Humoral and cellular immune responses eleven months after the third dose of BNT162b2 an mRNA-based COVID-19 vaccine in people with HIV – a prospective observational cohort study

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Summary

Background We investigated long-term durability of humoral and cellular immune responses to third dose of BNT162b2 in people with HIV (PWH) and controls.

Methods In 378 PWH with undetectable viral replication and 224 matched controls vaccinated with three doses of BNT162b2, we measured IgG-antibodies against the receptor binding domain of SARS-CoV-2 spike protein three months before third dose of BNT162b2, and four and eleven months after. In 178 PWH and 135 controls, the cellular response was assessed by interferon-γ (IFN-γ) release in whole blood four months after third dose. Differences in antibody or IFN-γ concentrations were assessed by uni- and multivariable linear regressions.

Findings Before the third dose the concentration of SARS-CoV-2 antibodies was lower in PWH than in controls (unadjusted geometric mean ratio (GMR): 0.68 (95% CI: 0.54–0.86, p = 0.002). We observed no differences in antibody concentrations between PWH and controls after four (0.90 (95% CI: 0.75–1.09), p = 0.285) or eleven months (0.89 (95% CI: 0.69–1.14), p = 0.346) after the third dose. We found no difference in IFN-γ concentrations four months after the third dose between PWH and controls (1.06 (95% CI: 0.71–1.60), p = 0.767).

Interpretation We found no differences in antibody concentrations or cellular response between PWH and controls up to eleven months after third dose of BNT162b2. Our findings indicate that PWH with undetectable viral replication and controls have comparable immune responses to three doses of the BNT162b2 vaccine.

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Keywords: BNT162b2; HIV; SARS-CoV-2; Immune response; Booster dose

Research in context

Evidence before this study

People with HIV (PWH) were prioritised for vaccination against SARS-CoV-2 in many countries. After the initial two-dose regimen of the mRNA-based COVID-19 vaccine, BNT162b2, we, and others, found robust antibody responses in PWH. However, antibody responses were impaired in PWH compared to controls. Waning immunity from the initial two-dose regimen was seen in PWH as well as in the general population, leading to the recommendation of a third dose. However, evidence of immunological responses after booster immunisation was sparse. Before undertaking this study, we searched PubMed, Scopus, Embase, and Web of Science using the search terms: (((“Acquired Immunodeficiency Syndrome” [Mesh]) OR “HIV” [Mesh]) OR (“AIDS” OR “hiv” OR “people living with hiv” OR “people with hiv” OR “PLWH” OR “PWH”)) AND ((((((“mrna covid-19 vaccine*”) OR (comirnaty)) OR (mRNA-1273)) OR (BNT162b2)) OR (“mrna vaccine*”))). We found no studies exceeding one month of follow-up after the third dose of an mRNA-based COVID-19 vaccine. Additionally, most available studies were of very low sample size.

Added value of this study

This study shows humoral immunity eleven months after vaccination with a third dose of an mRNA-based COVID-19 vaccine and cell-mediated immunity four months after the third dose in PWH. Our findings suggest that PWH with undetectable viral replication do not have impaired immune responses after the third dose of the mRNA-based vaccine, BNT162b2.

Implications of all the available evidence

This study adds to the evidence that PWH with undetectable viral replication produce robust immune responses to BNT162b2, and responses to the third dose in PWH are comparable to those seen in matched controls. However, attention to waning immunity over time and the emerging immune evasion from new SARS-CoV-2 variants as well as the risk of immune imprinting is necessary for the optimal timing of future booster vaccinations in PWH, as it is in the general population.

Introduction

People with HIV (PWH) were initially considered to be at an increased risk of severe coronavirus disease 2019 (COVID-19) despite receiving antiretroviral treatment (ART) and having suppressed viral replication.[1–4] Although ART improves immune functions, chronic inflammation and residual immune dysfunction may result in increased morbidity and mortality associated with non-AIDS comorbidities.[5–7] Because of this, many countries prioritised PWH for early COVID-19 vaccination.

After the initial two-dose regimen of the mRNA-based COVID-19 vaccine, BNT162b2, we, and others, found robust antibody responses in PWH. However, antibody responses were impaired in PWH compared to controls.[8–10] Real-world data has shown waning immunity from the initial two-dose regimen in both PWH and the general population, but with inferior maintenance of antibody responses in PWH.[11] A few recent studies have investigated immune responses up to six months after the administration of the third dose of BNT162b2 in PWH. These studies found comparable humoral and cellular responses between PWH and controls.[11–14] However, no studies have reported on the durability of the immune response to the third dose of mRNA-based COVID-19 vaccination in PWH exceeding six months.

In the present study, we examined the long-term durability of the humoral immune response to the mRNA-based COVID-19 vaccine, BNT162b2, in PWH and controls four and eleven months after the administration of the third dose, and the cellular immune response at four months after the third dose. In exploratory analyses, we investigated associations of HIV-related variables with these responses.

Methods

Study design and participants

All PWH followed at the HIV outpatient clinic at Copenhagen University Hospital, Rigshospitalet, aged 18 years or older, were invited to participate in this prospective observational study initiated between December 2020 and April 2021.[15,16] Healthcare professionals from Copenhagen University Hospital,

Rigshospitalet and Herlev-Gentofte Hospital, aged 18 years or older, were enrolled in a parallel study over the same time course and were included in the present study as controls.[15,17] The HIV status in the control group is unknown. However, the prevalence of HIV infection in Denmark is approximately 0.1%.[18] COVID-19 vaccines were offered as part of the Danish vaccination program. Participation in the study was voluntary and did not interfere with the vaccination strategy. All participants received at least three doses of the mRNA-based SARS-CoV-2 vaccine BNT162b2 during the study period and were excluded if they received other SARS-CoV-2 vaccine types. Only participants with a blood sample collected after their third dose were included.

All PWH and controls provided consent after receiving oral and written information. The study was approved by the Regional Scientific Ethics Committee of the Capital Region of Denmark (H-20079890) and was conducted in accordance with the Declaration of Helsinki.

Participants were invited for six blood sample collections between December 27, 2020 and October 25, 2022, starting at the time of their first vaccination and up to twenty months after the first vaccine dose, regardless of the administration of additional vaccine doses. Sampling time points were planned according to the time of the first vaccination and occurred at baseline, three weeks, two, six, twelve, and eighteen months after the first dose. Samples for assessment of cellular responses were collected to coincide with the twelve-month sample. For analyses of the response to the third dose, sample time points were recalibrated according to the time of administration of the third vaccine dose, with the date of the third dose being considered the baseline for the present analyses. A six-month sample was thus collected approximately three months before baseline, and twelve-, and eighteen-month samples were collected approximately four and eleven months after baseline, respectively. Similarly, the sample for assessment of cellular response was collected approximately four months after baseline.

For the PWH, data on the latest and nadir CD4+ T-cell counts, HIV viral load, and ART at the time of the first vaccination dose was collected from medical records at study entry. Data on body mass index (BMI) was collected on all participants through a questionnaire completed at study entry. Data on vaccination status, including the type of vaccine and date of administration, was collected for all participants via the Danish Vaccination Register, a national registry with mandatory registration of all administered vaccines in Denmark since 2015.[19]

Antibody measurements

All analyses are based on the SARS-CoV-2 ancestral strain. IgG antibodies specific for the receptor-binding domain (RBD) of the spike (S) protein were determined using an in-house ELISA.[16] Briefly, purified recombinant RBD was coated onto Nunc Maxisorp 384-well plates (Thermo Fisher Scientific, Massachusetts, USA) overnight in phosphate-buffered saline (PBS) (Rigshospitalet, Copenhagen, Denmark). Before adding the buffer-diluted patient serum, the wells were blocked for 1 h in PBS and Tween 20 (PBS-T, Merck, Darmstadt, Germany). RBD-bound IgG was detected using horseradish peroxidase conjugated polyclonal rabbit-anti-human IgG (Agilent Technologies, Santa Clara, CA, USA). Tetramethylbenzidine (TMB) One substrate (Kem-En-Tec, Taastrup, Denmark) was added, and the reaction was stopped using H_2SO_4. Optical density was measured by a Synergy HT absorbance reader (BioTek Instruments, Winooski, VT, USA). Plates were washed in PBS-T four times between each step. IgG concentrations were calculated in Arbitrary Units (AU)/mL. The threshold of a positive IgG response was 225 AU/mL. Samples with a value below 1 AU/mL were set to 1 AU/mL.[8,17,20]

To assess for previous infection with SARS-CoV-2, antibodies targeting the nucleocapsid (N) protein were determined in all samples by an electrochemiluminescence assay according to the manufacturer's instructions (Elecsys® Anti-SARS-CoV-2 Elecsys® assay, Roche Diagnostics, GmbH, Germany). The N protein is not a part of the original BNT162b2 vaccine and is thus used as a marker of natural infection.

Measurement of virus-neutralising capacity

We used a validated in-house ELISA-based assay to estimate the degree of inhibition by the ACE-2 host receptor and RBD interaction as a proxy for neutralising capacity, as described elsewhere.[15,21] This pseudo-neutralising assay correlates well with the gold standard plaque reduction neutralisation test (r = 0.9231).[21] In brief, recombinant ACE-2 ectodomain was coated onto Nunc Maxisorp 96-well plates in PBS overnight. For 1 h, a solution of patient serum, Pierce High Sensitivity Streptavidin-HRP (Thermo Fisher Scientific), and biotinylated recombinant RBD was incubated in non-binding 96-well plates. The mixtures of biotinylated RBD/Streptavidin-HRP and patient serum were transferred to the ACE-2 ectodomain-coated wells for 35 min. Between each step, the wells were washed three times with PBS-T. Plates were developed for 20 min. The threshold for assay positivity was set to 25% inhibition in 10% diluted serum based on a receiver operating characteristic (ROC) curve analysis to estimate the optimal cut-off between naturally infected convalescent sera and sera from individuals obtained before 2020.[8,16]

Interferon-γ release quantification

The cellular response to vaccination was assessed by measuring interferon-γ (IFN-γ) release after stimulation

with SARS-CoV-2 S1 peptides in fresh whole blood. A commercial kit was used according to the manufacturer's instructions, as previously described.[15,20] In brief, 4 mL of venous blood was collected in Lithium-Heparin coated tubes and aliquoted in 3 different tubes: one blank tube to measure unstimulated IFN-γ concentration, one tube containing SARS-CoV-2 S1 protein-specific peptides, and one tube containing a mitogen serving, as a positive control (product ET 2606-3003, EUROIMMUN, Lübeck, Germany). After incubation for 21 h at 37 °C, the samples were centrifuged at 12,000*g* for 10 min. IFN-γ concentrations were measured using an IFN-γ ELISA kit (product EQ 6841-9601, EUROIMMUN). Results from the unstimulated tubes were subtracted from the SARS-CoV-2 S1 peptide and mitogen tubes to estimate IFN-γ concentrations caused by SARS-CoV-2 S1 stimulation of whole blood and served as a proxy for a cellular response.

Statistics and modelling

The sample size for the present analysis was chosen pragmatically, with the inclusion of all PWH from the original cohort who met the inclusion criteria and were willing to continue their participation. PWH were matched to controls using a propensity score based on age, sex, number of volunteered samples, and the total number of vaccine doses. Propensity scores were estimated by logistic regression using the glm function of the stats package for R software (version 4.1.0 for Windows, R Foundation for Statistical Computing, Vienna, Austria) with the predict function from the same package being used to compute the propensity scores.[22] As a standard 1:1 matching would likely have resulted in a reduction of the sample size, we chose to use cardinality matching based on the propensity score,[23] using the cardinality method in the MatchIt package and a balance tolerance set to 0.1.[24] This approach provided us with a balanced control set without a large reduction in the available sample. Potential confounders from the available collected dataset were identified based on knowledge of factors that could potentially confound an association between HIV status and vaccine responses. Continuous data were reported as medians with interquartile range (IQR) or means with standard deviation (SD). Categorical data were reported as frequency counts and percentages. Differences between the study groups were assessed using the Mann–Whitney U test, unpaired t-test, or Chi-squared (χ^2) test, as appropriate. The normality of data was assessed by quantile-quantile plots and scatter plots of residuals and fitted values. Observed and predicted antibody and IFN-γ concentrations were reported as geometric mean concentrations (GMC) with 95% confidence intervals (CI), with geometric mean ratios (GMR) and 95% CI used to compare groups. Antibody and IFN-γ concentrations were $\log_{10}$ transformed and back transformed before being reported as appropriate.

To compare antibody concentrations, pseudo-neutralising capacity, or IFN-γ concentrations between PWH and controls, we fitted univariable and multivariable linear regressions at each time point. Median time from baseline to sample time was calculated for PWH and controls combined at each time point, and used for the prediction of mean antibody concentrations, pseudo-neutralising capacity, or IFN-γ concentrations as determined by the time-adjusted linear models. For sensitivity analyses, the presence of N-antibodies was included in the regression model at all time points, to compare antibody concentrations between PWH and controls with and without evidence of a previous infection. Similarly, a sensitivity analysis was performed at the eleven-month follow-up time in which all individuals who received more than three vaccine doses were excluded. Additionally, we re-fitted all regression models using the complete, unmatched, dataset with adjusted analyses including the propensity score as an additional independent variable. Univariable and multivariable linear regressions were fitted with either $\log_{10}$-transformed anti-RBD IgG concentrations, pseudo-neutralising capacity or $\log_{10}$-transformed IFN-γ concentrations as the dependent variable and HIV-status as the independent variable in the univariable models. We fitted a series of multivariable models in which we additionally included the nadir CD4 count, latest CD4 count or latest HIV viral load at the first dose as a second independent variable. Time since baseline was additionally included in the time adjusted multivariable models. Nadir CD4 count, latest CD4 count, and latest HIV viral load were included in the models as categorical variables as these did not meet the assumption of linearity. Nadir and the latest CD4 T-cell counts were categorised into four groups of <200, 200–349, 350–500, or >500 cells/μL, respectively. HIV viral load was categorised as <50 or ≥50 copies/mL, respectively. To visualise mean antibody concentrations and observed antibody concentrations, GMC of anti-RBD IgG was predicted by the linear model for each time point, at median time from baseline. Wilcoxon matched-pairs signed-rank test was applied to compare changes in antibody concentrations between time points. p-values < 0.05 were considered significant. All statistical analyses were performed in R using packages dplyr,[25] tidyr,[26] MatchIt,[24] stringr,[27] and ggsci.[28]

Role of the funding source

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Results

We included 378 PWH and 224 propensity score matched controls for our primary analyses; 89% of PWH and 94% of controls were male (p = 0.066). The median age at the time of the first vaccination was 56 years in PWH [IQR: 48, 63] and 55 years in controls [IQR: 44, 63] (p = 0.112). The median time between administration of the third dose (baseline) and the last time-point of sample collection (eleven months follow-up) was 328 days [IQR: 313, 339] in PWH and 320 days [IQR: 313, 325] in controls (p < 0.001). Assessed by the presence of N-antibodies, 7.1% of PWH and 9.4% of controls had shown evidence of a previous infection with SARS-CoV-2 three months prior to baseline, while 59% of PWH and 60% of controls had shown evidence of a previous infection by the time of the eleven-month follow-up visit. All PWH had HIV viral loads <200 copies/mL at the initiation of the vaccination program, and in 98% of PWH the HIV viral load was <50 copies/mL. The median CD4 count was 640 cells/μL [IQR: 490, 800]. Table 1 shows that PWH and controls were reasonably matched and potential confounders were well balanced for age, sex, number of samples available, and total number of vaccine doses. Additionally, the proportion of individuals who obtained N-antibodies during the study period was balanced between the groups. All characteristics of the study populations are presented in Table 1.

Three months prior to baseline, the concentration of anti-RBD antibodies was significantly lower in PWH than in controls (unadjusted GMR: 0.68 (95% CI: 0.54, 0.86), p = 0.002, Table 1, Fig. 1a). We observed no strong evidence to suggest that antibody concentrations differed between PWH and controls at either four- or eleven-months follow-up (unadjusted GMR (95% CI) at four months: 0.90 (0.75, 1.09, p = 0.285) at eleven months: 0.89 (0.69–1.14), p = 0.346, Table 1, Fig. 1a). When adjusting for time since the third dose, the GMR was: 0.94 (95% CI: 0.76, 1.17, p = 0.607), and 0.86 (95% CI: 0.67, 1.11, p = 0.257) at four and eleven months, respectively. In sensitivity analyses, participants with more than three vaccine doses at eleven-month follow-up were excluded (8 PWH and 3 controls) with similar results in both the unadjusted model and adjusted models (data not shown).

The time unadjusted anti-RBD IgG GMC increased from three months before to four months after the third dose in both PWH and controls (both p < 0.001, Table 1, Fig. 1a). From four to eleven months after the third dose, there was a decline in the GMC of anti-RBD IgG within both groups (p = 0.008 and p = 0.056 for PWH and controls, respectively, Table 1 and Fig. 1a). At eleven months after the third dose the GMC of anti-RBD IgG remained higher third dose than it was three months prior to the third dose in both PWH and controls (p < 0.001 and p < 0.001, Table 1 and Fig. 1a).

Evidence of a previous infection, assessed by the presence of N-antibodies, was significantly associated with higher GMC of anti-RBD IgG at all time points in both unadjusted and time-adjusted models (p < 0.001 for all, Fig. 1b). By the eleven-month follow-up 178/267 (66.7%) of PWH and 103/142 (72.5%) of controls had presented antibodies targeting the N-protein. Regardless of being a PWH or control the presence of N-antibodies was associated with a four-fold higher anti-RBD IgG concentration (GMR: 4.04 (95% CI: 3.25, 5.01), p < 0.001).

The mean capacity of the circulating antibodies to neutralise SARS-CoV-2 prior to baseline was 3.68 (95% CI: 0.41, 6.96) percent points lower in PWH than in controls (p = 0.028). We did not observe any significant differences in mean pseudo-neutralising capacity between PWH and controls at either four (unadjusted mean difference, controls *minus* PWH: 0.36 (95% CI: –0.65, 1.35), p = 0.485) or eleven 0.24 (95% CI: –0.65, 1.15), p = 0.588) months after the third dose. When adjusting for time since the administration of the third dose, the mean pseudo-neutralising capacity was 0.27 percent points (95% CI: –0.90, 1.43) lower in PWH than controls at four months (p = 0.652), and 0.26 percent points (95% CI: –0.66, 1.19) lower at 11 months (p = 0.577).

We investigated associations between HIV-related risk factors and antibody concentrations eleven months after the third dose. We found no associations of either nadir CD4, latest CD4 count at initiation of the vaccination program or HIV viral load at initiation of the vaccination program with lower antibody concentrations in both adjusted and unadjusted models (Table 2).

The cellular response was assessed four months after the administration of the third dose in a subset of 178 PWH and 135 controls. We did not find any significant difference in mean IFN-γ concentration between PWH and controls either before (GMR of IFN-γ in PWH compared to controls: 1.06 (95% CI: 0.71–1.60), p = 0.767) or after (1.05 (95% CI: 0.65–1.70), p = 0.830) adjustment for time since administration of the third dose. In subanalyses, we found that PWH with CD4 counts of <200 cells/μL (GMR: 0.03, 95% CI: 0.00, 0.55) and 200–349 cells/μL (GMR: 0.34, 95% CI: 0.11, 1.00) at the initiation of the vaccination program had lower IFN-γ concentrations than PWH with CD4 counts >500 cells/μL (Table 2), with no difference seen between those with CD4 counts of 350–500 and >500 cells/μL. Nadir CD4 count and HIV viral load at initiation of the vaccination program were not associated with lower IFN-γ concentrations in either unadjusted or adjusted models (Table 2).

Discussion

In this observational cohort study, we found similar humoral and cellular immune responses in PWH with undetectable viral replication and controls up to eleven months after receiving the third dose of the BNT162b2 vaccine. In exploratory analyses CD4 count <200 cell/μL at

	PWH n = 378	Controls n = 224	p-value
Age; years, median [IQR]	56 [48–63]	55 [44–63]	0.112
Male sex, n (%)	336 (88.9)	210 (93.8)	0.066
BMI, mean (SD)	25.3 (4.3)[a]	25.5 (3.7)[a]	0.548
Nadir CD4+ T-cell count, cells/μL, n (%)			
>500	45 (11.9)	–	–
350–500	57 (15.1)	–	–
200–349	95 (25.1)	–	–
<200	118 (31.2)	–	–
NA	63 (16.7)	–	–
Latest CD4+ T-cell count at first vaccine dose, cells/μL, n (%)			
>500	271 (71.7)	–	–
350–500	70 (18.5)	–	–
200–349	33 (8.7)	–	–
<200	4 (1.1)	–	–
Latest viral load at first vaccine dose, copies/mL, n (%)			
<50	367 (97.1)	–	–
≥50	11 (2.9)	–	–
cART use at first dose, n (%)	377 (99.7)	–	–
Days from 3rd dose, median [IQR]			
Three months before baseline	−82 [−101, −64]	−129 [−139, −99]	<0.001
4 months follow-up	119 [104, 136]	86 [78, 98]	<0.001
11 months follow-up	328 [313, 339]	320 [313, 325]	<0.001
No. of samples available at timepoint, n, (%)			
Three months before baseline	314 (83.1)	176 (78.6)	0.207
4 months follow-up	346 (91.5)	209 (93.3)	0.532
11 months follow-up	267 (70.6)	142 (63.4)	0.080
No. of vaccine doses at last visit, n (%)			
3, n, (%)	368 (97.4)	221 (98.7)	–
4, n, (%)	10 (2.6)	3 (1.3)	0.438
N-antibody-verified COVID-19 infection, n (%)			
Three months before baseline	27 (7.1)	21 (9.4)	0.411
By time of final sample	224 (59.3)	134 (59.8)	0.960
Anti-SARS-CoV-2-RBD IgG geometric mean concentration (GMC)–AU/mL (95% CI)			
Three months before baseline	1774 (1540–2044)	2589 (2143–3127)	0.002
4 months follow-up	16,407 (14,634–18,396)	18,160 (15,674–21,040)	0.285
11 months follow-up	12,027 (10,387–13,926)	13,552 (11,084–16,570)	0.346
IFN-γ geometric mean concentration (GMC)—mIU/mL (95% CI)			
4 months follow-up	1174 (898–1535)	1104 (812–1502)	0.767

PWH: people with HIV. [a]Information on BMI was not available in 31 (8.2%) of PWH and 32 (14.3%) of controls.

Table 1: **Characteristics of the study population.**

the initiation of the vaccination program was associated with lower IFN-γ concentrations four months after the third dose. We did not find any associations between HIV viral load or nadir CD4 counts with impaired humoral or cellular immune responses in this homogenous cohort.

Following the first two doses of BNT162b2, some studies found that impaired immune responses in PWH were correlated to very low CD4 nadir counts and/or detectable viral loads.[10] However, the few published studies on immune responses up to six month after the administration of the third vaccine dose have not been able to establish a correlation between impaired immune responses in PWH with low current or nadir CD4 counts.[13,29] Correspondingly, we could not establish an association between low nadir or current CD4 counts with impaired antibody responses. We did, however, find lower IFN-γ concentrations after spike peptide stimulation in whole blood from PWH with a CD4 count <200 cells/μL at initiation of the vaccination program. CD4+ and CD8+ T-cells are a major source of IFN-γ secretion in whole blood, and SARS-CoV-2-specific T-cell responses are essential for viral clearance.[30] However, we cannot infer that the T-cells present in this population were less effective in clearing the

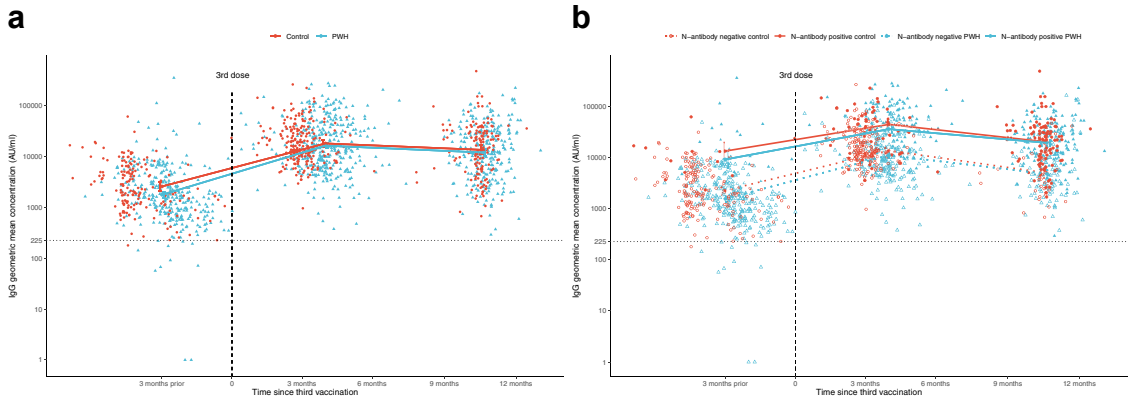

Fig. 1: **Observed anti-RBD IgG concentrations and geometric mean concentrations of anti-RBD IgG up to one year after the third BNT162b2 vaccination in people with HIV (PWH) and controls.** The figure shows the geometric mean concentration (GMC) of anti-RBD IgG predicted by linear regression in AU/mL on a $\log_{10}$ scale plotted on top of observed individual concentrations of anti-RBD IgG from PWH in blue triangles and controls in red circles. The dotted horizontal lines indicate the minimum threshold of an IgG response. Error bars indicate 95% confidence intervals. a) Composite predicted anti-RBD IgG GMC irrespective of previous infection status in PWH (blue) and controls (red). b) Predicted geometric mean concentrations of SARS-CoV-2 antibodies stratified by the presence of antibodies targeting the nucleocapsid protein as indication of a previous infection in PWH (blue) and controls (red). Solid line and filled circles/triangles indicate presence of N-antibodies. Dotted line and open circles/triangles indicate that N-antibodies were not detected.

SARS-CoV-2 virus, as a lower number of T-cells would also produce less IFN-γ in a given volume. Additionally, only very few participants in this study had CD4 counts <200 cells/μL at initiation of the vaccination program.

Waning antibody concentrations and decreased vaccine efficacy was seen after the initial two-dose regimen, leading to recommendations for an additional booster dose. Here, we similarly found decreasing antibody concentrations in PWH eleven months after the third dose of BNT162b2, although the decrease was not statistically significant in controls between the four- and eleven-month sample, the results were highly suggestive of decreasing antibody concentrations in the controls as well. However, despite a decrease, antibody concentrations were still considerably higher in both PWH and controls eleven months after the third dose than in the

	Anti-SARS-CoV-2-RBD IgG—unadjusted geometric mean ratio (95% CI)	p-value	Anti-SARS-CoV-2-RBD IgG—adjusted geometric mean ratio[a] (95% CI)	p-value	IFN-γ—unadjusted geometric mean ratio (95% CI)	p-value	IFN-γ—adjusted geometric mean ratio[a] (95% CI)	p-value
Nadir CD4 count, cells/μL								
>500	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
350–500	0.86 (0.45–1.63)	0.644	0.87 (0.46–1.65)	0.661	1.03 (0.36–2.89)	0.962	1.03 (0.36–2.89)	0.960
200–349	0.66 (0.36–1.19)	0.163	0.65 (0.36–1.19)	0.161	0.80 (0.31–2.04)	0.639	0.76 (0.30–1.94)	0.567
<200	1.09 (0.62–1.92)	0.771	1.09 (0.62–1.94)	0.762	1.16 (0.47–2.86)	0.742	1.67 (0.47–2.87)	0.735
Latest CD4+ T-cell count at first dose, cells/μL								
>500	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
350–500	1.38 (0.94–2.03)	0.099	1.37 (0.93–2.01)	0.108	1.02 (0.50–2.09)	0.947	0.99 (0.48–2.07)	0.993
200–349	1.04 (0.59–1.81)	0.901	1.04 (0.59–1.84)	0.883	0.34 (0.12–1.01)	0.052	0.34 (0.11–1.00)	0.051
<200	0.60 (0.18–2.05)	0.413	0.51 (0.14–1.78)	0.289	0.03 (0.00–0.53)	0.016	0.03 (0.00–0.55)	0.018
Latest viral load at first dose, copies/mL								
<50	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
≥50	1.48 (0.67–3.25)	0.326	1.41 (0.64–3.12)	0.394	1.63 (0.23–11.71)	0.625	1.72 (0.24–12.41)	0.589

[a]Model included propensity score and time since third dose as covariates in addition to each categorical HIV-related variable tested one at a time.

Table 2: **Associations between HIV-related variables and anti-SARS-CoV-2-RBD IgG antibody concentration at 11 months follow-up or IFN-γ concentration at four months follow-up.**

sample collected three months prior to the third dose, six months after initiation of the vaccination program, indicating a more robust antibody response after three doses. By the time of the final blood sampling, a high proportion of participants did, however, have evidence of a previous infection, as they presented antibodies against the N-protein of SARS-CoV-2, which is not part of the vaccine. While the presentation of N-antibodies is not a perfect measure of previous infection in vaccinated individuals it does provides a strong indication of such.[31] A previous infection was thus highly associated with increased antibody concentrations as reported in other studies.[17,31]

This study investigated long-term durability of the immune response to the third dose of BNT162b2 in PWH with up to eleven months follow-up. Our findings suggest that mRNA vaccines effectively facilitate humoral and cellular immune responses to SARS-CoV-2 in PWH. While an absolute benchmark for the protective level of antibodies has yet to be established, a couple of studies by the groups behind some of the original efficacy trials have found correlations between higher concentrations of neutralising antibodies and vaccine efficacy, in individuals vaccinated with the mRNA-based COVID-19 vaccine mRNA-1273 or the vector-based vaccine ChAdOx1 nCoV-19.[32,33] Additionally, several studies have found significant effectiveness in protection against symptomatic illness, and hospitalisation or death after vaccination with BNT162b2 in both PWH and the general population.[34] Thus, vaccination continues to be the key to prevention of COVID-19 in PWH as well as people without HIV. However, attention to the phenomenon of immune imprinting, where the production of antibodies cross-reacting with previously encountered variants overshadows the production of antibodies targeting new variants when encountering these, is pertinent in the development of future vaccine programs, as recent studies have raised awareness of the possibility for negative immune imprinting after booster vaccination against SARS-CoV-2.[35,36]

Our study has some limitations. The study was designed for the original two-dose regimen and thus humoral and cellular responses were assessed in response to the SARS-CoV-2 ancestral strain, although Omicron was the dominant strain at the time the third dose was administered. This limitation decreases the generalisability of our results to the Omicron dominating era, as neutralisation of new strains with serum from individuals vaccinated with the ancestral vaccine has shown less effective in neutralising the BA.4 and BA.5 strain,[37] however new virus strains will evolve, and the dominant strain will vary over time, to which variant updated vaccines have been, and will be developed, thus our results provide a good indication of the vaccine response in the time the vaccine was designed for. Additional limitations include that samples at the exact time of the third vaccine dose were not available. IFN-γ measurements were only available at one time-point and only after the third dose, and our study was not powered to assess protection against infection. Furthermore, the homogenous composition of the study cohort limits generalisability to populations of PWH with less controlled viremia and lower CD4 counts. Despite adjusting for age, sex, number of volunteered samples, and the total number of vaccine doses (through the propensity score), as well as the time since administration of the third dose, we cannot rule out the possibility that other, unmeasured, confounders remain. In particular, we were unable to adjust for the use of immunosuppressant therapies (although it is unlikely that these would be used differently in the two study populations) or the presence of comorbidities. Furthermore, we did not have access to information on symptom presentation or hospitalisation in infected individuals and were therefore not able to correlate the immune responses to disease severity. Our findings should be interpreted in the context of a well-treated cohort of PWH with good viral suppression and should not be inferred to PWH with ongoing viremia or very low CD4+ counts. Strengths of our study include a large study population with a long follow-up period and assessment of both humoral and cellular immune responses.

In contrast to results after the second dose of BNT162b2, we here found that antibody concentrations in PWH were comparable to those of controls eleven months after vaccination with the third dose of BNT162b2. Additionally, cellular responses four months after the third dose of BNT162b2 were comparable between PWH and controls. Our findings suggest that PWH with undetectable viral replication do not have impaired immune responses to the mRNA-based vaccines after the third dose, and could follow vaccination guidelines developed for the general population. However, attention to waning immunity and the emerging immune evasion from new SARS-CoV-2 variants as well as the risk of immune imprinting is necessary for the optimal timing of future booster vaccinations in PWH, as it is in the general population.

Contributors

All authors contributed to drafting the paper and revised the manuscript for important intellectual content. LDH, HB, PG, KI and SDN conceived and designed the study. LDH, LP-A, RBH, CBH, SRH, DLM, MP-H, KF, JG, KG, SRO, RF-S, ES, LH, HB, PG, KI, CS and SDN contributed to data collection and analysis. LDH and CS verified data and had full access to raw data. All authors had full access to summary data reported in this study. All authors read and approved the final version of the manuscript.

Data sharing statement

The datasets generated and analysed during the current study are available in de-identified format from the corresponding author on reasonable request.

Declaration of interests

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References

1 Brown AE, Croxford SE, Nash S, et al. COVID-19 mortality among people with diagnosed HIV compared to those without during the first wave of the COVID-19 pandemic in England. *HIV Med*. 2022;23:90.
2 Ambrosioni J, Blanco JL, Reyes-Urueña JM, et al. Overview of SARS-CoV-2 infection in adults living with HIV. *Lancet HIV*. 2021;8:e294–e305.
3 Rasmussen LD, Cowan S, Gerstoft J, et al. Outcomes following SARS-CoV-2 infection among individuals living with and without HIV; a Danish nationwide cohort study. *AIDS*. 2022;37(2):311–321. https://doi.org/10.1097/qad.0000000000003393. Publish Ah.
4 Almomen A, Cox J, Lebouché B, et al. Short communication: ongoing impact of the social determinants of health during the second and third waves of the COVID-19 pandemic in people living with HIV receiving care in a Montreal-based tertiary care center. *AIDS Res Hum Retroviruses*. 2022;38:359–362. https://home.liebertpub.com/aid.
5 Hove-Skovsgaard M, Zhao Y, Tingstedt JL, et al. Impact of age and HIV status on immune activation, senescence and apoptosis. *Front Immunol*. 2020;11:583569. https://doi.org/10.3389/FIMMU.2020.583569.
6 Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. SL-T, 2014 undefined *Lancet*. 2014;384:258–271.
7 Nakagawa F, Lodwick RK, Smith CJ, et al. Projected life expectancy of people with HIV according to timing of diagnosis. *AIDS*. 2012;26:335–343.
8 Heftdal LD, Knudsen AD, Hamm SR, et al. Humoral response to two doses of BNT162b2 vaccination in people with HIV. *J Intern Med*. 2021;291(4):513–518. https://doi.org/10.1111/JOIM.13419.
9 Yin J, Chen Y, Li Y, Wang C, Zhang X. Immunogenicity and efficacy of COVID-19 vaccines in people living with HIV: a systematic review and meta-analysis. *Int J Infect Dis*. 2022;124:212.
10 Hensley KS, Jongkees MJ, Geers D, et al. Immunogenicity and reactogenicity of SARS-CoV-2 vaccines in people living with HIV in the Netherlands: a nationwide prospective cohort study. *PLoS Med*. 2022;19:e1003979.
11 Costiniuk CT, Singer J, Lee T, et al. COVID-19 vaccine immunogenicity in people with HIV. *AIDS*. 2023;37:F1–F10.
12 Lapointe HR, Mwimanzi F, Cheung PK, et al. People with HIV receiving suppressive antiretroviral therapy show typical antibody durability after dual COVID-19 vaccination, and strong third dose responses. *J Infect Dis*. 2022;227(7):838–849. https://doi.org/10.1093/INFDIS/JIAC229. Published online June 7.
13 Lapointe HR, Mwimanzi F, Cheung PK, et al. Antibody response durability following three-dose COVID-19 vaccination in people with HIV receiving suppressive ART. *AIDS*. 2022;37(5):709–721. https://doi.org/10.1097/qad.0000000000003469. Publish Ah.
14 Griessbach A, Chammartin F, Abela IA, et al. Antibody response after third vaccination with mRNA-1273 or BNT162b2: extension of a randomized controlled SARS-CoV-2 noninferiority vaccine trial in patients with different levels of immunosuppression (COVERALL-2). *Open Forum Infect Dis*. 2023;10:ofad150. https://doi.org/10.1093/OFID/OFAD150.
15 Pérez-Alós L, Armenteros JJA, Madsen JR, et al. Modeling of waning immunity after SARS-CoV-2 vaccination and influencing factors. *Nat Commun*. 2022;13:1614.
16 Hansen CB, Jarlhelt I, Pérez-Alós L, et al. SARS-CoV-2 antibody responses are correlated to disease severity in COVID-19 convalescent individuals. *J Immunol*. 2021;206:109–117.
17 Hansen CB, Jarlhelt I, Hasselbalch RB, et al. Antibody-dependent neutralizing capacity of the SARS-CoV-2 vaccine BNT162b2 with and without previous COVID-19 priming. *J Intern Med*. 2021;290(6):1272–1274. joim.13366.
18 HIV. https://en.ssi.dk/surveillance-and-preparedness/surveillance-in-denmark/annual-reports-on-disease-incidence/hiv-2020; 2020. Accessed February 4, 2023.
19 Grove Krause T, Jakobsen S, Haarh M, Mølbak K. The Danish vaccination register. *Euro Surveill*. 2012;17:20155.
20 Hamm SR, Møller DL, Pérez-Alós L, et al. Decline in antibody concentration 6 months after two doses of SARS-CoV-2 BNT162b2 vaccine in solid organ transplant recipients and healthy controls. *Front Immunol*. 2022;13:564.
21 Bayarri-Olmos R, Idorn M, Rosbjerg A, et al. SARS-CoV-2 neutralizing antibody responses towards full-length spike protein and the receptor-binding domain. *J Immunol*. 2021;207(3):878–887. https://doi.org/10.4049/jimmunol.2100272.
22 R Core Team. *R a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2019. References - Scientific Research Publishing. https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=2631126. Accessed November 4, 2021.
23 Niknam BA, Zubizarreta JR. Using cardinality matching to design balanced and representative samples for observational studies. *JAMA*. 2022;327(2):173–174. https://doi.org/10.1001/jama.2021.20555.
24 Ho DE, Imai K, King G, Stuart EA. MatchIt: nonparametric preprocessing for parametric causal inference. *J Stat Softw*. 2011;42:1–28.
25 A grammar of data manipulation [R package dplyr version 1.0.7]. Published online June 18. https://cran.r-project.org/package=dplyr; 2021. Accessed November 4, 2021.
26 Tidy messy data [R package tidyr version 1.2.0]. Published online Feb 1. https://cran.r-project.org/package=tidyr; 2022. Accessed August 27, 2022.
27 Wickham H. Simple, consistent wrappers for common string operations [R package stringr version 1.4.1]. Published online Aug 20 https://cran.r-project.org/package=stringr; 2022. Accessed August 27, 2022.
28 Xiao N. Scientific journal and Sci-Fi themed color palettes for 'ggplot2' [R package ggsci version 2.9]. Published online May 14. https://cran.r-project.org/package=ggsci; 2018. Accessed August 27, 2022.
29 Vergori A, Cozzi-Lepri A, Matusali G, et al. SARS-CoV-2 omicron variant neutralization after third dose vaccination in PLWH. *Viruses*. 2022;14:1710. https://doi.org/10.3390/V14081710.
30 Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol*. 2022;23:186–193.
31 Navaratnam AMD, Shrotri M, Nguyen V, et al. Nucleocapsid and spike antibody responses following virologically confirmed SARS-CoV-2 infection: an observational analysis in the Virus Watch community cohort. *Int J Infect Dis*. 2022;123:104–111.
32 Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27:2032.
33 Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*. 2022;375:43.
34 Chambers C, Samji H, Cooper CL, et al. Coronavirus disease 2019 vaccine effectiveness among a population-based cohort of people living with HIV. *AIDS*. 2022;36:F17–F26. https://doi.org/10.1097/QAD.0000000000003405.
35 Chemaitelly H, Ayoub HH, Tang P, et al. Long-term COVID-19 booster effectiveness by infection history and clinical vulnerability and immune imprinting: a retrospective population-based cohort study. *Lancet Infect Dis*. 2023. https://doi.org/10.1016/S1473-3099(23)00058-0. Published online March.
36 King SM, Bryan SP, Hilchey SP, Wang J, Zand MS. First impressions matter: immune imprinting and antibody cross-reactivity in influenza and SARS-CoV-2. *Pathogens*. 2023;12:169. https://doi.org/10.3390/PATHOGENS12020169.
37 Tuekprakhon A, Nutalai R, Dijokaite-Guraliuc A, et al. Antibody escape of SARS-CoV-2 omicron BA.4 and BA.5 from vaccine and BA.1 serum. *Cell*. 2022;185:2422–2433.e13. https://doi.org/10.1016/j.cell.2022.06.005.