J Korean Med Sci. 2025 Mar 10;40(9):e28
https://doi.org/10.3346/jkms.2025.40.e28
eISSN 1598-6357·pISSN 1011-8934

JKMS

Original Article
Infectious Diseases

Neutralizing Activity and T-Cell Responses Against Wild Type SARS-CoV-2 Virus and Omicron BA.5 Variant After Ancestral SARS-CoV-2 Vaccine Booster Dose in PLWH Receiving ART Based on CD4 T-Cell Count

Received: Jun 14, 2024
Accepted: Oct 9, 2024
Published online: Nov 18, 2024

Address for Correspondence:
Yeon-Sook Kim, MD, PhD
Division of Infectious Diseases, Department of Medicine, Chungnam National University School of Medicine, 282 Munwha-ro, Jung-gu, Daejeon 35015, Republic of Korea.
Email: idalicekim@gmail.com

*Na Young Ha and Ah-Ra Kim contributed equally to this work.

ORCID iDs
Na Young Ha
https://orcid.org/0000-0003-3358-2548
Ah-Ra Kim
https://orcid.org/0000-0001-5610-3787
Hyeongseok Jeong
https://orcid.org/0000-0002-4539-079X
Shinhye Cheon
https://orcid.org/0000-0002-1783-121X
Cho Rong Park
https://orcid.org/0000-0001-8185-2384
Jin Ho Choe
https://orcid.org/0009-0001-1274-2197
Hyo Jung Kim
https://orcid.org/0009-0004-6998-8149

Na Young Ha[1,2*] **Ah-Ra Kim**[3*] **Hyeongseok Jeong**[1,4] **Shinhye Cheon**[1,4] **Cho Rong Park**[1] **Jin Ho Choe**[1] **Hyo Jung Kim**[1] **Jae Won Yoon**[1] **Miryoung Kim**[1] **Mi Yeong An**[1] **Sukyoung Jung**[5] **Hyeon Nam Do**[3] **Junewoo Lee**[3] **and Yeon-Sook Kim**[1,2,4]

[1]Emerging Infectious Diseases Research Institute, Chungnam National University Hospital, Daejeon, Korea
[2]Translational Immunology Institute, Chungnam National University, Daejeon, Korea
[3]Division of Clinical Research for Vaccine, Center for Vaccine Research, Korea National Institute of Infectious Diseases, Cheongju, Korea
[4]Division of Infectious Diseases, Department of Internal Medicine, Chungnam National University School of Medicine, Daejeon, Korea
[5]Department of Health Care Policy Research, Korea Institute for Health and Social Affairs, Sejong, Korea

ABSTRACT

Background: We evaluated severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)-specific humoral and cellular responses for up to 6 months after the 3rd dose of ancestral coronavirus disease 2019 (COVID-19) vaccination in people living with HIV (PLWH) and healthy controls (HCs) who were not infected with COVID-19.
Methods: Anti-spike receptor-binding domain IgG (anti-RBD IgG) concentrations using chemiluminescence immunoassay and neutralizing antibodies using focus reduction neutralization test (FRNT) were assessed at 1 week after each dose of vaccination, and 3 and 6 months after the 3rd dose in 62 PLWH and 25 HCs. T-cell responses using intracellular cytokine stain were evaluated at 1 week before, and 1 week and 6 months after the 3rd dose.
Results: At 1 week after the 3rd dose, adequate anti-RBD IgG (> 300 binding antibody unit /mL) was elicited in all PLWH except for one patient with 36 CD4 T-cell count/mm^3. The geometric mean titers of 50% FRNT against wild type (WT) and omicron BA.5 strains of SARS-CoV-2 in PLWH with CD4 T-cell count ≥ 500 cells/mm^3 (high CD4 recovery, HCDR) were comparable to HC, but they were significantly decreased in PLWH with CD4 T-cell count < 500/mm^3 (low CD4 recovery, LCDR). After adjusting for age, gender, viral suppression, and number of preexisting comorbidities, CD4 T-cell counts < 500/mm^3 significantly predicted a poor magnitude of neutralizing antibodies against WT, omicron BA.5, and XBB 1.5 strains among PLWH. Multivariable linear regression adjusting for age and gender revealed that LCDR was associated with reduced neutralizing activity (P = 0.017) and interferon-γ-producing T-cell responses (P = 0.049 for CD T-cell; P = 0.014 for CD8 T-cell) against WT, and strongly associated with more decreased cross-neutralization against omicron BA.5 strains (P < 0.001).

Jae Won Yoon
https://orcid.org/0000-0003-1316-7136
Miryoung Kim
https://orcid.org/0009-0008-2918-978X
Mi Yeong An
https://orcid.org/0000-0002-9124-2915
Sukyoung Jung
https://orcid.org/0000-0001-9200-2158
Hyeon Nam Do
https://orcid.org/0000-0002-9337-8859
Junewoo Lee
https://orcid.org/0000-0002-2923-5597
Yeon-Sook Kim
https://orcid.org/0000-0003-1142-5488

Funding

This research was supported by the Korea National Institute of Health (KNIH) research project (project No. 2021ER260600), and by the National Research Foundation (NRF) funded by the Korean government (No. 2021M3E5E3080533).

Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Kim YS. Data curation: Ha NY, Jeong H, Cheon S, Kim YS. Formal analysis: Ha NY, Jung S, Kim YS. Funding acquisition: Kim YS. Investigation: Ha NY, Kim AR, Jeong H, Cheon S, Park CR, Choe JH, Kim HJ, Yoon JW, Kim M, An MY, Do H, Lee JW, Kim YS. Methodology: Ha NY, Lee JW, Kim YS. Project administration: Kim YS. Resources: Ha NY, Jeong H, Cheon S, Lee JW, Kim YS. Software: Jung S, Kim YS. Supervision: Kim YS. Validation: Ha NY, Lee JW, Kim YS. Visualization: Ha NY, Kim YS. Writing - original draft: Ha NY, Kim AR, Kim YS. Writing - review & editing: Ha NY, Kim AR, Jeong H, Cheon S, Park CR, Choe JH, Kim HJ, Yoon JW, Kim M, An MY, Jung S, Do H, Lee JW, Kim YS.

Conclusion: HCDR demonstrated robust humoral and cell-mediated immune responses after a booster dose of ancestral SARS-CoV-2 vaccine, whereas LCDR showed diminished immune responses against WT virus and more impaired cross-neutralization against omicron BA.5 strain.

Keywords: COVID-19 Vaccines; HIV; Neutralizing Antibodies; Cellular Immunity

INTRODUCTION

People living with HIV (PLWH) are well known to have suboptimal immune responses to routine vaccines, which are associated with low CD4+ T-cell counts, uncontrolled HIV RNA levels, aging, and presence of multi-comorbidity.[1-3] While the incidence of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection in PLWH is likely influenced by social distancing, health status, and social inequalities, those with low CD4 counts or unsuppressed HIV viral loads are at increased risk of severe coronavirus disease 2019 (COVID-19) outcomes and also, have a high prevalence of comorbidities, such as cardiovascular diseases, diabetes, chronic respiratory diseases and obesity, which are associated with poorer COVID-19 outcomes.[4-6] A study analyzed clinical characteristics of COVID-19 hospitalized patients from 38 countries showed that PLWH had 15% increased risk of severe COVID-19 and were 38% more likely to die in the hospital compared with individuals without HIV.[7] Considering this, the World Health Organization (WHO) has sought to prioritize PLWH with advanced HIV or uncontrolled disease for vaccination and provide them with additional booster doses.[8] The updated 'WHO SAGE roadmap on the uses of COVID-19 vaccines in the context of OMICRON and substantial population immunity' (30 March 2023) places PLWH in the 'high priority' group for vaccination if they have a CD4 count < 200 cells/mm^3, an unsuppressed/detectable viral load or an opportunistic infection.[8]

Data on the immunogenicity elicited by COVID-19 vaccines in PLWH varies and reports conflicting results, reflecting the heterogeneous nature of immune competence within this population.[9-18] Several studies found no significant effect of HIV status on vaccine efficacy in PLWH with normal CD4 counts during the well-controlled status on antiretroviral therapy (ART),[9-12] but other studies observed significantly low antibody responses in PLWH with low CD4 counts and multiple comorbidities.[13-18] In addition, the emergence of omicron variants has raised serious concerns as its unusually high number of amino acid alterations in the spike protein likely contributes to significant immune escape and an increased reinfection risk or breakthrough infections following vaccination.[19,20] Taken together, previous studies mainly demonstrated vaccine-induced binding antibody responses and little is known about T-cell immunity and neutralizing effect against current VOCs such as omicron. Thus, further comprehensive cohort study of vaccine immunogenicity is needed to establish a vaccination policy for PLWH. Our study aimed to compare the longitudinal durability of vaccine-induced binding antibody responses, and the neutralizing effect against wild-type (WT) virus, omicron BA.5, and XBB.1.5 variants in PLWH and HIV-negative healthy controls (HCs) during the 3-dose vaccination. In addition, using intracellular cytokine stain (ICS), we analyzed the SARS-CoV-2 specific T-cell responses after the booster shot.

METHODS

Study design and participants

We enrolled a cohort of HIV-1 infected individuals (n = 62) who were stable on ART and under routine follow-up at Chungnam National University Hospital, Republic of Korea and a cohort of healthy controls (HCs) (n = 25). PLWH participants were then stratified by CD4 count at T0 into two groups according to the size of CD4 count recovery: CD4 T-cell count < 500 cell/mm^3: Low CD4 recovery (LCDR); CD4 T-cell count ≥ 500 cell/mm^3: High CD4 recovery (HCDR). PLWH who were actively undergoing cancer therapies or treatment for severe autoimmune diseases with systemic immunosuppressive therapy were also excluded. Individuals under the age of 18 and older than 80 years old at the time of enrollment were excluded, as were those with allergies to any vaccine ingredient and those who were pregnant or lactating. None of the participants had SARS-CoV-2 infection prior to enrollment and breakthrough SARS-CoV-2 infections during the vaccination were identified with self-reported rapid antigen tests and/or the presence of serum antibodies against SARS-CoV-2 nucleocapsid (N) as measured by the Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, Rotkreuz, Switzerland). All participants were vaccinated with mRNA (BNT162b2 and mRNA1273) or viral vector (ChAdOx1) vaccines according to the government's approved schedules. Sera for evaluating humoral immunity were collected before vaccination (T0), at 1 week after each dose (T2, T3, T5), and at 3 (T6) and 6 (T7) months after the 3rd shot peripheral blood mononuclear cells (PBMCs) for evaluating cellular immunity were prepared at 1 week before (T4), and 1 week after (T5), and 6 months (T7) after the 3rd dose of vaccination. The study design and various assay time points are shown in **Fig. 1A**.

Sample collection

Blood was collected in 5-mL SST tubes, centrifuged at 2,500 rpm for 10 minutes, aliquoted, and stored at −80°C for further experiments. PBMCs were isolated from blood collected in K_3EDTA or lithium heparin tubes by density gradient centrifugation. Briefly, blood was layered on a density gradient (Lymphoprep, STEMCELL Technologies), and PBMCs were separated by centrifuging at 2,000 rpm for 30 minutes. PBMCs were washed three times in phosphate-buffered saline (PBS) and subsequently frozen in liquid nitrogen in 90% fetal bovine serum with 10% DMSO (Sigma, St. Louis, MO, USA) until use in stimulation assays.

Anti-SARS-CoV-2 receptor-binding domain (RBD) IgG antibodies

We measured total binding antibodies against ancestral SARS-CoV-2 spike RBD and nucleocapsid in serum samples using the Elecsys Anti-SARS-CoV-2 S and Anti-SARS-CoV-2 assays respectively then measured on cobas e801 modular analyzers (Roche Diagnostics). All samples were processed according to the manufacturer's instructions and the cut-offs for anti-S and anti-N were 0.8 U/mL and 1.0 cut-off index respectively.

Focus reduction neutralization tests (FRNT)

Heat-inactivated serum samples were serially diluted then incubated with 100–150 focus-forming units of different strains of SARS-CoV-2 (WT, NCCP No. 43326; omicron BA.5, No. 43408; omicron XBB.1.5, No.43440) for 1 hour at 37°C. Antibody-virus complexes were added to Vero cell monolayers in 96-well plates and incubated for 5 hours at 37°C. Subsequently, cells were fixed with 10% formalin in PBS overnight at 4°C. Cells were washed and permeabilized with methanol and incubated with cross-reactive rabbit anti-SARS-CoV-N IgG (Sino Biological) for 1 hour at room temperature before adding HRP-conjugated goat anti-rabbit IgG (H+L) antibody (Bio-Rad, Hercules, CA, USA). SARS-CoV-2–infected cell foci were

https://doi.org/10.3346/jkms.2025.40.e28

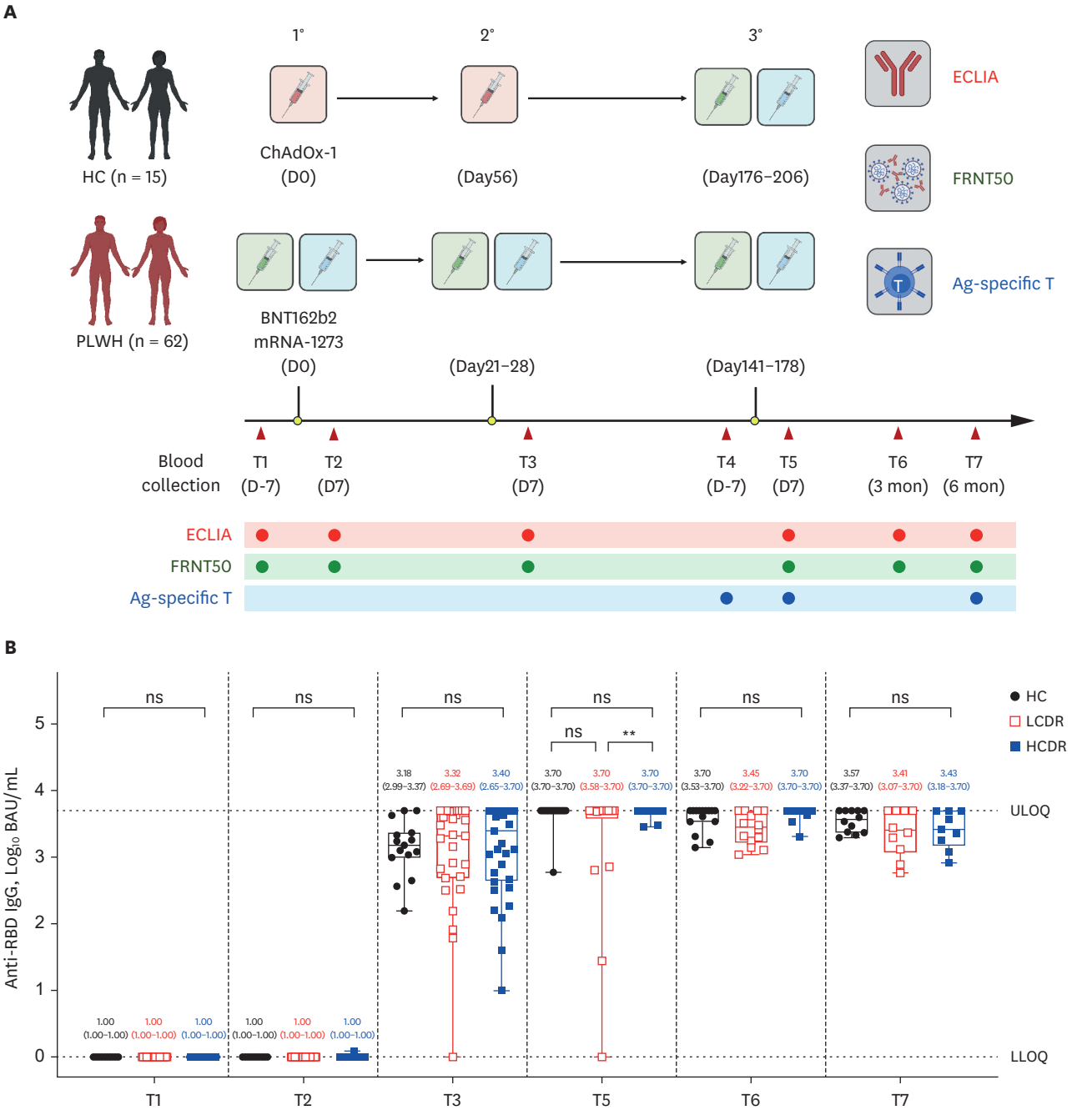

Fig. 1. Longitudinal analysis of anti-SARS-CoV-2 RBD IgG responses in people living with HIV and HCs. (**A**) Schematic of the study design and sampling points' details. (**B**) SARS-CoV-2 anti-RBD IgG levels against the ancestral wild type. Black solid circle represents for HC, red open square represents for LCDR, and blue solid square represents for HCDR. Statistical analysis was performed using the two-sided Kruskal-Wallis test with Dunns' multiple comparisons test.
HC = healthy control, PLWH = people living with human immunodeficiency virus, FRNT50 = 50% focus reduction neutralization test, Ag = antigen, LCDR = low CD4 recovery (CD4 T-cell count < 500 cells/mm^3), HCDR = high CD4 recovery (CD4 T-cell count ≥ 500 cells/mm^3), RBD = receptor-binding domain, Ig = immunoglobulin, BAU = binding antibody unit, SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2, HIV = human immunodeficiency virus, ns = not significant.
$^{**}P < 0.01$.

visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies, Shaker Heights, OH, USA). The 50% neutralizing dose titer was calculated using the Karber formula.

https://doi.org/10.3346/jkms.2025.40.e28

Stimulation for ICS

PBMCs were cultured in the presence of SARS-SoV-2 OLP pools (GenScripts) and 1 μg/mL anti-human CD28 and CD49d mAbs for 6 hours at 37°C. Brefeldin A (GolgiPlug; BD Biosciences, Franklin Lakes, NJ, USA) and monensin (GolgiStop; BD Biosciences) were added 2 hours after the initial stimulation. After incubation, cells were stained with fluorochrome-conjugated antibodies for specific surface markers for 30 minutes at 4°C. Surface marker-stained cells were fixed and permeabilized using the FoxP3 staining buffer kit (Invitrogen, Waltham, MA, USA), and then stained for intracellular markers for 30 minutes at 4°C. The following monoclonal antibodies were used for multi-color flow cytometry: anti-hCD4 BV605 (clone RPA-T4, cat# 565491, 1:100), anti-hCD3 BN785 (clone UCHT1, cat# 565491, 1:100), anti-hCD8 FITC (clone RPA-T8, cat# 555366, 1:50), anti-hIFN-γ PE-Cy7 (clone 4S.B3, cat# 557844, 1:100). Multi-color flow cytometry was performed using a BD Fortessa X-20 instrument, and then the data were analyzed in FlowJo software (FlowJo LLC, Ashland, OR, USA).

Statistical analyses

Descriptive analyses were presented as the median with the interquartile range (IQR) for continuous variables and frequency with proportion for categorical variables. Student's *t*-test, Kruskal-Wallis test, and χ^2 tests were performed to compare the means and proportions between groups.

A multivariable linear regression test was performed to evaluate the association between current CD4 T-cell count and the magnitude of immune response in PLWH after adjustment for the main confounders such as age, gender, achievement of viral suppression (< 50 vs. ≥ 50 copies/mL), and the number of chronic conditions (diabetes, cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver disease, malignancy receiving chemo or immunotherapy, conditions receiving immunosuppressive therapy). A different multivariable linear regression model, adjusted for age and gender, was fitted to evaluate the association between the magnitude of immune response and PLWH groups and HCs.

A $\log_{10}$ transformation was used for RBD-binding IgG and FRNT50 titers to make the data conform more closely to the normal distribution because the distribution of the immunogenic response parameters was positively skewed. Means and standard deviations in the $\log_{10}$ scale are presented.

All tests were 2-sided, with differences considered statistically significant at P value < 0.05. Analyses were performed using SAS statistical Software version 9.4 (SAS Institute, Cary, NC, USA). All figures were generated using GraphPad Prism 9.0 c.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of Chungnam National University Hospital (CNUH-IRB-2021-12-049) and all participants provided written informed consent.

RESULTS

Study cohort (participant characteristics)

A total of 62 PLWH and 25 HCs were enrolled in this study and received two doses of ChAdOx 1, BNT162b, or mRNA-1273 COVID-19 vaccine. Of these, 40 PLWH and 25 healthy controls

JKMS

received a third dose with BNT162b or mRNA-1273 vaccine. The median interval days (IQR) between 2nd and 3rd doses was 108 (97–125) in PLWH and 190 (167–192) in HC. The main characteristics of PLWH participants according to CD4 T-cell count at the initiation of vaccination and HCs are listed in **Table 1**. Briefly, PLWH were broadly similar in age, gender distribution, and number of chronic conditions between LCDR and HCDR groups, but the median years living with HIV (IQR) was 6 (1–12) in LCDR and 12 (6.5–18) HCDR (P = 0.021). All PLWH were on ART at the time of COVID-19 vaccination, and the median years of ART (IQR) were 6 (1–12) in LCDR and 9 (5–14.5) in HCDR (P = 0.018). The proportion of HIV-RNA < 50 copies/mL in PLWH was 75.9% (22/29) in LCDR and 87.9% (29/33) in HCDR. For HC group, 53.3% were male with a younger median age (IQR) 34 years (29–45) than PLWH, whose median age was 56 years (44–62). Anti-N IgG was detected in 4 patients in LCDR, 2 in HCDR, and 7 participants in HC throughout the study period, and they were excluded from the analysis.

Table 1. Main characteristics of people living with HIV and HC at the first dose of SARS-CoV-2 vaccine

Characteristics	PLWH with CD4 < 500 cells/mm³ (n = 29)	PLWH with CD4 ≥ 500 cells/mm³ (n = 33)	*P* value	HCs (n = 25)
Age, median (IQR), yr	53 (36–59)	58 (49–63)	0.055	34 (29–45)
Sex, male/female	28/1	27/6	0.074	9/16
CD4 T-cell count, median (IQR), cells/mm³	256 (205–320)	787 (706–877)	< 0.001	-
HIV RNA < 50 copies/mL	22 (75.9)	29 (87.9)	0.217	
Years living with HIV, median (IQR), yr	6 (1–12)	12 (6.5–18)	0.021	
Hepatitis C virus-Ab positivity	2 (6.9)	1 (3.0)	0.451	
At least 1 chronic condition	5 (17.2)	11 (33.3)	0.211	0
Diabetes	3 (13.8)	5 (15.2)	0.430	
Cardiovascular	0	1 (3.0)	0.532	
COPD/asthma	0	0	1.000	
Neurologic	1 (3.4)	2 (6.1)	0.549	
Renal	0	4 (12.1)	0.144	
Liver cirrhosis	1 (3.4)	0	0.468	
Malignancy receiving chemo or immunotherapy	0	0	1.000	
Conditions receiving immunosuppressive therapy	0	0	1.000	
Current ART	29 (100)	33 (100)	1.000	
Years of ART, median (IQR), yr	6 (1–12)	9 (5–14.5)	0.018	
Vaccine sequence			0.882	
3 doses of BNT162b or mRNA-1273	12 (41.4)	12 (36.4)		16
2 doses of ChAdOx1 +3rd dose of BNT162b or mRNA-1273	7 (24.1)	9 (27.3)		9
2 doses of BNT162b or mRNA-1273	8 (27.6)	8 (24.2)		0
2 doses of ChAdOx1	2 (6.9)	4 (12.1)		0
Blood collection				
T1 (before 1st vaccination)	23	33		10
T2 (1 wk after 1st vaccination)	27	33		15
T3 (1 wk after 2nd vaccination)	27	33		15
T4 (1 wk before 3rd vaccination)	18	19		15
T5 (1 wk after 3rd vaccination)	18	17		15
T6 (3 mon after 3rd vaccination)	14	16		15
T7 (6 mon after 3rd vaccination)	10	9		15
Anti-nucleocapsid IgG (+)				
T1	0	0		0
T2	0	0		0
T3	0	0		0
T4	0	1		0
T5	0	0		0
T6	3	0		0
T7	1	1		7

Values are presented as number (%) unless otherwise indicated.
HC = healthy control, SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2, PLWH = people living with human immunodeficiency virus, IQR = interquartile range, Ab = antibody, COPD = chronic obstructive pulmonary diseases, ART = antiretroviral therapy.

https://doi.org/10.3346/jkms.2025.40.e28

Longitudinal analysis of anti-SARS-CoV-2 RBD IgG antibodies (median serum Ab titer with IQR)

Both PLWH and HC groups showed a significant increase in the magnitude of anti-RBD IgG response against wild type (WT) virus after the 2nd vaccination ($P < 0.001$; **Fig. 1B**). Only one patient with low CD4 T-cell counts (36 cells/mm^3) who had not achieved viral suppression (569 copies/mL) among the LCDR group failed to show any immune response.

The highest levels of SARS-CoV-2 RBD IgG antibodies were observed at 1 week after the 3rd vaccination (T5) in all groups, and the geometric mean titer (GMT) was 3.28 (95% CI, 2.77–3.77) in LCDR, 3.67 (3.63–3.71) in HCDR, and 3.64 (3.50–3.77) in HCs ($P = 0.208$). At 6 months after the 3rd shot (T7), the binding antibody levels decreased to the levels similar to those at 1 week after the 2nd shot (T3) in all groups.

Longitudinal analysis of neutralizing antibodies

Neutralizing antibodies (nAbs) were measured using FRNT with WT SARS-CoV-2 virus, omicron BA.5, and omicron XBB.1.5 variants. Neutralization activity was substantially increased in all groups according to the number of vaccinations, peaking at 1 week after the 3rd dose (**Fig. 2A**) and then decreased to the levels originally elicited by two-dose vaccination in all groups at 6 months after the 3rd shot (T7).

SARS-CoV-2 ancestral vaccine elicited significant nAbs against WT in HCDR, comparable to HC. However, the LCDR group showed significantly decreased neutralization activity against WT, compared to HC at 1 week after the 2nd (T3) and 3rd vaccination (T5) ($P < 0.001$). The level of nAb response against WT at T5 was lower in HCDR than HC ($P = 0.004$) At T5, the GMT of 50% FRNT (FRNT50) against wild type (WT) virus was 3.38 (95% CI, 2.86–3.75) in LCDR, 3.80 (95% CI, 3.61–3.94) in HCDR, and 3.93 (95% CI, 3.43–4.19; $P = 0.110$) in HC. The mean (± standard deviation, SD) fold reduction in antibody titers from T5 to T6 was –0.244 (± 0.059) per month in LCDR, –0.216 (± 0.079) in HCDR, and –0.247 (± 0.080) in HC ($P = 0.586$). The mean (± SD) fold reduction from T6 to T7 was –0.247 (± 0.078) in LCDR, –0.292 (± 0.063) in HCDR, and –0.311 (± 0.009) in HC ($P = 0.276$).

Omicron BA.5-specific neutralization responses were significantly less elicited than those against WT virus at all timepoints for all groups (**Fig. 2 B**), and the immunologic responses in PLWH, especially in LCDR, were further impaired, compared to HC at T5 ($P < 0.001$). The GMT of FRNT50 against omicron BA.5 variant at T5 was 2.19 (1.60–2.57) in LCDR, 2.73 (2.28–3.05) in HCDR, and 3.11 (2.93–3.41) in HCs ($P = 0.002$). The mean log10 difference of nAbs between against BA.5 and WT was –1.19 (–1.26, –1.18) in LCDR, –1.07 (–1.33, –0.89) in HCDR, and –0.82 (–0.78, –0.5) in HCs.

Neutralizing activity against omicron XBB.1.5 elicited by ancestral SARS-CoV-2 vaccination was even poorer than that against omicron BA.5, and the GMT of FRNT50 was less than 2 in all groups (**Fig. 2C**).

SARS-CoV-2 specific T cell responses after the third vaccination

We found significantly increased frequencies of IFN-γ-producing CD4+ and CD8+T cells after WT-spike peptide stimulation in all groups from 1 week before the 3rd dose (T4) to 1 week after the 3rd dose (T5). At 6 months after the 3rd dose (T7), the frequencies of IFN-γ-producing CD4+ and CD8+T cells declined to levels similar to those before the 3rd vaccination.

https://doi.org/10.3346/jkms.2025.40.e28

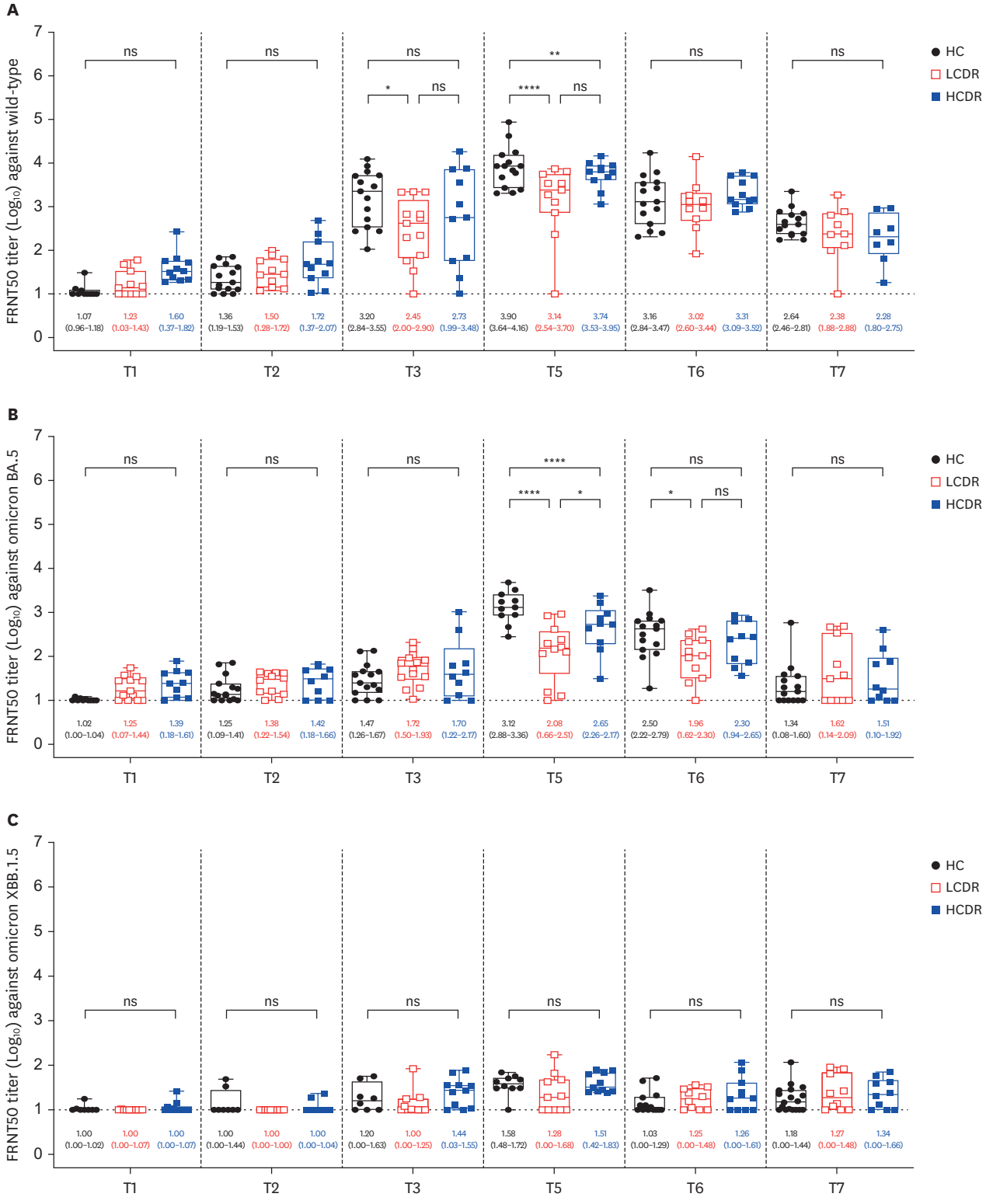

Fig. 2. Longitudinal Analysis of neutralizing activity against live wild-type (**A**), omicron BA.5 (**B**), and XBB.1.5 (**C**) variant virus in PLWH and HC. Neutralizing activity between PLWH and HC was compared at indicated time points. Black solid circle represents for HC, red open square represents for LCDR, and blue solid square represents for HCDR. Statistical analysis was performed using the two-sided Kruskal-Wallis test with Dunns' multiple comparisons test.
FRNT50 = 50% focus reduction neutralizing titer, HC = healthy control, HCDR = high CD4 recovery (CD4 T-cell count ≥ 500 cells/mm^3), LCDR = low CD4 recovery (CD4 T-cell count < 500 cells/mm^3), PLWH = people living with human immunodeficiency virus, ns = not significant.
$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

https://doi.org/10.3346/jkms.2025.40.e28

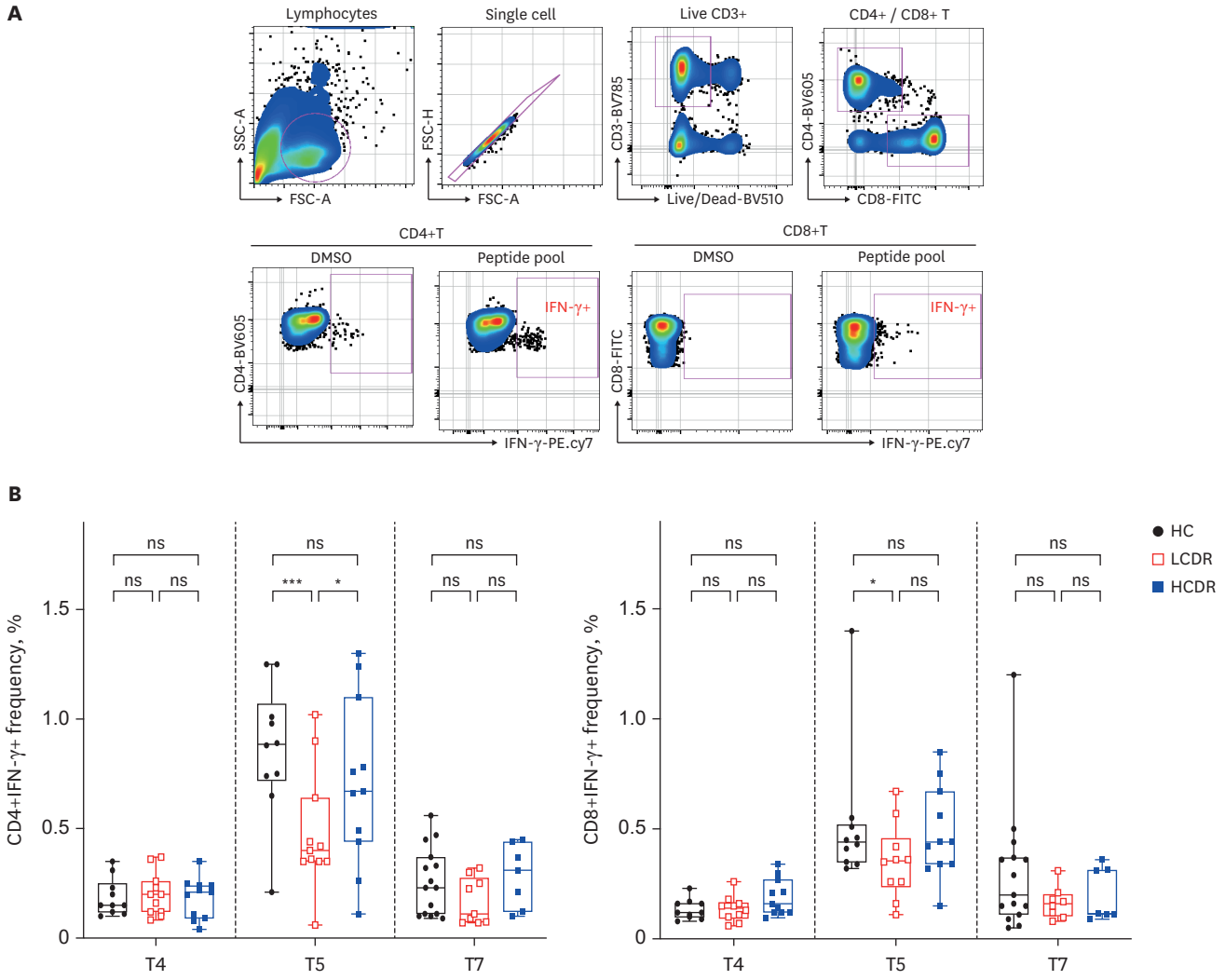

Fig. 3. T-cell responses induced by the booster vaccination in PLWH and HC. Frequency of IFNγ-producing CD4+ and CD8+T cells was compared at indicated time point. (**A**) Flow cytometry gating strategy. (**B**) The frequency of IFN-γ-producing CD4+ (left) and CD8+T cells (right). Two-way ANOVA corrected for multiple comparisons in (**B**).
DMSO = dimethylsulfoxide, HC = healthy control, HCDR = high CD4 recovery (CD4 T-cell count ≥ 500 cells/mm³), IFN-γ = interferon-gamma, LCDR = low CD4 recovery (CD4 T-cell count < 500 cells/mm³), PLWH = people living with human immunodeficiency virus, ns = not significant.
$^{*}P < 0.05$, $^{***}P < 0.001$.

The magnitude of IFN-γ-producing CD4+T-cell responses was diminished with statistical significance in LCDR, compared to those in HC ($P < 0.001$) and in HCDR ($P = 0.035$). IFN-γ-producing CD8+T-cell responses were also decreased in LCDR, compared to HC ($P = 0.014$) but, not different from HCDR (**Fig. 3**).

Role of CD4 T-cell count at the initiation of vaccination in predicting immunogenicity in PLWH

In PLWH, having the magnitude of anti-RBD IgG, nAb titers, and IFN-γ production as dependent covariates, CD4 T-cell count < 500/mm³ at the initiation of vaccination was associated with a significantly lower magnitude of nAbs against WT, omicron BA.5, and XBB 1.5 after adjusting for the main identified confounders (age, gender, achievement of viral suppression under 50 copies/mL, and number of chronic conditions) at 1 week after the 3rd dose of vaccine (**Table 2**).

https://doi.org/10.3346/jkms.2025.40.e28

Table 2. Analysis of CD4 T-cell count at the initiation of vaccination as an independent predictor of magnitude of immune response to vaccination among PLWH at T5

CD4 T-cell count, cells/mm³	Unadjusted			Adjusted[a]		
	Estimate	95% CI	*P* value	Estimate	95% CI	*P* value
RBD-binding IgG (BAU/mL)			0.116			0.169
< 500	−0.97	−2.2 to 0.25		−0.94	−2.31 to 0.42	
≥ 500	Ref			Ref		
FRNT50 (Wuhan)			0.039			0.035
< 500	−1.40	−2.73 to −0.08		−1.36	−2.6 to −0.11	
≥ 500	Ref			Ref		
FRNT50 (Omi BA.5)			0.044			0.031
< 500	−1.31	−2.57 to −0.04		−1.50	−2.83 to −0.16	
≥ 500	Ref			Ref		
FRNT50 (XBB 1.5)			0.044			0.049
< 500	−1.06	−2.09 to −0.03		−1.24	−2.48 to −0.01	
≥ 500	Ref			Ref		
CD4+ IFN-γ+			0.230			0.161
< 500	−0.39	−1.04 to 0.27		−0.53	−1.31 to 0.24	
≥ 500	Ref			Ref		
CD8+ IFN-γ+			0.152			0.507
< 500	−0.34	−0.81 to 0.14		−0.15	−0.64 to 0.33	
≥ 500	Ref			Ref		

PLWH = people living with human immunodeficiency virus, CI = confidence interval, RBD = receptor-binding domain, Ig = immunoglobulin, BAU = binding antibody unit, FRNT50 = 50% focus reduction neutralization test, IFN = interferon.
[a]Adjusted for age (years), gender, achievement of viral suppression (< 50 or > 50 copies/mL), and number of chronic conditions.

Comparisons of immunogenicity of SARS-CoV-2 vaccine between PLWH and HCWs

Using HC as the reference, after adjustment for age and gender by multivariable linear regression, a significant association between LCDR and a reduction in the magnitude of the immune response was found for nAb response against WT ($P = 0.017$) and omicron BA.5 ($P < 0.001$), and the frequencies of IFN-γ-producing CD4+ T-cells ($P = 0.049$) and CD8+ T-cells ($P = 0.014$) at 1 week after the 3rd vaccine (**Table 3**).

DISCUSSION

This study showed that a 3rd dose of ancestral SARS-CoV-2 vaccine elicited robust humoral and cell-mediated immune responses against WT strain in most PLWH receiving ART. The immunogenicity was strongly associated with the CD4 T-cell count at the initiation of vaccination, thus the immune responses in PLWH with CD4 T-cell count > 500 cells/mm³ were comparable to those observed in HC. In PLWH with low CD4 T-cell count, however, nAbs and T-cell responses were diminished with statistical significance even after adjusting for age and gender, compared to HC. Cross-neutralization against omicron BA.5 was also induced by ancestral SARS-CoV-2 vaccine in both PLWH and HC, but its magnitude was lower than that measured against WT, and it was more diminished in LCDR.

Our findings are consistent with previously reported data on the characterization of the humoral and T-cell responses to 3 doses of COVID-19 vaccines in PLWH receiving ART.[11,12,16-18] Antinori et al.[16] and Vergori et al.[17,18] reported immune response parameters including neutralizing antibodies and IFN-γ release were elicited in most of PLWH, although they were significantly lower in PLWH with current CD4 < 200 cells/mm³. Lapointe et al.[12] showed that there was no significant impact of HIV infection on antibody outcome measure in PLWH with high CD4 T-cell counts (median counts, 645) after adjustment for sociodemographic and health variables.

https://doi.org/10.3346/jkms.2025.40.e28

Table 3. Multivariable linear regression models of factors associated with magnitude of immune responses with different groups

CD4 T-cell count, cells/mm^3	Estimate	95% CI	*P* value
Anti-RBD IgG (BAU/mL)			
< 500	−0.64	−1.99 to 0.71	0.345
≥ 500	0.39	−0.91 to 1.69	0.550
Healthy control	Ref		
FRNT50 (Wuhan)			
< 500	−1.73	−3.13 to −1.34	0.017
≥ 500	−0.29	−1.59 to 1.02	0.659
Healthy control	Ref		
FRNT50 (Omi BA.5)			
< 500	−2.74	−4.1 to −1.37	< 0.001
≥ 500	−1.28	−2.61 to 0.04	0.057
Healthy control	Ref		
FRNT50 (XBB 1.5)			
< 500	−0.69	−1.79 to 0.42	0.214
≥ 500	0.30	−0.75 to 1.36	0.559
Healthy control	Ref		
CD4+ IFN-γ+			
< 500	−1.00	−1.49 to 0	0.049
≥ 500	−0.45	−0.94 to 0.5	0.541
Healthy control	Ref		
CD8+ IFN-γ+			
< 500	−0.64	−1.14 to −0.14	0.014
≥ 500	−0.39	−0.87 to 0.09	0.104
Healthy control	Ref		

CI = confidence interval, RBD = receptor-binding domain, Ig = immunoglobulin, BAU = binding antibody unit, FRNT50 = 50% focus reduction neutralization test, IFN = interferon.
[a]Adjusted for age (years) and gender.

Notably, cross-neutralization activity against omicron BA.5 induced by the ancestral SARS-CoV-2 vaccine in HCDR was not significantly different from that in HC after controlling for age and gender, although the magnitude was lower than that against WT. This demonstrates a certain degree of cross-reactive immunity achieved by the ancestral SARS-CoV-2 vaccine, though it is less protective in both PLWH and HC. In LCDR, however, nAb titers against omicron BA.5 were decreased with statistical significance compared to HC even after adjusting for age and gender. This suggests that the magnitude of cross-neutralization against BA.5 might be also associated with CD4 T-cell count. Our finding is in contrast to the data that neutralization activity against SARS-CoV-2 BA.5 strain was independent of the CD4 T-cell count demonstrated by Corma-Gómez et al.[21] However, their data showed that cross-neutralization activity against omicron B. 1, B. 1.617.2, BA.1 and BA.2 strains was significantly inferior among PLWH with CD4 T-cell count < 200 cells/mm^3.

It is suggested that vaccine-induced antibodies may wane more quickly in PLWH than in healthy individuals.[22] It has been reported that the CD4 count is critical in orchestrating an optimal immune response induced by a vaccine.[23-25] In PLWH, the CD4 T-cell count could diminish the differentiation of effective and long-lived memory B and T-cell immunity.[26,27] Indeed, the duration of seroprotection against *S. pneumoniae* and hepatitis B was reported to be shorter in PLWH than in otherwise healthy persons.[1] Therefore, monitoring of antibody levels and timing of revaccination in these patients was implicated by some experts.[1-3] Our study, however, showed that SARS-CoV-2-vaccine-induced neutralizing Abs against WT did not decline faster more quickly in PLWH even in LCDR. This finding contrasts with antibody responses elicited by other general vaccines in PLWH.[1]

https://doi.org/10.3346/jkms.2025.40.e28

T-cell responses were known to last for a longer duration after SARS-CoV-2 infection or vaccination in many studies.[26-28] Our study demonstrated robust IFN-γ-producing CD4+ and CD8+T cell responses after WT-spike peptide stimulation at 1 week after the 3rd dose vaccination, but they declined in both PLWH and HCs at 6 months after the 3rd vaccination. Reinscheid et al.[29] also showed spike-specific T-cells are activated after the 3rd vaccination, but it was followed by a contraction phase and lasts only for about 30–60 days.[29] They concluded the steep decline of spike-specific T-cell response caused by booster vaccination is in contrast to a more prolonged contraction reported for non-spike epitope-specific CD8+ T-cells after SARS-CoV-2 infection. Differences in individual HLA type, antigen-half life, innate immunity, co-stimulation, and cytokines are suggested to make the difference in virus-specific CD8+ T-cell kinetics induced by SARS-CoV-2 infection versus mRNA vaccination.[30-32]

Our study has several limitations. First, it was designed as an observational and nonrandomized study having no matched HIV-negative control group. However, the comparisons with HC were controlled for gender and age. Second, the sample size was so small that our data stratified the PLWH into two groups according to the size of CD4 count recovery more or less than 500 cells/mm^3. Recruiting PLWH with CD4 cell counts < 200 cells/μL was also challenging since most Korean PLWH receiving ART achieve good immunological control. Despite this challenge, the median CD4 T-cell count in LCDR of our cohort was 256 cells/μL (IQR, 205–320). Third, previous SARS-CoV-2 infection has been excluded by detecting anti-N IgG at each sampling point for asymptomatic participants. Since PLWH have a low possibility of revealing detectable anti-N IgG after COVID-19 than HC, testing anti-N IgG only could have missed previous COVID-19 episodes.[33] To the best of our knowledge, the present study is the first to characterize the humoral and cellular immune response and its durability against WT and omicron variants BA.5, and XBB.1.5. after a booster dose of SARS-CoV-2 vaccination among PLWH. Our findings may provide helpful information to guide public health interventions among immunocompromised population including PLWH.

In conclusion, a booster dose of ancestral SARS-CoV-2 vaccine elicited robust humoral and cell-mediated immune responses against WT virus and cross-neutralization against omicron BA.5 strain in PLWH with CD4 T-cell count ≥ 500 cells/mm^3 which was comparable to HC, whereas PLWH with low CD4 T-cell count showed diminished immune responses. PLWH, especially those with LCDR, should be provided with vaccine booster containing the recently circulating variant strains since the immune responses against the vaccine strains and potential cross-reaction against new variant ones are reduced among this group.

REFERENCES

1. Kernéis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boëlle PY. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. *Clin Infect Dis* 2014;58(8):1130-9. PUBMED | CROSSREF
2. Parmigiani A, Alcaide ML, Freguja R, Pallikkuth S, Frasca D, Fischl MA, et al. Impaired antibody response to influenza vaccine in HIV-infected and uninfected aging women is associated with immune activation and inflammation. *PLoS One* 2013;8(11):e79816. PUBMED | CROSSREF
3. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med* 2011;62(1):141-55. PUBMED | CROSSREF

4. Danwang C, Noubiap JJ, Robert A, Yombi JC. Outcomes of patients with HIV and COVID-19 co-infection: a systematic review and meta-analysis. *AIDS Res Ther* 2022;19(1):3. PUBMED | CROSSREF
5. Ambrosioni J, Blanco JL, Reyes-Urueña JM, Davies MA, Sued O, Marcos MA, et al. Overview of SARS-CoV-2 infection in adults living with HIV. *Lancet HIV* 2021;8(5):e294-305. PUBMED | CROSSREF
6. Geretti AM, Stockdale AJ, Kelly SH, Cevik M, Collins S, Waters L, et al. Outcomes of coronavirus disease 2019 (COVID-19) related hospitalization among people with human immunodeficiency virus (HIV) in the ISARIC World Health Organization (WHO) Clinical Characterization Protocol (UK): a prospective observational study. *Clin Infect Dis* 2021;73(7):e2095-106. PUBMED | CROSSREF
7. Bertagnolio S, Thwin SS, Silva R, Nagarajan S, Jassat W, Fowler R, et al. Clinical features of, and risk factors for, severe or fatal COVID-19 among people living with HIV admitted to hospital: analysis of data from the WHO Global Clinical Platform of COVID-19. *Lancet HIV* 2022;9(7):e486-95. PUBMED | CROSSREF
8. World Health Organization. Coronavirus disease (COVID-19) and people living with HIV. https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-(covid-19)-covid-19-and-people-living-with-hiv. Updated 2023. Accessed May 15, 2024.
9. Levy I, Wieder-Finesod A, Litchevsky V, Biber A, Indenbaum V, Olmer L, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in people living with HIV-1. *Clin Microbiol Infect* 2021;27(12):1851-5. PUBMED | CROSSREF
10. Brumme ZL, Mwimanzi F, Lapointe HR, Cheung PK, Sang Y, Duncan MC, et al. Humoral immune responses to COVID-19 vaccination in people living with HIV receiving suppressive antiretroviral therapy. *NPJ Vaccines* 2022;7(1):28. PUBMED | CROSSREF
11. Costiniuk CT, Singer J, Lee T, Langlois MA, Arnold C, Galipeau Y, et al. COVID-19 vaccine immunogenicity in people with HIV. *AIDS* 2023;37(1):F1-10. PUBMED | CROSSREF
12. Lapointe HR, Mwimanzi F, Cheung PK, Sang Y, Yaseen F, Speckmaier S, et al. Antibody response durability following three-dose coronavirus disease 2019 vaccination in people with HIV receiving suppressive antiretroviral therapy. *AIDS* 2023;37(5):709-21. PUBMED | CROSSREF
13. Noe S, Ochana N, Wiese C, Schabaz F, Von Krosigk A, Heldwein S, et al. Humoral response to SARS-CoV-2 vaccines in people living with HIV. *Infection* 2022;50(3):617-23. PUBMED | CROSSREF
14. Tau L, Turner D, Adler A, Marom R, Ahsanov S, Matus N, et al. SARS-CoV-2 humoral and cellular immune responses of patients with HIV after vaccination with BNT162b2 mRNA COVID-19 vaccine in the Tel-Aviv Medical Center. *Open Forum Infect Dis* 2022;9(4):ofac089. PUBMED | CROSSREF
15. Benet S, Blanch-Lombarte O, Ainsua-Enrich E, Pedreño-Lopez N, Muñoz-Basagoiti J, Raïch-Regué D, et al. Limited humoral and specific T-cell responses after SARS-CoV-2 vaccination in PLWH with poor immune reconstitution. *J Infect Dis* 2022;226(11):1913-23. PUBMED | CROSSREF
16. Antinori A, Cicalini S, Meschi S, Bordoni V, Lorenzini P, Vergori A, et al. Humoral and cellular immune response elicited by mRNA vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in people living with human immunodeficiency virus receiving antiretroviral therapy based on current CD4 T-lymphocyte count. *Clin Infect Dis* 2022;75(1):e552-63. PUBMED | CROSSREF
17. Vergori A, Cozzi Lepri A, Cicalini S, Matusali G, Bordoni V, Lanini S, et al. Immunogenicity to COVID-19 mRNA vaccine third dose in people living with HIV. *Nat Commun* 2022;13(1):4922. PUBMED | CROSSREF
18. Vergori A, Cozzi-Lepri A, Matusali G, Colavita F, Cicalini S, Gallì P, et al. SARS-CoV-2 omicron variant neutralization after third dose vaccination in PLWH. *Viruses* 2022;14(8):1710. PUBMED | CROSSREF
19. Tan ST, Kwan AT, Rodríguez-Barraquer I, Singer BJ, Park HJ, Lewnard JA, et al. Infectiousness of SARS-CoV-2 breakthrough infections and reinfections during the omicron wave. *Nat Med* 2023;29(2):358-65. PUBMED | CROSSREF
20. Ai J, Zhang H, Zhang Y, Lin K, Zhang Y, Wu J, et al. Omicron variant showed lower neutralizing sensitivity than other SARS-CoV-2 variants to immune sera elicited by vaccines after boost. *Emerg Microbes Infect* 2022;11(1):337-43. PUBMED | CROSSREF
21. Corma-Gómez A, Fernández-Fuertes M, Viñuela L, Domínguez C, Santos M, Fuentes-López A, et al. Reduced neutralizing antibody response to SARS-CoV-2 vaccine booster dose in people living with HIV with severe immunosuppression. *J Med Virol* 2023;95(3):e28602. PUBMED | CROSSREF
22. Levy I, Rahav G. The effect of HIV on COVID-19 vaccine responses. *Curr Opin HIV AIDS* 2023;18(3):135-41. PUBMED | CROSSREF
23. Alrubayyi A, Gea-Mallorquí E, Touizer E, Hameiri-Bowen D, Kopycinski J, Charlton B, et al. Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with HIV. *Nat Commun* 2021;12(1):5839. PUBMED | CROSSREF
24. Spinelli MA, Peluso MJ, Lynch KL, Yun C, Glidden DV, Henrich TJ, et al. Differences in post-mRNA vaccination SARSCoV-2 IgG concentrations and surrogate virus neutralization test response by HIV status

and type of vaccine: a matched case-control observational study. *Clin Infect Dis* 2022;75(1):e916-9. PUBMED | CROSSREF

25. Painter MM, Mathew D, Goel RR, Apostolidis SA, Pattekar A, Kuthuru O, et al. Rapid induction of antigen-specific CD4$^+$ T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity* 2021;54(9):2133-2142.e3. PUBMED | CROSSREF
26. Datwani S, Kalikawe R, Waterworth R, Mwimanzi FM, Liang R, Sang Y, et al. T-cell responses to COVID-19 vaccines and breakthrough infection in people living with HIV receiving antiretroviral therapy. *Viruses* 2024;16(5):661. PUBMED | CROSSREF
27. Arunachalam PS, Lai L, Samaha H, Feng Y, Hu M, Hui HS, et al. Durability of immune responses to mRNA booster vaccination against COVID-19. *J Clin Invest* 2023;133(10):e167955. PUBMED | CROSSREF
28. Pitiriga VC, Papamentzelopoulou M, Konstantinakou KE, Vasileiou IV, Konstantinidis AD, Spyrou NI, et al. Pronlonged SARS-CoV-2 T cell responses in a vaccinated COVID-19-naïve population. *Vaccines (Basel)* 2024;12(3):270. PUBMED | CROSSREF
29. Reinscheid M, Luxenburger H, Karl V, Graeser A, Giese S, Ciminski K, et al. COVID-19 mRNA booster vaccine induces transient CD8+ T effector cell responses while conserving the memory pool for subsequent reactivation. *Nat Commun* 2022;13(1):4631. PUBMED | CROSSREF
30. Mentzer AJ, O'Connor D, Bibi S, Chelysheva I, Clutterbuck EA, Demissie T, et al. Human leukocyte antigen alleles associate with COVID-19 vaccine immunogenicity and risk of breakthrough infection. *Nat Med* 2023;29(1):147-57. PUBMED | CROSSREF
31. Dan J, da Silva Antunes R, Grifoni A, Weiskopf D, Crotty S, Sette A. Observations and perspectives on adaptive immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2022;75 Suppl 1:S24-9. PUBMED | CROSSREF
32. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021;184(4):861-80. PUBMED | CROSSREF
33. Macías J, Fernández-Fuertes M, Oliver N, Corma-Gómez A, Real LM, Pineda JA. Lower probability of persistence of total anti-SARS-CoV-2 antibodies after COVID-19 among people living with HIV. *Clin Microbiol Infect* 2022;28(5):755-6. PUBMED | CROSSREF