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Article

T-cell Response and Antibody Production by Booster COVID-19 Vaccination in Japanese Patients with Chronic Kidney Disease Treated with Hemodialysis

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Abstract: Most studies on vaccines of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have focused on antibody, but cellular immunities are also critical. We aimed to evaluate the immune reactions of hemodialysis (HD) patients after the administration of the booster dose from the perspective of both humoral and cellular immunities. Hemodialysis patients (HD group) and age- and sex-matched non-dialysis individuals (control group) receiving three doses of BNT162b2 vaccine were measured for anti-SARS-CoV-2 immunoglobulin (IgG) and T-SPOT®.COVID test (T-SPOT) before, 3 weeks, and 3 months after the booster dose. The HD group had significantly higher SARS-CoV-2 IgG levels 3 weeks and 3 months after the booster dose than the control group, although both groups had no difference in SARS-CoV-2 IgG levels before the booster dose. Moreover, the HD group had significantly higher T-SPOT levels before and 3 weeks after the booster dose than the control group, but the difference was not significantly different 3 months after the booster dose. Furthermore, the incidence rates of local and systemic adverse reactions were significantly higher in the HD group than in the control group. HD patients obtained higher SARS-CoV-2 IgG levels and SARS-COV-2-specific T-cell responses after the booster dose than control.

Keywords: COVID-19; hemodialysis; vaccination; cellular immunity; humoral immunity; adverse reactions

1. Introduction

The global epidemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had devastating effects on healthcare, the economy, and society since 2020 [1]. Hemodialysis (HD) patients have more comorbidities and impaired immune function than healthy individuals, making them more susceptible to severe COVID-19, and the mortality rate of COVID-19 is approximately 10 times higher in HD patients than controls [2]. Vaccines are significantly important in preventing COVID-19 and its severe symptoms, and major nephrology societies recommend preferential vaccination of HD patients [3]. However, HD patients have lower antibody titers than controls after the primary series (two-dose series), and low antibody titers are related to a short dialysis time [4]. Moreover, the efficacy of the vaccine diminishes with time; in our study, the antibody titer in HD patients was 1,085 BAU/mL 2 weeks after the primary series but decreased to 212.3 BAU/mL 3 months later [4]. Therefore, booster doses are being administered worldwide. In contrast, although higher antibody titers are associated with both a lower risk of breakthrough infection and lower viral

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RNA copy numbers [5], there are no generally accepted clinical cutoff values for antibodies to protect against breakthrough infections or to prevent severe disease. Since some reports have indicated that antibody titers are not strongly associated with the prevention of severe COVID-19 in HD patients [6], cellular immunity is critical in those who fail to seroconvert [7]. Therefore, it is necessary to examine the efficacy of vaccines in terms of antibody titers and cellular immunity. T-SPOT®.COVID test (T-SPOT), an enzyme-linked immunospot (ELISpot) assay, identifies interferon-gamma (IFN-γ)-releasing T cells in response to stimulation with SARS-CoV-2 peptides and is highly accurate (area under the curve, 0.95) at differentiating confirmed infection with SARS-CoV-2-specific T cells, even several months after infection [8]. When quantifying and comparing the number of IFN-γ-releasing T cells in response to SARS-CoV-2, dividing by the median number of spots in response to the positive control can eliminate the impact of individual immune variations on T-SPOT. In addition, T-SPOT results are adjusted for lymphocyte count, which minimizes the influence of varying lymphocyte counts between patients [9] [10]. In this study, we aimed to evaluate the changes in the immune status of HD patients by the booster dose by measuring both antibody titer and cellular immunity.

2. Materials and Methods

We conducted a prospective multicenter study by the Infection Control Committee of the Japanese Society for Dialysis Therapy (JSDT). After receiving approval from the Ethics Committee of the JSDT (approval numbers 1–10), facilities that could recruit patients to participate in this study were enrolled from July 6 to July 31, 2021, on the JSDT website.

The conditions for enrollment in HD patients (HD group) were as follows: subjects who had received the primary series of the vaccine; had not been infected with SARS-CoV-2; had not been treated for any malignancy within 1 year; had not been treated with drugs, such as steroids, immunosuppressants, and immunomodulators; were scheduled to receive a booster dose of the BNT162b2 vaccine; and had provided written consent for this study. The control group was registered by open recruitment at Tokyo Saiseikai Central Hospital and its affiliated facilities by matching the number of enrolled dialysis patients in terms of age (in 10-year increments) and sex. Regarding the dialysis patients, the control group also comprised patients who met the conditions set for HD patients, in addition to having an estimated glomerular filtration rate of ≥ 45 mL/min/1.73 m^2.

SARS-CoV-2 immunoglobulin (IgG) antibody titers to the S1 subunit of the spike protein of SARS-CoV-2 (anti-S1 antibody titers) were measured using the Ortho-Clinical Diagnostics VITROS® Anti-SARS-CoV-2 IgG chemiluminescent immunoassay correlated with neutralizing antibodies at the following time points: 6 months after the primary series, 3 weeks after the booster dose, and 3 months after the booster dose. An antibody level of ≥ 17.8 BAU/mL was diagnosed as positive. Patients with symptomatic COVID-19 during the study period were also excluded. The antibody titers over time were compared between the two groups. We defined the variation rate as the change in anti-S1 antibody titer divided by the reference values, and we took the values of anti-S1 antibody titer 6 months after the primary series and 3 weeks after the third vaccination as the reference values:

3 weeks after the third vaccination – 6 months after the primary series /6 months after the primary series

3 months after the third vaccination – 3 weeks after the third vaccination))/3 weeks after the third vaccination

In addition, T-SPOT®.COVID test was performed on subjects aged between 50 and 80 years who provided consent for additional blood samples, and those from hospitals that required more than 6 h to transport the specimens to Tokyo Saiseikai Central Hospital were excluded (due to specimen preservation issues). T-SPOT®.COVID test was performed according to the manufacturer's protocol. Pretreatment peripheral blood samples were collected in heparinized tubes to isolate peripheral blood mononuclear cells

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(PBMCs). The isolated PBMCs were incubated in a microplate well as a positive control well with phytohemagglutinin (PHA), a negative control well with the medium, and wells containing SARS-CoV-2 peptides (CoV-A for spike protein and CoV-B for nucleocapsid protein). Plates were incubated for 16–20 h at 37°C with 5% CO_2, an anti-IFN-γ antibody conjugate was added, and the number of spot-forming cells (SFCs) was counted using ELISpot. Those with a negative control of ≥ 10 and a positive control of ≤ 20 and those with CoV-B of ≥ 8 (previous infection) were excluded from this study. Individuals with CoV-A minus negative control of ≥ 8 were diagnosed with T-SPOT as positive, those with CoV-A minus negative control of ≤ 7 were diagnosed as negative. The number of SFCs in CoV-A divided by the number of SFCs in PHA over time was compared between the two groups.

Furthermore, a questionnaire survey was conducted after the booster dose to investigate the presence of adverse reactions (pain, redness, swelling, pruritus, fatigue, headache, muscle pain, coldness, fever [> 37.5°C], arthralgia, nausea, diarrhea, stomachache, and anaphylaxis), which were compared between the control and HD groups.

The median values were compared using the Mann–Whitney U test. Frequencies between groups were compared using Fisher's exact test or chi-squared test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 9.

3. Results

In this study, 10 facilities (Tokyo Saiseikai Central Hospital, Harada Naika Clinic, Ozawa Clinic, Mizuno Clinic, Nakamura Clinic, Konan-no-sato, Shirogane-no-mori, Keifukuen, Oumori Nursing Home, and Kurara-Kaminoge) participated as the control group, and seven facilities (Shinagawa Dialysis Clinic, Meguro Station Building Clinic, Tokyo Saiseikai Central Hospital, Omiya Yoshizawa Clinic, Urawa Yoshizawa Clinic, Minami-Ooi Clinic, and Chuou Naika Clinic) participated as the HD group. In total, 103 and 194 subjects were recruited as the control and HD groups, respectively (Figure 1).

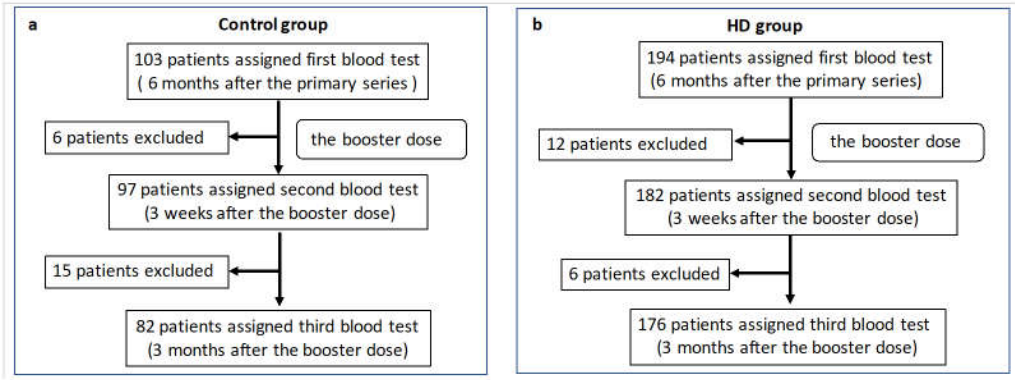

Figure 1. Trial profile. For the control group, blood samples were collected from 103 subjects 6 months after the primary series (2-6 M). Subsequently, one subject moved away, two were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and three did not wish to receive the booster dose. In total, six subjects withdrew from this study, and blood samples were collected from 97 subjects 3 weeks after the booster dose (3-3 W). Thereafter, 10 subjects moved away or resigned, three were infected with SARS-CoV-2, and two could not be contacted. In total, 15 subjects withdrew from this study, and blood samples were collected from 82 subjects 3 months after the booster dose (3-3 M) (a).
In the HD group, blood samples were collected from 194 subjects 6 months after the primary series (2-6 M). Subsequently, five subjects were infected with SARS-CoV-2, two moved away, two died, and three did not wish to receive additional vaccinations. In total, 12 subjects withdrew from this study, and blood samples were collected from 182 subjects 3 weeks after the booster dose (3-3 W). Thereafter, four patients were hospitalized, one moved away, and one died. In total, six patients withdrew from this study, and blood samples were taken from 176 subjects 3 months after the booster dose (3-3 M) (b).
HD, hemodialysis.

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Table 1. Characteristics of subjects.

	All			Males			Females		
	Control group (n=103)	HD group (n=194)	p-value	Control group (n=62)	HD group (n=126)	p-value	Control group (n=41)	HD group (n=68)	p-value
Males (n, (%))	68(61.8)	125(64.8)	0.79						
Age (year-old±SD)	65.4±11.6	67.0±11.1	0.31	64.8±11.5	67.0±11.5	0.78	66.5±11.7	66.6±10.2	0.78
BMI (kg/m²±SD)	23.6±	22.5±	**0.03**	24.1±4.1	22.8±3.9	**0.01**	22.8±4.3	22.0±4.8	**0.01**
Diabetes mellitus (n, (%))	17(16.3)*	79(40.9)	**<0.0001**	13(19.7)*	59(47.2)	**0.0002**	4(10.5)*	20(29.4)	**0.03**
Hypertension (n, (%))	48(46.2)*	81(42.0)	0.81	33(50.0)*	55(44.0)	0.54	15(39.5)*	26(38.2)	>0.99
Malignant tumor (n, (%))	11(10.6)*	29(15.0)	0.29	8(12.1)*	19(15.2)	0.66	3(7.9)*	10(14.7)	0.37
Cerebrovascular disease (n, (%))	3(2.9)*	42(21.8)	**<0.0001**	2(3.0)*	31(24.8)	**0.0001**	1(2.6)*	11(16.2)	0.052
Cardiovascular disease (n, (%))	4(3.8)*	37(19.2)	**<0.0001**	4(6.1)*	27(21.6)	**0.007**	0(0)*	10(14.7)	**0.013**
COPD (n, (%))	8(7.7)*	11(5.7)	0.63	5(7.6)*	3(2.4)	0.13	3(7.9)*	8(11.8)	0.74
	*n=104			*n=66			*n=38		

BMI; Body Mass Index,
COPD; Chronic Obstructive Pulmonary Disease,
HD; Hemodialysis
*: n=107 due to not available of data for 6 patients
Age and BMI are shown as average±SD

3.1. Anti-S1 antibody titers

The characteristics of each group are shown in Table 1.

Anti-S1 antibody titers 6 months after the primary vaccination series in the HD group were not significantly different from those in the control group ($p > 0.05$). However, the HD group had significantly higher anti-S1 antibody titers 3 weeks ($p = 0.003$) and 3 months after the booster dose ($p = 0.0004$) than the control group (Figure 2a–c).

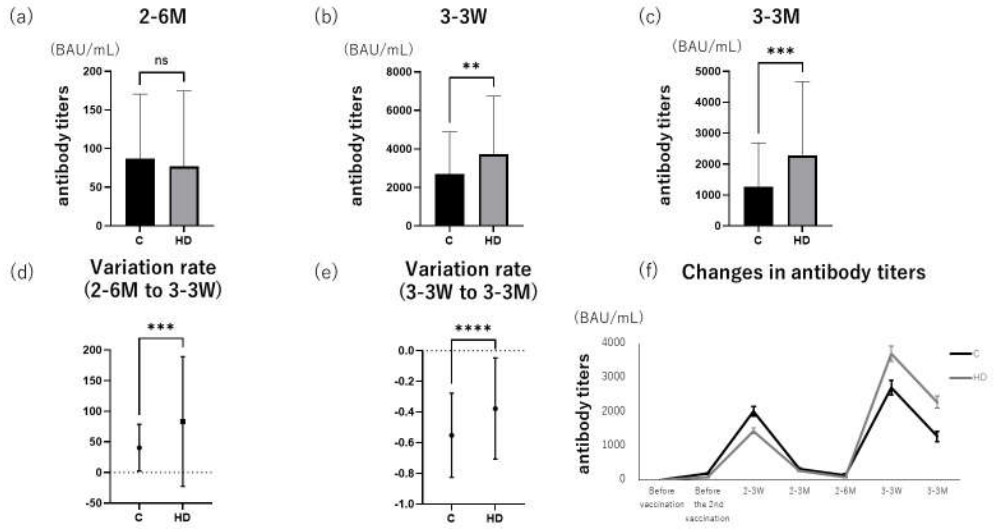

Figure 2. Comparison of antibody titers between the control and HD groups. Antibody titers in the control and HD groups were compared at three time points: (a) 6 months after the primary series (control, n = 103; HD, n = 194), (b) 3 weeks after the booster dose (control, n = 97; HD, n = 182), and (c) 3 months after the booster dose (control, n = 82; HD group, n = 176). No significant difference was observed between the two groups 6 months after the primary series; however, antibody titers 3 weeks and 3 months after the booster dose were significantly higher in the HD group ($p = 0.003$ and $p = 0.0004$, respectively) than in the control group. The rate of change was significantly greater in the HD group from 6 months after the primary series to 3 weeks after the booster dose ($p < 0.0001$) than in the control group (d) and significantly lower in the HD group from 3 weeks after the booster

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dose to 3 months after the booster dose ($p < 0.0001$) than in the control group (e). The time course of antibody titers from before the first vaccination to 3months after the booster dose is shown in f.
$^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$, $^{****}P \le 0.0001$
C, control; HD, hemodialysis
2-6 M, 6months after the primary series; 3-3 W, 3 weeks after the booster dose; 3-3 M, 3 months after the booster dose

In addition, the variation in anti-S1 antibody titers (from 6 months after the primary series to 3 weeks after the booster dose) was significantly higher in the HD group than in the control group ($p < 0.0001$). Furthermore, the variation in anti-S1 antibody titers (from 3 weeks after the booster dose to 3 months after the booster dose) was significantly lower in the HD group than in the control group ($p < 0.0001$) (Figure 2d–e). The time course of antibody titers from the first vaccination to 3 months after the booster dose is shown in Figure 2f. There were 29 withdrawals from the study between blood collection 6 months after the primary series and 3 months after the booster dose, which may have caused a selection bias. Therefore, antibody titers were reevaluated only in those who completed blood collection until 3 months after the third dose, showing results similar to those shown in Figure 2 (Figure S1).

3.2. T-SPOT®.COVID test

In total, 48 subjects were recruited as the control group (age, 63.4 ± 7.8; male, 66.7%), and 66 patients were recruited as the HD group (age, 69.2 ± 4.8; male, 57.8%) (Table S1). The same results were obtained as above for antibody titers 6 months after the primary series and 3 weeks and 3 months after the booster dose (Figure S2). The T-SPOT results were calculated by dividing the number of SFCs in CoV-A by the number of SFCs in the positive control to eliminate the effect of individual immune variability on T-SPOT. Cases with a CoV-B spot count of ≥ 8 were excluded as cases of infection. As a result, the HD group had a significantly larger number of SFCs 6 months after the primary series ($p = 0.007$) and 3 weeks after the booster dose ($p = 0.004$) than the control group. However, SFCs in the HD group were not significantly different from those in the control group 3 months after the booster dose ($p > 0.05$) (Figure 3a–c).

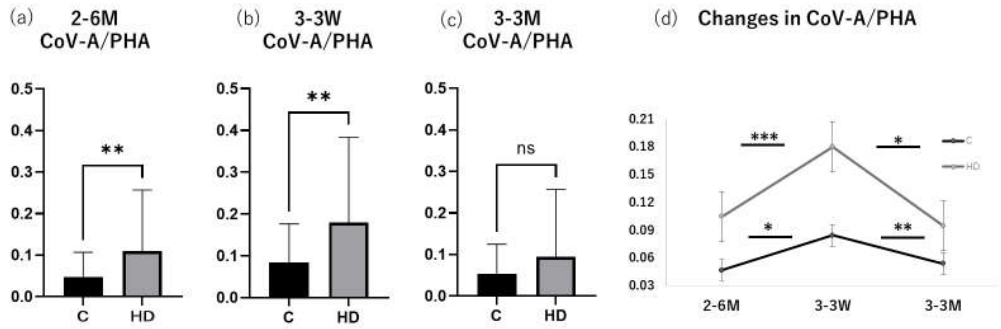

Figure 3. Changes in T-cell response. The number of CoV-A spots in the T-SPOT COVID divided by the number of spots in the positive control was compared between the two groups (control and HD) 6 months after the primary series (a), 3 weeks after the booster dose (b), and 3 months after the booster dose (c). CoV-A/PHA was significantly higher in the HD group 6 months after the primary series (control, n = 47; HD, n = 65) and at 3 weeks after the booster dose (control, n = 45; HD, n = 65) than in the control group ($p = 0.007$ and $p = 0.004$, respectively). No significant difference was observed between the two groups 3 months after the booster dose (control, n = 45; HD, n = 63) ($p = 0.12$).
In each group, the change in the CoV-A/PHA ratio of the T-SPOT was also examined (d).
In both the control and HD groups, the values at 3 weeks after the booster dose were significantly higher than those at 6 months after the primary series. The values at 3 weeks after the booster dose were significantly higher than those at 3 months after the booster dose.

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*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001
C, control; HD, hemodialysis
2-6 M, 6months after the primary series; 3-3 W, 3 weeks after the booster dose; 3-3 M, 3 months after the booster dose
PHA, phytohemagglutinin as a positive control

The number of SFCs at each time point was compared using one-way analysis of variance, and Tukey's multiple comparison test was used to assess the difference between them. In the control group, the number of SFCs at 3 weeks after the booster dose was higher than those at 6 months after the primary series (p = 0.02) and at 3 months after the booster dose (p = 0.008). In addition, in the HD group, the number of SFCs at 3 weeks after the booster dose was higher than those at 6 months after the primary series (p = 0.0004) and at 3 months after the booster dose (p = 0.01) (Figure 3d). When the percentage of negative T-SPOT was compared between the control and dialysis groups 6 months after the primary series and 3 weeks and 3 months after the booster dose, the control group had significantly higher percentage of negative T-SPOT level than the HD group 6 months after the primary series. At 3 weeks and 3 months after the booster dose, it tended to be higher in the control group than in the HD group, although there was no significant difference between them (Table 2).

Table 2. Negative rate of T-SPOT COVID-19.

	Control(%)	HD(%)	*p*-value
2-6M	66.7 *1	43.1*4	0.02
3-3W	35.0 *2	29.0*5	0.66
3-3M	37.2 *3	24.2*6	0.19

*1n=45, *2n=40, *3n=43
*4n=65, *5n=63, *6n=62

The correlation between T-SPOT results and antibody titers was examined for each of the three measurement points in each group (Figure 4).

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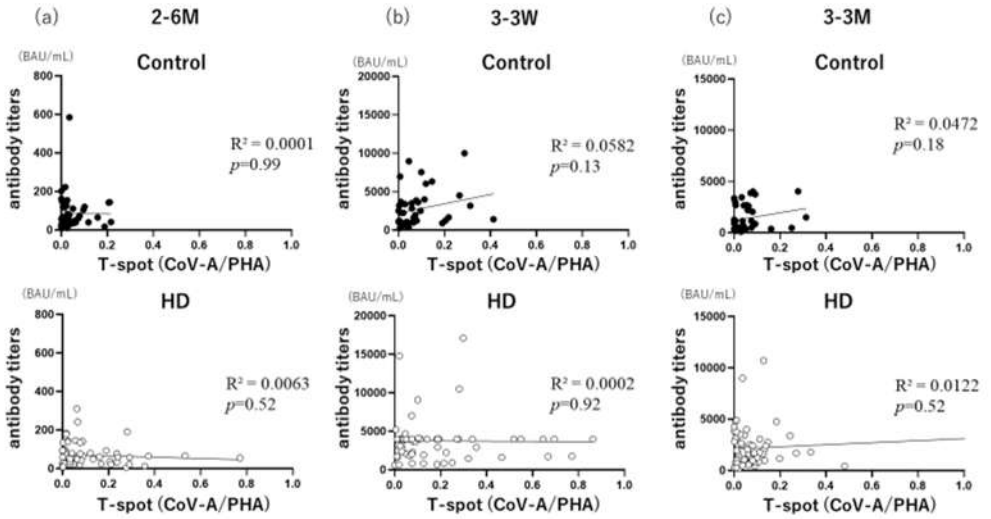

Figure 4. Correlation between T-SPOT (CoV-A/PHA) and antibody titers. The correlation between T-SPOT COVID and antibody titers was examined at each of the three measurement points in each group. Pearson's product-ratio correlation coefficient was used to calculate the R^2 and p-values. No significant correlations were observed 6 months after the primary series (a), 3 weeks after the booster dose (b), or 3 months after the booster dose (c).
2-6 M, 6months after the primary series; 3-3 W, 3 weeks after the booster dose; 3-3 M, 3 months after the booster dose; PHA, phytohemagglutinin as a positive control.

No significant correlations were observed 6 months after the primary series, 3 weeks after the booster dose, or 3 months after the booster dose. Table 3 shows the contingency table of positive or negative antibody levels and positive or negative T-SPOT values.

Table 3. Contingency Table for T-SPOT and SARS-CoV-2 IgG .

Control:2-6M				HD:2-6M			
		SARS-CoV-2 IgG				SARS-CoV-2 IgG	
		+(%)	−(%)			+(%)	−(%)
T-SPOT	+(%)	34.78	0.00	T-SPOT	+(%)	50.00	6.25
	−(%)	54.35	10.87		−(%)	39.06	4.69
Control:3-3W				HD:3-3W			
		SARS-CoV-2 IgG				SARS-CoV-2 IgG	
		+(%)	−(%)			+(%)	−(%)
T-SPOT	+(%)	65.00	0.00	T-SPOT	+(%)	70.97	0.00
	−(%)	35.00	0.00		−(%)	29.03	0.00
Control:3-3M				HD:3-3M			
		SARS-CoV-2 IgG				SARS-CoV-2 IgG	
		+(%)	−(%)			+(%)	−(%)
T-SPOT	+(%)	62.79	0.00	T-SPOT	+(%)	75.81	0.00
	−(%)	37.21	0.00		−(%)	24.19	0.00

T-SPOT Positive (+): CoV-A－Negative control≧8, T-SPOT Negative (－): CoV-A－Negative control ≦7
SARS-CoV-2 IgG Positive (+) ≧ 17.8 BAU/mL, SARS-CoV-2 IgG Negative (－)<17.8 BAU/mL,

A total of 10.94% of the dialysis patients had negative antibody levels 6 months after the primary series, but 57% (6.25%/10.94%) tested positive for T-SPOT.

Regarding the adverse reactions, the incidence rates of pruritus, fatigue, and muscle pain were significantly higher in the HD group ($p = 0.008$, $p = 0.005$, and $p = 0.009$, respectively) than in the control group after the booster dose. When the two groups were further compared according to sex, the incidence rates of systemic adverse reactions (fatigue,

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muscle pain, coldness, and fever) were significantly higher in the HD group than in the control group (p = 0.04, p = 0.008, p = 0.004, and p = 0.001, respectively). In contrast, in women, the incidence rates of local adverse reactions (pain and pruritus) were significantly higher in the HD group than in the control group (p = 0.05 and p = 0.01, respectively) (Table 4).

Table 4. Adverse reactions after vaccination.

All

	Local n (%)				Systemic n (%)									
	pain	redness	swelling	pruritus	fatigue	Headache	muscle pain	coldness	fever	arthralgia	nausea	diarrhea	Stomach ache	anaphylaxis
Control (n=87)	42(48.3)	7(8.0)	5(5.7)	5(5.7)	11(12.6)	6(6.9)	9(10.3)	6(6.9)	10(11.5)	3(3.4)	0(0)	2(2.3)	0(0)	0(0)
HD (n=184)	104(56.5)	18(9.8)	24(13.0)	33(17.9)	52(28.3)	18(9.8)	44(23.9)	28(15.2)	45(24.5)	20(10.9)	7(3.8)	2(1.1)	5(2.7)	0(0)
p-value	0.24	0.82	0.09	**0.008**	**0.005**	0.5	**0.009**	0.08	0.015	0.06	0.1	0.59	0.18	>0.99

Male

	Local n (%)				Systemic n (%)									
	pain	redness	swelling	pruritus	fatigue	headache	muscle pain	coldness	fever	arthralgia	nausea	diarrhea	Stomach ache	anaphylaxis
Control (n=51)	23(45.1)	1(0.2)	1(0.2)	1(0.2)	5(9.8)	1(0.2)	3(5.9)	0(0)	2(3.9)	1(0.2)	0(0)	0(0)	0(0)	0(0)
HD (n =121)	57(47.1)	9(7.4)	11(9.1)	11(9.1)	30(24.8)	6(5.0)	27(22.3)	17(14.0)	29(24.0)	11(9.1)	4(3.3)	2(1.7)	3(2.5)	0(0)
p-value	0.87	0.29	0.11	0.11	**0.04**	0.68	**0.008**	**0.004**	**0.001**	0.11	0.32	>0.99	0.56	>0.99

Female

	Local n (%)				Systemic n (%)									
	pain	redness	swelling	pruritus	fatigue	headache	muscle pain	coldness	fever	arthralgia	nausea	diarrhea	Stomach ache	anaphylaxis
Control (n=36)	19(52.8)	6(16.7)	4(11.1)	4(11.1)	6(16.7)	5(13.9)	6(16.7)	6(16.7)	8(22.2)	2(5.6)	0(0)	2(5.6)	0(0)	0(0)
HD (n=63)	47(74.6)	10(15.9)	13(20.6)	22(34.9)	22(34.9)	12(19.0)	17(27.0)	11(17.5)	16(25.4)	9(14.3)	3(4.8)	0(0)	2(3.2)	0(0)
p-value	**0.05**	>0.99	0.28	**0.01**	0.07	0.59	0.32	>0.99	0.81	0.32	0.55	0.13	0.53	>0.99

HD; hemodialysis

4. Discussion

To investigate the effect of booster vaccination with BNT162b2 in HD patients, immune responses to vaccination were analyzed for both cellular and humoral immunities at the following time points: 6 months after the primary series, 3 weeks after the booster dose, and 3 months after the booster dose. Although most studies on the effects of vaccines have focused on humoral immunity, only antibody levels, this study analyzed the effects of vaccines from both perspectives. In our study, antibody levels in the HD group were higher than those in the control group 3 weeks and 3 months after the booster dose, and T-SPOT, a measure of SARS-COV-2-specific T-cell response, was significantly higher in the dialysis group than in the control group 6 months after the primary series and 3 weeks after the booster dose.

HD patients show lower vaccine antibody titers and decline rapidly for influenza and hepatitis B vaccines [11-13]. In the COVID-19 vaccine, antibody titers were significantly lower in the HD group than in the control group after the primary series of BNT162b2 vaccination [14,15] . Our previous study also showed that dialysis patients had significantly lower antibody levels than controls up to 3 months after the primary series [4]. Our present study is contrary to our prediction of lower antibody levels in HD patients. Simon et al. conducted a similar study on the increase in antibody levels after a booster dose. This study examined SARS-CoV-2-specific antibody titers in controls and HD patients 6–8 weeks after the third vaccine and reported no significant difference in antibody titers between controls and HD responders. However, the controls were younger and more likely to be female than the HD group, although antibody titers are higher in younger people and women than in older people and men [16]. In our study, age- and sex-matched studies were likely evaluated more accurately, resulting in higher antibody titers after the

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booster dose in the HD group than in controls. To date, no immunological studies have been conducted to determine why antibody titers are higher in HD patients than in controls. Thus, the reason behind this remains unclear. However, we hypothesize that B cells differentiate into antibody-producing cells under antigen stimulation and with the assistance of helper T cells, resulting in the production of antibodies. During this process, the priming mechanisms of T and B cells may be abnormal in HD patients; however, once sufficiently primed, they may respond adequately to the vaccine. Further immunological studies are required.

Most studies have focused on antibodies as tools for humoral immunity. However, the T-cell response generally precedes the antibody response because of its necessity for priming B cells and is maintained for a longer period than the antibody response [17]. This has been demonstrated in studies comparing T-cell responses and antibody titers in patients infected with SARS-CoV-2. In some cases, a strong T-cell response occurs, even in the absence of antibody production [18]. Based on previous studies, this study also examined T-cell responses. Although T-cell responses after the first and primary series were not examined in our study, Clarke et al. reported that T-cell responses after the primary series were significantly lower in HD patients than in the control group [19]. This is consistent with the fact that end-stage kidney disease and uremia are associated with T-cell exhaustion and suppression of IFN-γ production. However, Bernard et al. reported that T-cell responses after the primary series were equivalent between HD patients and controls [20]. Clarke noted that these differences may be due to differences in the peptide pool used in the ELISpot assay and the threshold used to define a positive result [19]. Our data at 6 months after the primary series showed that T-SPOT level was significantly higher and the negative rate of T-SPOT was significantly lower in the HD group than in the control group. The detailed reasons for these findings remain unclear, but dialysis patients have a higher percentage of circulating T cells with interleukin (IL)-2 receptors (IL-2R) and that they maintain high levels of plasma-soluble IL-2R, resulting in a chronic preactivation state of T cells [21]. This may explain why the T-cell response was higher even 6 months after the primary series. In the dialysis group, the originally activated T-cell response was further enhanced by the booster dose, and 3 weeks after the booster dose, T-SPOT level was still significantly higher in the dialysis group than in the control group, indicating that high cellular immunity is enhanced in the dialysis patients. These results are similar to those reported by Bruminhent et al. [22]. They examined the number of SARS-CoV-2-specific IFN-γ-producing T cells against S1 protein using the ELISpot assay from Mabtech and found that the number of IFN-γ-producing T cells after the booster dose was not significantly different between the control and HD groups. However, the median value was higher in the HD group after the booster dose than in the control group. Our study was age- and sex-matched, which may have made this difference significant.

Moreover, correlations between antibody levels and T-SPOT levels were examined in our study to investigate the relationship between cellular and humoral immunities. Previous reports of cases of SARS-CoV-2 infection have shown that high antibody levels correlate with high cellular immunity [23]. However, no correlation was observed in our study, and several dialysis patients with negative antibody levels also showed T-cell responses, which may play an important role in the prevention of severe disease, as mentioned by McMahan et al. [7]. Thus, the fact that both cellular and humoral immunities were significantly higher in the dialysis group than in the control group at 3 weeks and 3 months after the booster dose was considered helpful for the prevention of severe disease.

Comparing the age-specific mortality rates of the general population and dialysis patients in Japan from January 6 to June 2, 2022 (period of Omicron), the mortality rates of the general population were 0.18%, 0.94%, and 3.36% in the 60s, 70s, and 80s, respectively, whereas those in dialysis patients in their 60s, 70s, and 80s accounted for 2.40%, 4.30%, and 7.21%, respectively, indicating that the prognosis of dialysis patients remains worse than that of the general population. However, among dialysis patients who received the booster dose, the mortality rates were 0%, 1.14%, and 2.76% for those in their 60s, 70s, 80s, respectively, similar to the rates in the general population [24,25]. As of late May, the

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booster dose rate for those aged ≥ 65 years in the general population was 89.2%. Our results may explain the fact that the mortality rate of dialysis patients has improved after the booster dose compared to the controls. However, given that the innate immune system is lower in dialysis patients compared to controls, higher levels of cellular immunity and antibody levels than in controls may be necessary to protect dialysis patients from infection.

Regarding adverse reactions from the booster dose, systemic adverse reactions were observed to be significantly higher in dialysis patients than in those after the primary series [4]. In dialysis patients, the change in antibody titers before and after the booster dose was significantly higher, suggesting that the vaccine-induced immune response was stronger. This is hypothesized to be the reason for the stronger adverse reactions observed in dialysis patients.

As a limitation, no study has been conducted to determine whether the results of the antibody titer of the present vaccine apply to the results of neutralizing activity. In addition, cellular immunity was evaluated only by T-SPOT, and no other cellular immunity analyses were performed. Further investigation of the mechanism as to why the antibody titer was higher in the dialysis group after the booster dose than in the control group and why the T-cell response was higher in the dialysis group than in the control group is required.

5. Conclusions

HD patients obtained higher antibody titers and SARS-COV-2-specific T-cell responses 3 weeks after the booster dose than control.

Author Contributions: AY, MR and YK designed this study with the approval of the Infection Control Committee of JSDT. AY, MR, KKikuchi, TK, and KS contributed to the participant enrollment. KKatayama measured the antibody titers of patients. AY, MT and EO summarized the clinical information and analyzed the data. MK made significant contributions as a statistical expert. AY, MT, and MR drafted the manuscript. EO, KKikuchi, TK, KS, MK, KKatayama, YU, NO, YK, HK, TS, YT, JT, KH, YN, NHasegawa, NHanafusa, FH, KM, SW, HN, and YT modified the manuscript. All the authors have read and approved the final manuscript.

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Institutional Review Board Statement: The Ethics Committee of the Japanese Society for Dialysis Therapy (JSDT) approved this study (approval number 1-10).

Informed Consent Statement: All participants provided written informed consent for inclusion in this study.

Data Availability Statement: The datasets generated and analyzed during this study are available from the corresponding author on reasonable request.

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