

IMMUNE RESPONSE TO SARS-COV-2 IN PATIENTS WITH CHRONIC HIV INFECTION

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ABSTRACT

Introduction. Data for the long-term effects of SARS-CoV-2/HIV co-infection on immune restoration, as well as the level of post-exposure and post-vaccination immunity at the current stage of SARS-CoV-2 pandemic in HIV+ individuals is still scarce. We assessed SARS-CoV-2-specific immune responses, and the effects of SARS-CoV-2 infection on the immune recovery in HIV+cART+ patients with different exposure history.

Materials and methods. HIV+cART+ patients 9 (2-18) months after mild/moderate COVID-19 and completed immunization with anti-SARS-CoV-2 vaccine (n=13, group A), convalescent, not immunized (n=11, group B), or with no history of exposure to SARS-CoV-2 (n=11, group C) were included in the study. CD4AC and CD4/CD8 ratio were determined before and after the documented/probable contact with SARS-CoV-2 by 4-color flow cytometry (TRUCount, MultiTest, FACSCanto II). Virus-specific immunity was characterized by the SARS-CoV-2 specific IFN γ production (SARS-CoV-2 IGRA, Euroimmun) and the levels of RBD-IgG ((Euroimmun ELISA).

Results. SARS-CoV-2 specific T-cell and IgG responses were highly correlated and present, respectively, in

92% and 100%; 64% and 54%, 36% and 50% from group A, B and C patients. SARS-CoV-2 specific IFN γ +T cells and RBD-IgG were significantly higher in the group with hybrid exposure (A) as compared to convalescent (B) and asymptomatic (C) patients. No significant difference existed between background and actual CD4AC (mean 836 vs 799 cells/ μ l, $p > 0.05$, Mann-Whitney), and the CD4/CD8 ratio significantly increased in the group with hybrid exposure (0.92 vs 1.07, $p < 0.01$, paired T-test).

Conclusion. Over 80% of tested HIV+ individuals have mounted a SARS-CoV-2 specific immune response. Immunization and hybrid exposure provide a durable and significantly stronger SARS-CoV-2-specific immune response as compared to mild/asymptomatic infection, without affecting the long-term immune recovery

Key words HIV/SARS-CoV-2 coinfection, immune recovery, SARS-CoV-2-specific response

INTRODUCTION

Although people living with HIV (PLWH) comprise a small percent of the global population, they represent a particularly vulnerable group due to the direct immunosuppressive effect of HIV as well as the premature immune aging associated with the life-long chronic infection.

COVID-19 pandemics raised specific questions regarding the immune response of PLWH to SARS-CoV-2. According to recent statistics, while PLWH do not have a higher chance of being infected after contact with SARS-CoV-2, the rate of hospital admission and lethality, when adjusted to age and sex are significantly higher among PLWH with COVID-19 as compared to the HIV-negative population (1, 2, 3). Even in the settings of contemporary antiretroviral therapy (cART) and undetectable HIV viral load, PLWH are prone to premature immune aging (4) and when compared to the uninfected population of the same age group have a higher frequency of comorbidities such as cardiovascular diseases, renal failure and diabetes (5). HIV infection is also indicated as an independent factor for increased mortality (6). All these factors were listed as predispositions for worse COVID-19 infection outcome (7). Further, on, PLWH are a legitimate target for anti-SARS-CoV-2 vaccination (8)

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though the long-term post-immunization memory in this specific population and in the settings of genetically evolving virus are scarcely studied.

From 3 January 2020 to 4 January 2023 1,292,224 cases of SARS-CoV-2 infection, and 38,108 deaths due to COVID-19 were registered in Bulgaria. With a rate of 548.2 COVID-19-related deaths per 100000 Bulgaria is ranking second worldwide after Peru. At the same time, the rate of completed vaccinations hardly approaches 30% (29.87% as of 10 December 2022 (9).

An estimated number of 3690 HIV+ individuals currently live in Bulgaria. In 2021, 1766 or over 97% of those registered were on cART (10). Almost 3 years after the first COVID-19 case in Bulgaria, data about the level of post-exposure and post-vaccination immunity among PLWH in Bulgaria is still limited. In this study we assessed the SARS-CoV-2-specific immune responses in HIV+ patients receiving cART (HIV+ART+), with different exposure history regarding SARS-CoV-2.

MATERIAL AND METHODS

A total of 35 HIV+ patients were included in this study, and distributed in the following three groups: (A, n=13) convalescent after confirmed SARS-CoV-2 infection, and vaccinated against SARS-CoV-2; (B, n=11) convalescent after confirmed SARS-CoV-2 infection, not vaccinated; (C, n=11) neither having been infected, nor vaccinated. The demographic and epidemiological characteristics of patients are shown in Table 1. Patients were selected randomly during the routine follow-up visits at the Immune Deficiency Department of the Specialized Hospital for infectious Diseases, Sofia. This study was reviewed and approved by the institutional review board of NCIPD and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all donors and patients.

Whole blood samples in heparinized vacutainer tubes were obtained during routine immune monitoring of HIV+ART+ patients in October 2022. Samples from group A and B patients were acquired during the first regular check-up visit following a SARS-CoV-2 infection. The closest to the date of SARS-CoV-2 infection pre-exposure data (CD4 AC and

CD4/CD8 index) were obtained from the National Database for HIV patients monitoring.

The absolute count of CD4+ T lymphocytes (CD4AC), and CD4/CD8 index were determined using 4-color staining, lysis-no-wash protocol (MultiTest, TRUCount) and single platform analysis. Samples were acquired with 3-laser FACSCanto II (BD Biosciences) and analyzed with FACSDiva v.6.1.3.

SARS-CoV-2 - specific antibody responses were measured in plasma with semi-quantitative ELISA. For evaluation of receptor binding domain-specific human IgG (RBD-IgG) antibodies, microtiter wells coated with the relevant domains of spike protein were used (Anti-SARS-CoV-2 ELISA (IgG), Euroimmun, Germany). Results were presented as the ratio between the extinction of the patient sample and the extinction of the calibrator (Es/Ec). According to the manufacturer's instructions, concentrations of IgG corresponding to a ratio greater than 1.1 (Es/Ec) were considered positive, and those below 0.8 – negative. SARS-CoV-2-specific IFN- γ response was measured with Quantitative interferon-gamma release assay (IGRA), according to manufacturer's instructions. (Quan-T-Cell SARS-CoV-2 & Quan-T-Cell ELISA, Euroimmun, Germany).

Briefly 0.5 ml human heparinized blood were distributed in each of three stimulation tubes: blank (negative control), IGRA TUBE (containing a peptide pool derived from SARS-CoV-2 spike protein, suitable for both CD4+ and CD8+T stimulation) and IGRA STIM (positive control with T-cell mitogen). Samples were incubated for 24h at 37 °C. After the incubation, 100 μ l of heparinized plasma from each tube were used immediately or stored at -20°C for later measurement. Samples diluted 1:5 in sample buffer, together with 6 calibrators, as per instruction manual, were transferred to ELISA plate and incubated for 120 min, followed by 5 wash rounds, and subsequent incubations with biotin (30min), conjugate (30 min) and substrate (20 min), with intermediate washings after each incubation. Extinction was measured at 450 nm and 620 nm. Samples concentrations were calculated in mIU/ml based on a 4log calibration curve. The results were interpreted as follows: (negative: below 100 mIU /ml; borderline: between 100 and 200 mIU/ml; positive: above 200 mIU/ml)

Table 1. Patients enrolled in the study

Patients groups		A	B	C	P*
Number		13	11	11	>0.05
Type of exposure	Vaccinated	+	-	-	
	Infected	+	+	-	
Sex	M/F	12/1	7/4	10/1	
Age (years)	Mean	46	39	44	>0.05
	Range	34-68	26-53	34-66	
Time on ART (months)	Mean	73	89	66	>0.05
	range	3 - 144	3 - 242	0 - 130	
CD4AC before last exposure (cells/ μ kl)	Mean	836	600*	800**	>0.05
	Range	191-1468	355-933	538 - 1164	
CD4/CD8 ratio before exposure	Mean	0.92	1.13	1.16**	>0.05
	Range	0.28 - 1.36	0.34 - 2.67	0.7 – 2.52	
Time since exposure (months)	Mean	10	9	-	>0.05
	range	2-18	3-12	-	

*Two patients were excluded due to very recent therapy start

**For Group C, data obtained prior to March 2020 or earliest in record for those diagnosed after March 2020, were used P values were determined using ANOVA one-way analysis.

Statistics and Software

We used nonparametric paired Mann-Whitney test and Kruskal-Wallis ANOVA for multiple comparisons analysed through GraphPad Prism 9. P values <0.05 were considered significant.

RESULTS

First, we compared the baseline parameters of the studied groups. As detailed in Table 1, patients were mostly male. The studied groups did not differ significantly in age, either: mean 46 (34 – 68), 39 (26-53) and 44 (34-66) years for groups A, B and C, respectively (Kruskal-Wallis ANOVA, $p>0.05$). The time of exposure was within the past 18 months: average (min-max) 10 (2–18) for group A, and 9 (3-13) for group B. The last CDAC and CD4/CD8 measured before SARS-CoV-2 infection for groups A and B and those closest to March 2020 for group C were also compared, and were not significantly different (Kruskal-Wallis ANOVA, $p>0.05$) (**Table 1**).

To evaluate SARS-CoV-2 specific responses, we measured SARS-CoV-2 specific IFN γ + T cells, alongside with RBD - specific IgG antibodies. SARS-CoV-2-specific

IFN γ + T cells were detected in 92% of group A, 63% of Group B and 36% of group C patients. The rates of IgG-mediated virus specific responses were as follows: 100% in group A, 54% in group B and 50% (one patient excluded due to insufficient material) in group C, (**Fig.1**).

The individual SARS-CoV-2 specific IFN- γ responses were significantly stronger in group A as compared to groups B and C: average (min – max) 2185 (195 – 6667) mIU/ml vs. 434 (0 - 2575) mIU/ml, $p<0.001$ and vs. 822 (0 - 3012) mIU/ml, $p<0.05$ respectively, (**Fig.2**) A quantitative SARS-CoV-2 IGRA was used (Euroimmun). Dotted lines denote negative (below 100 mIU/ml) and borderline responses (between 100-200 mIU/ml) (* $p<0.05$, *** $p<0.001$, Mann-Whitney).

SARS-CoV-2-specific RBD-binding IgG also differed significantly between group A and groups B and C. The average (min-max) RBD-IgG index was 5.3 (1.24 - 7.25) for group A, vs. 2.0 (0.39 - 6.1), $p< 0.01$ and vs. 2.4 (0.17 - 7.45) $p<0.001$ for group B and group C, respectively (**Fig.3**). In addition, a very strong positive correlation was established between T-cell

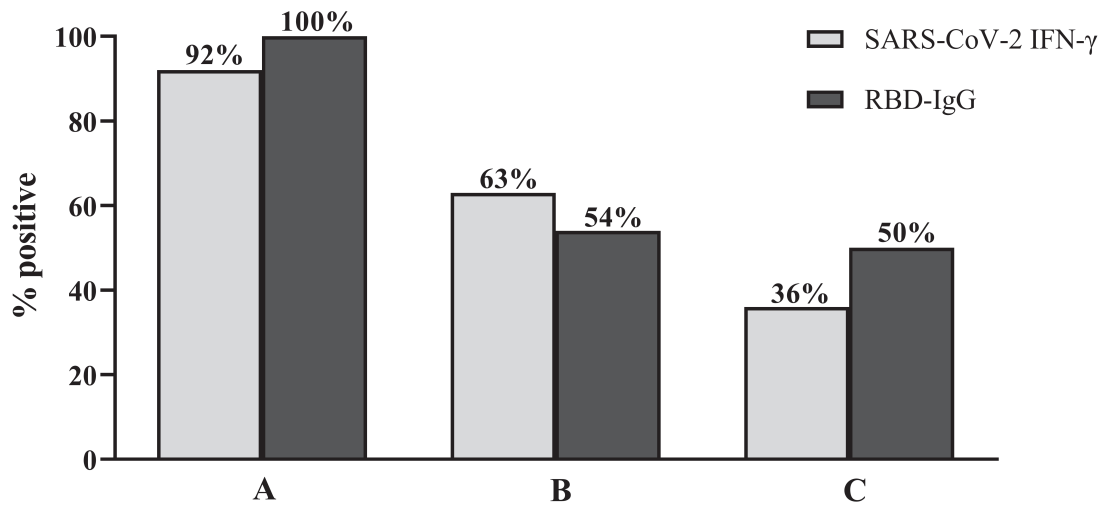


Figure. 1. Rates of SARS-CoV-2-specific IFN γ + T-cell and RBD-IgG responses in the studied patients' groups.

and IgG – mediated virus specific responses for all studied patients ($R= 0.8, p<0.001$, data not shown). Noteworthy, in group C that had no history of previous infection and/or vaccination we detected 4 patients with strong virus-specific responses most likely due to asymptomatic SARS-CoV-2 infections.

A semi-quantitative ELISA test (Euroimmun). Dotted lines denote negative (below 0.8), and borderline (between 0.8 - 1.1) responses (* $p<0.05$, *** $p<0.001$ Mann–Whitney).

In fact, the strength of both T- and IgG virus-specific responses was quite heterogeneous, mostly in group A. Therefore, we checked the effect of several possible factors on the strength of virus-specific responses: the time after infection/vaccination, CD4AC before

exposure, CD4AC at the time of evaluation. However, no correlation was found between the level of IFN γ +T cells and the time after exposure, nor between the level of IFN γ +T cells and CD4AC at baseline or at the time of evaluation (for groups A and B), $p>0.05$, data not shown.

To evaluate a possible effect of SARS-CoV-2 natural exposure and/or vaccination on the immune restoration in the studied groups, CD4AC and CD4/CD8 ratio were determined in the same blood samples as for the evaluation of virus-specific responses, and were compared with the corresponding baseline values. Average (min-max) CD4AC at the time of evaluation were 799 (224-1469); 551 (118-790), and 735 (283-1068) for groups A, B and C, respectively,

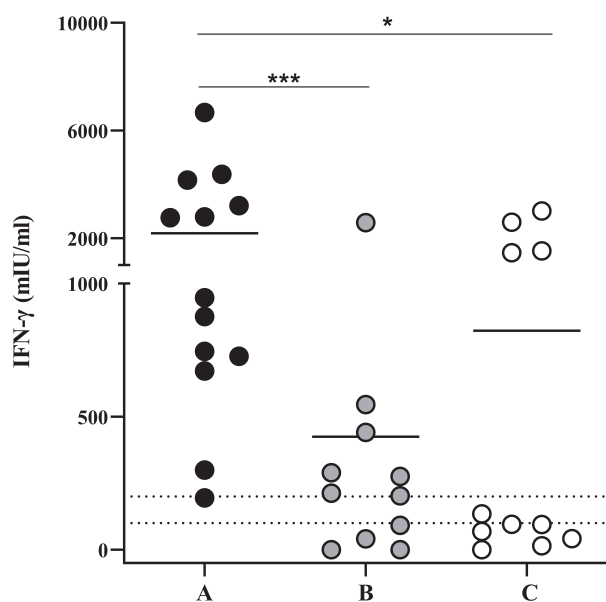


Figure.2. Individual values of SARS-CoV-2 specific IFN- γ response.

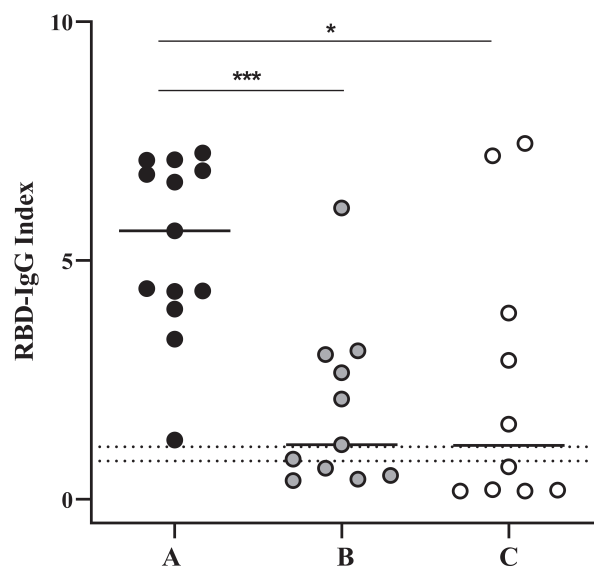


Figure.3. Individual values of SARS-CoV-2 specific RBD-binding IgG response

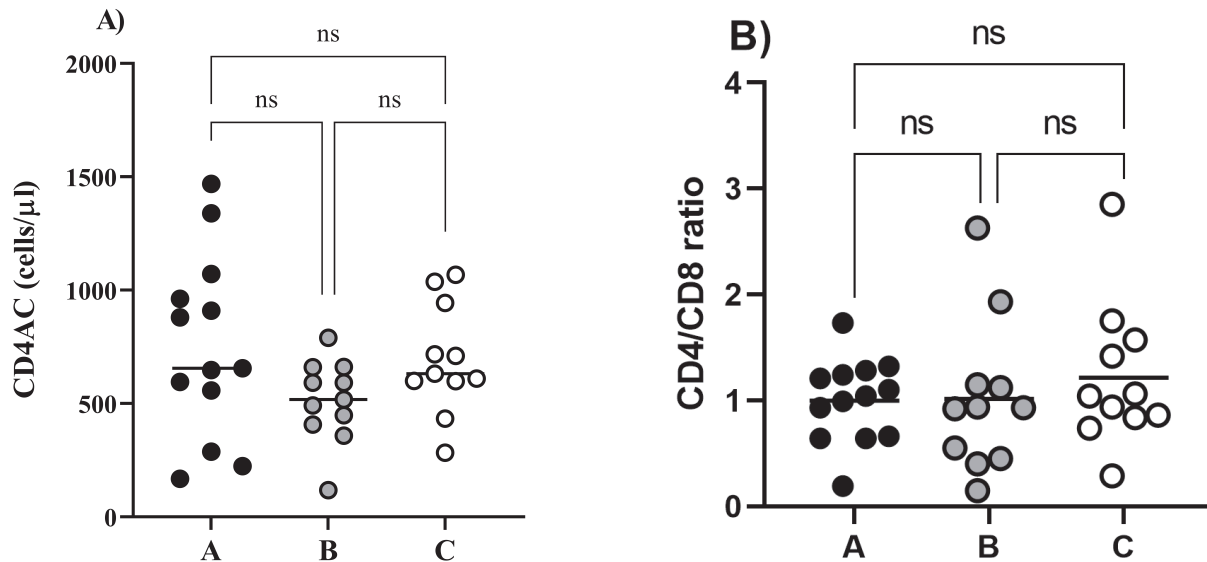


Figure.4. Individual CD4AC (A) and CD4/CD8 values (B) measured in the same samples that were used to determine SARS-CoV-2 specific T and IgG responses.

with no significant differences between them ($p > 0.05$, one-way ANOVA), Fig.4A. The average, min-max values for CD4/CD8 ratio did not differ significantly, either 1.00 (0.19 – 1.73) for group A, 1.01 (0.15-2.63) for group B, and 1.21 for group C (0.29-2.85) ($p > 0.05$, ns, Kruskal-Wallis ANOVA.) (**Fig.4B**)

We also compared the last CD4AC and CD4/CD8 measured prior to the exposure (or to March 2020 for group C) to the values obtained from the current samples. Four patients who had started (resumed) cART at the time of exposure were excluded from analysis in order to avoid a possible bias due to the effect of treatment (**Fig.5A, B**). CD4AC measured before the documented exposure (for groups A and B) or before March 2020 (for group C) and CD4AC determined at the time of the study were not significantly different (751 vs. 702, $p > 0.05$, Mann-Whitney test). At the same time, a tendency of increase was observed for CD4/CD8 ratios during the study due to a significant increase of CD4/CD8 in group A (patients that were both naturally exposed and vaccinated): mean 0.92 vs. 1.07, (Mann-Whitney test, $p < 0.01$) (**Fig.5C**). Thus, mild to moderate SARS-CoV-2 co-infection did not seem to perturb CD4AC restoration by cART. Importantly, immunization seemed to have a beneficial effect on the low level immune activation, as witnessed by an increasing CD4/CD8 ratio.

DISCUSSION

In this study, we evaluated the SARS-CoV-2 specific response in HIV+ patients on ART, two and a half years after the spread of SARS-CoV-2 in Bulgaria, and up to 13 months after a documented exposure. We established that an important part of the tested patients displayed correlated T cell and IgG-mediated virus-specific immunity. These immune responses did not correlate with the time after exposure. A large study measuring SARS-CoV-2 specific responses in PLWH after natural exposure have shown stable IgG responses between 90 - 120 days post-infection (10), and another study confirmed 76.7% positive IgG responses and 77.7% positive IFN- γ responses 10 months after natural exposure (12). Our data extend these observations to 12 months, confirming that chronic controlled HIV infection does not preclude the development of a lasting anti-SARS-CoV-2 response. Moreover, we detected significant T-cell and IgG-mediated virus specific response in 4 patients without history of infection or vaccination, most probably reflecting recent asymptomatic exposure. Therefore, HIV+ patients with mild to moderate immune deficiency are perfectly capable to mount an efficient response to SARS-CoV-2.

In addition, we compared the rate and strength of SARS-CoV-2 responses and showed that combined natural exposure and vaccination produced significantly stronger and durable responses, as compared to infection only. In fact, all patients

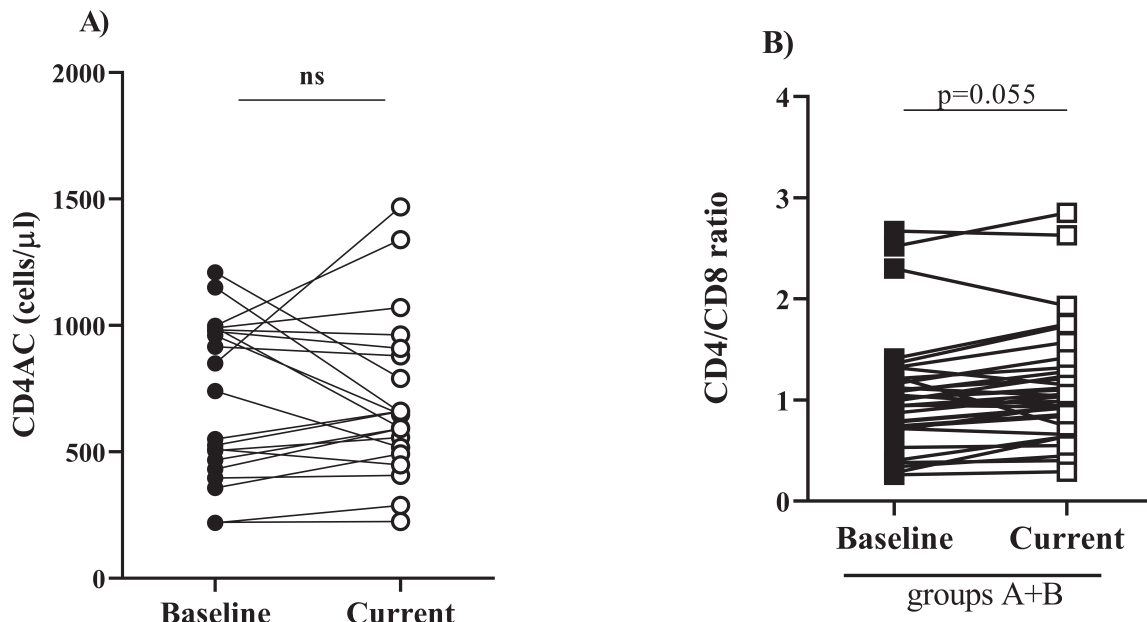


Figure.5. Comparison of CD4AC in groups A+B (A) and CD4/CD8 ratio in groups A+B (B), and group A only (C) before exposure and in the same samples used to determine specific T- and IgG-responses. ** $p < 0.01$, paired T-test

from group A showed virus-specific IFN- γ + T and/or RBD-binding IgG response vs. only 5 out of 11 patients from group B. In fact, a number of studies have compared SARS-CoV-2-specific responses elicited after simple vaccination vs. infection in HIV-donors, giving priority to natural responses (13). Some recently published studies demonstrated that protection associated with hybrid immunity is superior to the one elicited by simple vaccination, due to a distinct immune memory landscape and a greater polyfunctional potential of spike-specific CD4 T cells (14, 15). A study among vaccinated PLWH showed that, cellular immune responses did not differ between vaccinated and convalescent PLWH while, anti-RBD IgG was higher in vaccinated PLWH compared with PLWH who had recovered from COVID-19 infection (16). To our knowledge, this is the first study comparing hybrid exposure to infection only in PLWH, further strengthening the idea that anti-SARS-CoV-2 immunization is efficient, and important in the settings of chronic HIV infection. Another important aspect of our study was the relation between the immune status and SARS-CoV-2 infection. We demonstrated that the presence and strength of SARS-CoV-2 specific responses was not associated with CD4AC at baseline, nor at the time of evaluation. Although all studied patients were on cART, their CD4AC varied between 191 and 1468

cells/ml before exposure and 224 and 1429 cells/ μ l at the time of study. According to our results, moderate immune deficiency (CD4AC between 200 and 350 cells/ μ l) did not preclude efficient response to SARS-CoV-2. Thus a patient from group A who had started cART less than six months before the study with severe immune deficiency (CD4 AC = 76), responded to SARS-CoV-2 (CD4AC= 284 at the time of the study). On the other hand, two other patients from group B with one treated for more than 12 months, with CD4AC in the range of 303-593, and 85-283 during the study, failed to develop SARS-CoV-2 specific memory responses. These observations remind that immune restoration is a complex process that is not completely characterized by CD4AC measurement, and the generation of immune memory is seriously affected in the settings of chronic HIV infection. We have also demonstrated that immunization/natural exposure to SARS-CoV-2 did not affect immune restoration under cART, at least in the majority of cases. Importantly, while CD4AC was not significantly affected, we observed a significant amelioration of CD4/CD8 ratio specifically in the group with hybrid exposure.

The CD4/CD8 ratio is a well-recognized marker of low-level immune activation, the latter inevitably associated with increased activity of Treg, accelerated differentiation of effector T cells, and impaired

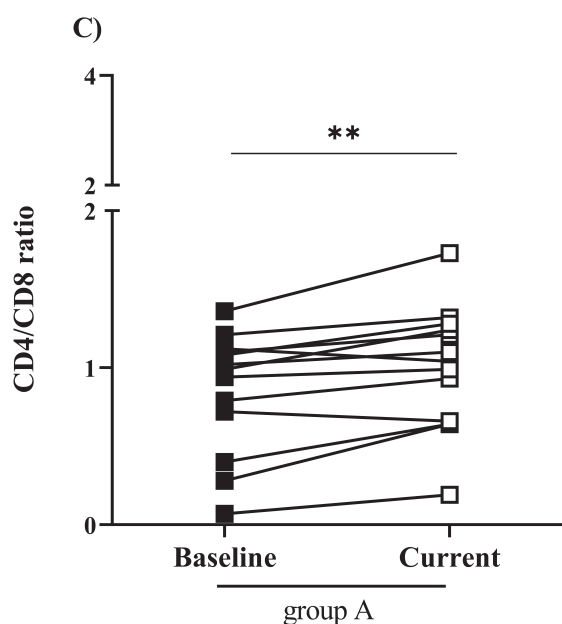


Figure.5, C.

generation of immune memory (17). In this aspect, anti-SARS-CoV-2 vaccines might boost not only the immediate protection but also, importantly, the generation of long-lasting memory for SARS-CoV-2. A major limitation of this study is the small number of participants, as well as some missing data on the exact dates of SARS-CoV-2 infection, and vaccination, as variables that may impact our findings. We also lack information about the patients viral load levels.

In conclusion, the higher rates of complications and mortality among PLWH hospitalized with COVID-19 warrants prioritization of vaccination policies for this risk group. This is especially important in a country with low vaccination coverage, and high rate of newly diagnosed HIV patients with advanced immune deficiency as Bulgaria.

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