antibody titer significantly decreased from 2896 [IQR, 1110–4358] AU/mL 4 weeks after the second dose to 220 [IQR, 127–411] AU/mL 1 week before the third dose (p<0.001). After the

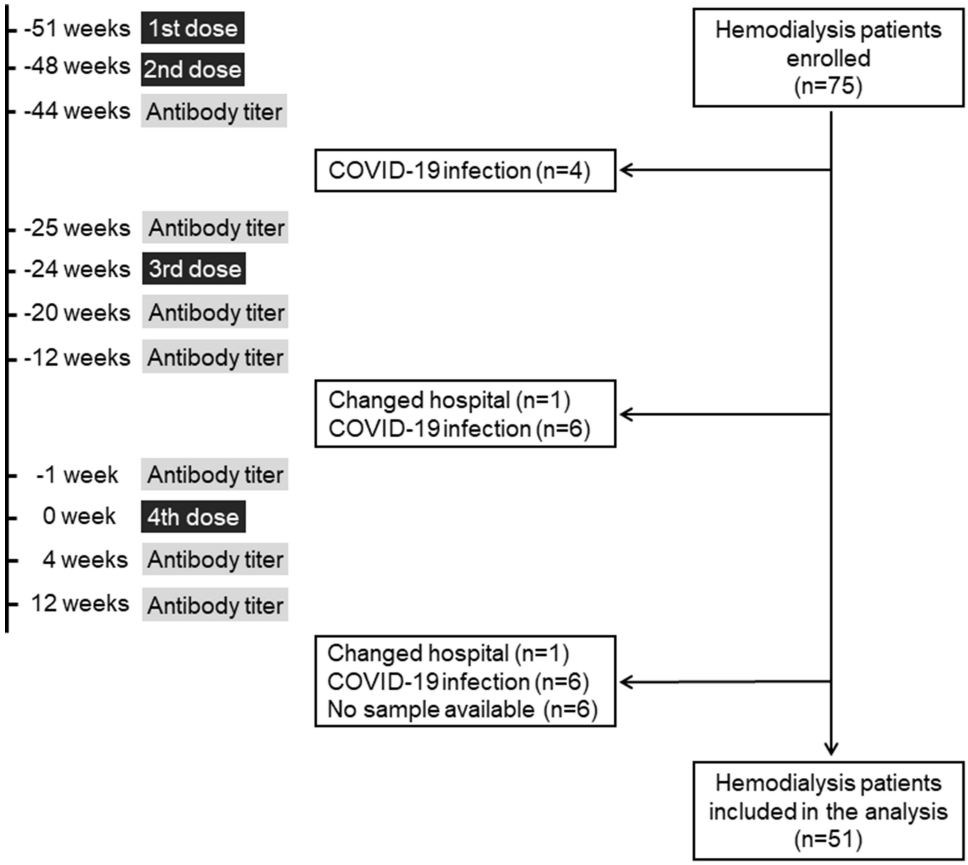

Figure 1 Study flow chart.
Abbreviation: COVID-19, coronavirus disease 2019.

Table 1 Demographic and Clinical Characteristics

	Hemodialysis Patients (n=51)
Age (year)	70.7 ± 12.5
Male sex (number, %)	31 (60.8%)
Body mass index (kg/m^2)	22.6 ± 4.2
Hemodialysis vintage (year)	4.0 [2.0–8.0]
Past or current smoking (number, %)	10 (19.6%)
Alcohol drinking (number, %)	11 (21.6%)
Hypertension (number, %)	32 (62.7%)
Diabetes mellitus (number, %)	22 (43.1%)
Autoimmune disease (number, %)	6 (11.8%)
Allergic disease (number, %)	15 (29.4%)
Previous hepatitis B virus infection (number, %)	8 (15.7%)
Previous hepatitis C virus infection (number, %)	2 (3.9%)
Previous syphilis infection (number, %)	3 (5.9%)
Renin–angiotensin system inhibitor (number, %)	17 (33.3%)
Statin (number, %)	16 (31.4%)
Erythropoiesis-stimulating agent (number, %)	44 (86.3%)
Hypoxia-inducible factor prolyl hydroxylase inhibitor (number, %)	4 (7.8%)
Iron supplement (number, %)	29 (56.9%)
Zinc supplement (number, %)	4 (7.8%)
Phosphate binder (number, %)	41 (80.4%)
Vitamin D analog (number, %)	40 (78.4%)
Calcimimetic (number, %)	19 (37.3%)
Corticosteroid (number, %)	3 (5.9%)
Albumin (g/dL)	3.5 ± 0.4
White blood cell count (/μL)	6066 ± 1670
Lymphocyte count (/μL)	1145 ± 378
Hemoglobin (g/dL)	10.7 ± 1.2
Platelet count (×10^4/μL)	18.7 ± 6.1
Blood urea nitrogen (mg/dL)	57.2 ± 14.0
Creatinine (mg/dL)	10.2 ± 2.9
Sodium (mEq/L)	139.2 ± 3.2
Potassium (mEq/L)	4.6 ± 0.6
Chloride (mEq/L)	101.4 ± 3.5

(*Continued*)

Table 1 (Continued).

	Hemodialysis Patients (n=51)
Total calcium (mg/dL)	8.3 ± 0.5
Phosphate (mg/dL)	4.9 ± 1.1
Magnesium (mg/dL)	2.5 ± 0.3
Uric acid (mg/dL)	6.8 ± 1.1
Total cholesterol (mg/dL)	152.5 ± 39.9
C-reactive protein (mg/dL)	0.27 [0.11–0.95]
Intact-parathyroid hormone (pg/mL)	147.8 ± 57.3
β2 microglobulin (mg/L)	28.6 ± 6.3
Ferritin (ng/mL)	256.8 ± 207.7
Transferrin saturation (%)	28.5 ± 21.4
Zinc (μg/dL)	58.8 ± 12.4
Glycated hemoglobin (%)	5.8 ± 0.9
Glycoalbumin (%)	19.5 ± 3.9
Normalized protein catabolism rate (g/kg/day)	0.73 ± 0.20
Single pool Kt/V	1.50 ± 0.27

Abbreviation: Kt/V, urea clearance.

third dose, the titer significantly increased to 18,000 [IQR, 11,000–32,500] AU/mL 4 weeks after the third dose ($p<0.001$), then significantly decreased to 5903 [IQR, 2469–15,000] AU/mL 1 week before the fourth dose ($p<0.001$). After the fourth dose, the titer significantly increased to 30,000 [IQR, 14,000–56,000] AU/mL 4 weeks after the fourth

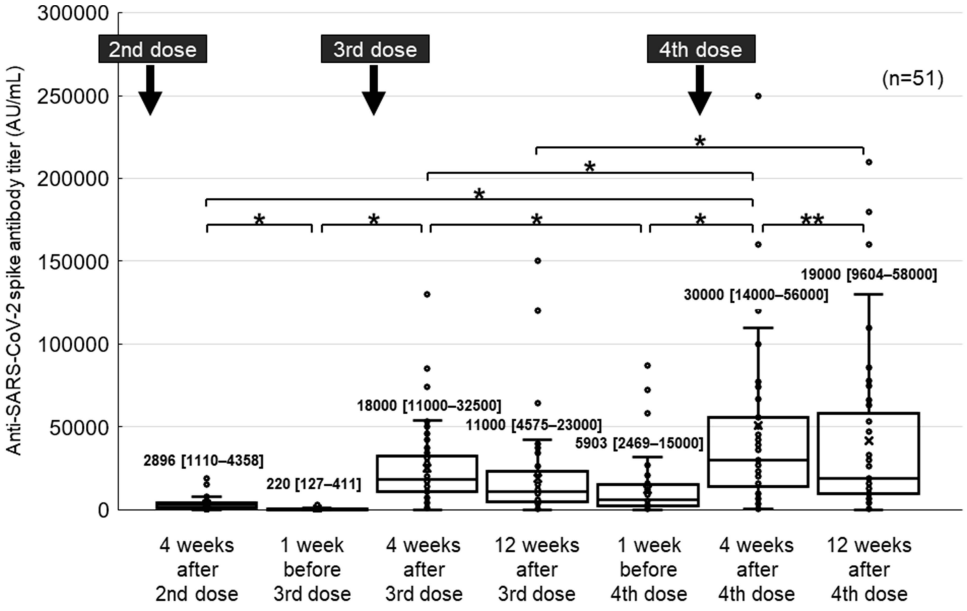

Figure 2 Change in the median anti-SARS-CoV-2 spike antibody titers in hemodialysis patients (n = 51). Values are presented as medians [interquartile ranges].
Notes: *p < 0.001; **p < 0.01.
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

dose (p<0.001), then significantly decreased to 19,000 [IQR, 9604–58,000] AU/mL 12 weeks after the fourth dose (p<0.01). The anti-SARS-CoV-2 spike antibody titer was significantly higher 4 weeks after the fourth dose compared with 4 weeks after the third dose (30,000 [IQR, 14,000–56,000] vs 18,000 [IQR, 11,000–32,500] AU/mL, p<0.001) and 4 weeks after the second dose (30,000 [IQR, 14,000–56,000] vs 2896 [IQR, 1110–4358] AU/mL, p<0.001). The anti-SARS -CoV-2 spike antibody titer was significantly higher 12 weeks after the fourth dose than 12 weeks after the third dose (19,000 [IQR, 9604–58,000] vs 11,000 [IQR, 4575–23,000] AU/mL, p<0.001).

Figure 3 shows the change in the mean anti-SARS-CoV-2 spike antibody titers. The anti-SARS-CoV-2 spike antibody titer significantly decreased from 3434 ± 3498 AU/mL 4 weeks after the second dose to 362 ± 465 AU/mL 1 week before the third dose (p<0.001). After the third dose, the titer significantly increased to 24,924 ± 23,501 AU/mL 4 weeks after the third dose (p<0.001), then significantly decreased to 12,099 ± 17,283 AU/mL 1 week before the fourth dose (p<0.001). After the fourth dose, the titer significantly increased to 50,622 ± 61,987 AU/mL 4 weeks after the fourth dose (p<0.001), then remained unchanged 12 weeks after the fourth dose (50,622 ± 61,987 to 41,687 ± 50,054 AU/mL, p=1.00). The anti-SARS-CoV-2 spike antibody titer was significantly higher 4 weeks after the fourth dose compared with 4 weeks after the third dose (50,622 ± 61,987 vs 24,924 ± 23,501 AU/mL, p<0.005) and 4 weeks after the second dose (50,622 ± 61,987 vs 3434 ± 3498 AU/mL, p<0.001). The anti-SARS-CoV-2 spike antibody titer was significantly higher 12 weeks after the fourth dose than 12 weeks after the third dose (41,687 ± 50,054 vs 18,951 ± 27,287 AU/mL, p<0.005).

Among patients taking corticosteroids, the anti-SARS-CoV-2 spike antibody titer significantly increased from 8908 [IQR, 5196–17,954] AU/mL 1 week before the fourth dose to 29,000 [IQR, 17,197–39,500] AU/mL 4 weeks after the fourth dose (p<0.05), then tended to decrease to 18,000 [IQR, 10,282–32,500] AU/mL 12 weeks after the fourth dose (p=0.47).

Factors Associated with the Anti-SARS-CoV-2 Spike Antibody Titer After the Fourth Dose of BNT162b2 mRNA COVID-19 Vaccine

According to simple linear regression analyses, the log-anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose was significantly correlated with log-hemodialysis vintage, erythropoiesis-stimulating agent use, hypoxia-inducible factor prolyl hydroxylase inhibitor use, single pool Kt/V, and the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (Table 2), whereas the log-anti-SARS-CoV-2 spike antibody titer 12 weeks after the fourth dose

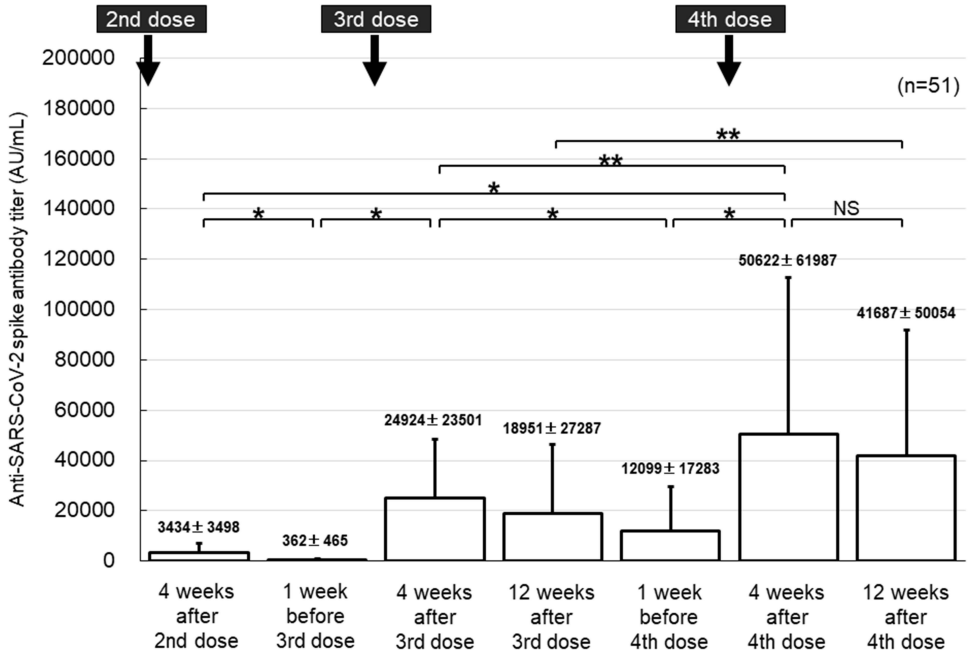

Figure 3 Change in the mean anti-SARS-CoV-2 spike antibody titers in hemodialysis patients (n = 51). Values are presented as means ± standard deviations.
Notes: *p < 0.001; **p < 0.005.
Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NS, not significant.

Table 2 Simple and Multiple Linear Regression Analyses of the Variables Correlated with Log-Anti-SARS-CoV-2 Spike Antibody Titer 4 Weeks After the Fourth BNT162b2 mRNA COVID-19 Vaccination

Variables	Simple Linear Regression Analysis		Multiple Linear Regression Analysis	
	Standard Coefficient	P value	Standard Coefficient	P value
Age (year)	−0.176	0.22		
Male sex (yes vs no)	0.249	0.08		
Body mass index (kg/m^2)	0.166	0.24		
Log-hemodialysis vintage (year)	−0.281	0.046	−0.025	0.74
Past or current smoking (yes vs no)	0.223	0.12		
Alcohol drinking (yes vs no)	0.127	0.37		
Diabetes mellitus (yes vs no)	−0.034	0.81		
Hypertension (yes vs no)	0.012	0.93		
Allergic disease (yes vs no)	0.123	0.39		
Autoimmune disease (yes vs no)	−0.145	0.31		
Previous hepatitis B virus infection (yes vs no)	0.013	0.93		
Previous hepatitis C virus infection (yes vs no)	0.116	0.42		
Previous syphilis infection (yes vs no)	−0.212	0.14		
Corticosteroid (yes vs no)	−0.048	0.74		
Renin–angiotensin system inhibitor (yes vs no)	0.081	0.57		
Statin (yes vs no)	0.069	0.64		
Erythropoiesis-stimulating agent (yes vs no)	−0.282	0.045	0.087	0.31
Hypoxia-inducible factor prolyl hydroxylase inhibitor (yes vs no)	0.306	0.029	0.217	0.011*
Iron supplement (yes vs no)	−0.075	0.61		
Zinc supplement (yes vs no)	−0.180	0.21		
Phosphate binder (yes vs no)	−0.106	0.46		
Vitamin D analog (yes vs no)	−0.070	0.63		
Calcimimetic (yes vs no)	0.030	0.83		
Albumin (g/dL)	0.002	0.99		
White blood cell count (/μL)	0.139	0.33		
Lymphocyte count (/μL)	0.078	0.59		
Hemoglobin (g/dL)	0.172	0.23		
Platelet count (×10^4/μL)	0.005	0.97		
Blood urea nitrogen (mg/dL)	−0.213	0.13		
Creatinine (mg/dL)	0.018	0.90		
Sodium (mEq/L)	−0.149	0.30		

(*Continued*)

Table 2 (Continued).

Variables	Simple Linear Regression Analysis		Multiple Linear Regression Analysis	
	Standard Coefficient	P value	Standard Coefficient	P value
Potassium (mEq/L)	−0.209	0.14		
Chloride (mEq/L)	0.020	0.89		
Total calcium (mg/dL)	−0.080	0.58		
Phosphate (mg/dL)	−0.042	0.77		
Magnesium (mg/dL)	−0.073	0.61		
Uric acid (mg/dL)	−0.148	0.30		
Total cholesterol (mg/dL)	−0.220	0.12		
Log-C-reactive protein (mg/dL)	0.131	0.36		
Intact-parathyroid hormone (pg/mL)	0.186	0.19		
β2 microglobulin (mg/L)	−0.035	0.81		
Ferritin (ng/mL)	−0.112	0.43		
Transferrin saturation (%)	−0.079	0.58		
Zinc (μg/dL)	0.002	0.99		
Glycated hemoglobin (%)	0.268	0.20		
Glycoalbumin (%)	0.125	0.55		
Normalized protein catabolism rate (g/kg/day)	−0.219	0.12		
Single pool Kt/V	−0.379	0.006	−0.102	0.18
Log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth BNT162b2 mRNA COVID-19 vaccination (AU/mL)	0.858	<0.001	0.810	<0.001*

Note: *p<0.05.
Abbreviations: COVID-19, coronavirus disease 2019; Kt/V, urea clearance; Log, logarithm; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

was significantly correlated with blood urea nitrogen, potassium, normalized protein catabolism rate, single pool Kt/V, and the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (Table 3). Multiple linear regression analyses were performed using variables that showed significant correlations with the log-anti-SARS-CoV-2 spike antibody titers 4 and 12 weeks after the fourth dose in simple linear regression analyses. These analyses revealed hypoxia-inducible factor prolyl hydroxylase inhibitor use (standard coefficient [β]=0.217, p=0.011) and the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (β=0.810, p<0.001) were independently correlated with the log-anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose (Table 2), whereas only the log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose (β=0.677, p<0.001) was independently correlated with the log-anti-SARS-CoV-2 spike antibody titer 12 weeks after the fourth dose (Table 3).

Comparison of Patients' Characteristics Between Hemodialysis Patients Not Infected with COVID-19 and Those Infected with COVID-19 After the Third or Fourth Dose

Table 4 shows patients' characteristics in hemodialysis patients not infected with COVID-19 and those infected with COVID-19 after the third or fourth dose. There was no significant difference in patients' characteristics between hemodialysis patients not infected with COVID-19 and those infected with COVID-19 after the third or fourth dose.

Table 3 Simple and Multiple Linear Regression Analyses of the Variables Correlated with Log-Anti-SARS-CoV-2 Spike Antibody Titer 12 Weeks After the Fourth BNT162b2 mRNA COVID-19 Vaccination

Variables	Simple Linear Regression Analysis		Multiple Linear Regression Analysis	
	Standard Coefficient	P value	Standard Coefficient	P value
Age (year)	−0.052	0.72		
Male sex (yes vs no)	0.250	0.08		
Body mass index (kg/m^2)	0.057	0.69		
Log-hemodialysis vintage (year)	−0.264	0.06		
Past or current smoking (yes vs no)	0.153	0.29		
Alcohol drinking (yes vs no)	0.121	0.40		
Diabetes mellitus (yes vs no)	0.147	0.31		
Hypertension (yes vs no)	0.071	0.62		
Allergic disease (yes vs no)	0.101	0.48		
Autoimmune disease (yes vs no)	−0.114	0.43		
Previous hepatitis B virus infection (yes vs no)	0.165	0.25		
Previous hepatitis C virus infection (yes vs no)	0.056	0.69		
Previous syphilis infection (yes vs no)	−0.254	0.07		
Corticosteroid (yes vs no)	−0.067	0.64		
Renin–angiotensin system inhibitor (yes vs no)	0.082	0.57		
Statin (yes vs no)	−0.021	0.89		
Erythropoiesis-stimulating agent (yes vs no)	−0.262	0.06		
Hypoxia-inducible factor prolyl hydroxylase inhibitor (yes vs no)	0.218	0.12		
Iron supplement (yes vs no)	0.038	0.79		
Zinc supplement (yes vs no)	−0.201	0.16		
Phosphate binder (yes vs no)	−0.091	0.53		
Vitamin D analog (yes vs no)	−0.077	0.59		
Calcimimetic (yes vs no)	−0.070	0.62		
Albumin (g/dL)	−0.041	0.77		
White blood cell count (/μL)	0.057	0.69		
Lymphocyte count (/μL)	0.008	0.95		
Hemoglobin (g/dL)	0.152	0.29		
Platelet count (×10^4/μL)	−0.047	0.75		
Blood urea nitrogen (mg/dL)	−0.339	0.015	−0.015	0.90
Creatinine (mg/dL)	−0.068	0.64		
Sodium (mEq/L)	−0.098	0.49		

(*Continued*)

Table 3 (Continued).

Variables	Simple Linear Regression Analysis		Multiple Linear Regression Analysis	
	Standard Coefficient	P value	Standard Coefficient	P value
Potassium (mEq/L)	−0.280	0.046	−0.071	0.52
Chloride (mEq/L)	0.089	0.54		
Total calcium (mg/dL)	−0.062	0.67		
Phosphate (mg/dL)	−0.166	0.24		
Magnesium (mg/dL)	0.008	0.96		
Uric acid (mg/dL)	−0.185	0.19		
Total cholesterol (mg/dL)	−0.146	0.31		
Log-C-reactive protein (mg/dL)	0.166	0.24		
Intact-parathyroid hormone (pg/mL)	0.112	0.44		
β2 microglobulin (mg/L)	−0.096	0.51		
Ferritin (ng/mL)	0.025	0.86		
Transferrin saturation (%)	−0.116	0.42		
Zinc (μg/dL)	−0.045	0.76		
Glycated hemoglobin (%)	0.210	0.31		
Glycoalbumin (%)	0.095	0.65		
Normalized protein catabolism rate (g/kg/day)	−0.282	0.045	−0.070	0.68
Single pool Kt/V	−0.423	0.002	−0.131	0.40
Log-anti-SARS-CoV-2 spike antibody titer 1 week before the fourth BNT162b2 mRNA COVID-19 vaccination (AU/mL)	0.752	<0.001	0.677	<0.001*

Note: *p<0.05.
Abbreviations: COVID-19, coronavirus disease 2019; Kt/V, urea clearance; Log, logarithm; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4 Comparison of Patients' Characteristics Between Hemodialysis Patients Not Infected with COVID-19 and Those Infected with COVID-19 After the Third or Fourth Dose

	Hemodialysis Patients not Infected with COVID-19 (n=51)	Hemodialysis Patients Infected with COVID-19 After the Third or Fourth Dose (n=12)	P value
Age (year)	70.7 ± 12.5	76.8 ± 14.4	0.14
Male sex (number, %)	31 (60.8%)	5 (41.7%)	0.33
Body mass index (kg/m^2)	22.6 ± 4.2	20.6 ± 3.4	0.15
Hemodialysis vintage (year)	4.0 [2.0–8.0]	3.0 [3.0–12.3]	0.57
Past or current smoking (number, %)	10 (19.6%)	2 (16.7%)	1.00
Alcohol drinking (number, %)	11 (21.6%)	1 (8.3%)	0.43

(*Continued*)

Table 4 (Continued).

	Hemodialysis Patients not Infected with COVID-19 (n=51)	Hemodialysis Patients Infected with COVID-19 After the Third or Fourth Dose (n=12)	P value
Hypertension (number, %)	32 (62.7%)	10 (83.3%)	0.31
Diabetes mellitus (number, %)	22 (43.1%)	6 (50.0%)	0.75
Autoimmune disease (number, %)	6 (11.8%)	1 (8.3%)	1.00
Allergic disease (number, %)	15 (29.4%)	7 (58.3%)	0.09
Previous hepatitis B virus infection (number, %)	8 (15.7%)	2 (16.7%)	1.00
Previous hepatitis C virus infection (number, %)	2 (3.9%)	1 (8.3%)	0.48
Previous syphilis infection (number, %)	3 (5.9%)	0 (0.0%)	1.00
Renin–angiotensin system inhibitor (number, %)	17 (33.3%)	7 (58.3%)	0.18
Statin (number, %)	16 (31.4%)	5 (41.7%)	0.51
Erythropoiesis-stimulating agent (number, %)	44 (86.3%)	12 (100.0%)	0.33
Hypoxia-inducible factor prolyl hydroxylase inhibitor (number, %)	4 (7.8%)	0 (0.0%)	1.00
Iron supplement (number, %)	29 (56.9%)	8 (66.7%)	0.75
Zinc supplement (number, %)	4 (7.8%)	0 (0.0%)	1.00
Phosphate binder (number, %)	41 (80.4%)	10 (83.3%)	1.00
Vitamin D analog (number, %)	40 (78.4%)	11 (91.7%)	0.43
Calcimimetic (number, %)	19 (37.3%)	5 (41.7%)	1.00
Corticosteroid (number, %)	3 (5.9%)	0 (0.0%)	1.00
Anti-SARS-CoV-2 spike antibody titer 4 weeks after the second BNT162b2 mRNA COVID-19 vaccination (AU/mL)	2896 [1110–4358]	1606 [608–2531]	0.14
Anti-SARS-CoV-2 spike antibody titer 1 week before the third BNT162b2 mRNA COVID-19 vaccination (AU/mL)	220 [127–411]	188 [127–492]	0.83

Abbreviations: COVID-19, coronavirus disease 2019; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Clinical Outcome of Hemodialysis Patients Infected with COVID-19 After the Third or Fourth Dose

Table 5 shows the timing of infection and clinical outcome of hemodialysis patients infected with COVID-19 after the third dose and the fourth dose. Among hemodialysis patients infected with COVID-19 after the third dose, two patients were hospitalized and three patients died. Anti-SARS-CoV-2 spike antibody titer 1 week before the third dose was not different between survivor and non-survivor patients (p=0.66). Among hemodialysis patients infected with COVID-19 after the fourth dose, no patient was hospitalized but one patient died. Anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose was not different between survivor and non-survivor patients (p=0.59). Hospitalization rate tended to be lower in hemodialysis patients infected with COVID-19 after the fourth dose than in those infected with COVID-19 after the third dose (0.0% vs 33.3%, p=0.46). Mortality rate tended to be lower in hemodialysis patients infected with COVID-19 after the fourth dose than in those infected with COVID-19 after the third dose (16.7% vs 50.0%, p=0.55).

Table 5 Timing of Infection and Clinical Outcome of Hemodialysis Patients Infected with COVID-19 After the Third Dose (**A**) and the Fourth Dose (**B**)

(A) **Hemodialysis patients infected with COVID-19 after the third dose**						
Patient Number	**Age (Years)**	**Sex**	**Timing of Infection**	**Hospitalization**	**Clinical Outcome**	**Anti-SARS-CoV-2 Spike Antibody titer 1 Week Before the Third BNT162b2 mRNA COVID-19 Vaccination (AU/mL)**
1	88	Female	1 week after the third dose	Yes	Alive	401
2	86	Female	6 weeks after the third dose	Yes	Death	7
3	66	Male	18 weeks after the third dose	No	Death	198
4	78	Male	19 weeks after the third dose	No	Alive	1078
5	98	Female	22 weeks after the third dose	No	Death	793
6	85	Female	23 weeks after the third dose	No	Alive	85
(**B**) **Hemodialysis patients infected with COVID-19 after the fourth dose**						
Patient number	**Age (Years)**	**Sex**	**Timing of Infection**	**Hospitalization**	**Clinical Outcome**	**Anti-SARS-CoV-2 Spike Antibody Titer 1 Week Before the Fourth BNT162b2 mRNA COVID-19 Vaccination (AU/mL)**
1	83	Female	4 weeks after the fourth dose	No	Alive	1088
2	89	Male	7 weeks after the fourth dose	No	Alive	531
3	69	Female	8 weeks after the fourth dose	No	Death	13,000
4	50	Male	9 weeks after the fourth dose	No	Alive	1702
5	75	Female	11 weeks after the fourth dose	No	Alive	14,000
6	55	Male	12 weeks after the fourth dose	No	Alive	9540

Abbreviations: COVID-19, coronavirus disease 2019; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Discussion

In the present study, we found that the anti-SARS-CoV-2 spike antibody titer was cumulatively increased following four doses of COVID-19 vaccine in Japanese patients undergoing hemodialysis. Also, hypoxia-inducible factor prolyl hydroxylase inhibitor use and the anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose of COVID-19 vaccine were correlated with the anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose. In contrast, only the anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose was correlated with the anti-SARS-CoV-2 spike antibody titer 12 weeks after the fourth dose.

Recently, several studies have investigated the anti-SARS-CoV-2 spike antibody response to the fourth dose of BNT162b2 mRNA COVID-19 vaccine in patients undergoing hemodialysis.[9–12] One of these studies reported that anti-SARS-CoV-2 spike antibody level declined by 87% over 5 months after the second dose and by 68% over 4 months after the third dose.[12] This study also showed that the anti-SARS-CoV-2 spike antibody level increased 74.8-fold after the third dose and 4.8-fold after the fourth dose, and that the anti-SARS-CoV-2 spike antibody level was higher 1 month after the fourth dose (37,000 AU/mL) compared with 1 month after the second (2501 AU/mL) and third (24,000 AU/mL) doses.[12] In the present study, the anti-SARS-CoV-2 spike antibody level declined by 92% within 5 months after the second dose and by 67% within 5 months after the third dose. The anti-SARS-CoV-2 spike antibody level increased 81.8-fold and 5.1-fold after the third and fourth doses, respectively. The anti-SARS-CoV-2 spike antibody level was higher 1 month after the fourth dose (30,000 AU/mL) compared with 1 month after the second (2896 AU/mL) and third

(18,000 AU/mL) doses. These results indicate that the anti-SARS-CoV-2 spike antibody titer cumulatively increases following four doses of COVID-19 vaccine in patients undergoing hemodialysis, although the antibody titer declines with time. Further research is necessary to determine the optimal dose and schedule of COVID-19 vaccine doses to maintain an adequate anti-SARS-CoV-2 spike antibody titer in hemodialysis patients.

In the present study, the anti-SARS-CoV-2 spike antibody titer 1 week before the fourth dose of COVID-19 vaccine was positively correlated with that at 4 and 12 weeks after the fourth dose. Several studies reported that the anti-SARS-CoV-2 spike antibody level before the third dose of COVID-19 vaccine was positively associated with the anti-SARS-CoV-2 spike antibody level after the third dose in patients undergoing hemodialysis.[14–16] These results indicate that the degree of humoral immunity against SARS-CoV-2 before COVID-19 vaccination may influence the anti-SARS-CoV-2 spike antibody response after COVID-19 vaccination in hemodialysis patients. Further research is necessary to clarify the relationship between the anti-SARS-CoV-2 spike antibody level before and after COVID-19 vaccination in patients undergoing hemodialysis.

Hypoxia-inducible factor has pleiotropic effects on various systems, including erythropoiesis, iron metabolism, and the immune response by activating transcription of its target genes.[17] It has been reported that hypoxia-inducible factor activation induced by hypoxia conditions promoted the antibody production and proliferation of germinal center B cells through increased glycolytic metabolism and mitochondrial biogenesis.[18] In the present study, hypoxia-inducible factor prolyl hydroxylase inhibitor use was positively correlated with the anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose of COVID-19 vaccine. By contrast, the anti-SARS-CoV-2 spike antibody titer 4 weeks after the fourth dose was not associated with transferrin saturation, ferritin, or hemoglobin concentration. These findings suggest that hypoxia-inducible factor activation may enhance the humoral immune response to COVID-19 vaccination in patients undergoing hemodialysis, independent of its effects on anemia and iron metabolism. However, further data are needed to clarify the role of hypoxia-inducible factor prolyl hydroxylase inhibitor in humoral immune response to COVID-19 vaccination in hemodialysis patients because the number of patients taking hypoxia-inducible factor prolyl hydroxylase inhibitor in our study was really small (n=4). Further studies are required to investigate whether hypoxia-inducible factor prolyl hydroxylase inhibitor augments the anti-SARS-CoV-2 spike antibody response in hemodialysis patients after COVID-19 vaccination.

In the present study, anti-SARS-CoV-2 spike antibody titer 1 week before the third dose was not different between hemodialysis patients not infected with COVID-19 and those infected with COVID-19 after the third or fourth dose. In addition, anti-SARS-CoV-2 spike antibody was not different between survivor and non-survivor patients infected with COVID-19. However, mortality rate showed a tendency to be lower in hemodialysis patients infected with COVID-19 after the fourth dose than in those infected with COVID-19 after the third dose. Our study results suggest that the fourth dose of COVID-19 vaccine might reduce COVID-19-related mortality in hemodialysis patients infected with COVID-19. Further research is necessary to clarify the effect of the fourth dose of COVID-19 vaccine on clinical outcome of COVID-19 in hemodialysis patients.

This study has seven limitations. First, the patients were recruited from a single institution, which limits the external validity of the results. Second, the number of patients was less, restricting generalization of the findings. Third, there was no control group; therefore, it is unclear whether the anti-SARS-CoV-2 spike antibody level after the fourth dose of COVID-19 vaccine differs between patients undergoing hemodialysis and healthy controls. Fourth, patients with asymptomatic COVID-19 may have been included in this study, although we excluded patients who developed COVID-19. Fifth, we cannot determine whether the fourth dose is effective against the currently circulating COVID strain because there was no data of patients who did not receive the fourth dose. Sixth, we did not measure anti-SARS-CoV-2 spike protein receptor binding domain antibody titer, which is more important to evaluate the efficacy of COVID-19 vaccine. Seventh, we did not assess the cellular immune response, which is also important for protection against COVID-19. Therefore, large-scale multicenter studies are necessary to validate the present study findings.

In conclusion, hypoxia-inducible factor prolyl hydroxylase inhibitor use and the anti-SARS-CoV-2 spike antibody titer before the fourth dose of COVID-19 vaccine were associated with the anti-SARS-CoV-2 spike antibody titer after the fourth dose in Japanese hemodialysis patients.

Ethical Approval

This study was approved by the Ethical Committee of Mizue Yuai Clinic (MYC 2021-01) and conducted in accordance with the basic principles contained in the Helsinki Declaration.

Informed Consent

Written informed consent was gained from all study participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest related to this study.

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