Humoral and cellular immune response elicited by mRNA vaccination against SARS-CoV-2 in people living with HIV (PLWH) receiving antiretroviral therapy (ART) according with current CD4 T-lymphocyte count

Andrea Antinori[1*], Stefania Cicalini[1], Silvia Meschi[2], Veronica Bordoni[3], Patrizia Lorenzini[1], Alessandra Vergori[1], Simone Lanini[1], Lidya De Pascale[1], Giulia Matusali[2], Davide Mariotti[3], Alessandro Cozzi Lepri[4], Paola Galli[5], Carmela Pinnetti[1], Roberta Gagliardini[1], Valentina Mazzotta[1], Ilaria Mastrorosa[1], Susanna Grisetti[1], Francesca Colavita[2], Eleonora Cimini[3], Elisabetta Grilli[1], Rita Bellagamba[1], Daniele Lapa[2], Alessandra Sacchi[3], Alessandra Marani[5], Carlo Cerini[1], Caterina Candela[1], Marisa Fusto[1], Vincenzo Puro[6], Concetta Castilletti[2], Chiara Agrati[3], Enrico Girardi[7], Francesco Vaia[5], for the HIV-VAC Study Group†.

[1]Clinical Department, HIV/AIDS Unit, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

[2]Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

[3]Laboratory of Cellular Immunology and Clinical Pharmacology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

[4]Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CREME), Institute for Global Health, UCL, London, UK.

[5]Health Direction, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

[6]Risk Management, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

[7]Clinical Epidemiology, National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Roma, Italy.

*Corresponding Author:

Andrea Antinori, MD

Clinical Department of Infectious Diseases and Research, HIV/AIDS Unit

National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS

Via Portuense 292, 00149 Roma, Italy

e-mail: andrea.antinori@inmi.it

HIV-VAC study group † listed in the acknowledgment section

Summary: Humoral and cell-mediated immune response against SARS-CoV-2 were elicited in PLWH, significantly poorer in those with CD4 T-cell <200/mm^3 versus those with >500 cell/mm^3 and HIV-negative controls; immune response in PLWH with a CD4 T-cell >500/mm^3 was comparable to HIV-negative population.

Abstract

Background

Data on SARS-CoV-2 vaccine immunogenicity in PLWH are currently limited. Aim of the study was to investigate immunogenicity according to current CD4 T-cell count.

Methods

PLWH on ART attending a SARS-CoV-2 vaccination program, were included in a prospective immunogenicity evaluation after receiving BNT162b2 or mRNA-1273. Participants were stratified by current CD4 T-cell count (poor CD4 recovery, PCDR: <200/mm^3; intermediate CD4 recovery, ICDR: 200-500/mm^3 high CD4 recovery, HCDR: >500/mm^3). RBD-binding IgG, SARS-CoV-2 neutralizing antibodies (nAbs) and IFN-γ release were measured. As control group, HIV-negative healthcare workers (HCWs) were used.

Findings

Among 166 PLWH after 1 month from the second dose, detectable RBD-binding IgG were elicited in 86.7% of PCDR, 100% of ICDR, 98.7% of HCDR, and a neutralizing titre ≥1:10 elicited in 70.0%, 88.2% and 93.1%, respectively. Compared to HCDR, all immune response parameters were significantly lower in PCDR. After adjusting for confounders, current CD4 T-cell <200/mm^3 significantly predicted a poor magnitude of anti-RDB, nAbs and IFN-γ response. As compared with HCWs, PCDR elicited a consistently reduced immunogenicity for all parameters, ICDR only a reduced RBD-binding antibody response, whereas HCDR elicited a comparable immune response for all parameters.

Conclusion

Humoral and cell-mediated immune response against SARS-CoV-2 were elicited in most of PLWH, albeit significantly poorer in those with CD4 T-cell <200/mm^3 versus those with >500 cell/mm^3 and HIV-negative controls. A decreased RBD-binding antibody response than HCWs was also observed in PLWH with CD4 T-cell 200-500/mm^3, whereas immune response elicited in PLWH with a CD4 T-cell >500/mm^3 was comparable to HIV-negative population.

Key words: HIV; AIDS; anti-SARS-CoV-2 vaccine; Immunogenicity

INTRODUCTION

Effective vaccination strategy currently represents the main control measure of pandemic[1], particularly in highly vulnerable people at high risk of severe COVID-19[2].

In people living with HIV (PLWH), despite the early antiretroviral therapy (ART) beneficial effects[3], they may persistently experience a chronic immune dysregulation[4], causing a not fully restored immune health[5].

Observational studies suggested that COVID-19 may have a worse prognosis in PLWH compared to HIV-negative population, with an increased risk of mortality[6-7]. Recent data suggested that a higher risk of severe COVID-19 in PLWH may be associated with poor neutralising antibody (nAbs) titres and this might reflect a diminished antibody response to SARS-CoV-2 natural infection[8]. These data are consistent with the observation that HIV infection may favor a poor serological response to vaccines for viral agents, such as influenza[9] or hepatitis B[10].

At present, few data have been published on the immunogenicity of SARS-CoV-2 vaccination in PLWH. Preliminary data in PLWH from a single-arm open-label study from a large, controlled, phase 2/3 randomized trial in UK, showed that ChAdOx1-nCoV-19 vaccine, given as prime-boost dosing 4–6 weeks apart, was safe and produced consistent immune responses in PLWH on ART and with CD4 counts above 350 cells/mm^3 [11]. Similarly, the interim results from a randomized, double-blind, placebo-controlled phase 1b/2 trial on the safety and immunogenicity of the same adenovirus-vectored vaccine in South Africa, showed comparable safety and immunogenicity between PLWH, with a median count of 695 cell/mm^3, and HIV-negative people[12].

Three observational studies were published on mRNA vaccines in PLWH. In the first study, 98% of HIV-infected individuals enrolled in a prospective evaluation of BNT162b2 vaccine with a mean CD4 count of 700 cell/ mm^3 showed a RBD-binding IgG response detectable at a median of 18 days after the second dose[13]. In a small size prospective study, on PLWHs with a median of 913 CD4 T-cells/mm^3 receiving BNT162b2, a robust humoral and cellular immune response, comparable to that observed in healthy donors, was observed[14]. Finally, in another small size prospective study, HIV-infected individuals receiving BNT162b2 or mRNA-1273, of whom 86% with a CD4 count >200 cell/mm^3, developed high titers of anti-RBD antibodies[15]. Nonetheless, in these studies, the information about the immunogenicity of SARS-CoV-2 vaccination in PLWH with low current CD4 T-cell count is lacking, and the value of this marker in predicting vaccine response in HIV-infected people has not been yet estimated.

Aim of the present study was to evaluate the immunogenicity of a SARS-CoV-2 vaccination with a mRNA vaccine (BNT1622b or mRNA-1273) in PLWH, according with current CD4 T-cell count, and estimate this variable as predictor of immune response to vaccination.

METHODS

Study design and population

On March 24, 2021, as part of the Nationwide Mass Vaccination Program in Italy, the National Institute for Infectious Diseases Lazzaro Spallanzani in Rome started a vaccination campaign against SARS-CoV-2 in PLWH, according to the Ministry of Health recommendations, primarily targeted to fragile individuals, e.g. those with a previous AIDS or a current CD4 T-cell count <200/mm^{3}or comorbidities. In the following months, vaccination campaign has been extended to all PLWH.

The HIV-VAC study is an observational study on the outcomes of SARS-CoV-2 vaccination in PLWH. According to the protocol, demographic, epidemiologic, clinical and laboratory characteristics of PLWH undergoing vaccination were collected. The main study outcomes are: a) prevalence and magnitude of anti-Spike RBD-binding antibodies response after vaccination; b) prevalence and magnitude of neutralizing activity and cell-mediated immune response after vaccination (only in a subgroup of participants). By protocol, following written informed consent, blood samples were collected for all PLWH enrolled at the time of first dose (baseline, T0), before the second dose (T1) and 1 month (T2) after the second dose; the study will continue with further evaluation timepoints after the second dose. The study was approved by the Scientific Committee of the Italian Drug Agency (AIFA) and by the Ethical Committee of the Lazzaro Spallanzani Institute, as National Review Board for COVID-19 pandemic in Italy (approval number 323/2021).

Here we present results on immunogenicity (humoral, neutralizing and cell-mediated response) at T1 and T2 of follow-up. The study population consisted of PLWH who completed the 2-dose schedule with BNT162b2 or mRNA-1273 vaccines up to July 20, 2021 and consecutively enrolled at the immunogenicity sub-study. Individuals with a SARS-CoV-2 infection diagnosis, defined by a RT-PCR positive on the nasopharyngeal swab, or positivity to anti-N and/or to anti-Spike receptor-binding domain (anti-S/RBD) antibodies at T0, or to anti-N at T1 or T2, were excluded for the present analysis. An unmatched control group of health care workers (HCWs), vaccinated with BNT162b2 who underwent the same schedule of blood sample collection, enrolled in another surveillance study[16], were included as external controls.

Laboratory procedures

Two commercial chemiluminescence microparticle antibody assays (CMIA), the SARS-CoV-2 specific anti-N, and the anti-S/RBD tests (ARCHITECT SARS-CoV-2 IgG, and ARCHITECT SARS-CoV-2 IgG II Quantitative, Abbott Laboratories, Wiesbaden, Germany respectively,) were performed on ARCHITECT® i2000sr (Abbott Diagnostics, Chicago, IL, USA) and used according to manufacturer's instruction; Index >1.4 and Binding Antibody Units (BAU)/mL ≥7.1 are considered positive, respectively.

Micro-neutralization assay (MNA) was performed as previously described, using SARS-CoV-2/Human/ITA/PAVIA10734/2020, as challenging virus[17]. Briefly, serum samples were heat-inactivated at 56°C for 30 minutes, and titrated in duplicate in 7 two-fold serial dilutions (starting dilution 1:10). Equal volumes (50µl) of serum and medium containing 100 $TCID_{50}$ SARS-CoV-2 were mixed and incubated at 37 °C for 30

min. Serum-Virus mixtures were then added to sub-confluent Vero E6 cell monolayers and incubated at 37°C and 5% CO_2. After 48 hours, microplates were observed by light microscope for the presence of cytopathic effect (CPE). To standardize inter-assay procedures, positive control samples showing high (1:160) and low (1:40) neutralizing activity were included in each assay session. Serum from the National Institute for Biological Standards and Control, UK (NIBSC) with known neutralization titer (Research reagent for anti-SARS-CoV-2 Ab NIBSC code 20/130) was used as reference in MNA.

We studied IFN-γ and IL-2 production in response to Spike stimulation as a surrogate of specific T-cell function. Briefly, whole blood was stimulated in vitro at 37°C (5% CO2) with a pool of peptides covering the sequence of SARS−CoV−2 spike protein (SARS−CoV−2 PepTivator® Prot_S1, Prot_S, and Prot_S+, Miltenyi Biotec, Germany). After 16-20 hours of incubation, plasma was harvested and stored at -80°C until use. IFN-γ levels were measured by an automatic ELISA (ELLA, protein simple), and the IFN-γ values obtained from the stimulated samples were subtracted from the unstimulated-control value. The Staphylococcal enterotoxin B (SEB) was used as positive control. The detection limit of these assays was 0.17 pg/ml and 0.54 pg/ml for IFN-γ and IL-2 respectively.

Statistical analysis

PLWH included in the present analysis were stratified into three groups according to the degree of immune recovery: 1) patients with current CD4 T-cell <200/mm^3 (poor CD4 recovery, PCDR); 2) patients with current CD4 T-cell between 200 and 500 cell/mm^3 (intermediate CD4 recovery, ICDR); 3) patients with current CD4 T-cell >500/mm^3 (high CD4 recovery, HCDR). Descriptive statistics were presented as median with interquartile range (IQR) for continuous variables and frequency with proportion for categorical variables. For the comparison over time within each group, parameters at T1 and T2 were compared with baseline level using paired Wilcoxon sign-rank test, and paired proportions were compared with McNemar test. The overall responses at times T1 and T2 have been also compared by gender. For the comparison between groups, Kruskal-Wallis test was performed to determine if groups were significantly different on all continuous variables considered. Specifically, Dunn's test with Bonferroni correction was used for pairwise multiple comparisons of each parameter between any pairs. Chi-square test was used for comparison of proportions. Moreover, a multivariable linear regression model was fitted to evaluate the association between current CD4 T-cell count or CD4/CD8 ratio and the magnitude of immune response after adjustment for main confounders such as age, years of HIV infection, CD4 nadir, level of HIV-RNA (<50 vs. >50 copies/mL), type of mRNA vaccination and presence of previous or current malignancy. Further, a different multivariable linear regression model, adjusted for gender and age, was fitted to control the association between the magnitude of immune response (anti-S/RBD, nAbs titres, and IFN-γ) and PLWH groups and HCWs. Since the distribution of data was positively skewed, a logarithmic transformation was performed for RBD-binding IgG, nAbs titres, IFN-γ and IL2, to make the data conform more closely to the normal distribution and to improve the model fit. Finally, linear regression was used to investigate the correlation between the CD4 count and CD4/CD8 ratio at T0 and level

of each parameter at T2. A 2-sided P value <0.05 was considered statistically significant. Analyses were performed by STATA v15.1.

RESULTS

Study population

N=166 PLWH were included in the analysis (PCDR=32; ICDR=56; HCDR=78). The main characteristics of HIV-infected participants according to current CD4 T cell count at vaccination were reported in Table 1. The three groups significantly differed for years of HIV infection, previous AIDS diagnosis, current or previous malignancy, CD4 nadir, CD4/CD8 ratio. All HIV patients were on ART at time of SARS-CoV-2 vaccination, and the three groups significantly differed for duration of ART exposure. Proportion of PLWH with HIV-RNA lower than 50 copies/mL was 68.8% in PCDR, 92.9% in ICDR, and 100% in HCDR (p<0.001). No significant difference was observed for main non-infectious comorbidities in the three groups. As vaccination, 93 (57%) received BNT162b2, and 70 (43%) mRNA-1273. As control group, 169 unmatched HCWs were included: 71.6% female with a median age of 42 years (IQR 32-53). All HCWs received BNT162b2 as vaccine.

RBD-binding IgG response and neutralizing antibody (nAb) response after vaccination in PLWH

We first compared RBD-binding IgG responses by gender and we found no evidence for an association. Median changes (IQR) at T1 were 98.4 (20.6-254.5) in males vs. 86.7 (20.6-4010.3) in females (p=0.97) (Supplementary Table 1). The corresponding figures at T2 were 1360.5 (521.9-2357.7) vs 1142.9 (736.0-1923.2), p=0.78. A significant increase of magnitude of RBD-binding IgG response from time of priming dose (T0) to time of the second dose (T1), and at 1 month after the second dose (T2) was observed for all PLWH groups (Figure 1). After the priming dose of vaccine (T1), immunogenicity measured by RBD-binding IgG response was significantly lower in PCDR than ICDR (p=0.011) and HCDR (p<0.001), as well as lower in ICDR than HCDR (p=0.004) (Supplementary Figure S1a). After 1 month from the second dose (T2), a detectable RBD-binding IgG response was elicited in 86.7% of PCDR, 100% of ICDR and 98.7% of HCDR (PCDR vs ICDR, p=0.014; PCDR vs HCDR, p=0.021; ICDR vs HCDR, p=1.0) (Table 2). The level of RBD-binding IgG (BAU/mL) response at T2 were lower in PCDR than ICDR (p=0.029) and HCDR (p<0.001), but not different between ICDR and HCDR (p=0.184) (Figure 2a). At T2, nAbs response against SARS-CoV-2 (defined as a titre >1:10) was elicited in 70.0% of PCDR, 90.8% of ICDR and 90.9% of HCDR (PCDR vs ICDR, p=0.041; PCDR vs HCDR, p=0.002; ICDR vs HCDR, p=0.356) (Table 2). Magnitude of nAbs titres [MNA reciprocal of dilution], were lower in PCDR than HCDR (p=0.001), but not in PCDR than ICDR (p=0.150) and in ICDR than HCDR (p=0.239) (Figure 2b). A significant correlation between RBD-binding IgG at time T2 and nAbs was found by non-parametric Spearman correlation coefficient (r=0.85, p<0.001) (Supplementary Figure S2a).

Spike specific T-Cell response after vaccination in PLWH

We first compared T-cell responses by gender and we found no evidence for an association. Median changes at T1 were 24.3 (0.01-159.6) in males vs. 49.8 (4.3-116.1) in females (p=0.42) (Supplementary Table 1). The corresponding figures at T2 were 220.7 (51.2-441.6) vs 122.7 (76.4-358.8), p=0.51. In contrast, the data carried some evidence that the IL-2 response was greater in females vs. males. Median changes at T1 were 54.5 (4.2-151.8) vs. 159.6 (25.3-235.6) p=0.03 and 150.8 (61.5-353.5) vs. 215.2 (99.5-523.4) at T2 (p=0.17). A significant increase of specific T cell response (IFN- γ and IL-2 production after Spike peptide stimulation) from time of priming dose (T0) to time of the second dose (T1), and at 1 month after the second dose (T2) was observed for all PLWH groups (Figure 3), except for IFN- γ production at T1, which was not different from baseline in PCDR group. After 1 month from the second dose (T2), IFN- γ release after stimulation was significantly lower in PCDR than ICDR (p=0.007) and HCDR (p<0.001), but not different between ICDR and HCDR (p=0.557) (Figure 4a). Median (IQR) values of IL-2 release after stimulation was lower in PCDR than HCDR (p=0.006) but not between PCDR and ICDR (p=0.171) and between ICDR and HCDR (p=0.570) (Figure 4b). A positive correlation between IFN-γ and IL-2 was observed (Pearson, r=0.428, P<0.0001), suggesting a coordinated response (Figure 5a); this correlation was confirmed as significant in PCDR and ICDR, but only as marginal in HCDR (Figure 5b-5d). A significant correlation between RBD-binding IgG BAU/mL at time T2 and IFN-γ pg/mL was also found by non-parametric Spearman correlation coefficient (r=0.16, p=0.004) (Supplementary Figure S2b).

Role of current CD4 T cell count and of CD4/CD8 ratio on predicting immunogenicity

In PLWHs, a significant correlation between CD4 T cell/mm^3 and magnitude of RBD-binding IgG (Figure 6a), nAbs (Figure 6b) and IFN-γ release (Figure 6c) was observed by simple linear regression. Having magnitude of RBD-binding IgG production, nAbs titre and IFN-γ release as dependent covariates, current CD4 count <200 cell/mm^3 was associated with a significantly lower magnitude of immune response, after adjusting for the main identified confounders (age, years of HIV infection, CD4 nadir, HIV-RNA <50 vs ≥50 copies/mL, type of mRNA vaccine and previous or current malignancy) (Table 3). CD4/CD8 ratio was associated only with increasing magnitude of RBD-binding IgG production after multivariable adjustment (Table 3).

Comparisons of immunogenicity of vaccine-between PLWH and HCWs

Compared to HCWs, the proportion of a detectable RBD-binding IgG response after 1 month from second dose, was lower in PCDR (p<0.001), but comparable in ICDR (p=1.0) and in HCDR (p=0.313) (Table 2), and the median values of RBD-binding IgG response after 1 month from second dose of mRNA vaccine was significantly lower for all PLWHs groups [HCWs vs PCDR (p<0.001), HCW vs ICDR (p<0.001), HCWs vs HCDR (p=0.031)] (Figure 2a). Comparing the nAbs of PLWHs to that of HCWs, the proportion of nAbs vaccine responders was significantly lower in PCDR (p<0.001) and in ICDR (p=0.019), but not in HCDR (p=0.116) (Table 2), and the magnitude of nAbs response significantly lower in PCDR (p<0.001), only marginally in ICDR (p=0.050) and not different in HCDR (p=1.000) (Figure 2b). Compared to HCWs, the median (IQR) value of IFN-γ release after

stimulation was lower in PCDR (p<0.001), in ICDR (p=0.020), but not in HCDR (p=0.528) (Figure 4a), and the median (IQR) value of IL-2 release after stimulation lower in PCDR (p=0.024) but not in ICDR (p=1.000) or in HCDR (p=0.932) (Figure 4b). Having HCWs as reference, after adjustment for gender and age by multivariable linear regression, a significant association between PCDR and reduction of magnitude of immune response was found for all three parameters (MNA p<0.001; RBD-binding IgG p<0.001; IFN-γ p<0.001). ICDR was associated only with a significant reduction of RBD-binding antibodies, whereas no significant association, after multivariable adjustment, was observed for HCDR (Table 4).

DISCUSSION

According to these findings, SARS-CoV-2 vaccination with a mRNA vaccine induced a robust humoral and cell-mediated immune response in most of PLWH receiving ART. Notably, this immunogenicity was strongly related to CD4 T cell count at the time of vaccination, thus in PLWH with current CD4-T cell count above 500 cells/mm^3, the immune response after the second dose was comparable, for humoral and cell-mediated immunity, to that found in HCWs. These results are consistent with previously published data on immunogenicity after adenovirus-vectored[11-12] or mRNA vaccines[13-15] in PLWH on ART and high CD4 T-cell count. In contrast, we found no evidence for an association with gender with the exception of IL-2 responses which appeared to be larger in females vs. males. A well-integrated immune response represents the main goal of vaccination strategies, and we showed that mRNA vaccination of PLWH with high CD4 T-cell count was able to induce a coordinated immune response, seen in recovered patient after natural SARS-CoV-2 infection[18], and as described in HCWs[16].

Nevertheless, in PLWH with poor CD4 recovery, we observed a significant reduced response to SARS-CoV-2 vaccination compared both to immunologically restored HIV people and HIV-negative controls. In PLWH with a current CD4 T-cell count <200 cell/mm^3, a neutralizing activity was detectable only in 70%. This proportion might be remarkable in the light of severe and persistent immunologic dysregulation, although substantially lower than that observed in PLWH with high CD4 T-cell count and HIV-negative control.

Correlate of protections of vaccines against COVID-19 are currently unclear[19], as studies evaluating the impact of an impaired immunological response to vaccines on the risk of SARS-CoV-2 infection and symptomatic COVID-19 are lacking. To our knowledge, the present study is the first characterizing immune response to SARS-CoV-2 vaccination in advanced PLWHs, and may provide useful information for answering the question of what vaccine strategy is feasible in this vulnerable population.

A low CD4/CD8 ratio was also suggested as factor associated with increased innate and adaptive immune activation, immune-senescent phenotype[20] and also to a poor magnitude of SARS-CoV-2-specific responses[21]. In our analysis, CD4/CD8 ratio was independently associated only with RBD-binding production after vaccination, but not to neutralizing or cell-mediated response, although we cannot exclude a residual effect.

Our data highlighted that PLWH seem to display a better immune response after SARS-CoV-2 vaccination than observed for other immunosuppressed populations as solid organ transplant recipients[22]: 35% of renal

transplant recipients developed nAbs after BNT162b2 vaccination[23], and similar results were observed in those receiving rituximab[24],poorer rates than 70% observed in PLWH with very low latest CD4 T-cell count. A reasonable explanation of this discrepancy may be due to the different mechanisms of immunosuppression in the two populations. Transplant recipients or patients receiving anti-CD20 therapy experience a strong active pharmacological immunosuppression due to the ongoing treatment; in contrast, the effective ART able to suppress HIV replication can allow a partial restoration of functional immune response also in patients with a still low CD4 T-cell count.

Main limitation of our study was the observational, not randomized nature of the design, lacking of a matched HIV-negative control group. However, the comparisons with HCWs were controlled for gender and age. Moreover, the short duration of current follow up, made us still unable to give appropriate information on waning of protective immune response after SARS-CoV-2 vaccination in PLWH. Although females were under-represented in our sample we were able to detect significant differences in IL-2 responses comparing males with females.

Despite these limitations, the results of the present study may serve to select subpopulation of PLWH poorly responder to SARS-CoV-2 vaccination for additional dose strategies, as recently recommended in those with low CD4 count[25], and currently investigated in other immunosuppressed people[26]. In the context of the current debate on benefit-risk of booster dose, it was suggested that an additional dose could be delivered to immunocompromised individuals without an adequate immune response to standard schedule[27]. Our data support that PLWH with current CD4 T-cells <200 cell/mm^3 should receive additional dose; this dose could be reasonably offered also to PLWH with a CD4 count between 200 and 500 cell/mm^3, taking into account both dysregulation and poor immune response observed than HIV-negative controls.

In conclusions, the present study supports the hypothesis that mRNA vaccination would be able to elicit a robust humoral and cellular immune response against SARS-CoV-2 in most of PLWH receiving ART, particularly in those with full immune recovery after suppressive therapy. Nevertheless, this immune response to vaccination is significantly poorer in those with current CD4 T-cell count <200 cell/mm^3, suggesting that chronic persistent dysregulation in ART-treated population may affect the effector immune response to this vaccination. The implications of these findings as correlates of protection of SARS-CoV-2 vaccination in PLWH should be further investigated.

NOTES

Acknowledgements

The authors gratefully acknowledge nurse staff, all the patients and all members of the *HIV-VAC Study Group*:

Chiara Agrati, Alessandra Amendola, Andrea Antinori, Francesco Baldini, Rita Bellagamba, Aurora Bettini, Licia Bordi, Veronica Bordoni, Marta Camici, Caterina Candela, Rita Casetti, Concetta Castilletti, Carlo Cerini, Stefania Cicalini, Francesca Colavita, Sarah Costantini, Flavia Cristofanelli, Alessandro Cozzi Lepri, Claudia D'Alessio, Alessia De Angelis, Federico De Zottis, Lydia de Pascale, Massimo Francalancia, Marisa Fusto, Roberta Gagliardini, Paola Gallì, Enrico Girardi, Giulia Gramigna, Germana Grassi, Elisabetta Grilli, Susanna Grisetti, Denise Iafrate, Simone Lanini, Daniele Lapa, Patrizia Lorenzini, Alessandra Marani, Erminia Masone, Ilaria Mastrorosa, Davide Mariotti, Stefano Marongiu, Giulia Matusali, Valentina Mazzotta, Silvia Meschi, Annalisa Mondi, Stefania Notari, Sandrine Ottou, Jessica Paulicelli Luca Pellegrino, Carmela Pinnetti, Maria Maddalena Plazzi, Adriano Possi, Vincenzo Puro, Alessandra Sacchi, Eleonora Tartaglia, Francesco Vaia, Alessandra Vergori.

Authors'Contributions

AA, SL and AV conceptualized, designed the study and wrote the protocol. AA, SC, CA and CC wrote the first draft of the manuscript and referred to appropriate literature. AA, FV, EG, CA, CC and VP conceived, supervised the study and contributed to data interpretation. PL and ACL were the main responsible persons for formal data analysis and also contributed to the article drafting. MF and LDP were responsible for data curation; AV, CP, VM, RG, IM revised the manuscript content, reviewed and edited the manuscript. SM, GM, FC, DL performed all the serology tests and neutralization assays; VB, DM, EC,AS performed all the T-cell function tests; LDP, CC and CC performed and supervised the anti-SARS-CoV-2 vaccination campaign at INMI for HIV-positive individuals and enrolled participants; SG, EG, RB enrolled participants and reviewed the manuscript. All authors agreed with and approved the final version of the manuscript.

Ethics Commettee Approval

The study was approved by the Scientific Committee of the Italian Drug Agency (AIFA) and by the Ethical Committee of the Lazzaro Spallanzani Institute, as National Review Board for COVID-19 pandemic in Italy (approval number 323/2021).

Data sharing

Anonymized participant data will be made available upon reasonable requests directed to the corresponding author. Proposals will be reviewed and approved by investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

Funding

This work was supported by SARS-CoV-2 Surveillance and Response Program by the Lazio Region Health Authority; Ministero della Salute, Programma CCM 2020; Ministero della Salute, Ricerca Corrente - Linea 1 e Linea 2; Ministero della Salute, COVID-2020-12371675; European Commission, European Virus Archive - GLOBAL, grants no. 653316 and no. 871029. ACL was funded by EU under HORIZON Europe Program (Grant Agreement 101046016).

Declarations of Interests

The corresponding author is responsible for submitting a competing interests statement on behalf of all authors of the paper. Outside of this submitted work: Andrea Antinori has served as a paid consultant to Gilead Sciences, Janssen-Cilag, Merck, GlaxoSmithKline, Astra Zeneca, Roche, and ViiV Healthcare and received research institutional grants from Gilead Sciences, Janssen-Cilag and ViiV Healthcare and received payment or honoraria from Gilead Science and ViiV Healthcare and received support for attending meetings and/or travel from ViiV Healthcare and AbbVie; Alessandra Vergori received institutional grant from Gilead Sciences, speakers' honoraria/educational activities for Merck Sharp and Dohme and Janssen-Cilag, advisor for Janssen-Cilag; Enrico Girardi received institutional grants for Gilead Sciences, Italian Ministry of Health, and Mylan, personal fees from Gilead Sciences and ViiV, and consulting fees from Mylan; Roberta Gagliardini reports payments to their institution from Gilead, consulting MSD, Gilead, Jansssen, ViiV, and Thera technologies, speakers' honoraria/educational activities for ViiV Healthcare, Merck Sharp and Dohme and Gilead Sciences, advisor for ViiV Healthcare and Janssen-Cilag. Carmela Pinnetti received personal fee from Gilead-Sciences for a case presentation and a travel grant from Gilead and served on an advisory board for Janssen-Cilag. Stefania Cicalini reports consulting fees paid to self from Viiv, Jansen-Cilag, MSD, Gilead; payment or honoraria from Viiv, Jansen-Cilag, MSD, and Gilead; support for attending meetings and/or travel from Viiv, Jansen-Cilag, MSD, and Gilead; and participation in a Data Safety Monitoring Board of Advisory Board from Viiv, Jansen-Cilag, MSD, and Gilead. Ballagamba Rita reports consulting fees from VIIV, GILEAD, and MSD, payment or honoraria from GILEAD, MSD, and VIIV, support for attending meetings and/or travel gilead and Janseen-cilad and participation on a Data Safety Monitoring Board or Advisory Board from viiv, gilead, and Janssen-cilad- MSD. Alessandro Cozzi-Lepri reports grants or contracts paid to UCL from Icona Foundation Study and from EuCARE project funded by the EU under the HORIZON Europe programme, GA n. 6944397, consulting fees from Fondazione Smith Kline. The other co-authors declare no conflicts of interests outside the submitted work.

REFERENCES

1. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med 2021;384(15):1412-1423. doi: 10.1056/NEJMoa2101765. Epub 2021 Feb 24. PMID: 33626250; PMCID: PMC7944975.
2. Koff WC, Schenkelberg T, Williams T, et al. Development and deployment of COVID-19 vaccines for those most vulnerable. Sci Transl Med 2021;13(579):eabd1525. doi: 10.1126/scitranslmed.abd1525. PMID: 33536277.
3. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med 2015;373(9):795-807. doi: 10.1056/NEJMoa1506816. Epub 2015 Jul 20. PMID: 26192873; PMCID: PMC4569751.
4. Paiardini M, Müller-Trutwin M. HIV-associated chronic immune activation. Immunol Rev 2013;254(1):78-101. doi: 10.1111/imr.12079. PMID: 23772616; PMCID: PMC3729961.
5. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet 2013;382(9903):1525-33. doi: 10.1016/S0140-6736(13)61809-7. Epub 2013 Oct 23. PMID: 24152939; PMCID: PMC4058441.
6. Geretti AM, Stockdale AJ, Kelly SH, et al. Outcomes of COVID-19 related hospitalization among people with HIV in the ISARIC WHO Clinical Characterization Protocol (UK): a prospective observational study. Clin Infect Dis. 2020 Oct 23:ciaa1605. doi: 10.1093/cid/ciaa1605. Epub ahead of print. PMID: 33095853; PMCID: PMC7665382.
7. Bhaskaran K, Rentsch CT, MacKenna B, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. Lancet HIV 2021;8(1):e24-e32. doi: 10.1016/S2352-3018(20)30305-2. Epub 2020 Dec 11. PMID: 33316211; PMCID: PMC7773630.
8. Spinelli MA, Lynch KL, Yun C, et al. SARS-CoV-2 seroprevalence, and IgG concentration and pseudovirus neutralising antibody titres after infection, compared by HIV status: a matched case-control observational study. Lancet HIV 2021;8(6):e334-e341. doi: 10.1016/S2352-3018(21)00072-2. Epub 2021 Apr 29. PMID: 33933189; PMCID: PMC8084354.
9. Tebas P, Frank I, Lewis M, et al. Poor immunogenicity of the H1N1 2009 vaccine in well controlled HIV-infected individuals. AIDS 2010;24(14):2187-92. doi: 10.1097/QAD.0b013e32833c6d5c. PMID: 20616698.
10. Collier AC, Corey L, Murphy VL, et al. Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. Ann Intern Med 1988;109(2):101-5. doi: 10.7326/0003-4819-109-2-101. PMID: 2968064.
11. Frater J, Ewer KJ, Ogbe A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. Lancet HIV 2021;8(8):e474-e485. doi: 10.1016/S2352-3018(21)00103-X. Epub 2021 Jun 18. PMID: 34153264; PMCID: PMC8213361.
12. Madhi S, Koen A, Cutland C, et al. ChAdOx1 nCoV-19 (AZD1222) Vaccine in People Living With and Without HIV. Lancet HIV. Published online: August 17, 2021. doi:https://doi.org/10.1016/S2352-3018(21)00157-0

13. Levy I, Wieder-Finesod A, Litchevski V et al. Immunogenicity and Safety of the BNT162b2 mRNA COVID-19 Vaccine in People Living with HIV-1. Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.07.031.
14. Woldemeskel BA, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living with HIV. Clin Infect Dis 2021 Jul 22:ciab648. doi: 10.1093/cid/ciab648. Epub ahead of print. PMID: 34293114.
15. Ruddy JA, Boyarsky BJ, Bailey JR, et al. Safety and antibody response to two-dose SARS-CoV-2 messenger RNA vaccination in persons with HIV. AIDS 2021 Jul 8. doi: 10.1097/QAD.0000000000003017. Epub ahead of print. PMID: 34261097.
16. Agrati C, Castilletti C, Goletti D, et al. Coordinate Induction of Humoral and Spike Specific T-Cell Response in a Cohort of Italian Health Care Workers Receiving BNT162b2 mRNA Vaccine. Microorganisms. 2021;9(6):1315. doi: 10.3390/microorganisms9061315. PMID: 34208751; PMCID: PMC8235087.
17. Matusali G, Colavita F, Lapa D, et al. SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. Viruses. 2021 Apr 10;13(4):655. doi: 10.3390/v13040655. PMID: 33920222; PMCID: PMC8069482.
18. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell 2020;183(4):996-1012.e19. doi: 10.1016/j.cell.2020.09.038. Epub 2020 Sep 16. PMID:33010815; PMCID: PMC7494270.
19. Corbett KS, Nason MC, Flach B, et al. Immune correlates of protection by mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. Science, 373 (6561), eabj0299. doi:10.1126/science.abj0299.
20. Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 2014;10(5):e1004078. doi: 10.1371/journal.ppat.1004078. PMID: 24831517; PMCID:PMC4022662.
21. Alrubayyi A, Gea-Mallorquí E, Touizer E, et al. Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with HIV. bioRxiv 2021.02.15.431215; doi: https://doi.org/10.1101/2021.02.15.431215.
22. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. JAMA 2021;325(17):1784-1786. doi:10.1001/jama.2021.4385. PMID: 33720292; PMCID: PMC7961463.
23. Hod T, Ben-David A, Olmer L, et al. Humoral Response of Renal Transplant Recipients to the BNT162b2 SARS-CoV-2 mRNA Vaccine Using Both RBD IgG and Neutralizing Antibodies. Transplantation. 2021 Jul 6. doi: 10.1097/TP.0000000000003889. Epub ahead of print. PMID: 34310101.
24. Moor MB, Suter-Riniker F, Horn MP, et al. Humoral and cellular responses to mRNA vaccines against SARS-CoV-2 in patients with a history of CD20 B-cell-depleting therapy (RituxiVac): an investigator-initiated, single-centre, open-label study. Lancet Rheumatol. 2021 Sep 7. doi:10.1016/S2665-9913(21)00251-4. Epub ahead of print. PMID: 34514436; PMCID:PMC8423431.

25. British HIV Association (BHIVA). BHIVA statement on JCVI recommendations for a third COVID-19 vaccine dose. September, 3, 2021. https://www.bhiva.org/BHIVA-statement-on-JCVI-recommendations-for-a-third-COVID-19-vaccine-dose.
26. Hall VG, Ferreira VH, Ku T, et al. Randomized Trial of a Third Dose of mRNA-1273 Vaccine in Transplant Recipients. N Engl J Med. 2021 Aug 11. doi: 10.1056/NEJMc2111462. Epub ahead of print. PMID:34379917.
27. Krause PR, Fleming TR, Peto R, et al. Considerations in boosting COVID-19 vaccine immune responses. Lancet Published Online September 13, 2021 https://doi.org/10.1016/ S0140-6736(21)02046-8.

Figure 1. Change of RBD-binding IgG response (BAU/mL) in PLWHs from time of priming dose (T0), to time of second dose (T1) and at 1 month after the second dose (T2).

Figure 2. Humoral response in PLWHs and HCWs after the priming and the second dose of BNT162b2 or mRNA-1273 vaccine.

Figure 3 Increase of cell-mediated immunogenicity in PLWHs from T0 to T2, expressed as pg/mL of IFN- γ or IL-2 release at the time of priming dose (T0), at time of the second dose (T1) and at 1 month after the second dose (T2).

Figure 4. Cell-mediated immunogenicity in PLWHs and HCWs at 1 month after the second dose (T2). Immune response is expressed as median (IQR) pg/mL values of release of IFN- γ and IL-2 after SARS-Cov-2 Spike peptide stimulation.

Figure 5. Scatterplots of the association between IFN-γ pg/mL and IL-2 pg/mL production in blood sample of PLWHs collected 1 month after the second dose of SARS-CoV-2 mRNA vaccination (T2). IFN- γ pg/mL and IL-2 pg/mL production in overall PLWHs population. (Pearson's, r= 0.427 p<0.001) **(Figure 5a)**; IFN- γ pg/mL and IL-2 pg/mL production in PLWHs with Severe Immunodeficiency (SID) (Pearson's, r=0.80; p<0.001); **(Figure 5b).** IFN-γ pg/mL and IL-2 pg/mL production in PLWHs with Minor Immunodeficiency (MID) (Pearson's, r=0.71; p<0.001) **(Figure 5c)**; IFN- γ pg/mL and IL-2 pg/mL production in PLWHs with No Immunodeficiency (NID) (Pearson's, r=0.48; P <0.001) **(Figure 5d).** All P values were calculated by linear regression (r, Pearson's correlation coefficient).

Figure 6. Scatterplots of the association between CD4 cell T count/mm^3 at the time of priming dose of mRNA vaccine (T0) and RBD-binding IgG response, nAb response, and IFN- γ production at T2 in PLWHs. CD4 T cell count was performed at T0, and blood samples were collected for immunologic response 1 month after the dose of SARS-CoV-2 mRNA vaccination (T2). RBD-binding IgG response (BAU/mL) at T2 and current CD4 cell count/mm^3 at T0. (rho=0.44; p<0.001) **(Figure 6a);** nAb MNA reciprocal dilution at T2 and current CD4 cell count/mm^3 at T0. (rho=0.37; p<0.001) **(Figure 6b)**; IFN-γ release after S-peptide stimulation (pg/mL) at T2 and current CD4 cell count/mm^3 at T0. (rho=0.38; p<0.001) **(Figure 6c).** (rho, Spearman's rank correlation coefficient)

Table 1. Main characteristics of PLWHs (n=166) at time of priming dose of SARS-CoV-2 vaccination according with current CD4 T cell count (cell/mm^3).

	PLWHs with current CD4 <200 (PCDR)	PLWHs with current CD4 200-500 (ICDR)	PLWHs with current CD4 >500 (HCDR)	p-value
	N=32	N=56	N=78	
Gender, female, n (%)	8 (25.0)	9 (16.1)	10 (12.8)	0.290
Age, years, median (IQR)	57 (52-60)	54 (46-59)	54 (46-59)	0.105
Years of HIV infection, median (IQR)	22.2 (2.9-30.8)	9.2 (1.8-25.7)	11.0 (5.8-24.8)	0.033
Previous AIDS diagnosis, n (%)	12 (37.5)	26 (46.4)	37 (47.4)	<0.001
Current or previous malignancy, n (%)	2 (6.3)	14 (25.0)	9 (11.5)	0.030
HCV-Ab positivity, n (%)	12 (37.5)	18 (32.1)	17 (21.8)	<0.021
HIV-RNA <50 copies/ml, n (%)	22 (68.8)	52 (92.9)	78 (100.0)	<0.001
Nadir CD4 T cell/mm^3, median (IQR)	49 (23-122)	63 (29-150)	174 (68-280)	<0.001
Current CD4 T cell/mm^3, median (IQR)	140 (100-163)	335 (245-441)	727	<0.00

			(585-856)	1
Current CD8 T cell/mm^3, median (IQR)	671 (503-1030)	694 (505-1196)	859 (640-1139)	0.112
CD4/CD8 T cell ratio, median (IQR)	0.16 (0.12-0.26)	0.44 (0.28-0.69)	0.90 (0.67 - 1.17)	<0.001
At least 1 comorbidity, n (%)	11 (34.4)	16 (30.2)	23 (15.4) 12 (19.4)	0.876
- Cardiovascular	6 (18.8)	14 (25.0)	6 (7.7)	0.378
- Neurologic	2 (6.3)	3 (5.4)	1 (1.3)	0.862
- Renal	1 (3.1)	1 (1.8)	1 (1.3)	0.805
- Diabetes	2 (6.3)	1 (1.8)	1 (1.3)	0.283
- Chronic Obstructive Pulmonary Disease/asthma	3 (9.4)	3 (5.5)	11 (14.1)	0.254
- Liver cirrhosis	4 (12.5)	18 (32.1)	13 (16.7)	0.040
Current antiretroviral therapy, n (%)	32 (100)	56 (100)	78 (100)	1.000
Years of HIV therapy, median (IQR)	13.7 (1.4-21.7)	6.4 (1.8-14.7)	10.1 (5.0-14.0)	0.190
Type of vaccine administered, n (%) • **BNT162b2** • **mRNA-1273**	 22 (68.8) 10 (31.3)	 38 (67.9) 18 (32.1)	 35 (44.9	 0.010

			) 43 (55.1)	
Abbreviations: PCDR, poor CD4 recovery; ICDR, intermediate CD4 recovery; HCDR, high CD4 recovery				

Table 2. Proportion of participants anti-RBD and neutralization responder to BNT162b2 or mRNA-1273 vaccination. Responder was defined as with a detectable RBD-binding IgG response and with a nAbs titre at MNA ≥1:10, respectively) at 1 month after the second dose (T2) in the three PLWHs groups and in Health Care Workers (HCWs).

	Anti-RBD response		Neutralization (nAb) response	
	Detectable RBD-binding IgG (%)*	Median (IQR) BAU/mL of RBD-binding IgG**	nAB ≥10 (reciprocal dilution at MNA) (%)^	Median (IQR) reciprocal dilution values at MNA§
PCDR	26/30 (86.7)	507 (212-1143)	21/30 (70.0)	30 (5-80)
ICDR	53/53 (100)	1477 (471-2056)	45/51 (88.2)	40 (10-160)
HCDR	76/77 (98.7)	1782 (989-2769)	67/72 (93.1)	80 40-160)
HCWs	168/168 (100)	2353 (1378-3758)	72/73 (98.6)	80 (40-160)

Abbreviations: PCDR, poor CD4 recovery; ICDR, intermediate CD4 recovery; HCDR, high CD4 recovery; HCWs, health care workers

*Comparisons between HIV groups by Chi-square test: PCDR vs ICDR, P=0.014; PCDR vs HCDR, P=0.021; ICDR vs HCDR, P=1.0. Comparisons of PLWHs with HCWs by Fisher's exact test: PCDR vs HCWs, P <0.001; ICDR vs HCWs, P=1.0; HCDR vs HCWs, P=0.313.

** Comparisons between HIV groups by Kruskal-Wallis test P<0.001; by Dunn's test with Bonferroni adjustment for multiple comparisons: PCDR vs ICDR, P=0.029; PCDR vs HCDR, P<0.001; ICDR vs HCDR, p=0.184. Comparisons of PLWHs with HCWs by Kruskal-Wallis test p<0.001; by Dunn's test with Bonferroni adjustment: PCDR vs HCWs, p<0.001; ICDR vs HCWs, p<0.001; HCDR vs HCWs, p=0.031.

^ Comparisons between HIV groups by Chi-square test: PCDR vs ICDR, p=0.041; PCDR vs HCDR, p=0.002; ICDR vs HCDR, p=0.356. Comparisons of PLWHs with HCWs by Fisher's exact test: PCDR vs HCWs, p<0.001; ICDR vs HCWs, p=0.019; HCDR vs HCWs, p=0.116.

§ Comparisons between HIV groups by Kruskal-Wallis test p<0.001; by Dunn's test with Bonferroni adjustment for multiple comparisons: PCDR vs ICDR, p=0.150; PCDR vs HCDR, p=0.001; ICDR vs HCDR, p=0.239. Comparisons of PLWHs with HCWs by Kruskal-Wallis test p<0.001; by Dunn's test with Bonferroni adjustment: PCDR vs HCWs, p<0.001; ICDR vs HCWs, p=0.05; HCDR vs HCWs, p=1.0.

Table 3. Analysis of current CD4 T cell count and CD4/CD8 ratio strata as independent predictors of magnitude of immune response to vaccination among PLWHs. Results of univariable and multivariable linear regression analysis having as outcome (dependent variable): **a)** RBD-binding IgG; **b)** nAb titre; **c)** IFN-γ). All analyses are based on logarithmic units. Significant associations are in bold.

a) Dependent variable: RBD-binding IgG (BAU/mL);						
		Crude			**Adjusted***	
	Beta	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
Current CD4 T cell count (cell/mm^3)						
<200	**-0.66**	**-0.92 -0.41**	**<0.001**	**-0.64**	**-0.94 -0.34**	**<0.001**
200-500	-0.19	-0.41 0.03	0.092	-0.16	-0.39 0.07	0.182
>500	ref			ref		
*adjusted for age, years of HIV infection, CD4 nadir, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy						
		Crude			**Adjusted****	
	Beta	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
CD4/CD8 ratio, per 0.5 increase	**0.24**	**0.13 0.34**	**<0.001**	**0.16**	**0.01 0.30**	**0.033**
**adjusted for age, years of HIV infection, current CD4 T cell count, CD4 nadir, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy						
b) Dependent variable: nAb titre (reciprocal dilution at MNA)						
		Crude			**Adjusted***	
Current CD4 T cell count (cell/mm^3)	**Beta**	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
<200	**-0.52**	**-0.78 -0.25**	**<0.001**	**-0.41**	**-0.70 -0.12**	**0.006**
200-500	-0.20	-0.43 0.02	0.076	-0.08	-0.31 0.15	0.497
>500	ref			ref		
*adjusted for age, years of HIV infection, CD4 nadir, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy						
		Crude			**Adjusted****	
	Beta	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
CD4/CD8 ratio, per 0.5 increase	0.17	0.05 0.28	0.002	0.06	-0.09 0.20	0.426
**adjusted for age, years of HIV infection, CD4 nadir, current CD4 T cell count, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy						
c) Dependent variable: IFN- γ pg/mL)						
		Crude			**Adjusted***	
Current CD4 T cell count (cell/mm^3)	**Beta**	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
<200	**-1.03**	**-1.36 -0.69**	**<0.0001**	**-0.74**	**-1.13 -0.34**	**<0.001**
200-500	-0.14	-0.42 0.14	0.335	-0.03	-0.28 0.34	0.850
>500	ref			ref		
*adjusted for age, years of HIV infection, CD4 nadir, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy						

	Crude			Adjusted**		
	Beta	**95%CI**	**p-value**	**Beta**	**95%CI**	**p-value**
CD4/CD8 ratio, per 0.5 increase	0.15	0.02 0.28	0.020	-0.05	-0.19 0.10	0.544

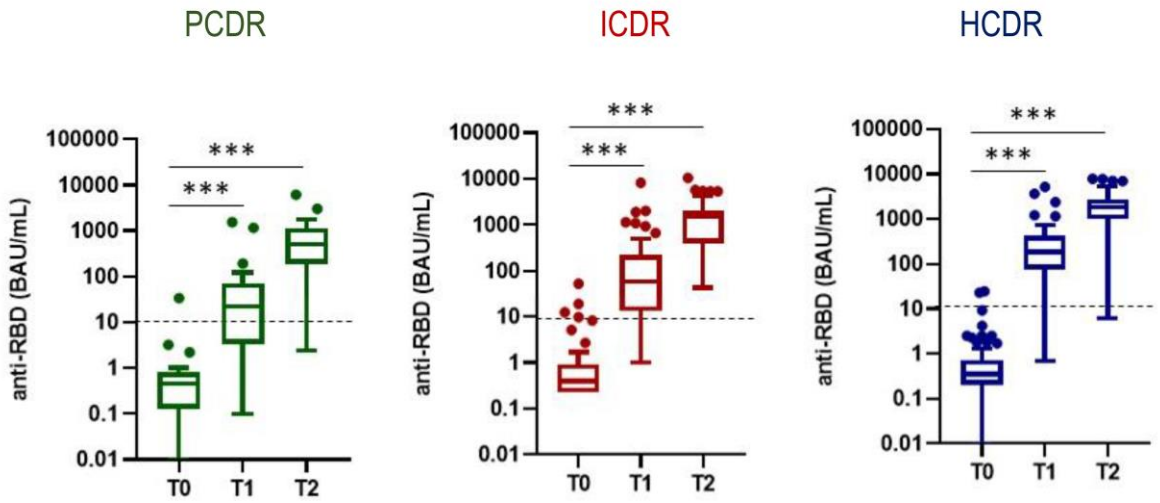
**adjusted for age, years of HIV infection, CD4 nadir, current CD4 T cell count, HIV-RNA undetectable (<50 vs ≥50 copies/mL), type of mRNA vaccine (BNT162n2 or mRNA-1273), previous or current malignancy

Table 4. Multivariable linear regression models of factors associated with magnitude of RBD-binding IgG response, nAB response at MNA, and IFN- γ release after S-peptide stimulation with different groups Results were adjusted for gender and age by means of three separate multivariable linear regression models.

Dependent variable: RBD-binding IgG (BAU/mL)* at 1 month after the second dose				
	Beta	**95%CI**		**p-value**
PCDR	-0.69	-0.89	-0.49	<0.001
ICDR	-0.23	-0.40	-0.06	0.008
HCDR	-0.05	-0.21	0.10	0.485
HCWs	ref			
Dependent variable: MNA (reciprocal of dilution, log_2)* at 1 month after second dose				
	Beta	**95%CI**		**p-value**
PCDR	-0.43	-0.71	-0.16	0.002
ICDR	-0.14	-0.39	0.11	0.261
HCDR	0.05	-0.18	0.27	0.695
HCWs	ref			
Dependent variable: IFN- γ (pg/mL)* at 1 month after second dose				
	Beta	**95%CI**		**p-value**
PCDR÷	-1.05	-1.33	-0.78	<0.001
ICDR	-0.20	-0.43	0.02	0.077
HCDR÷	0.08	-0.29	0.13	0.446
HCWs	ref			

Abbreviations:PCDR, poor CD4 recovery; ICDR, intermediate CD4 recovery; HCDR, high CD4 recovery; HCWs, health care workers MNA = microneutralization assay; anti-RBD = anti receptor binding domain antibodies; IFN- γ interferon gamma;. * all values are expressed as log_{10}

Figure 1

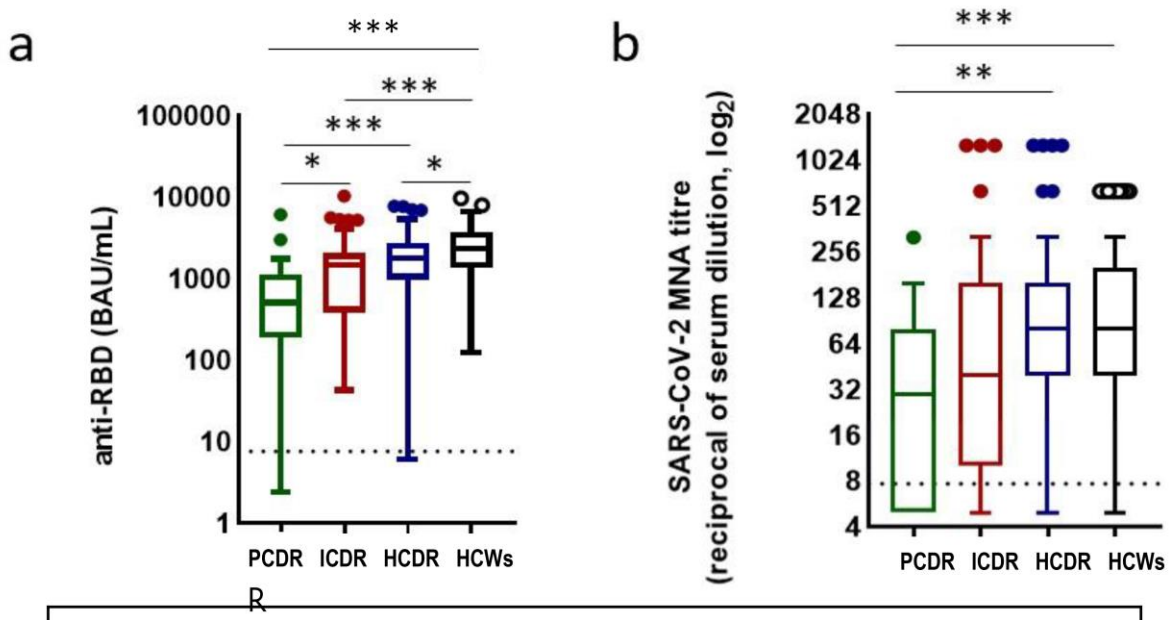


Median	0.5	21.9	507	0.4	59	1477	0.4	183	1782
IQR	0.2; 0.8	3.7; 68.5	212; 1143	0.3; 0.9	15; 214	471; 2056	0.2; 0.7	77; 421	989; 2769

***p<0.001 by paired Wilcoxon test

Abbreviations: PCDR, poor CD4 recovery:current CD4 T cell count <200 cell/mm^3; ICDR: intermediate CD4 recovery: current CD4 T cell count 200-500 cell/mm^3; HCDR, high CD4 recovery: current CD4 T cell count >500 cell/mm^3

Figure 2



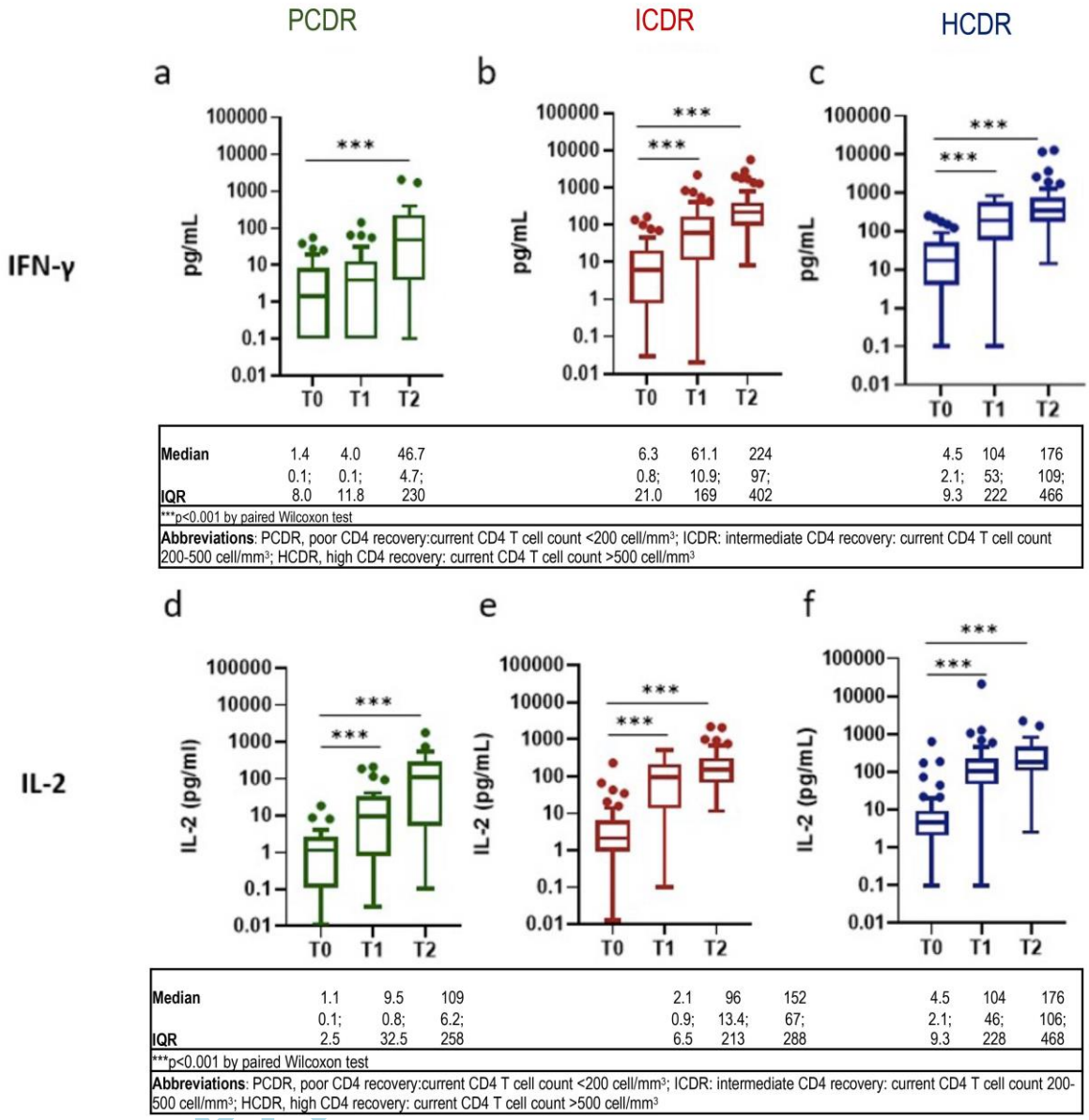
R

Median	507	1477	1782	2353	30	40	80	80
IQR	212; 1143	471; 2056	989; 2769	1378; 3758	5; 80	10; 160	40; 160	40; 160

P<0.001 at Kruskall-Wallis test; *p<0.05; **p<0.01; ***p<0.001

Abbreviations: PCDR, poor CD4 recovery:current CD4 T cell count <200 cell/mm^3; ICDR: intermediate CD4 recovery: current CD4 T cell count 200-500 cell/mm^3; HCDR, high CD4 recovery: current CD4 T cell count >500 cell/mm^3 ; HCWs, health care workers

Figure 3



	PCDR			ICDR			HCDR		
Median	1.4	4.0	46.7	6.3	61.1	224	4.5	104	176
	0.1;	0.1;	4.7;	0.8;	10.9;	97;	2.1;	53;	109;
IQR	8.0	11.8	230	21.0	169	402	9.3	222	466

***p<0.001 by paired Wilcoxon test

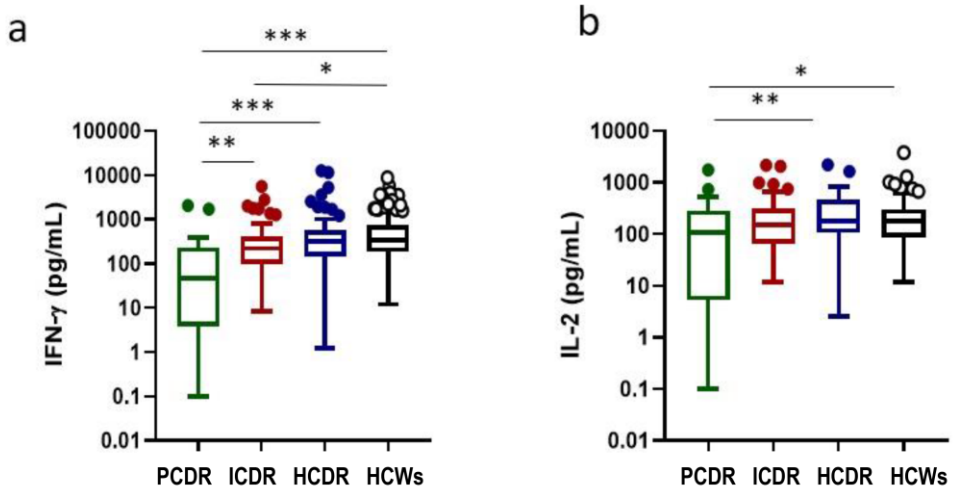
Abbreviations: PCDR, poor CD4 recovery:current CD4 T cell count <200 cell/mm^3; ICDR: intermediate CD4 recovery: current CD4 T cell count 200-500 cell/mm^3; HCDR, high CD4 recovery: current CD4 T cell count >500 cell/mm^3

	PCDR			ICDR			HCDR		
Median	1.1	9.5	109	2.1	96	152	4.5	104	176
	0.1;	0.8;	6.2;	0.9;	13.4;	67;	2.1;	46;	106;
IQR	2.5	32.5	258	6.5	213	288	9.3	228	468

***p<0.001 by paired Wilcoxon test

Abbreviations: PCDR, poor CD4 recovery:current CD4 T cell count <200 cell/mm^3; ICDR: intermediate CD4 recovery: current CD4 T cell count 200-500 cell/mm^3; HCDR, high CD4 recovery: current CD4 T cell count >500 cell/mm^3

Figure 4



Median	46.7	223.6	315	343		109	152	176	177
IQR	4.7; 230	97.2; 402.2	148.5; 552.9	188; 715		6.2; 258	67; 288	109; 466	87; 304
P<0.001 at Kruskall-Wallis test; test; *p<0.05; **p<0.01; ***p<0.001					p=0.019 at Kruskall-Wallis				

Abbreviations: PCDR, poor CD4 recovery:current CD4 T cell count <200 cell/mm^3; ICDR: intermediate CD4 recovery: current CD4 T cell count 200-500 cell/mm^3; HCDR, high CD4 recovery: current CD4 T cell count >500 cell/mm^3 ; HCWs, health care workers

Figure 5

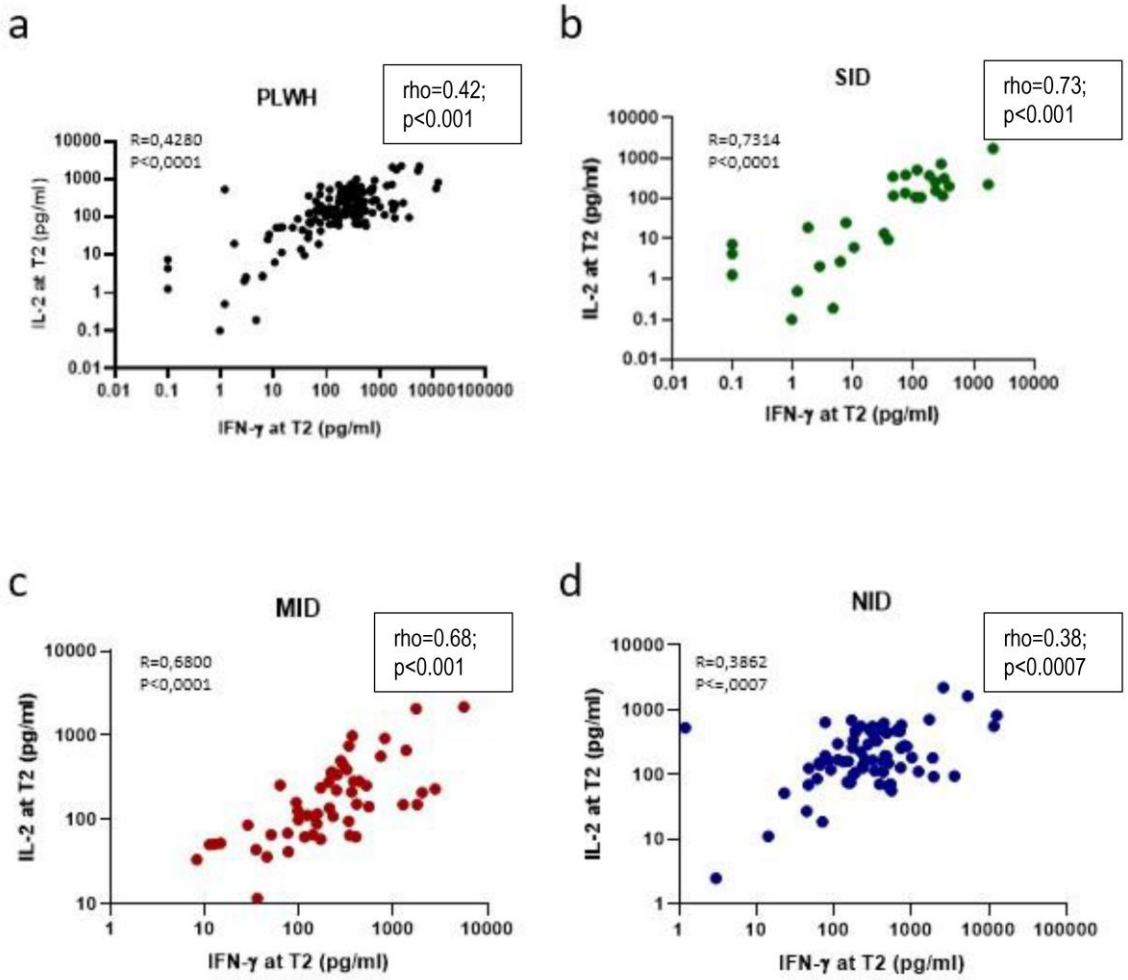


Figure 6