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# The humoral virus-neutralizing response resulting from "CHADOX1 S" in persons living with HIV in Bulgaria

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## Abstract

The widespread propagation of SARS-CoV-2 necessitated the development of vaccines, both for healthy individuals and for people with comorbidities. The aim of this article was to present data on the post-vaccination virus-neutralizing response in people living with HIV (PLHIV) in Bulgaria, two months after the second dose of ChAdOx1 S vector vaccine. Totally 40 PLHIV had been vaccinated, 36/40 received a full vaccination course and finally, 32/40 participated in the study. The results were compared to 19 "healthy controls", also with a completed vaccination protocol. All 32 patients were on antiretroviral therapy at the time of vaccination, most of them (27/32, 84%) had undetectable viral loads and the remaining 5/32 (15%) had a temporary spike up to 400 copies/ml. CD4+ T-lymphocytes counts in 31 of PLHIV group were >350 cells/mm3; one of them had 190 CD4+ T cells/mm3, but this did not affect the antibody response (93.29%). Sera of all participants were tested by blocking ELISA with the "SARS-CoV-2 cPassTM GenScript neutralizing antibody detection kit". The results expressing the % of neutralization were interpreted as follows: <30% - were considered negative, and those >= 30% - positive. Positive results were also found in the "control" group, where 10/19 examined showed >90% presence of neutralization antibodies. This study confirms the high efficacy of the ChAdOx1S vector vaccine and the excellent humoral response in the PLHIV. The number of patients included in the study is not high, but the results, together with the literature data demonstrate the effectiveness and the need for priority vaccination of PLHIV.

**Keywords:** SARS-CoV-2; COVID-19; Vaccines; PLHIV; Immunodeficiency; Virus-neutralizing antibodies

## 1. Introduction

Over the past couple of years, much of the attention of medical professionals was devoted to managing the newly emerging COVID-19 pandemic. The cause of the pandemic, as it became clear, was the SARS-CoV-2 virus - a novel RNA-containing virus from the Coronaviridae family. Systematic approaches regarding diagnosis and treatment were subsequently developed, but the main focus on a faster and more effective means of disease prevention remained. The first vaccines were administered at the end of 2020 after the use approval by the European Commission and the European Medicines Agency (EMA) [1]. Currently, five approved vaccines are available in Bulgaria: two are nucleoside-modified mRNA based, two are vector based with and one being an inactivated viral vaccine.

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The subject of this article is the ChAdOx1 S vaccine against SARS-CoV -2 (Vaxzevria) [**Error! Reference source not found.**]. Regarding this vaccine's effectiveness, a large-scale study found an overall effectiveness of 70.4% (95·8% CI 54·8–80·6; 30 [0·5%] of 5807 and 101 [1·7%] of 5829) [2]. Additionally, a good safety profile, as measured by the incidence of serious adverse effects, was reported. From data published sofar, similar levels of neutralizing antibody titers are observed in the different age groups after the second dose of the vaccine (at day 42 post-vaccination 18–55 years, 193 [IQR 113–238], n=39; 56–69 years, 144 [119–347], n=20 and ≥70 years, 161 [73–323], n=47; p=0·40) [3]. Another study involved 12,390 individuals, including people living with HIV (PLHIV) aged 18-55. Mandatory conditions for participation included being on antiretroviral therapy (ART), having undetectable viral loads (VL), and a CD4 level >350 cells/ μL. The study is currently ongoing and is expected to assess the immune response after the vaccination depending on the patient's immune status, age and CD4 T-lymphocyte count [4].

In the course of the COVID-19 pandemic, the risk factors for a severe course of the disease became clear: male gender, age over 50 years, the presence of chronic comorbidities, as well as laboratory data on established lymphopenia, thrombocytopenia, increased levels of the enzymes LDH, ALT, AST, Cr and D-dimer [5]. Compared to the general population, PLHIV are known to be at higher risk of developing infectious diseases with possible severe complications.

The risk factors for the severe course (including fatal outcome) in PLHIV were labeled: the lack of adequate viral suppression, an advanced stage of HIV infection, and CD4+ counts below 350 cells/μL were among the defined [6,7]. Such data, as well as the demonstrated safety and efficacy of vaccines encouraged us to carry out prophylactic vaccination for this population. At present, there is no clear consensus on the preferred vaccine and vaccine regimen for PLHIV. Recommendations differ depending on the country and it is striking that in more countries from Central and Eastern Europe, the PLHIV population is considered with priority for vaccination. Currently, only three countries (Greece, Serbia and the Czech Republic) reported for developing national vaccination recommendations especially for PLHIV [8].

## 2. Material and methods

During the period February - May 2021, the first dose of ChAdOx1 S was administered to 40 PLHIV. 3/40 developed a mild form of COVID-19 shortly after the first dose. 36/40 completed the vaccination protocol in full. Peripheral venous blood was taken after two months for evaluation in 32/36. During the same period, 21 non-HIV-infected individuals with a completed vaccination protocol were included in the study serving as a control group. All 32 PLHIV were on antiretroviral therapy (ART) at the time of vaccination. Most of them (27/32; 84%) had an undetectable HIV viral load (VL), and the remaining 5 (15%) had a temporary spike in the VL level in the range of 65 to 400 HIV-1 RNA copies/ml.

The principle of the ELISA method used in this study was based on blocking the interaction of the recombinant RBD fragment of the S - protein of the SARS-CoV-2 virus and the human $ACE_2$ receptor ($hACE_2$). The virus-neutralizing antibodies in the samples bound the recombinant RBD fragment and the mixture was then added to a plate preloaded with the $hACE_2$ protein. Finally, the absorbance at 450 nm, an inverse dependence of the titer of the bound virus-neutralizing antibodies against SARS-CoV-2, was documented. To ensure the validity of the results, the test included positive and negative controls. The results were interpreted as follows:

- >= 30 % - Positive, indicating the presence of a neutralizing antibodies against SARS-CoV–2;
- < 30 % - Negative, indicating the absence or a level of neutralizing antibody below the detection limit of this test [9].

### 2.1. Statistical methods

All qualitative variables are presented as frequencies and percentages whilst numerical as the median and interquartile range (25th and 75th percentile). Pearson chi-square test is used to compare the distribution of qualitative variables' categories between the groups. Average values differences between two groups are compared by using Mann-Whitney U test. The results are considered as significant if $p<0.05$.

## 3. Results

All PLHIV included in the study were on ART at the time of vaccination and follow-up. The majority had undetectable viral loads (27/32; 84%). The data regarding the number of CD4+ T-lymphocytes counts in 31 of the examined patients were >350 cells/$mm^3$ and in only one of the patients the count was <200 cells/$mm^3$ - namely 190 cells/$mm^3$. The immune response in patients with a more severe form of immunodeficiency (CD4+<200 cells/$mm^3$) also resulted in virus-neutralizing antibody titers of 93.29%. The following table shows the data of the PLHIV, the majority of them,

according to the questionnaire, are men who have sex with men (MSM). The average age of the included patients was 40.7 years, all living in the city of Sofia (Table 1).

The control group consisted of 21 individuals with a completed vaccination protocol and the same ELISA method was applied to evaluate the virus-neutralizing antibodies. Their median age was 47 years (median 46, ranging from 19 to 76), 12/21 (57.1%) were women and 7/21 were men, all from the city of Sofia. Peripheral venous blood was taken from 19 of the individuals. Patients from both groups had certain differences in terms of gender and age. The gender difference between HIV patients and healthy controls was significant ($p<0.001$) (**Fig.1**). The two groups also had a different median age, but this did not prove to be statistically significant difference ($p=0.063$) (**Table 2**).

**Table 1** Individual virus-neutralizing titer (VNT%) in the group of people living with HIV (PLHIV) with their immune status (CD4+T-ly number) and viral load (VL) at the time of immunization with Vaxzevria

| PLHIV | Age | Sex | CD4+T-ly cells/mm3 | VL (copies/ml) | VNT % |
|---|---|---|---|---|---|
| Patient 1 | 42 | M | 1180 | undetectable | 96.99% |
| Patient 2 | 31 | M | 850 | undetectable | 91.84% |
| Patient 3 | 37 | M | 997 | undetectable | 75.60% |
| Patient 4 | 39 | M | 600 | undetectable | 56.78% |
| Patient 5 | 36 | M | 750 | undetectable | 94.10% |
| Patient 6 | 56 | M | 739 | 93.2 copies | 97.11% |
| Patient 7 | 40 | M | 1 000 | undetectable | 97.05% |
| Patient 8 | 47 | F | 500 | 200 copies | 96.99% |
| Patient 9 | 40 | F | 900 | undetectable | 93.29% |
| Patient 10 | 44 | M | 190 | undetectable | 93.29% |
| Patient 11 | 35 | M | 569 | undetectable | 96.30% |
| Patient 12 | 41 | M | 900 | undetectable | 95.80% |
| Patient 13 | 37 | M | 710 | undetectable | 97.05% |
| Patient 14 | 44 | M | 1300 | 50 copies | 91.41% |
| Patient 15 | 56 | M | 535 | undetectable | 96.93% |
| Patient 16 | 48 | M | 600 | undetectable | 94.92% |
| Patient 17 | 39 | M | 1200 | 400 copies | 78.42% |
| Patient 18 | 30 | M | 800 | undetectable | 80.39% |
| Patient 19 | 40 | M | 1498 | undetectable | 96.55% |
| Patient 20 | 32 | M | 1400 | undetectable | 96.93% |
| Patient 21 | 60 | M | 590 | undetectable | 68.38% |
| Patient 22 | 59 | M | 412 | undetectable | 62.86% |
| Patient 23 | 37 | M | 575 | 65 copies | 96.36% |
| Patient 24 | 35 | M | 1130 | undetectable | 97.05% |
| Patient 25 | 31 | M | 565 | undetectable | 97.05% |
| Patient 26 | 46 | M | 505 | undetectable | 76.50% |
| Patient 27 | 53 | M | 757 | undetectable | 96.93% |
| Patient 28 | 30 | M | 530 | undetectable | 95.57% |
| Patient 29 | 40 | M | 675 | undetectable | 96.11% |
| Patient 30 | 40 | M | 900 | undetectable | 96.17% |

| | | | | | |
|---|---|---|---|---|---|
| Patient 31 | 51 | M | 800 | undetectable | 96.11% |
| Patient 32 | 45 | F | 900 | undetectable | 95.81% |

In patients 6, 8, 14, 17 and 23 a slight VL increase above the level of detection was found, without an impact on virus-neutralizing antibody response. All of them demonstrated a positive response and in 4/32 the result exceeded 90%. Patients 21 and 22 (aged 60 and 59 years, respectively) had a lower response rate of 68.38% and 62.86%, respectively.

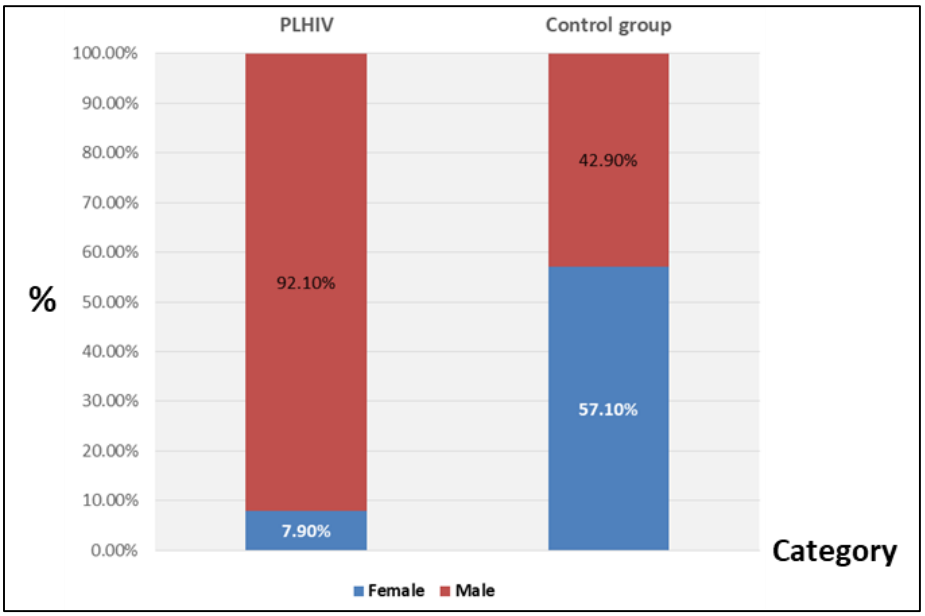


**Figure 1** Sex distribution of participants from PLHIV and the control group (p<0.001)

**Table 2** Age distribution of participants from PLHIV and healthy control group (p=0.063)

| | **Patients** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **PLHIV** | | | | **Control group** | | | |
| | **Mean** | **Median** | **Min** | **Max** | **Average** | **Median** | **Min** | **Max** |
| Age | 40.7 | 39.5 | 29 | 60 | 47 | 46 | 19 | 76 |

**Table 3** Post-vaccinal reactions in PLHIV and healthy controls (p>0.05). NA; not applicable

| | | **PLHIV** | | **Control group** | | **p** |
|---|---|---|---|---|---|---|
| | | **n** | **%** | **n** | **%** | |
| **Gender** | Male | 35 | 92.1% | 9 | 42,9% | <0.001 |
| | Female | 3 | 7.9% | 12 | 57,1% | |
| **First vaccine shot** | No | 0 | 0.0% | 0 | 0,0% | |
| | Yes | 38 | 100.0% | 21 | 100,0% | |
| **Second vaccine shot** | No | 5 | 13.2% | 0 | 0,0% | |
| | Yes | 33 | 86.8% | 21 | 100% | |
| **Post vaccinal reactions first shot** | No | 3 | 7.9% | **NA** | | |
| | Yes | 35 | 92.1% | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Post vaccinal reactions second shot** | No | 13 | 38.2% | | |
| | Yes | 21 | 61.8% | | |

The most commonly reported adverse reaction was pain at the application site, which occurred in 20/32 participants (62%). 3/32 reported no adverse events after vaccination (9%). The other most frequently reported reactions were: myalgia in 6/32 patients (18%), fever - in 15/32 (46%) and headache - in 7/32 (21%). The second dose of the vaccine was given 4-12 weeks after the first, with the majority of patients following the recommended longer interval between the two doses. Lower rates of adverse events after the second vaccination was observed; 11/32 (34%). The most common complaint was pain at the application site - in 16/32 (50%), followed by myalgia in 3/32 (9%), fever in 2/32 (6%), and headache in one patient in 1/32 (3%). The highest number of adverse effects after vaccination were reported in the first 48 hours. Adverse events were mild to moderate and did not occur after two weeks post-vaccination, demonstrating the safety and low reactogenicity of the vaccines in the cohorts involved [10]. No statistically significant difference in post-vaccination reactions after the second dose($p>0.05$) was observed between the PLHIV and control groups (**Table 3**).

## 4. Discussion

The obtained data clearly demonstrates the impact of vaccination using ChAdOx1 S in PLHIV on the subsequent VL. 86.8% of the tested population had a viral load below the detection threshold. The single VL spikes detected in 5 cases (13.2%) did not correlate with a poorer humoral immune response. All patients had a positive (>30%) virus-neutralizing response with 26/32 having >90% (80%), 4/32 patients having a result between 70-90% (11%) and only 3/32 patients showing neutralization in the range 50-70% (9%). A comparative analysis was made with the result obtained from the tested sera of the individuals in the control group. The results were positive as expected (>30%) and in 10/19 controls they were even > 90% (51%). 6/19 patients had a result between 70-90% (31%), 3/19 people developed antibodies in the range of 50-70% (14%) and only 1/19 has a result below the 50%, namely 42.99%. The person with this lowest score was a 49-year-old woman who reported no comorbidities or immune related disorders. The results of the virus-neutralizing titers (VNT) in both groups are presented on **Fig. 2**. We compared the median virus-neutralizing antibody titers of the two groups and found that they were significantly different ($p=0.029$) (**Fig.3**). It is noteworthy that they are higher in PLHIV compared to their distribution in the control group.

Regarding adverse events, a study investigated responses in 1,569 people vaccinated with the ChAdOx1 vaccine. Local pain, fever, general malaise, and myalgia were reported as the primary adverse events which resolved up to 2 days post-vaccination. Palpitations, chest pain, diarrhoea, abdominal pain have also been reported, but at significantly lower rates[11]. In our patient cohort, no such complaints were registered. In another study, 981 healthcare workers vaccinated with two doses of the ChAdOx1 vaccine were followed at 48 hours, 8th, 15th, 22nd, 28th days post vaccination. The highest number of adverse events after the vaccination were reported during the first 48 hours, which was also observed in the patients in this study. Adverse events were mild to moderate and did not occur two weeks after administration of both doses, demonstrating the safety and low reactogenicity of the vaccines in the people involved [10]. Similar results were observed in this study.

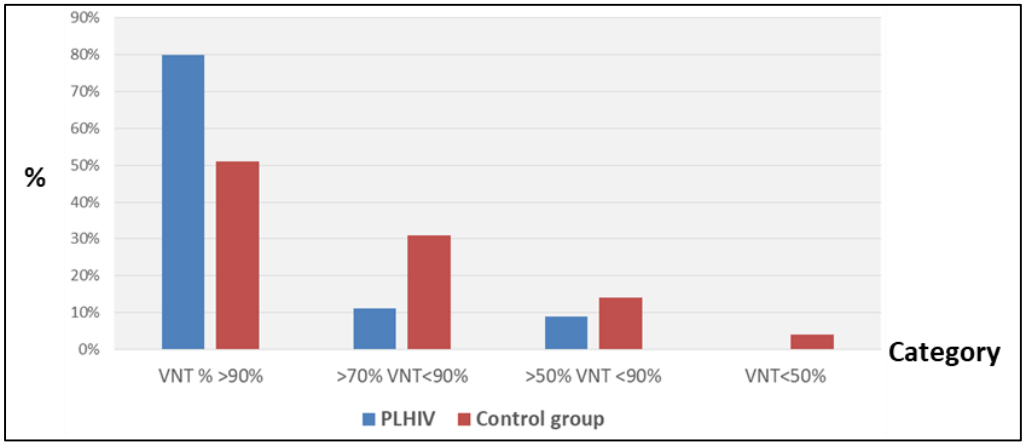

**Figure 2** Virus-neutralizing antibodies titers (VNT) - %

Overall, the results obtained in this study of PLHIV are conclusive in terms of both good efficacy and low to moderate reactogenicity after the administration of the Vaxzervia vaccine against COVID-19. Attention has been drawn to immunodeficiency in PLHIV as a major risk factor for severe progression of COVID-19, even with adequate VL suppression of HIV [6]. In our study, only one patient with low CD4 cell counts was included. However, the expectations for ineffective humoral immune response turned out to be false, with recordings of VNT at 93.23%. Nonetheless, further studies on vaccines' effectiveness in the immunodeficient population are necessary as a case report cannot lead to statistically significant conclusions. Considering the follow-up in the control group and the lack of statistically significant differences in its favor, it can be concluded that the humoral postvaccination response in immunosuppressed patients does not differ from that obtained in the general population. Despite the lack of clear consensus regarding preferred vaccines for priming and boosting doses in PLHIV, our data together with the increased need for hospitalization for COVID-19 among PLHIV and the higher mortality among the hospitalized; confirms the need for priority vaccination in this specific population. [**Error! Reference source not found.**].

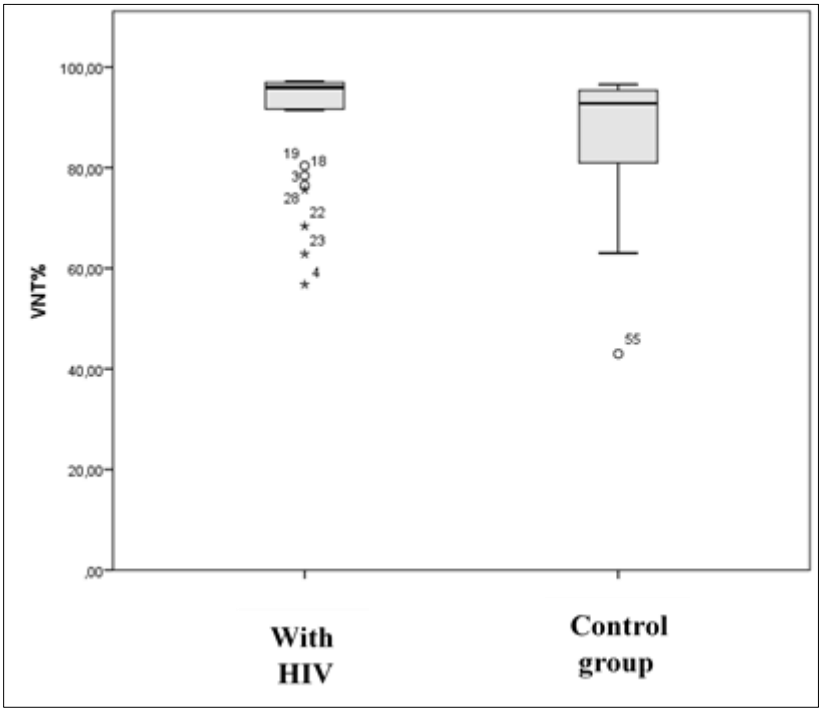


**Figure 3** Virus-neutralizing antibodies titers (VNT % ) in PLHIV and healthy control groups

## 5. Conclusion

All PLHIV demonstrated a positive virus-neutralizing response and the comparison of the median neutralizing titers of the two groups showed the titers were significantly higher (p=0.029) in PLHIV compared to controls. Post-vaccinal reactions after the first and second shots were mild to moderate in both groups (p>0.05), demonstrating the safety and low reactogenicity of the vaccine in all participants.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

*Statement of ethical approval*

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

*Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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