

# DOCTORAL THESIS

SARS-CoV-2 spike protein antibody titers 6 months after SARS-CoV-2 mRNA vaccination among patients undergoing hemodialysis in Japan

(日本人の血液透析患者における SARS-CoV-2 mRNA ワクチン接種後の  
抗SARS-CoV-2 スパイクタンパク質抗体価に関する検討)

September, 2023

(2023年9月)

Daisuke Kanai

金井 大輔

Yokohama City University Graduate School of Medicine

横浜市立大学 大学院医学研究科 医科学専攻 病態制御内科学

( Doctoral Supervisor : Kouichi Tamura, Professor )

( 指導教員 : 田村 功一 教授 )



# SARS-CoV-2 spike protein antibody titers 6 months after SARS-CoV-2 mRNA vaccination among patients undergoing hemodialysis in Japan

Daisuke Kanai<sup>1</sup> · Hiromichi Wakui<sup>1</sup> · Tatsuya Haze<sup>2</sup> · Kengo Azushima<sup>1</sup> · Sho Kinguchi<sup>1</sup> · Shunichiro Tsukamoto<sup>1</sup> · Tomohiko Kanaoka<sup>1</sup> · Shingo Urate<sup>1</sup> · Yoshiyuki Toya<sup>1</sup> · Nobuhito Hirawa<sup>3</sup> · Hideaki Kato<sup>4</sup> · Fumimasa Watanabe<sup>5</sup> · Kanako Hanaoka<sup>5</sup> · Masaaki Hanaoka<sup>5</sup> · Hiroshi Mitsuhashi<sup>5</sup> · Satoshi Yamaguchi<sup>6</sup> · Toshimasa Ohnishi<sup>5</sup> · Kouichi Tamura<sup>1</sup>

Received: 23 March 2022 / Accepted: 26 May 2022 / Published online: 25 June 2022  
© The Author(s), under exclusive licence to The Japanese Society of Nephrology 2022

## Abstract

**Background** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination is shown to prevent severe illness and death in hemodialysis (HD) patients, but the immune response to vaccines is reduced in this population. This study compared SARS-CoV-2 spike protein antibody titers between HD patients and healthy controls in Japan for up to 6 months following vaccination.

**Methods** A multi-institutional retrospective study at five clinics in Japan was conducted using 412 HD patients and 156 healthy controls who received two doses of the BNT162b2 (Pfizer-BioNTech) mRNA vaccine. Anti-SARS-CoV-2 spike protein S1 IgG antibody titers were measured at 1, 3, and 6 months after the second dose. The attenuation speed was calculated as slope (i.e.,  $-\beta$ ) using a linear mixed-effects model toward the log-transformed antibody titers.

**Results** The HD group had significantly lower month 1 antibody titers (Ab-titer-1) than the controls, and these remained lower through month 6 (95% CI: 2617.1 (1296.7, 5240.8) vs. 7285.4 (4403.9, 11,000.0) AU/mL at Ab-titer-1, and 353.4 (178.4, 656.3) vs. 812.0 (498.3, 1342.7) AU/mL at Ab-titer-6 ( $p < 0.001$ , respectively)). Lower log Ab-titer-1 levels in the HD group were significantly associated with a lower log Ab-titer-6 (0.90 [0.83, 0.97],  $p < 0.001$ ). The  $-\beta$  values in the HD patients and healthy controls were  $-4.7 \pm 1.1$  and  $-4.7 \pm 1.4$  ( $\text{year}^{-1}$ ), respectively.

**Conclusion** SARS-CoV-2 spike protein antibody titers were significantly lower in HD patients than in healthy controls at 1 (peak) and 6 months after the second vaccination. Low peak antibody titers contributed to low 6-month antibody titers.

**Keywords** SARS-CoV-2 · Antibody · Hemodialysis · vaccine · BNT162b2

---

Daisuke Kanai, Hiromichi Wakui and Tatsuya Haze are equally contributed to this work.

---

✉ Kouichi Tamura  
tamukou@med.yokohama-cu.ac.jp

<sup>1</sup> Department of Medical Science and Cardiorenal Medicine, Graduate School of Medicine, Yokohama City University, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan

<sup>2</sup> Center for Nobel and Exploratory Clinical Trials (Y-NEXT), Yokohama City University, Yokohama, Kanagawa, Japan

<sup>3</sup> Department of Nephrology and Hypertension, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan

<sup>4</sup> Infection Prevention and Control Department, Yokohama City University Hospital, Yokohama, Kanagawa, Japan

<sup>5</sup> Kohsaikai Kamiooka Jinsei Clinic, Yokohama, Kanagawa, Japan

<sup>6</sup> Kohsaikai Yokohama Jinsei Hospital, Yokohama, Kanagawa, Japan

## Introduction

Since January 2020, the pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a global public health emergency. Several types of vaccine against SARS-CoV-2 have been developed, of which mRNA vaccines are the most widely used because they can efficiently prevent severe illness, hospitalization, and death [1, 2]. The mRNA vaccines are primarily administered in a two-dose schedule, but humoral immunity gradually declines, resulting in reduced protection against COVID-19 [3, 4]. Therefore, a third booster vaccination has been administered in many countries [5, 6].

Patients with chronic kidney disease (CKD), especially those with end-stage kidney disease (ESKD) undergoing maintenance hemodialysis (HD), are at high risk of COVID-19 severity and death [7, 8]. Although patients undergoing HD are often excluded from vaccine trials for safety reasons, the results of many cohort studies have shown that SARS-CoV-2 mRNA vaccination reduces the risk of COVID-19 infection, severe illness, and death in patients receiving dialysis [9, 10]. Therefore, SARS-CoV-2 mRNA vaccination is now recommended for patients undergoing HD. However, patients with ESKD generally have a weak immune response to vaccines. Patients with ESKD have a reduced ability to produce antibodies to vaccine antigen owing to combined abnormalities of B cells, T cells, and antigen-presenting cells [11, 12]. For example, post-vaccination antibody positivity has been reported to be 44% versus 96% for hepatitis B virus and 40% versus 65% for seasonal influenza in patients with ESKD versus healthy controls, respectively [13, 14].

Similarly, immune responses to COVID-19 mRNA vaccines are weak in patients with ESKD [15]. Neutralizing antibody titers and SARS-CoV-2 spike protein antibody titers are reported to be lower in HD patients compared with those in healthy controls 1 to 2 months after SARS-CoV-2 mRNA vaccination [16–18], and importantly, both antibody titers have strong correlation [19–21]. Although there are few reports of long-term observation of post-vaccination antibody titers in patients with ESKD, studies in Israel and Germany have reported that dialysis patients had lower antibody titer levels 6 months after vaccination compared with healthy individuals [22, 23]. However, to our knowledge, there are no reports of antibody titers being examined up to 6 months after SARS-CoV-2 mRNA vaccination in patients receiving HD in Asia, including in Japan.

This was a multi-institutional retrospective cohort study. We aimed to examine post-vaccination antibody titers over time in patients with ESKD undergoing maintenance HD and healthy controls in Japan. SARS-CoV-2 spike protein antibody titers were examined 1 and 6 months after the

second dose of the BNT162b2 (Pfizer-BioNTech) mRNA vaccine in 412 patients undergoing HD and 156 health care workers. Additionally, we compared the attenuation speed of antibody titers after vaccination between HD patients and healthy controls.

## Materials and methods

### Study design and participants

We conducted a multi-institutional retrospective study at five clinics (Kamiooka Jinsei Clinic, Yokohama, Japan; Yokohama Jinsei Hospital, Yokohama; Bunko Jin Clinic, Yokohama; Kanazawa Clinic, Yokohama; and Oppama Jinsei Clinic, Yokosuka, Japan). These clinics offered free testing opportunities to measure SARS-CoV-2 spike protein antibody titers for patients undergoing HD and health care workers at the clinics. The testing was not conducted as a research project, but as a health checkup service provided by these clinics for those who wanted to know their antibody titers, with a total of two or three opportunities before and/or after the vaccinations. These clinics also declared that the testing for antibody titers was only for those who requested it, and patients and staff would never be penalized for not taking the test. The informed consent was clinically obtained by their physicians before each test. Not all participants attended all of the testing opportunities because it was not mandatory. The measurement of antibody titers was outsourced to an external laboratory and the blood samples were immediately discarded by the laboratory after measurement. The results were notified to the participants themselves and recorded in their medical records for patients undergoing HD and in health care information for medical staff. After we designed this study, we retrospectively collected the data from these medical records. This process was described in our research protocol and approved by the Ethics Committees of Yokohama City University Hospital (IRB approval number: F 220,200,037). We enrolled 840 participants, divided into a group who underwent HD three times a week (i.e., the hemodialysis group) and a group of healthy health care workers (i.e., the control group). All participants were aged over 20 years, had received the first vaccination between April and July 2021, and had received the second vaccination between May and August 2021, according to the Japanese government COVID-19 vaccination strategy. All participants had been vaccinated according to the vaccination schedule specified in the BNT162b2 (Comirnaty®, Pfizer-BioNTech) vaccine summary of product characteristics.

We excluded seven participants with documented SARS-CoV-2 infection before or during the study. We also excluded 265 participants 1) who had been treated for cancer within the past 1 year; 2) who had been taking any steroids

or immuno-suppressants within the past 1 year; 3) who had been under peritoneal dialysis or renal transplantation; 4) who had been diagnosed with immunodeficiency syndrome; 5) whose records included missing values; or 6) whose anti-S1 immunoglobulin (Ig) G antibody titers  $\leq 50$  AU/mL at Month 1 ( $n = 8$ ; all in the HD group) [24]. Finally, we selected 412 HD patients and 156 controls.

## Measurement

### Anti-S1 IgG antibody

Titers of anti-SARS-CoV-2 spike protein S1 IgG antibody were serologically measured at 1, 2, 3, and 6 months after the second dose of vaccine (i.e., Month 1, Month 2, Month 3, and Month 6) in the hemodialysis group. Titers for Month 1, Month 3, and Month 6 were available in the control group. Measurement was not conducted at all time points, but titers at Month 1 and Month 6 were mandatory for inclusion in the current study. We used the Abbott Architect SARS-CoV-2 IgG II Quant chemiluminescent microparticle immunoassay to detect IgG antibodies to the receptor-binding domain of the S1 subunit of the SARS-CoV-2 spike protein, per the manufacturer's instructions. The reportable measurement range of the assay is 6.8–80,000 AU/mL. As for within-laboratory precision, the coefficient of variation is reported to be between 4.2% and 5.1% [24]. Levels under the detection limit for the antibody were considered equivalent to 6.79 AU/mL in this study because the detection limit was 6.8 AU/mL.

### Others

In the HD group, blood samples were collected on the first dialysis day of the week, 3 days after the previous dialysis day. The dry weight was used as body weight in the hemodialysis group, which was determined by each physician, depending on the clinical findings. Body mass index (BMI) was calculated by dividing body weight (kg) by height (m) squared. For details of the other collected data, see Text S1 [25]. The clinical history and dialysis prescription were obtained from the medical records. Information about use of vitamin D, serum albumin levels, hemoglobin levels, and Kt/V is also collected, since these items are reportedly associated with SARS-CoV-2 spike protein antibody titer after the vaccination in patients undergoing HD [22, 23, 26, 27]. Data for the control group were unavailable in this study, except for anti-S1 IgG antibody titers, age, sex, height, body weight, and history of SARS-CoV-2 infection.

## Statistical analysis

First, we compared the antibody titers at Month 6 (Ab-titer-6) between the HD group and the control group using the Mann–Whitney  $U$  test. Because of the skewed distribution of the titers, we performed analysis of covariance (ANCOVA) to detect the difference in log-transformed Ab-titer-6 with multi-variable adjustment. Model 1 included age, sex, and BMI. Model 2 additionally included these covariates and log-transformed antibody titers at Month 1 (log Ab-titer-1).

Second, we assumed that the changes in antibody titers follow the exponential equation, which is known as the “one-compartment model,” [26] as follows:

$$A(t) = \alpha e^{(-\beta t)} \quad (1)$$

where  $A(t)$  equals the serum titer level at an arbitrary time  $t$ ,  $\alpha$  is a constant that equals the titer at  $t = 0$ , and  $\beta$  is a constant known as the elimination rate constant. By natural logarithm transformation of Eq. 1:

$$\log A(t) = \log \alpha - \beta t \quad (2)$$

According to Eq. 2, we can calculate  $\beta$  using a linear regression model with log-transformed antibody titers as a slope. Therefore, we used a mixed-effects linear regression model with random intercepts and random slopes on the log-transformed antibody titers and calculated the slope (i.e.,  $-\beta$ ) for each individual case. The onset of time ( $t = 0$ ) was defined as Month 1 in the model. We compared this slope ( $-\beta$ ) between the HD group and the control group using ANCOVA with multi-variable adjustment. Model 1 was unadjusted. Model 2 was adjusted by age, sex, and BMI. Plasma half-life ( $t = T_{1/2}$ ) was calculated using  $\beta$  [28]:

$$T_{1/2} = \log 2 \cdot \beta^{-1} \quad (3)$$

We also explored the association of clinical or laboratory characteristics related to HD treatment (e.g., Kt/V, use of vitamin D) with Ab-titer-1 or Ab-titer-6 in the HD group (see Text S1).

We compared the antibody titers at Month 1 (Ab-titer-1) between the HD group and control group using ANCOVA. As a sensitivity analysis, we used a non-linear mixed-effects regression model to directly estimate the constants in Eq. 1 without using log-transformation (see Text S1).

The assumption for normality and homoscedasticity was visually inspected using histograms and normal quantile–quantile plots. Possible violations of the assumptions in the regressions were examined by visually inspecting scatter plots of residuals and predicted values. All analyses were performed with R version 4.0.4 (The R Project for Statistical Computing, Vienna, Austria) using the default strings of

**Table 1** Characteristics of participants at baseline

	Overall ( <i>n</i> = 568)	HD group ( <i>n</i> = 412)	Control group ( <i>n</i> = 156)	<i>p</i> -value <sup>†</sup>
Age, years	62.5 ± 16.3	69.2 ± 12.2	45.0 ± 12.4	< 0.001*
Women, <i>n</i> (%)	236 (41.5)	117 (28.4)	119 (76.3)	< 0.001*
Height, cm	162.8 ± 8.8	163.2 ± 9.1	161.5 ± 7.8	< 0.05*
Weight, kg‡	60.5 ± 13.3	61.7 ± 13.8	57.3 ± 11.4	< 0.001*
Body mass index, kg/m <sup>2</sup>	22.8 ± 4.6	23.2 ± 5.0	21.9 ± 3.4	< 0.001*
Clinical data, history of disease, and prescription of dialysis in HD group ( <i>n</i> = 412)				
History of hypertension, <i>n</i> (%)		221 (53.6)		
History of diabetes, <i>n</i> (%)		192 (46.6)		
History of dyslipidemia, <i>n</i> (%)		48 (11.7)		
History of cardiovascular disease, <i>n</i> (%)		212 (51.5)		
Cause of dialysis				
Diabetes, <i>n</i> (%)		161 (39.1)		
Hypertension, <i>n</i> (%)		69 (16.7)		
Glomerular nephritis, <i>n</i> (%)		117 (28.4)		
Others or unknown, <i>n</i> (%)		65 (15.8)		
Type of dialysis				
Hemodialysis, <i>n</i> (%)		52 (12.6)		
Online–hemodiafiltration, <i>n</i> (%)		360 (87.4)		
Vascular access				
Arteriovenous fistula, <i>n</i> (%)		388 (94.2)		
Arteriovenous graft, <i>n</i> (%)		20 (4.9)		
Others, <i>n</i> (%)		4 (1.0)		
Duration of dialysis, years		4.8 (2.3, 10.2)		
Kt/V urea, per week		1.2 (1.1, 1.4)		
Time of dialysis at once, hours		4.0 (4.0, 4.0)		
Area of membrane used in dialysis, m <sup>2</sup>		2.2 (1.8, 2.5)		
Hemoglobin, g/dL		11.3 ± 1.1		
Total protein, g/dL		6.5 ± 0.5		
Albumin, g/dL		3.8 ± 0.3		

Data are expressed as means ± standard deviation, median (quartiles) for skewed variables, and counts (percentages). \**p* < 0.05. †HD group vs. control group via Welch's *t* test or Fisher's exact test where appropriate. ‡Dry weight was used as body weight in the HD group. HD hemodialysis

the nlme 3.1–152, lme4 1.1–26, and car 3.0–10 packages. Significance was defined as *p* < 0.05 using two-sided tests.

## Results

The final analytic sample for the primary analyses included 412 patients in the HD group (mean ± standard deviation [SD] of age was 69.2 ± 12.2 years and 28.4% were female patients), and 156 health care workers in the control group (mean ± SD of age was 45.0 ± 12.4 years and 76.3% were female individuals). Clinical history, laboratory data, and prescription of dialysis in the HD group are shown in Table 1. In total, 221 (53.6%) HD patients had a history of hypertension and 192 (46.6%) had a history of diabetes;

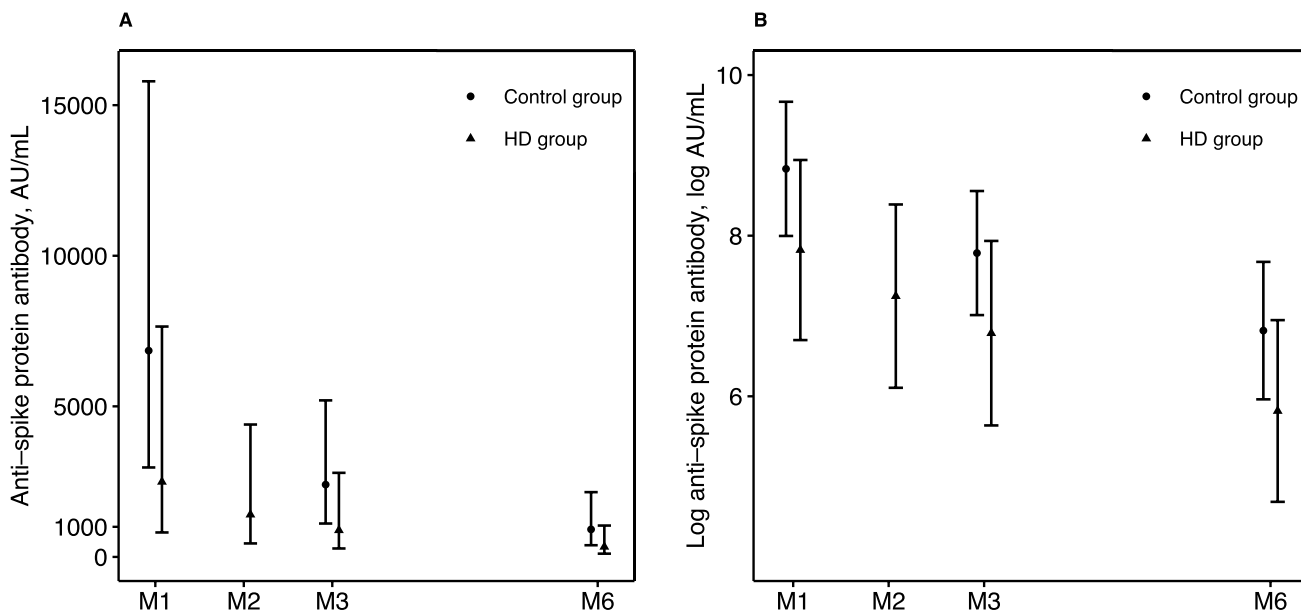
87.4% had been treated with the online hemodiafiltration method.

The SARS-CoV-2 spike protein antibody titers and positivity rate (i.e., ≥ 50 AU/mL) at each follow-up point are shown in Table 2 and Table S1, respectively. The HD group showed significantly lower levels of Ab-titer-6: median (95% confidence interval [CI]) 353.4 (178.4, 656.3) AU/mL versus 812.0 (498.3, 1342.7) AU/mL, (*p* < 0.001). 24 (5.8%) participants in the HD group showed seronegative levels at Month 6 whereas all control participants remained seropositive until Month 6 (Table S1). Log-transformed Ab-titer-6 levels were 5.8 ± 1.1 AU/mL and 6.8 ± 0.9 AU/mL, respectively. After multi-variable adjustment, the adjusted mean difference in log-transformed Ab-titer-6 between the HD group and the control group was −0.28 (−0.48, −0.08) (*p* < 0.001). Using back-transformation of this difference, we calculated the

**Table 2** Changes in titers of SARS–CoV–2 spike protein antibody from the 2nd vaccination

Antibody, AU/mL	HD group		Control group		Adjusted mean difference in log-transformation	
	Absolute values	Log-transformed	Absolute values	Log-transformed	Model 1	Model 2
at Month 1	2617.1 (1296.7, 5240.8) [n=412]	7.8 ± 1.1 [n=412]	7285.4 (4403.9, 11,000.0) [n=156]	8.8 ± 0.8 [n=156]	-0.45** (-0.72, -0.17)	
at Month 2	1568.4 (686.2, 2960.4) [n=327]	7.2 ± 1.1 [n=327]				
at Month 3	958.1 (451.5, 1756.2) [n=406]	6.8 ± 1.1 [n=406]	2197.3 (1323.3, 3541.3) [n=148]	7.8 ± 0.8 [n=148]		
at Month 6	353.4 (178.4, 656.3) [n=412]	5.8 ± 1.1 [n=412]	812.0 (498.3, 1342.7) [n=156]	6.8 ± 0.9 [n=156]	-0.60*** (-0.89, -0.32)	-0.28** (-0.48, -0.08)

The antibody titers 1, 2, 3, or 6 months after the second dose vaccination are shown in absolute values (median [quartiles]) and in natural logarithm form (mean ± standard deviation). Adjusted mean difference was estimated by analysis of covariance with the adjustment for age, sex, body mass index (Model 1) and additionally for log-Ab-titer-1 (Model 2). Diagonalized lines indicate that data were not available or comparison tests were not conducted in this study. \*\**p* < 0.01, \*\*\**p* < 0.001



**Fig. 1** Changes in the titers of anti-SARS-CoV-2 spike protein antibody. **A** Exponential changes in the absolute values of anti-SARS-CoV-2 spike protein antibodies are shown. Dots and error bars show the back-transformed means ± standard deviation of antibody titers in the control group (circular dots) and in the HD group (triangular dots) from the natural logarithmic form. **B** Linear changes in the nat-

ural log-transformed titers of SARS-CoV-2 spike protein antibodies are shown. Dots and error bars show the means ± standard deviation of log-transformed antibody titers in the control group (circular dots) and the HD group (triangular dots). M1–M6=Month 1 – Month 6, which means 1–6 months after the second vaccination, respectively. *HD* hemodialysis

adjusted ratio of Ab-titer-6 between the two groups (i.e., the HD group [AU/mL] per the control group [AU/mL]), which was estimated as 0.76 (0.62, 0.93). At Month 1, the HD group showed significantly lower levels of Ab-titer-1: median (95% CI) 2617.1 (1296.7, 5240.8) AU/mL versus 7285.4 (4403.9, 11,000.0) AU/mL. The adjusted mean difference in Ab-titer-1 was -0.45 (-0.72, -0.17), (*p* < 0.01). Fig. 1 shows the exponential changes in titers and linear changes in the log-transformed levels.

Using a linear mixed-effects model toward the log-transformed titers in the overall group, we calculated each individual's slope (i.e., -β) and intercept (i.e., logα). Table 3 shows the estimates between the HD group and the control group. The estimated -β values in the HD group and control group were -4.7 ± 1.1 and -4.7 ± 1.4 (year<sup>-1</sup>), respectively. In both the unadjusted and adjusted models, no significant difference in the slope (-β) was found between the two groups. Each plasma half-life (*t* = T<sub>1/2</sub>) estimated in the



**Table 3** Comparison of slope for SARS-CoV-2 spike protein antibody from one month after the 2nd vaccination

	Intercept (log $\alpha$ ), log AU/mL	Slope ( $-\beta$ ), log AU/mL/year	Adjusted mean difference	
			Model 1 (Unadjusted)	Model 2 (Adjusted)
Control group ( <i>n</i> = 412)	8.7 ± 0.7	− 4.7 ± 1.4	(REF)	(REF)
HD group ( <i>n</i> = 156)	7.7 ± 1.1	− 4.7 ± 1.1	0.06 (−0.17, 0.28)	− 0.21 (− 0.54, 0.12)

Using the log-transformed titers of SARS-CoV-2 spike protein antibody 1, 2, 3, and 6 months after the second dose vaccination, we calculated each individual's intercepts (log  $\alpha$ ) and slopes ( $-\beta$ ) by mixed-effect linear regression model, where  $\alpha$  is an estimated constant which equals the antibody titer at  $t=0$  (i.e., at Month 1), and  $-\beta$  is a constant known as an elimination rate constant. Using the estimated individual's slopes as outcome, we performed analysis of covariance to calculate the adjusted mean difference between the HD group and the control group. Model 1 is unadjusted. Model 2 is adjusted for age, sex, and body mass index. \* $p < 0.05$

adjusted model was 1.70 (1.49, 1.99) months in the HD group and 1.78 (1.59, 2.02) months in the control group, respectively. We also constructed a non-linear mixed-effects regression model as a sensitivity analysis. Results were similar in terms of comparable  $-\beta$  values between the HD group and control group, with a minimal random effect:  $\alpha = 4309.1$  (AU/mL),  $-\beta = -5.1$  (year<sup>-1</sup>),  $T_{1/2} = 1.64$  (months) in the HD group and  $\alpha = 9388.6$  (AU/mL),  $-\beta = -5.1$  (year<sup>-1</sup>),  $T_{1/2} = 1.64$  (months) in the control group. The estimated exponential curves of the titers in the two groups, which were well fitted for the actual observed titers, are shown in Figure S1.

In exploratory analyses, older age, lower hemoglobin level, and lower albumin level were related to a lower level of log Ab-titer-1 in both the unadjusted and adjusted models (Table S2). Use of vitamin D was significantly associated with a higher level of log Ab-titer-1 (estimates: 0.23 [0.02, 0.44]),  $p < 0.05$ ). Concerning the association with Ab-titer-6, lower Ab-titer-1 was significantly associated with a lower log Ab-titer-6 in both the unadjusted and adjusted models (estimates per 1 SD: 0.90 [0.83, 0.97],  $p < 0.001$ ). Similar to the association with log Ab-titer-1, older age and lower albumin level were associated with lower levels of log Ab-titer-6 in the adjusted model using age, sex, and BMI. However, after adjustment for the Ab-titer-1, these did not reach statistical significance (Table S3).

## Discussion

To the best of our knowledge, this is the first report in Japan to compare the SARS-CoV-2 spike protein antibody titers between patients undergoing HD and healthy individuals over a long period, up to 6 months after the second dose of SARS-CoV-2 mRNA vaccine. Additionally, in the present study, we examined the attenuation speed (slope) of SARS-CoV-2 spike protein antibody titer after the second vaccination in HD patients and healthy controls. The results

showed that SARS-CoV-2 spike protein antibody titers in HD patients were significantly lower than those in healthy controls at 6 months as well as at 1 month (peak time) after the second vaccination. Intriguingly, the attenuation speed of SARS-CoV-2 spike protein antibody titers after vaccination was not obviously different between HD patients and healthy controls. Furthermore, multivariate analysis revealed that low peak antibody titers (1 month after the second vaccination) contributed to the low antibody titers at 6 months in HD patients. Collectively, these results suggest that SARS-CoV-2 spike protein antibody titers may be maintained over a longer period if peak levels of antibody titer after vaccination can be raised sufficiently in HD patients. An increase in antibody titers with a third booster vaccine may be the key to protection against COVID-19 among patients undergoing HD.

In individuals with no history of SARS-CoV-2 infection, anti-SARS-CoV-2 spike protein antibody titers reach their peak 2 weeks to 1 month after the second dose of the BNT162b2 vaccine [29–31]. In HD patients, the increase in antibody titer after vaccination is delayed by approximately 1 week compared with that in healthy controls but reaches its peak well past 1 month after the second vaccination [15, 31]. HD patients are less likely to have an increase in SARS-CoV-2 spike protein antibody titer after vaccination compared with healthy individuals. Peak levels of SARS-CoV-2 spike protein antibody titer in HD patients after vaccination are reportedly around 1/9 to 1/3 those in healthy individuals [17, 18, 26, 32]. In the present study among Japanese patients receiving HD, antibody titers 1 month after the second vaccination were significantly lower than those in healthy controls and were approximately one-third those of healthy individuals.

In patients undergoing HD, the results of previous studies have demonstrated that use of immunosuppressive and anti-cancer agents, age, low serum albumin, low lymphocyte count, and low Kt/V are associated with lower peak levels of SARS-CoV-2 spike protein antibody titer after

vaccination [16, 17, 26, 32–34]. Additionally, calcitriol treatment is reportedly associated with SARS-CoV-2 spike protein IgG concentration 1 month after the second dose of the BNT162b2 vaccine in HD patients [27]. As a molecular mechanism by which vitamin D enhances the immune response to vaccines, dendritic cells activated by stimulation via toll-like receptors after vaccination are further activated by vitamin D and migrate to lymphoid tissues to stimulate antigen-specific T and B cells, resulting in sufficient antibody production [35, 36]. In the present study, SARS-CoV-2 spike protein antibody titers 1 month after the second vaccination were positively correlated with use of vitamin D, serum albumin and hemoglobin levels and negatively correlated with age. However, Kt/V was not associated with peak antibody titer 1 month after vaccination in the present study, which may be owing to differences in patient backgrounds, including therapeutic condition and health insurance systems for HD between Israel, Western countries, and Japan.

With respect to long-term humoral immunity after vaccination, several studies among health care workers have reported that SARS-CoV-2 spike protein antibody titers and neutralizing antibodies progressively decline up to 6 months after vaccination [3, 4, 37, 38]. Studies of HD patients in Germany showed that SARS-CoV-2 spike protein antibody titers peaked 1 month after the second vaccination in dialysis patients and declined to approximately 50% of peak values 3–4 months later [5, 23, 39]. The results of the present study showed that 6 months after the second dose of the BNT162b2 vaccine, SARS-CoV-2 spike protein antibody titers were markedly decreased (approximately one-seventh) compared with the peak value 1 month after vaccination.

There are few reports directly comparing antibody titers between patients receiving HD and healthy controls over a long period. A recent study in Israel reported that SARS-CoV-2 spike protein antibody titers in dialysis patients 6 months after the second dose of the BNT162b2 vaccine were significantly lower (approximately one-third) than those in healthy controls [40]. Consistent with this previous report, the results of the present study demonstrated that SARS-CoV-2 spike protein antibody titers in Japanese HD patients were significantly lower (approximately one-third) than those in healthy controls 6 months after receiving the second dose of the BNT162b2 vaccine. With respect to factors associated with long-term antibody titers, studies among health care workers have shown that low antibody titers 6 months after vaccination are related to age, immunosuppressive medications, low initial doses of vaccine, and low peak values 1 month after vaccination [3, 38]. In dialysis patients, age, use of immunosuppressive agents, low albumin levels, and low hemoglobin levels are reportedly associated with low antibody titers 6 months after vaccination [22, 23]. In our study, age and low albumin levels were associated with low antibody titers 6 months after receiving the second

shot of the BNT162b2 vaccine. However, these factors were no longer associated with antibody titers at 6 months, after adjustment for antibody titers at 1 month. Combined with the present results that attenuation speed did not differ between HD patients and healthy controls, low peak levels after vaccination may contribute to low antibody titers over the long term in HD patients.

The relationship between COVID-19 mRNA vaccine antibody titers and prevention of severe illness is controversial. In patients aged 60 years or older, the rate of severe illness due to SARS-CoV-2 infection reportedly increased 1.8 (95% CI 1.1–2.9) 6 months after the second vaccination compared with 3 months after the vaccination [41]. On the other hand, mRNA vaccines have the effects of conferring cellular immunity and immune memory in addition to humoral immunity. Cellular immunity is known to suppress the severity of SARS-CoV-2 infection independent of anti-SARS-CoV-2 spike protein IgG levels [42]. Several studies have reported that the preventive effects of mRNA vaccine on severe illness sustain even after a marked decrease in SARS-CoV-2 spike protein antibody titers [43, 44]. Therefore, the results of the present study, which showed lower antibody titers in response to mRNA vaccination in patients undergoing HD compared to controls, could not conclude that the effects of mRNA vaccination on prevention of the severity of SARS-CoV-2 infection is inferior in patients undergoing HD compared to controls.

The present study had some limitations. First, participants' backgrounds differed significantly between the two groups. Especially, the age distribution and the gender are very different between the HD patient group and the control group. To select medical staff as the control group is an important limitation. Second, this study had a retrospective study design. Third, it was difficult to exclude asymptomatic participants infected with SARS-CoV-2. Fourth, only adult patients undergoing HD (not less than 20 years old) were included. Fifth, the only vaccine investigated was BNT162b2 (Pfizer-BioNTech); patients who received other mRNA vaccines were not included. Sixth, we examined SARS-CoV-2 spike protein antibody titers but not neutralizing antibodies or cellular immunity. Therefore, further studies are needed to elucidate the immunoprotective effects of SARS-CoV-2 vaccines against COVID-19 in patients undergoing HD.

In conclusion, the present study findings demonstrated that SARS-CoV-2 spike protein antibody titers in patients undergoing HD in Japan were significantly lower than those in healthy controls 6 months after vaccination. In HD patients, low antibody titers 6 months after vaccination were associated with low peak levels whereas the attenuation speed of antibody titers did not differ from that of healthy controls. Taken together, these results indicate the



importance of higher peak antibody titers in HD patients. Given the fact that low hemoglobin and albumin levels were found to be associated with low peak antibody titers, it is important for HD patients to improve their nutrition and address anemia when receiving vaccination. Furthermore, in dialysis patients, SARS-CoV-2 spike protein antibody titers are reported to increase markedly by approximately 25-fold after the third booster dose of BNT162b2 vaccine compared with the second dose [45]. Therefore, a sufficient increase in antibody titers with a third booster vaccine may be the key to protection against COVID-19 in patients receiving HD.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10157-022-02243-8>.

**Acknowledgements** We thank the medical staff of Kohsaikai Kami-oooka Jinsei Clinic, Yokohama Jinsei Hospital, Kohsaikai Bunkojin Clinic, Kohsaikai Kanazawa Clinic, and Kohsaikai Oppama Clinic for their support of this study.

## Declarations

**Conflict of interest** The authors have declared that no conflict of interest exists.

**Informed consent** Because the present study was a retrospective and non-interventional study, we adopted an opt-out method instead of written informed consent. We provided patients with the opportunity to opt out by explaining the purpose of the study and the required individual data for the study by posting the poster at five clinics (Kamioooka Jinsei Clinic, Yokohama, Japan; Yokohama Jinsei Hospital, Yokohama; Bunko Jin Clinic, Yokohama; Kanazawa Clinic, Yokohama; or Oppama Jinsei Clinic, Yokosuka, Japan).

**Human and animal rights** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committees of Yokohama City University Hospital (IRB approval number F 220200037).

## References

1. Thomas SJ, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. *N Engl J Med.* 2021;385(19):1761–73.
2. Pilishvili T, et al. Effectiveness of mRNA Covid-19 vaccine among U S Health Care Personnel. *N Engl J Med.* 2021;385(25):e90.
3. Levin EG, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med.* 2021;385(24):e84.
4. Notarte KI, et al. Characterization of the significant decline in humoral immune response six months post-SARS-CoV-2 mRNA vaccination: a systematic review. *J Med Virol.* 2022;94(7):2939–61.
5. Petrelli F, et al. Third dose of SARS-CoV-2 vaccine: a systematic review of 30 published studies. *J Med Virol.* 2022;94(6):2837–44.
6. Arbel R, et al. BNT162b2 vaccine booster and mortality due to covid-19. *N Engl J Med.* 2021;385(26):2413–20.
7. Chung EYM, et al. Incidence and outcomes of COVID-19 in people with CKD: a systematic review and meta-analysis. *Am J Kidney Dis.* 2021;78(6):804–15.
8. De Meester J, et al. Incidence, characteristics, and outcome of COVID-19 in adults on kidney replacement therapy: A Region-wide Registry Study. *J Am Soc Nephrol.* 2021;32(2):385–96.
9. El Karoui K, et al. Vaccination and COVID-19 dynamics in dialysis patients. *Clin J Am Soc Nephrol.* 2022;17(3):395–402.
10. Sibbel S, et al. Real-World Effectiveness and Immunogenicity of BNT162b2 and mRNA-1273 SARS-CoV-2 vaccines in patients on hemodialysis. *J Am Soc Nephrol.* 2022;33(1):49–57.
11. Kato S, et al. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol.* 2008;3(5):1526–33.
12. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol.* 2013;9(5):255–65.
13. Ghadiani MH, et al. Response rates to HB vaccine in CKD stages 3–4 and hemodialysis patients. *J Res Med Sci.* 2012;17(6):527–33.
14. Mastalerz-Migas A, et al. Immune response to influenza vaccine in hemodialysis patients with chronic renal failure. *Adv Exp Med Biol.* 2013;756:285–90.
15. Carr EJ, et al. Review of early immune response to SARS-CoV-2 vaccination among patients with CKD. *Kidney Int Rep.* 2021;6(9):2292–304.
16. Van Praet J, et al. Predictors and dynamics of the humoral and cellular immune response to SARS-CoV-2 mRNA vaccines in hemodialysis patients: A Multicenter Observational Study. *J Am Soc Nephrol.* 2021;32(12):3208–20.
17. Grupper A, et al. Humoral response to the pfizer BNT162b2 vaccine in patients undergoing maintenance hemodialysis. *Clin J Am Soc Nephrol.* 2021;16(7):1037–42.
18. Yanay NB, et al. Experience with SARS-CoV-2 BNT162b2 mRNA vaccine in dialysis patients. *Kidney Int.* 2021;99(6):1496–8.
19. Robbiani DF, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature.* 2020;584(7821):437–42.
20. Nam M, et al. Evaluation of humoral immune response after SARS-CoV-2 vaccination using two binding antibody assays and a neutralizing antibody assay. *Microbiol Spectr.* 2021;9(3):e0120221.
21. Ramos A, et al. Assessing SARS-CoV-2 neutralizing antibodies after BNT162b2 vaccination and their correlation with SARS-CoV-2 IgG Anti-S1, Anti-RBD and Anti-S2 serological titers. *Diagnostics.* 2022;12(1):205.
22. Berar-Yanay N, et al. Waning humoral response 3–6 months after vaccination with the SARS-COV-2 BNT162b2 mRNA vaccine in dialysis patients. *J Clin Med.* 2021;11(1):64.
23. Boedecker-Lips SC, et al. Six-Month Follow-Up after Vaccination with BNT162b2: SARS-CoV-2 antigen-specific cellular and humoral immune responses in hemodialysis patients and kidney transplant recipients. *Pathogens (Basel, Switzerland).* 2022;11(1):67.
24. Abbott Core Laboratory: SARS-CoV-2 Immunoassays: Advancing diagnostics of COVID-19. Available at: <https://www.corelaboratory.abbott/int/en/offerings/segments/infectious-disease/sars-cov-2>. Accessed March 8, 2022
25. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol.* 1993;4(5):1205–13.
26. Danthu C, et al. Humoral Response after SARS-CoV-2 mRNA vaccination in a cohort of hemodialysis patients and kidney transplant recipients. *J Am Soc Nephrol.* 2021;32(9):2153–8.
27. Zitt E, et al. The safety and immunogenicity of the mRNA-BNT162b2 SARS-CoV-2 vaccine in hemodialysis patients. *Front Immunol.* 2021;12:704773.

28. Atkinson AJ Jr, et al. Clinical pharmacokinetics. *Annu Rev Pharmacol Toxicol.* 1979;19:105–27.
29. Bayart JL, et al. Waning of IgG, total and neutralizing antibodies 6 months post-vaccination with BNT162b2 in healthcare workers. *Vaccines.* 2021;9(10):1092.
30. Kato H, et al. Antibody titers against the Alpha, Beta, Gamma, and Delta variants of SARS-CoV-2 induced by BNT162b2 vaccination measured using automated chemiluminescent enzyme immunoassay. *J Infect Chemother.* 2022;28(2):273–8.
31. Attias P, et al. Antibody response to the BNT162b2 vaccine in maintenance hemodialysis patients. *Kidney Int.* 2021;99(6):1490–2.
32. Angel-Korman A, et al. Diminished and waning immunity to COVID-19 vaccination among hemodialysis patients in Israel: the case for a third vaccine dose. *Clin Kidney J.* 2022;15(2):226–34.
33. Frantzen L, et al. Efficacy of the BNT162b2 mRNA Covid-19 vaccine in a hemodialysis cohort. *Nephrol Dial Transplant.* 2021;36(9):1756–7.
34. Broseta JJ, 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(4):571–81.
35. Enioutina EY, et al. TLR ligands that stimulate the metabolism of vitamin D3 in activated murine dendritic cells can function as effective mucosal adjuvants to subcutaneously administered vaccines. *Vaccine.* 2008;26(5):601–13.
36. Enioutina EY, et al. TLR-induced local metabolism of vitamin D3 plays an important role in the diversification of adaptive immune responses. *J Immunol.* 2009;182(7):4296–305.
37. Padoan A, et al. Neutralizing antibody titers six months after Comirnaty vaccination: kinetics and comparison with SARS-CoV-2 immunoassays. *Clin Chem Lab Med.* 2022;60(3):456–63.
38. Đaković Rode O, et al. Decline of Anti-SARS-CoV-2 IgG antibody levels 6 months after complete BNT162b2 vaccination in healthcare workers to levels observed following the first vaccine dose. *Vaccines.* 2022;10(2):153.
39. Tillmann FP, et al. Long-term trajectories of SARS-CoV-2 neutralizing antibodies and predictive value of first dose vaccination-induced IgG-antibodies in hemodialysis patients. *Int Urol Nephrol.* 2021. <https://doi.org/10.1007/s11255-021-03076-2>.
40. Hsu CM, et al. Seroreponse to SARS-CoV-2 Vaccines among Maintenance Dialysis Patients over 6 Months. *Clin J Am Soc Nephrol.* 2022;17(3):403–13.
41. Goldberg Y, Mandel M, Bar-On YM, et al. Waning immunity after the BNT162b2 vaccine in Israel. *N Engl J Med.* 2021;385(24):e85.
42. Liu J, et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron. *Nature.* 2022;603(7901):493–6.
43. Tartof SY, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *Lancet.* 2021;398(10309):1407–16.
44. Chemaitelly H, et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. *N Engl J Med.* 2021;385(24):e83.
45. Bensouna I, et al. SARS-CoV-2 antibody response after a third dose of the BNT162b2 vaccine in patients receiving maintenance hemodialysis or peritoneal dialysis. *Am J Kidney Dis.* 2022;79(2):185–92.e1.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## 論文目録

### I 主論文

SARS-CoV-2 spike protein antibody titers 6 months after SARS-CoV-2 mRNA vaccination among patients undergoing hemodialysis in Japan

Kanai, D., Wakui, H., Haze, T., Azushima, K., Kinguchi, S., Tsukamoto, S., Kanaoka, T., Urate, S., Toya, Y., Hirawa, N., Kato, H., Watanabe, F., Hanaoka, K., Hanaoka, M., Mitsuhashi, H., Yamaguchi, S., Ohnishi, T., Tamura, K.:

雑誌名 : Clinical and Experimental Nephrology vol.26, issue 10, page 988-996, 2022.

### II 副論文

Blunted humoral immune response to the fourth dose of BNT162b2 COVID-19 vaccine in patients undergoing hemodialysis

Kanai, D., Wakui, H., Hanaoka, M., Haze, T., Azushima, K., Shinoda, S., Tsukamoto, S., Taguchi, S., Kinguchi, S., Kanaoka, T., Toya, Y., Hirawa, N., Kato, H., Watanabe, F., Hanaoka, K., Mitsuhashi, H., Yamaguchi, S., Ohnishi, T., Tamura, K.:

雑誌名 : Clinical and Experimental Nephrology, 2023. Online ahead of print.

### III 参考論文

Improved Immune Response to the Third COVID-19 mRNA Vaccine Dose in Hemodialysis Patients

Kanai, D., Wakui, H., Haze, T., Azushima, K., Kinguchi, S., Kanaoka, T., Toya, Y., Hirawa, N., Kato, H., Uneda, K., Watanabe, F., Hanaoka, K., Hanaoka, M., Mitsuhashi, H., Yamaguchi, S., Ohnishi, T., Tamura, K.:

雑誌名 : Kidney International Reports vol.7, issue 12, page 2718-2721, 2022.