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Humoral and cellular immunogenicity of COVID-19 vaccine boosters in participants with advanced HIV disease

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SUMMARY

Background: People living with HIV (PLWH) who experience advanced immunosuppression are susceptible to severe COVID-19 and demonstrate compromised vaccine responses due to low CD4 counts and uncontrolled HIV viral load. Although vaccine boosters enhance immunity in the general population, their immunogenicity in individuals with advanced HIV remains inadequately characterised.
Methods: This study evaluated the humoral and cellular immunogenicity of COVID-19 vaccine boosters in 41 individuals with advanced HIV at baseline and 4 weeks post-vaccination. Binding antibodies, neutralising antibodies, antibody-dependent cellular cytotoxicity (ADCC), as well as spike-specific CD4+ and CD8+ T-cell responses were quantified and characterised.
Results: Booster vaccination was found to increase binding antibody titres (8.0-fold) and neutralising activity (3.9-fold), even among participants with CD4 counts < 100 cells/mm³, although absolute responses remained lower than the controls. ADCC activity also modestly increased post-vaccination (2.1-fold). Spike-specific CD4+ T-cell responses increased in magnitude (0.001% to 0.160%, p=0.0001) and responder frequency (49% to 83%, p=0.0167) post-vaccination, while CD8+ T-cell responses remained low. Compared to the controls, PLWH had similar magnitudes of spike-specific CD4+ T-cell responses but significantly lower CD8+ T-cell responses.
Conclusion: COVID-19 vaccine boosters enhance immunity in PLWH, however, the responses remain suboptimal compared to immunocompetent individuals, emphasising the need for tailored vaccination strategies.

Introduction

The rapid emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 caused significant concern regarding populations that are particularly vulnerable to infection and poor clinical outcomes, such as immunosuppressed individuals, including people living with HIV (PLWH).[1,2] HIV infection progressively depletes CD4+ T-cell counts, thereby compromising cellular

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immunity and heightening susceptibility to opportunistic infections. In the context of coronavirus disease 2019 (COVID-19), individuals with advanced HIV disease, i.e., CD4+ T-cell counts < 200 cells/mm³, were shown to be at significantly increased risk of severe illness and hospitalisation compared to people not living with HIV (PNLWH).[3,4] Furthermore, PLWH not receiving antiretroviral therapy (ART), with unsuppressed HIV viral loads (VLs) and low CD4+ T-cell counts, face even greater odds of severe COVID-19 outcomes.[5,6] This heightened vulnerability is largely attributable to the compromised immune function that characterises advanced stages of HIV infection.[2,7]

While ART enables viral suppression and promotes the restoration of CD4+ T-cell levels,[8,9] PLWH may continue to experience persistent immune activation and systemic inflammation.[10] This chronic immune dysregulation not only affects their long-term response to HIV treatment but may also influence their outcomes when confronted with infections or vaccines. Reportedly, PLWH with well-controlled HIV do not appear to be at significantly greater risk for adverse COVID-19 outcomes compared to PNLWH. Indeed, emerging evidence suggests that well-managed PLWH with high CD4+ T-cell counts may experience similar, or in some cases, even more favourable, COVID-19 outcomes relative to those without HIV.[4,6,11] This underscores the critical importance of maintaining effective HIV control through consistent adherence to ART and immune system preservation to mitigate COVID-19-related risks in this population.

This is particularly important for individuals with advanced immunosuppression, who are at heightened risk for breakthrough infections and prolonged viral shedding.[2,12,13] Given the ongoing evolution of SARS-CoV-2 and the documented decline in vaccine-induced immunity over time, ensuring timely and equitable access to booster vaccinations remains essential for sustaining long-term protection in this vulnerable population.

Very little is, however, known about the immunogenicity of COVID-19 vaccines in people with untreated or advanced HIV, especially in the highest-burden settings, such as sub-Saharan Africa. Therefore, in the current study, the humoral and cellular immunogenicity of COVID-19 vaccine boosters in PLWH with advanced HIV infection seen at a tertiary hospital in the Tshwane District, South Africa, was evaluated. We hypothesised that COVID-19 vaccine boosters would enhance immune responses in individuals with advanced HIV disease; however, these responses would be less robust compared to the controls, due to the underlying immune dysregulation associated with HIV. Conducting this study in a South African cohort offers critical insights into the efficacy of booster vaccinations in a population at increased risk of COVID-19.

Table 1
Demographics and clinical characteristics of the participants.

	PLWH	Control A	Control B
Total (*n*)	41	9	26
Age (median, IQR)	43 (34–50)	42 (24–69)	41 (34–54)
Sex *n* (%)			
Male	24 (59%)	2 (22%)	7 (27%)
Female	17 (41%)	7 (88%)	19 (73%)
Vaccination *n* (%)[a]	41 (100%)	9 (100%)	0 (0%)
Pfizer	27 (66%)	9 (100%)	
JnJ	14 (34%)	0 (0%)	
HIV infection *n* (%)			
HIV positive	41 (100%)		
Viraemic	37 (90%)		
CD4+ T-cell count (cells/mm³) (median, IQR)	104 (47–161)		
Viral load (copies/mL) (median, IQR)	3480 (702–35200)		
Aviraemic[b]	4 (10%)		
CD4+ T-cell count (cells/mm³) (median, IQR)	41 (4–132)		
ART[c]	39 (95%)		
ART initiation (months)	19 (5–57)		
ART regimen *n* (%)			
Tenofovir/lamivudine/dolutegravir	26 (63%)		
Zidovudine/lamivudine/dolutegravir	3 (7%)		
Abacavir/lamivudine/dolutegravir	2 (5%)		
Zidovudine/lamivudine/lopinavir/ritonavir	2 (5%)		
Zidovudine/lamivudine/atazanavir/ritonavir	1 (2%)		
Efavirenz	1 (2%)		
Co-morbidities *n* (%)	11 (27%)	0 (0%)	8 (31%)
Hypertension	5 (12%)		
Chronic kidney disease	3 (7%)		
Rheumatoid arthritis	1 (2%)		
Gastrointestinal disease	1 (2%)		
Eczema	1 (2%)		
Anaemia	1 (2%)		
None	30 (73%)		
Concomitant treatment *n* (%)	23 (56%)		
Cotrimoxazole	15 (37%)		
Pyridoxine	6 (15%)		
Isoniazid	4 (10%)		
Amlodipine	3 (7%)		
Hydrochlorothiazide	3 (7%)		
Methotrexate	3 (6%)		
Tramadol	2 (5%)		
None	18 (44%)		

[a] Received booster vaccination during the study,
[b] HIV viral load below the limit of detection i.e., < 40 copies/mL,
[c] Antiretroviral therapy (ART).

Methods

Study design and participants

This study was conducted at the Steve Biko Academic Hospital (SBAH) COVID-19 vaccination site in Pretoria, South Africa, between 03 February 2023 and 27 December 2023. The study included adults (≥18 years) with advanced HIV disease who opted to receive a COVID-19 vaccination of their choice. Eligibility criteria required a confirmed diagnosis of advanced HIV (i.e. CD4+ T-cell count < 200 cell/mm³ or World Health Organization [WHO] Stage 4 disease) and qualification for an additional vaccination dose according to the South African National Department of Health's guideline, regardless of prior vaccination status. This included individuals who were unvaccinated, those who had received one or two doses of the Johnson & Johnson (JnJ) (Janssen) (Ad26. COV2. S) or Pfizer (BNT162b2) vaccines, those who had received heterologous booster doses, and individuals eligible for a second booster. Exclusion criteria included individuals under 18 years of age, those hospitalised with severe illness, fully vaccinated individuals, and those participating in other vaccine trials. Demographic and clinical data, including medical history, co-morbidities, vaccination history, and prior SARS-CoV-2 infection, were extracted from hospital records and the National Health Laboratory Services (NHLS, Tshwane District, Pretoria, South Africa) database. A total of 41 PLWH were enroled in the study. For each participant, HIV VLs were determined using a polymerase chain reaction (PCR)-based diagnostic assay, Abbott Alinity (NHLS). Absolute blood CD4+ T-cell counts were determined by the PanLeucoGating (PLG) method (NHLS). Blood samples were collected pre-vaccination (baseline [BL]) and one-month (4 weeks [4 W]) post-vaccination. Additionally, blood samples were collected from nine participants without HIV vaccinated as part of the study by van der Mescht et al.[14] (Control A, Table 1) and at a single time point from 26 participants without HIV with a similar age range as the cohort of PLWH (Control B, Table 1), recruited from the staff and students at the University of Pretoria, Pretoria, South Africa. Sodium heparin tubes (CPT™, BD Biosciences, Franklin Lakes, NJ, USA) were

used for the collection of cryopreserved peripheral blood mononuclear cells (PBMCs) and ethylenediaminetetraacetic acid (EDTA)-containing tubes (BD Biosciences) were used for plasma collection.

SARS-CoV-2 spike enzyme-linked immunosorbent assay

SARS-CoV-2 D614G full spike proteins were expressed in HEK293F suspension cells via polyethylenimine (PEI)-MAX transfection (Polysciences, Warrington, PA, USA). The cells were then incubated for six days at 37 °C with 70% humidity and 10% CO_2. The proteins were then purified via nickel affinity and size-exclusion chromatography before being stored at −80 °C until use. A commercial recombinant nucleocapsid protein (Catalogue no. BA25-C; BioTech Africa, Cape Town, RSA) was used as the antigen in nucleocapsid enzyme-linked immunosorbent assays (ELISAs). High-binding 96-well plates (Sigma-Aldrich, St. Louis, MO, USA) were coated with 2 μg/mL of spike protein or nucleocapsid and incubated at 4 °C overnight. The plates were then washed and incubated further in a blocking buffer containing 5% skim milk, 0.05% Tween 20, and 1x phosphate-buffered saline (PBS) for up to two hours. Vaccine plasma samples were diluted 1:100 in blocking buffer, added to the plates, and incubated for one hour. An immunoglobulin G (IgG)-specific secondary antibody, diluted to 1:3000 in blocking buffer, was added to the plates for one hour, followed by 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (Thermo Fisher Scientific, Waltham, MA, USA). To stop the reaction, 1 M H_2SO_4 was added, and the absorbance was measured spectrophotometrically at a wavelength of 450 nm. The monoclonal antibodies CR3022 and AIRU946-A6 (an in-house SARS-CoV-2 control) were used as positive controls, and palivizumab (RSV mAb; MedImmune LLC, Gaithersburg, MD, USA) was used as a negative control.

Pseudovirus-based neutralisation assay

Serum samples were tested for neutralisation using a vesicular stomatitis virus (VSV)-based neutralisation assay, which is not affected by ART.[15] Heat-inactivated serum samples underwent five-fold serial dilutions in 96-well plates before the addition of the SARS-CoV-2 pseudovirus. The serum-virus complexes were mixed with Vero E6 cells and incubated for 20–24 h. The SARS-CoV-2 D614G pseudotyped virus was generated by transfecting Expi293F cells with the D614G spike plasmid, along with a VSVΔG plasmid and incubated for 72 h. Infection was detected via luminescence of the luciferase gene. Neutralising titres were calculated as the reciprocal serum dilution corresponding to the 50% inhibitory dose (ID_{50}) and 80% (ID_{80}) levels.

Antibody dependent cellular cytotoxicity

Plasma antibodies from the vaccine groups were screened for their ability to cross-link and activate the FcγRIIIa (CD16) receptor on Jurkat and SARS-CoV-2 spike-expressing cells, which served as a proxy for antibody-dependent cellular cytotoxicity (ADCC). HEK293 T-cells were transfected with 5 μg of SARS-CoV-2 ancestral variant spike (D614G) plasmids using 1 mg/mL PEI-MAX 40,000 (Polysciences), then incubated for two days at 37 °C. Spike expression in the HEK293 T-cells was confirmed by detecting binding of CR3022, 946-A6, and P2B-2F6 with anti-IgG allophycocyanin (APC) staining, as measured by flow cytometry. Spike-transfected cells (1×10^5 per well) were incubated with heat-inactivated plasma (final dilution of 1:100) or monoclonal antibodies (final concentration of 100 μg/mL) in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (Pen/Strep) (Gibco, Gaithersburg, MD, USA) for one hour at 37 °C. Jurkat-Lucia™ NFAT-CD16 cells (InvivoGen, San Diego, CA, USA) were then added at a concentration of 2×10^5 cells/well, and the cells were incubated for 24 h at 37 °C and 5% CO_2. Then, 20 μL of the supernatant was transferred to a white 96-well plate containing 50 μL of reconstituted QUANTI-Luc™ (InvivoGen) secreted luciferase. The plate was read immediately on a Victor 3 luminometer with a 1-s integration time. Additionally, we added 1x cell stimulation cocktail (Thermo Fisher Scientific) and 2 μg/mL ionomycin in R10 as controls to induce the transgene and confirm sufficient expression of the Fc receptor. Relative light units (RLUs) were normalised between runs using monoclonal antibodies CR3022 and 946-A6.

T-cell stimulation and flow cytometry staining

Prior to stimulation, cryopreserved PBMCs were thawed, washed and rested for four hours in RPMI 1640 medium supplemented with 10% heat inactivated FBS. After resting, cells were seeded in a 96-well plate at a concentration of 1×10^6 cells/well and stimulated with two commercial SARS-CoV-2 S and S1 spike peptide pools (1 μg/mL, Miltenyi Biotec, Gaithersburg, MD, USA). The SARS-CoV-2 peptide pools are made up of 15-mer sequences with an overlap of 11 amino acids, encompassing the entire SARS-CoV-2 spike protein. They are derived from the Wuhan-1 strain and span the N-terminal S1 domain of SARS-CoV-2 from amino acid 1 to 692, as well as most of the C-terminal S2 domain. Unstimulated samples were included as negative controls. Co-stimulatory antibodies against CD28 (28.2, Invitrogen, Carlsbad, CA, USA) and CD49d (9F10, Invitrogen) were used at a final concentration of 1 μg/mL each and Brefeldin A (Invitrogen) at 10 μg/mL. After 16 h of stimulation, cells were washed, stained with Live-or-Dye Fixable Stain (NextGen Molecular Supplies, Midrand, RSA), then fixed and permeabilised using the PerFix-nc Kit (Beckman Coulter, Brea, CA, USA). The cells were then stained intracellularly using the DuraClone IF T Activation tubes (Beckman Coulter), HLA-DR BV650 (L243, Biolegend, San Diego, CA, USA) and Perforin PE Dazzle (dG9, Biolegend). After staining, cells were washed and fixed again with the PerFix-nc Kit (Beckman Coulter). Samples were acquired on the CytoFlex (Beckman Coulter) using CytExpert software and analysed using FlowJo (v10, FlowJo LLC, Ashland, OR, USA). Cells were gated on singlets, lymphocytes, live CD3+, CD4+ and CD8+ T-cells (Supplementary Figure 2). Results are expressed as the frequency of spike-specific CD4+ T-cells expressing interferon-gamma (IFN-γ) tumour necrosis factor-alpha (TNF-α), and interleukin (IL)-2. Cytokine responses presented are background subtracted values (from the frequency of the cytokine produced in unstimulated cells). A positive cytokine response was defined as twice the cytokine frequency of the unstimulated samples and at least 5 events in the stimulated samples.

Statistical analysis

All statistical analyses were performed in Prism (v9; GraphPad Software Inc., San Diego, CA, USA) and data from at least two independent experiments per assay were used for analyses. The Mann-Whitney *U* test and Wilcoxon matched pairs test were used for continuous variables for unmatched and paired samples, respectively, and nonparametric tests were used for all comparisons. In the case of categorical variables, Pearson's chi-square, Fisher's exact test and the Kruskal-Wallis test were used as indicated. Simplified Presentation of Incredibly Complex Evaluations (SPICE, v6.1) was used to analyse the co-expression profiles of antigen-responding CD4+ and CD8+ T-cells. Pestle (v2) was used for background subtraction and generating SPICE data. For all statistical analyses, a p-value of < 0.05 was considered statistically significant. Where values were below the detection limit they were imputed as half the limit of detection. Details of statistical analyses performed for each experiment are described in the figure legends. Our sample size of 40 within each group allowed us to estimate a 3-fold change at 80% power, assuming a type 1 error rate of 5% and a standard deviation of

0.75 on the log10 scale. Sample size calculations have been based on predicted fold increases in responses four weeks after vaccination compared to the baseline immediately prior to vaccination.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis and interpretation, or writing of the report.

Results

Clinical characteristics of the participants

A summary of the demographic and clinical characteristics of the study participants is presented in Table 1. The study cohort consisted of 41 PLWH, with a median age of 43 years (IQR: 34–50). The sex distribution was 59% male (*n*=24) and 41% female (*n*=17). All participants (100%) were vaccinated, with 66% (*n*=27) receiving the Pfizer vaccine and 34% (*n*=14) receiving the JnJ vaccine. Ninety percent (*n*=37) of participants were viraemic and 10% (*n*=4) aviraemic. Among the viraemic participants, the median CD4+ T-cell count was 104 cells/mm^3 (IQR: 47–161), and the median VL was 3480 copies/mL (IQR: 2702–35,200). For aviraemic participants, the median CD4+ T-cell count was 41 cells/mm^3 (IQR: 4–132). The majority of the participants (95%, *n*=39) were on ART, with a median duration of 19 months (IQR: 5–57) since ART initiation. ART adherence in the study was based on self-report. The most common ART regimen included tenofovir disoproxil fumarate/lamivudine/dolutegravir, used by 63% (*n*=26) of participants. Twenty-seven percent (*n*=11) of participants had at least one co-morbid condition, with the most prevalent co-morbidities being hypertension (12%, *n*=5) and chronic kidney disease (7%, *n*=3). Concomitant treatments were reported in 56% (*n*=23) of participants. The most common co-treatments were cotrimoxazole (37%, *n*=15) and pyridoxine (15%, *n*=6). Supplementary Figure 1 summarises the availability and inclusion of samples from the study participants for each immunological assay.

The study also included a total of 35 control participants: Control group A was used for the antibody-related comparisons and Control group B for the T-cell work (Table 1, Supplementary Figure 1). Control A did not have stored PBMC samples and therefore could not be used for flow cytometry analysis. Control B did not have stored plasma and could not be included in the antibody assays. The median ages of the Control A and B participants were 42 years (IQR: 24–69) and 41 years (IQR: 34–54), respectively. The sex distribution for Control A participants was 22% male (*n*=2) and 88% female (*n*=7), and 27% male (*n*=7) and 73% female (*n*=19) for Control B individuals. All of the Control A participants (*n*=9) were vaccinated with Pfizer. Thirty-one percent (*n*=8) of the Control B participants had a co-morbid condition that was well controlled.

Antibody responses following booster vaccination

To assess antibody immunity to SARS-CoV-2 in PLWH following booster vaccination, we measured binding antibodies and neutralising antibodies in 40 of the study participants and ADCC in a subset of 38 individuals. For binding antibodies, booster vaccination was associated with a significant 8.0-fold increase in titres from a geometric mean titre (GMT) area under the curve (AUC) of 2989 at BL to 23,909 at 4 W post-vaccination. In comparison, we observed a 5.7-fold-increase in titres in the Control A group (Table 1), from a GMT AUC of 6563 to 37,593 (Fig. 1A). To evaluate the functional capacity, we assayed neutralisation using a VSV-based pseudotyped virus neutralisation assay that is not subject to interference by antiretroviral drugs. As with binding antibodies, we observed a significant increase in titres following booster vaccination, with GMT ID_{50} increasing 3.9-fold from the BL of 163 to 636 at 4 W post-vaccination (Fig. 1B). Control titres similarly increased from a GMT of 74 to 722. For ADCC, booster vaccination resulted in a more moderate, but nonetheless significant 2.1-fold boost from 118 to 253 (Fig. 1C). A sub-analysis was performed to correct for gender, and we found no differences in binding and neutralising antibodies between males and females in either the controls or PLWH (data not shown).

We next compared the magnitude of the boost in binding antibodies, partitioning those individuals who were viraemic at enrolment from those who were not. In viraemic participants, a significant 9.2-fold boost in titres was observed, from a GMT AUC of 2512 to 23,003. Though a similar magnitude of boost was observed in the aviraemic PLWH (3.8-fold boost from 1048 to 3935) this did not achieve significance, likely due to the small numbers (Fig. 1D). Comparison of the post-boost titres between viraemic and non-viraemic participants showed no significant difference, though the small numbers of non-viraemic participants limit our ability to make definitive conclusions. We found no correlation between antibody responses and HIV VL (data not shown). Similarly, when partitioning by CD4+ T-cell count greater than or less than 100 cells/mm^3 at enrolment, significant boosts were observed in both groups. In PLWH with CD4+ T-cell counts < 100 cells/mm^3, a 7.4-fold boost was observed, with binding antibodies of 1511 boosted to 11,180 at 4 weeks post-boost. In those PLWH with CD4+ T-cell counts ≥100 cells/mm^3, GMT AUC was boosted 10-fold from 3451 to 34,738 (Fig. 1E). Of note, at BL and 4 W, titres were higher in PLWH with CD4+ T-cell counts ≥100 cells/mm^3, suggesting that immunocompromise reduces antibody responses at both time-points. Overall, vaccine boosters drive higher antibody responses in PLWH, even in the context of advanced HIV disease, though the response is muted in PLWH with a CD4+ T-cell count < 100 cells/mm^3.

Lastly, we compared the effect of a Pfizer versus JnJ vaccine boost, in participants who had previously experienced a SARS-CoV-2 infection (defined as being positive for antibodies to nucleocapsid) compared to those with no anti-nucleocapsid antibodies. We show that BL binding and neutralising antibodies were 5–10 times higher in individuals with prior SARS-CoV-2 infection, compared to those without evidence of prior infection (Fig. 2A and B). A Pfizer booster resulted in a 12.2-fold increase in binding antibodies in SARS-CoV-2 naïve participants, compared to a 9.3-fold boost in SARS-CoV-2 exposed individuals. Those who received a JnJ boost (a smaller proportion than Pfizer, Table 1) experienced a 13.6-fold increase in binding antibodies if SARS-CoV-2 naïve (*n*=3) compared to a 3.4-fold increase in titres in SARS-CoV-2 exposed individuals (Fig. 2A). All boosts were significant except for the JnJ SARS-CoV-2 naïve group, likely because of the very small numbers. However, the small numbers preclude firm conclusions. A similar pattern was observed for the smaller subset tested for neutralising antibodies with BL titres higher in SARS-CoV-2 exposed individuals and increasing in all groups following a booster, regardless of platform or SARS-CoV-2 status.

SARS-CoV-2 vaccination in PLWH increases the quantity of spike-specific CD4+ T-cells

To evaluate the specific immunity to SARS-CoV-2 in PLWH following booster vaccination, the frequency of spike-specific CD4+ and CD8+ T-cells (producing IFN-γ, TNF-α, and IL-2) was measured at BL and 4 W post-vaccination. We found that spike-specific CD4+ T-cell responses were detectable in 49% of the PLWH at BL (Fig. 3A). At 4 W, there was a significant increase in the magnitude of spike-specific CD4+ T-cells (medians 0.001% to 0.160%, p=0.0001) in 70% of the PLWH (Fig. 3A, C). In addition to this, the frequency of responders also increased from 49% to 83%, p=0.0167 (Fig. 3A). In contrast, spike-specific CD8+ T-cell responses were not readily detectable in the PLWH, with only 12% of participants exhibiting a CD8+ T-cell response at BL (Fig. 3B). Furthermore, at 4 W, only 20% of the PLWH

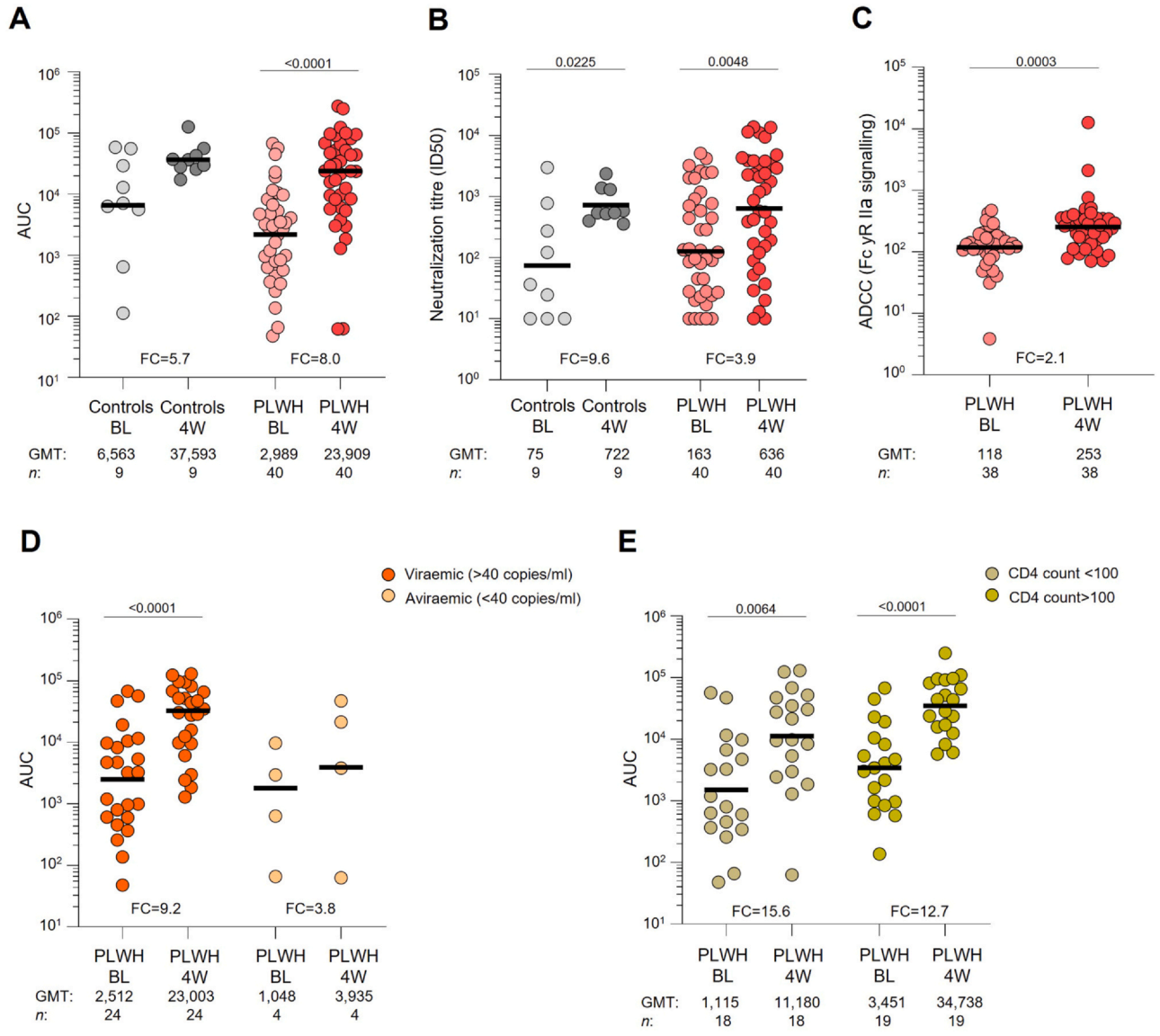

Fig. 1. Magnitude of spike-specific antibody responses following booster vaccination. (A, B) Spike-specific IgG binding titres (AUC) and D614G neutralisation titres (ID_{50}) in PLWH (n=40) and Controls (n=9) at baseline (BL) and 4 weeks (4 W) after vaccination. (C) ADCC activity in PLWH (n=38) following booster vaccination. (D) Spike-specific binding antibody titres in viraemic vs aviraemic PLWH and (E) PLWH with different CD4+ T-cell counts measured at BL and 4 W. Fold-change (FC) in the geometric mean (GMT) is indicated at the bottom of each graph. Statistical significance was calculated using a Mann-Whitney U test. Only statistically significant p-values ($p < 0.05$) are shown. Black horizontal bars indicate the GMT.

demonstrated an increase in their CD8+ T-cell responses (Fig. 3C), resulting in no significant differences in the magnitude of spike-specific CD8+ T-cells (medians 0.001% to 0.001%, p=0.6719) and frequency of responders (12% to 20%, p=0.4404).

Subsequently, in order to identify any potential differences in vaccine-induced immunity in PLWH compared to PNLWH, spike-specific CD4+ and CD8+ T-cell responses were measured in the Control B group (Table 1) at a single timepoint (Fig. 3D, E). We observed that spike-specific CD4+ T-cell responses were readily detected in all control participants (Fig. 3D). While there was a significant difference in the frequency of CD4+ T-cell responders between PNLWH and PLWH at BL (100% vs 49%, $p < 0.0001$), the magnitude of spike-specific CD4+ T-cells was similar between the two groups (Fig. 3D). In contrast to this, spike-specific CD8+ T-cells in the PLWH were significantly diminished in magnitude compared to the Control B group at BL (medians 0.022% vs 0.001%, p=0.0006) and 4 W (medians 0.22% vs 0.001%, p=0.0039) (Fig. 3E). A sub-analysis was performed to correct for gender, and we found no differences in CD4+ and CD8+ T-cell responses between males and females in either the controls or in PLWH (data not shown). We also did not find any correlation between CD4+ and CD8+ T-cell responses and HIV VL (data not shown).

Next, we evaluated the impact of Pfizer versus JnJ booster dose on spike-specific CD4+ and CD8+ T-cell responses (supplementary Figures 3A and B). A significant increase in the magnitude of spike-specific CD4+ T-cells was observed following both the Pfizer (medians 0.019 vs 0.141, p=0.0342) and JnJ (medians 0.001 vs 0.311, p=0.0156) booster doses (supplementary Figure 3A). Conversely, spike-specific CD8+ T-cells did not exhibit a significant increase post either Pfizer or JnJ booster vaccines (supplementary Figure 3B).

Variations in the quality of spike-specific T-cells in PLWH

Having established that SARS-CoV-2 vaccination significantly influenced the quantitative presence of circulating spike-specific CD4+ T-cells in our study cohort, we subsequently examined

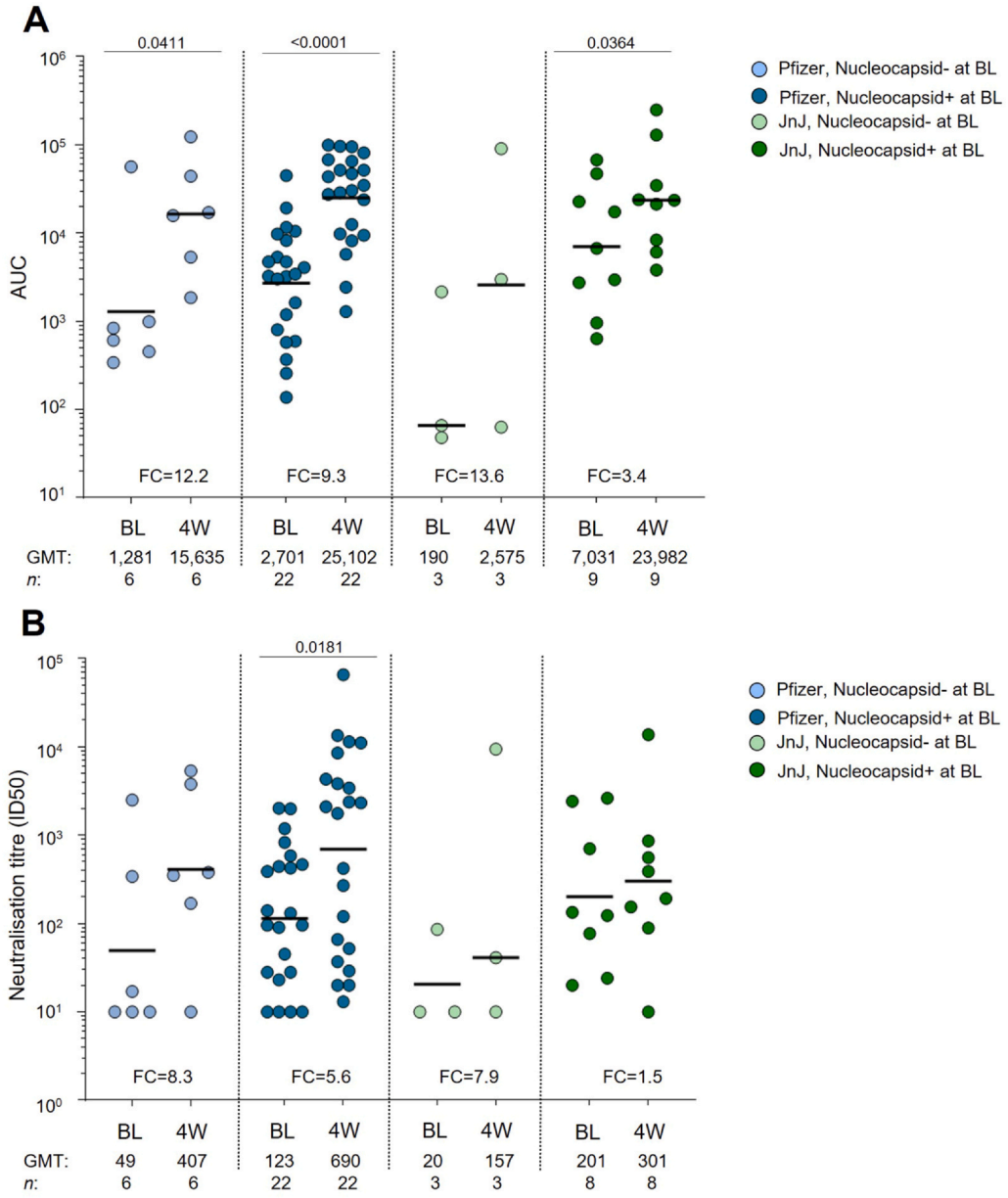

Fig. 2. Antibody responses to Pfizer versus JnJ booster vaccination. (A) Binding antibody titres (ID_{50}) and (B) D614G specific neutralisation were measured at baseline (BL) and 4 weeks (4 W) after vaccination in PLWH receiving either the Pfizer or JnJ booster. Participants that had previously been infected by SARS-CoV-2 are nucleocapsid positive. Fold-change (FC) in the geometric mean (GMT) is indicated at the bottom of each graph. Statistical significance was calculated using a Mann-Whitney *U* test. Only statistically significant p-values ($p < 0.05$) are shown. Black horizontal bars indicate the GMT.

whether the virus influenced their functional profiles. We first compared the polyfunctional profile of spike-specific CD4+ T-cells at BL and 4 W (supplementary Figure 3C). Spike-specific CD4+ T-cell responses at the two timepoints consisted mainly of mono-functional cells (TNF-α) (~27%), cells co-expressing three cytokines (IFN-γ+TNF-α+IL-2+) (24%) and TNF-α+IL-2+ dual-functional cells (~23%). While no significant differences were observed in the overall cytokine profiles of spike-specific CD4+ T-cells between the two time points, a slight decrease in IFN-γ+IL-2+ cells and an increase in IFN-γ+ cells were noted at 4 W (medians 0.8% vs 0%, p=0.0242; and medians 0% vs 3.9%, p=0.0166, respectively). Due to the limited number (12%) of CD8+ T-cell responders at BL within the PLWH group, analysis of the polyfunctional profiles of spike-specific CD8+ T-cells at this time point was not feasible.

Next, we compared the polyfunctional profile of spike-specific CD4+ and CD8+ T-cells between the control individuals and the PLWH at 4 W (Fig. 4). PLWH exhibited fewer IFN-γ+TNF-α+ and more TNF-α+IL-2+ dual-functional CD4+ T-cells. Overall, there was a significant difference in the cytokine profiles of spike-specific CD4+ T-cells between the PLWH and the controls at 4 W (p=0.036) (Fig. 4A). Although not significantly different, the spike-specific CD8+ T-cells of PLWH at 4 W had a more mono-functional cytokine profile in comparison to the controls (Fig. 4B). Altogether, these findings suggest that spike-specific CD8+ T-cells are markedly reduced in people with HIV compared to those without HIV.

Differential activation and cytolytic profiles of T-cells in PLWH

Finally, to determine the phenotypic characteristics of CD4+ and CD8+ T-cells between the control group and the PLWH, we measured the activation and cytolytic markers, human leucocyte antigen (HLA)-DR and perforin, respectively (Fig. 5). Analysis of HLA-DR and

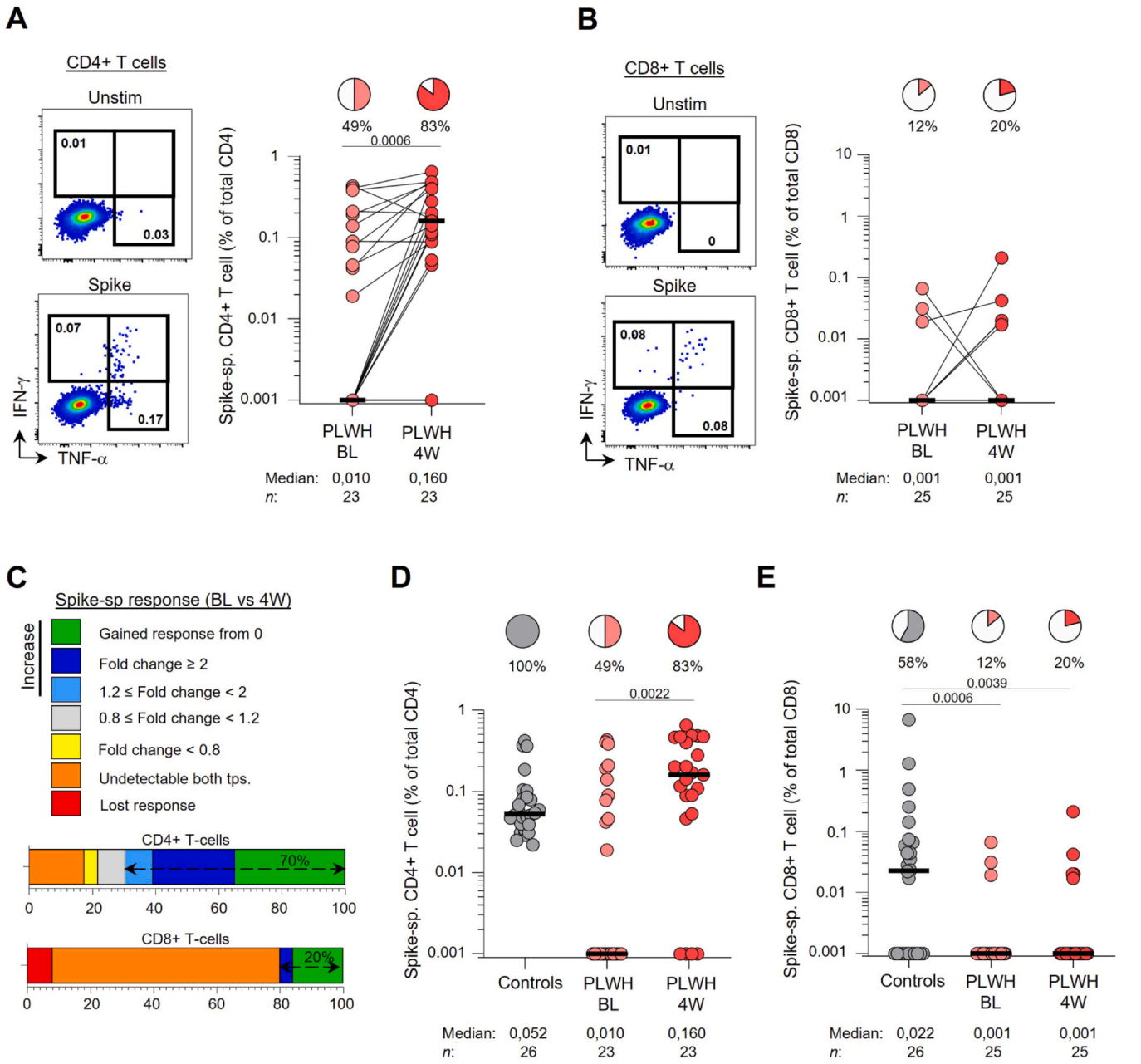

Fig. 3. The magnitude of spike-specific CD4+ T-cells. (A, B) Frequency of total spike-specific CD4+ and CD8+ T-cells in PLWH (n=25) at baseline (BL) and 4 weeks (4 W) after booster vaccination. (C) Fold change in the frequency of spike-specific CD4+ and CD8+ T-cells between BL and 4 W. Statistical significance was calculated using a Wilcoxon matched-pairs test. (D, E) The frequency of total spike-specific CD4+ T-cells and CD8+ T-cells in control participants and PLWH. Statistical significance was calculated using a one-way ANOVA with Dunn's correction. Only statistically significant p-values ($p < 0.05$) are shown. Black horizontal bars indicate the median values.

perforin expression showed no significant differences in the frequencies of activated and cytolytic spike-specific CD4+ T-cells between the control group and PLWH (Fig. 5A and B). Conversely, when examining the overall CD4+ T-cell compartment, a higher frequency of total HLA-DR+ CD4+ T-cells was observed in the PLWH at BL (medians 0.99% vs 5.47%, $p < 0.0001$) and 4 W (medians 0.99% vs 5.62%, $p < 0.0001$), along with an increased frequency of perforin+ CD4+ T-cells at both timepoints: BL medians 0.32% vs 1.04% (p=0.0007) and 4 W medians 0.32% vs 1.27% (p=0.0005), respectively, when compared to control individuals (Fig. 5C, D). Similarly, analysis of the overall CD8+ T-cell compartments also indicated a higher frequency of total HLA-DR+ CD8+ T-cells in the PLWH at BL (medians 3.27% vs 14.3%, $p < 0.0001$) and 4 W (medians 3.27% vs 15.5%, $p < 0.0001$), as well as an increased frequency of perforin+ CD8+ T-cells at both timepoints (medians 23.8% vs 45.0%, $p < 0.0001$) and (medians 23.8% vs 46.4%, p=0.0012), respectively (Fig. 5E, F).

Discussion

Since March 2020, South Africa has recorded over 4 million cases of SARS-CoV-2 infection.[16] Whilst the vast majority of the population has now developed immunity, either vaccine-induced or from prior infection or both,[17,18] there is a small proportion of the population that remains vulnerable to moderate and severe COVID-19. This includes individuals with severe immunocompromise, such as those with advanced HIV disease. In South Africa, despite the extensive HIV treatment programme, an estimated 2 million PLWH may not be accessing ART or are experiencing treatment failure. This number is

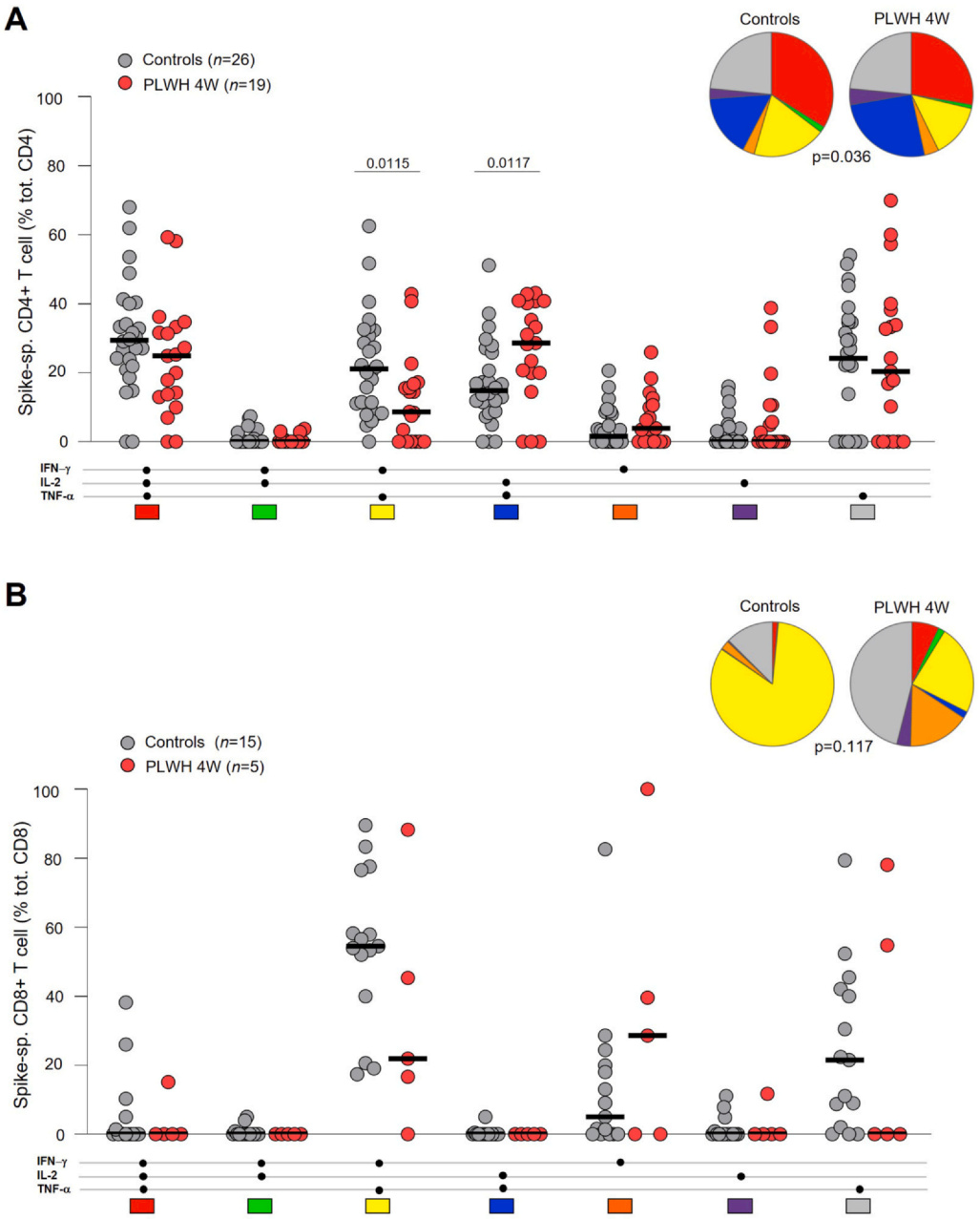

Fig. 4. The function of spike-specific T-cells. (A, B) Poly-functional profile of spike-specific (A) CD4+ and (B) CD8+ T-cells in control participants and PLWH at 4 W. The coloured bars (underneath) and pie chart slices represent different cytokine combinations produced by spike-specific T-cells (e.g., a red bar/slice represents cells producing IFN-γ, TNF-α and IL-2). Black horizontal bars indicate the median values. Statistical significance was calculated using a Mann-Whitney *U* test.

likely to increase due to recent funding cuts to the South African HIV response.

While several previous studies have assessed the antibody responses to COVID-19 vaccination and boosting, most of these studies have been performed in PLWH who are clinically well-controlled. The effectiveness of vaccines in PLWH who have advanced HIV disease is not well-established. In addition to their heightened personal risk, this population is of global interest due to several case studies indicating that incomplete immune reconstitution or uncontrolled HIV viraemia in immunocompromised individuals can lead to accelerated viral evolution, prolonged viral shedding, and the emergence of variants of concern.[19–21] While PLWH on ART with suppressed VLs typically develop adaptive immune responses, those with low CD4+ T-cell counts show reduced SARS-CoV-2-specific T-cell responses, impairing viral clearance. Unvaccinated PLWH with uncontrolled HIV often have weakened neutralising antibody responses to variants, increasing non-responder rates and enabling extended viral replication.[21] Prolonged SARS-CoV-2 infection in immunocompromised hosts, including PLWH, can last weeks to months, during which time intra-host viral evolution occurs, accumulating immune escape mutations such as S:E484K and S:N501Y.[22,23] This suggests that immunocompromised individuals may act as reservoirs for new variants. Consequently, viral shedding duration in PLWH may exceed the standard 14-day isolation period, with cases documenting shedding beyond 28 days, linking immunodeficiency to extended infectiousness.[24]

It has been suggested that PLWH on ART with well-controlled HIV VLs and preserved CD4+ T-cell counts can generate robust SARS-CoV-2-specific antibodies.[3,20,25–28] However, these individuals may experience a delay in the development of neutralising antibodies

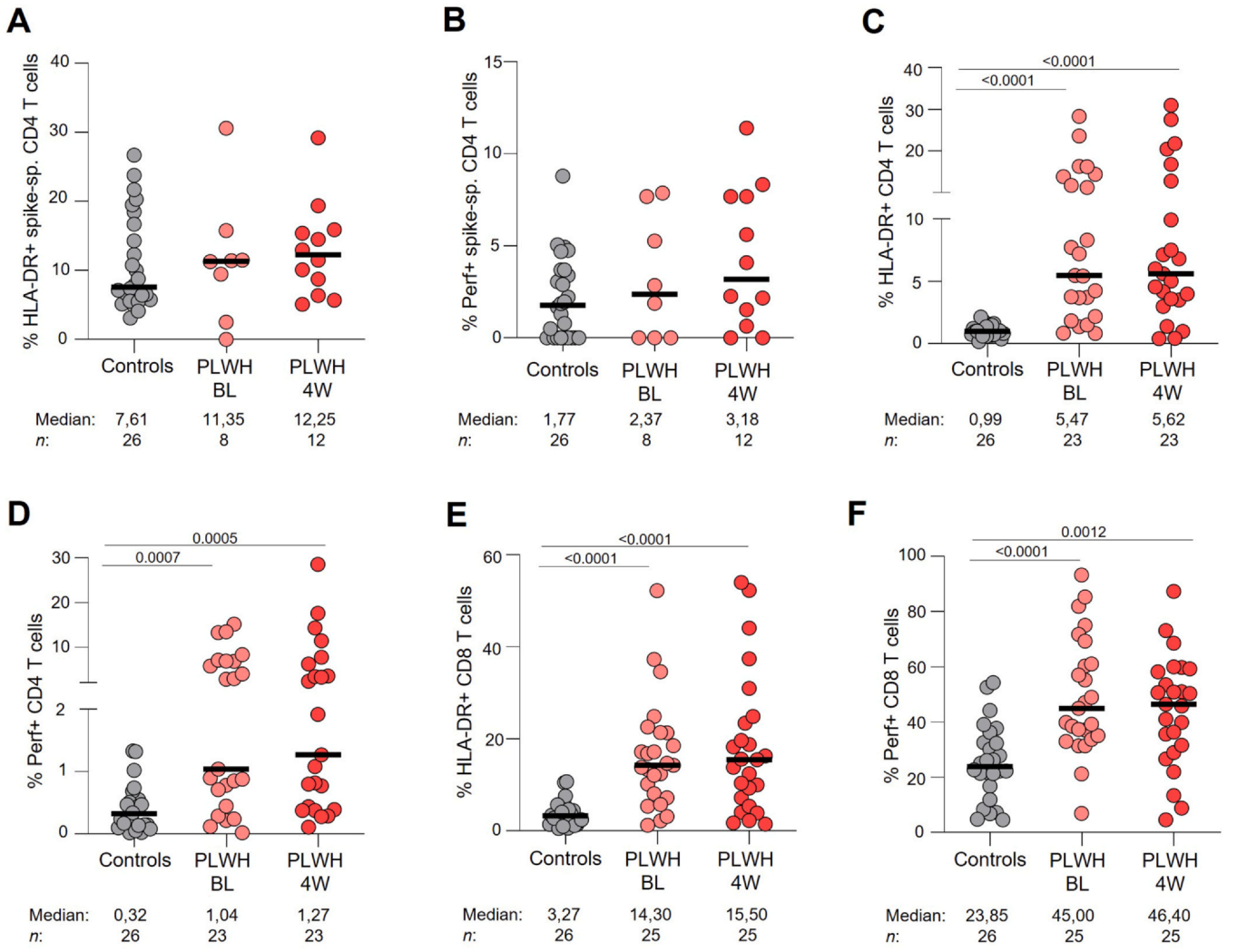

Fig. 5. The phenotype of spike-specific T-cells. (A, B) Frequency of HLA-DR+ and perforin+ spike-specific CD4+ T-cells and (C–F) total HLA-DR+ and perforin+ CD4+ and CD8+ T-cells in control participants and PLWH. Statistical significance was calculated using a one-way analysis of variance (ANOVA) with Dunn's correction. Only statistically significant p-values ($p < 0.05$) are shown. Black horizontal bars indicate the median values.

and exhibit a lower frequency of spike-specific memory B-cells compared to their uninfected counterparts.[29] Booster doses have been demonstrated to significantly enhance both humoral and cellular immunity, resulting in elevated levels of neutralising antibodies and more effective T-cell responses.[30–34] Notably, these booster vaccinations can help mitigate initial deficits in vaccine-induced immunity among PLWH, thereby offering improved protection against severe COVID-19 outcomes, including hospitalisation and complications from emerging variants.

In the current study, we show that even people living with advanced HIV have significant increases in binding, neutralisation and ADCC responses following booster vaccination, achieving values comparable to those observed in people without HIV. Importantly, however, even though PLWH with a CD4+ T-cell count less than 100 cells/mm^3 had a significant boost in antibody responses, levels were lower than that of control individuals at both time points investigated. This is similar to the findings of Han et al.[35] who reported lower antibody responses in PLWH after receiving an inactivated COVID-19 vaccine, although, in their case, PLWH with CD4+ T-cell counts ≤350 cells/mm^3 had poor antibody responses. PLWH with lower CD4+ T-cell counts specifically tend to produce reduced levels of anti-receptor binding domain (RBD) IgG antibodies following initial COVID-19 vaccine doses, relative to those with higher CD4+ T-cell counts.[1,31]

Our observations are also consistent with more recent studies that demonstrate the benefits of additional vaccine doses for PLWH. Notably, a longitudinal cohort study by Casado et al. observed progressive increases in antibodies and memory B-cells with successive boosters, but also noted reduced memory B-cell generation and higher post-booster infection rates in PLWH with lower CD4+ T-cell counts or those who received non-mRNA primary regimens.[36] Similarly, a study comparing humoral responses to ancestral and Omicron lineages of the virus found that, although boosters significantly elevate antibody titres, responses to Omicron remain comparatively weaker in PLWH than to earlier lineages.[37] This underscores the necessity for booster strategies and variant-updated vaccines in this vulnerable population.

Other authors have also reported that lower baseline CD4+ T-cell counts, reduced CD4/CD8 ratios, and higher VLs are associated with lower seroconversion rates.[38,39] Our study could not confirm the latter, but it should be noted that our study had a small number of aviraemic participants. These findings also apply to mRNA vaccines, which have been reported to produce reduced antibody titres in PLWH, albeit to varying degrees depending on the individual's CD4+ T-cell count and the variant of the virus, such as Omicron, they are exposed to.[40]

With regards to T-cell responses, while cytotoxic T-cell responses appear to be preserved in PLWH with controlled viraemia,

individuals with HIV viraemia tend to develop weaker or less diverse SARS-CoV-2-specific T-cell responses.[20,27,41] Similarly, PLWH with lower CD4+ T-cell counts have been reported to have less robust T-cell responses.[27,28,42]

In the current study, nearly half of the PLWH exhibited detectable spike-specific CD4+ T-cell responses at BL. Conversely, spike-specific CD8+ T-cell responses were detectable in only 12% of the PLWH, and booster vaccination enhanced neither the magnitude of these responses nor the frequency of responders. While the magnitude of spike-specific CD4+ T-cell responses was comparable to that of the control participants, this was not observed for CD8+ T-cell responses or the frequency of responders. The type of booster vaccine, whether Pfizer or JnJ, did not affect these responses. These results corroborate previous studies showing that while CD4+ T-cell responses are often preserved by ART, cytotoxic CD8+ T-cell responses are impaired in the context of advanced HIV.[27] Similarly, a more recent study by Chen et al. reported enhanced $CD4^+$ T-cell responses following third and subsequent doses of the vaccine, while also highlighting persistent deficits among participants with low CD4+ T-cell counts, especially consistently lower $CD8^+$ T-cell responses compared to HIV-negative controls.[43]

Based on these findings, we hypothesise that HIV may be impairing the function of these spike-specific CD8+ T cells, causing them to not respond to stimulation. Moreover, the few cells that do respond seem to exhibit reduced polyfunctionality.

A large proportion of spike-specific CD4+ T-cells (33%) in PLWH exhibited a mono-functional TNF-α+ profile at BL; however, more than half (53%) were poly-functional, comprising cells expressing either two or three cytokines. At 4 W post-vaccination, an increase in mono-functional IFN-γ-producing cells was noted. This profile differed from that of the control participants, who exhibited a higher prevalence of IFN-γ+TNF-α+ and a lower prevalence of TNF-α+IL-2+ dual-functional CD4+ T-cells. Although the small sample size precluded statistical analysis of the functional profile of spike-specific CD8+ T-cell responses in PLWH, these responses appeared to have a more mono-functional cytokine profile compared to that of the control participants.

The implications of a vaccine-induced T-cell response being mono-functional rather than poly-functional are significant concerning the efficacy and quality of the immune response elicited by a vaccine. Poly-functional T-cells provide a coordinated response involving various signalling pathways and cytokines, which is more effective in comprehensively engaging the immune system. A mono-functional response may not elicit such extensive immune engagement, potentially leading to less effective pathogen clearance.[44] Poly-functional T-cells, capable of secreting multiple cytokines simultaneously, contribute to a more robust and efficient immune response, and are therefore often used as markers of immune activity.[45] Vaccines that induce long-lived, poly-functional memory T-cells (both CD4+ and CD8+) are further desirable as they suggest a durable immune response capable of providing long-term protection. Poly-functional responses tend to correlate with the presence of these memory populations, whereas mono-functional responses might indicate a shorter-lived immunity.[46,47] Vaccine studies demonstrate that poly-functional T-cell responses are often associated with enhanced protective effects. For instance, poly-functional responses are crucial in augmenting the quality and efficacy of the immune response against pathogens,[48] as observed in some COVID-19 vaccines that drive poly-functional T-cell responses.[49] Additionally, a study by Rakshit et al. showed that poly-functional CD4+ T-cells were strongly correlated with the production of neutralising antibodies following vaccination with either COVISHIELD or COVAXIN.[50] They describe this relationship as a hallmark of the immunity induced by both vaccines in individuals with prior COVID-19 exposure.[50] This suggests that poly-functional CD4+ T-cells play a crucial role in inducing antibody production, a conventional function of CD4+ T-cells. Overall, while mono-functional T-cell responses may still confer some level of immunity, poly-functional responses are generally more favourable as they provide a more comprehensive immunological defence, thereby improving vaccine outcomes.

The functional profile of CD4+ and CD8+ T-cell responses to COVID-19 vaccines or boosters in individuals with advanced HIV is a developing area of research. Within the context of immune response variability across different conditions, factors such as aging and medication regimens significantly influence T-cell responses post-vaccination, offering a parallel understanding in PLWH. For example, elements affecting T-cell homoeostasis, including thymic function and CD161+ T-cells, have been shown to substantially impact T-cell responses following vaccination in elderly populations. Analogous findings can be observed in studies involving other immunocompromised groups, such as haematopoietic stem cell transplant recipients, who frequently demonstrate diminished immune responses to COVID-19 vaccines. Insights into the timing of vaccination, lymphocyte counts, and other determinants of immune responses may inform strategies to enhance vaccine efficacy in PLWH.[51,52]

The interaction between innate immunity and mRNA vaccines has demonstrated that the initial response involves stromal pro-inflammatory reactions and type I interferon responses, which are essential for the subsequent activation of T-cells. These mechanisms may be pivotal in enhancing vaccine efficacy in PLWH by addressing deficiencies in adaptive immune responses.[53] This understanding highlights the importance of developing tailored vaccine strategies for individuals with advanced HIV, emphasising the enhancement of both humoral and cellular responses. This could potentially be achieved through vaccine modifications, booster doses, or adjunct therapies that effectively modulate immune responses in this population.

The boosting effect in our study was observed in both SARS-CoV-2 naïve and SARS-CoV-2 exposed individuals, based on nucleocapsid seropositivity. We concede that we may be underestimating the degree of prior SARS-CoV-2 exposure, as PLWH generally mount lower / delayed responses, and waning of such responses to undetectable levels may have occurred in this study.[54] The preference of participants and their clinicians for Pfizer vaccine boosts over JnJ boosts limited the conclusions that could be made regarding the comparison of the two platforms. Some groups contain limited numbers of participants, which may influence statistical significance. We also measured functional responses (neutralisation and ADCC) in smaller subsets of participants compared to binding antibodies. Our study lacks variant-specific neutralisation data and does not include T-cell and antibody data for time points beyond 4 weeks; this limits our study's ability to address durability, which is particularly relevant in individuals with advanced HIV due to potentially faster waning of immune responses. The high proportion of participants with detectable VLs despite ART use in our study may indicate non-adherence or emerging drug resistance; however, we lack adherence or resistance data to confirm this. Previous studies show that there is a link between HIV viraemia and weaker or less diverse SARS-CoV-2-specific T-cell responses, particularly $CD8^+$T-cell responses.[27,28,42] Our dataset included too few $CD8^+$ responders for us to meaningfully assess this relationship between HIV VL and CD8+ T cell responses within our cohort.

Conclusion

The variability in vaccine efficacy among PLWH underscores the importance of ongoing monitoring and research to inform vaccination strategies in relevant subgroups of PLWH. While mRNA vaccines show relatively high immunogenicity rates in PLWH, consistent with the general population, disparities remain based on individual immune status.[33] Continued boosters are suggested to enhance

immunity, especially against variants that may exhibit immune evasion. Booster uptake, however, remains a challenge due to vaccine hesitancy and administrative barriers, with lower uptake noted among marginalised individuals.[55]

Future research should explore the optimal timing for booster vaccinations, considering whether personalised dosing intervals would benefit individuals with very low CD4+ T-cell counts or unsuppressed HIV VL. Longitudinal studies are needed to examine the durability of both antibody and T-cell responses, particularly booster-induced immunity over a 6–12-month period, to determine when immunity wanes and re-boosting is required. Additionally, studies investigating strategies to enhance $CD8^+$ T-cell responsiveness in people living with advanced HIV, such as alternative vaccine platforms or adjuvanted formulations, could also provide valuable insights.

Our findings have implications for vaccine policy and clinical practice. National programmes may need to prioritise more frequent boosters or differentiated vaccination schedules for people living with advanced HIV disease. Incorporating CD4+ T-cell count and VL status into booster eligibility criteria could significantly enhance protection against severe COVID-19. Furthermore, supporting ART adherence remains equally critical, given the relationship between HIV control and vaccine responsiveness.

Author contributions

FA and NS obtained funding to support the study, FA, PLM, TMR, VU and NS designed the study. FA, VU, KT and NS recruited study participants, IS and KT collected the data, RN, DTC, MAM, TP, PK, NS and NM performed experiments. RN, CC, TP, PK, NS, NM, SIR and TM analysed the data, RN, CC, SIR, TM, FA, PLM and TMR interpreted the data. TR assisted with statistical analysis. RN, CC, DTC, HCS, FA, PLM and TMR wrote the manuscript, with all authors contributing to providing critical feedback.

Ethics statement

Ethical approval was obtained from the University of Pretoria's Faculty of Health Sciences Research Ethics Committee (UP Ethics 680/2021, 234/2022, 332/2024). All participants provided written informed consent.

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Data availability

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Paperpal (v5.24.6, by Editage) in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2026.106676.

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