Increased risk of false-positive HIV ELISA results after COVID-19

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Objective: From the first-generation options available in 1985, tests to detect HIV-1 specific antibodies have increased its sensitivity and specificity. HIV-1 and SARS-CoV-2 surface glycoproteins present a certain degree of homology and shared epitope motifs, which results of relevance as both pandemics coexist. Here, we aimed to evaluate the rate of false-positive HIV serology results among individuals with COVID-19 diagnosis and in vaccinated individuals.

Design: A retrospective analysis of the samples stored at the Infectious Disease Biobank in Argentina from donors with previous COVID-19 diagnosis or anti-SARS-CoV-2 vaccination.

Methods: Plasma samples were analyzed using Genscreen Ultra HIV Ag-Ab. In those with a positive result, the following assays were also performed: ELISA lateral flow Determine Early Detect; RecomLine HIV-1 & HIV-2 IgG and Abbott m2000 RealTime PCR for HIV-1 viral load quantification. In all samples, the presence of anti-SARS-CoV-2 IgG antibodies was evaluated by ELISA using the COVIDAR kit. Statistical analysis was done using Pearson's and Fisher's exact chi-squared test; Mann–Whitney and Kruskal–Wallis tests.

Results: Globally, the false-positive HIV ELISA rate was 1.3% [95% confidence interval (95% CI) 0.66–2.22; $\chi^2 = 4.68$, $P = 0.03$, when compared with the expected 0.4% false-positive rate]. It increased to 1.4% (95% CI 0.70–2.24, $\chi^2 = 5.16$, $P = 0.02$) when only samples from individuals with previous COVID-19 diagnosis, and to 1.8% (95% CI 0.91–3.06, $\chi^2 = 7.99$, $P = 0.005$) when only individuals with detectable IgG SARS-CoV-2 antibodies were considered.

Conclusion: This higher occurrence of HIV false-positive results among individuals with detectable antibodies against Spike SARS-CoV-2 protein should be dispersed among virology testing settings, health providers, and authorities.

AIDS 2023, **37**:947–950

Keywords: COVID-19, false positive, HIV, SARS-CoV-2, serology

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Received: 25 November 2022; revised: 18 January 2023; accepted: 26 January 2023.

DOI:10.1097/QAD.0000000000003507

Introduction

Since the beginning of COVID-19 pandemic in December 2019, there have been 636 089 587 reported cases worldwide [1] and 12.7 billion of anti-SARS CoV2 vaccines have been administered globally (covering 67.9% of the total population) [2]. This pandemic coexists with the HIV pandemic, with more than 38.4 million of people with HIV around the world [3]. In Argentina, the number of reported COVID-19 cases is 9 708 420, 40 987 935 persons were vaccinated against COVID-19, and 140 000 individuals are infected with HIV [4].

Tests to detect HIV-1 specific antibodies have increased its sensitivity and specificity from the first-generation test available in 1985 to the fourth-generation ELISA used nowadays. The presence of comorbidities (e.g. autoimmune diseases, other viral infections) or pregnancy can increase the number of false-positive results [5–7], but globally the rate is 0.4% [8].

HIV and SARS-CoV surface glycoproteins present a certain degree of homology and shared epitope motifs [9]. Indeed, there have been several reports in 2020 and 2021 of false-positive results in HIV screening assays in individuals with acute or past SARS CoV-2 infection [10–14].

False-positive results in HIV ELISA test have also been reported during COVID-19 vaccine trials in Australia [15].

The aim of this study was to evaluate the rate of false-positive HIV serology results among individuals with COVID-19 diagnosis with and without detectable SARS-CoV-2 antibodies and as well as in vaccinated individuals with two doses of Sputnik V vaccine and with no evidence of previous infection with SARS CoV-2.

Materials and methods

BBEI (Biobanco de Enfermedades Infecciosas) collected blood samples from individuals with confirmed COVID-19 diagnosis between April 9, 2020, and August, 2021. The biobank received blood donations from 1078 donors with confirmed COVID-19 diagnosis, noninfected close contact, and vaccinated individuals. Samples from individuals who disclosed an HIV-positive status were excluded, as well as those from noninfected close contacts and those from individuals who were vaccinated against COVID but have a previous COVID-19 diagnosis. Six hundred and seventy-four individuals with COVID-19 diagnosis (in acute and convalescent phase) with detectable SARS-CoV-2 antibodies in plasma (Group 1), 200 individuals with COVID-19 diagnosis and not detectable SARS-CoV-2 antibodies in plasma (Group 2), and 47 vaccinated individuals (Group 3) were included in the present study. After signing the consent, all individuals donated 30 ml of peripheral blood in EDTA containing tubes (BD Vacutainer; BD, Franklin Lakes, New Jersey, USA).

Group 1 and 2 samples were taken within 15 days (acute), 60 days (early convalescent), and after 60 days (late convalescent) from symptom onset or molecular SARS-CoV-2 diagnosis. Vaccinated individuals (Group 3) received two doses of Sputnik V vaccine (nonreplicative viral vector -Ad26-Ad5- Gamaleya Research Institute of Epidemiology and Microbiology) and samples were taken 14 days after the second dose.

Plasma samples were analyzed using Genscreen Ultra HIV Ag-Ab (Batch 2A0138; Bio-Rad, Marnes-la-Coquette, France). This assay allows us to detect p24 antigen and HIV-1 and HIV-2 antibodies. In those samples that yielded a positive result, the following assays were also performed: ELISA lateral flow Determine Early Detect (Batch:143773k200; Abbott Diagnostics, Chiba, Japan) for the detection of HIV-1 antibodies; RecomLine HIV-1 & HIV-2 IgG—(Batch LHI122001; MIKROGEN GmbH, Neuried, Germany) for the identification of specific antibodies against the individual antigens of HIV-1 and HIV-2 (ENV proteins HIV-1: gp120, gp41; ENV proteins HIV-2: gp105, gp36; GAG proteins: p24, p17; POL proteins: p51, p31), and Abbott m2000 RealTime PCR for HIV-1 viral load quantification.

In all samples, the presence of anti-SARS-CoV-2 IgG antibodies was evaluated by ELISA using the COVIDAR kit (Laboratorio Lemos S.R.L, Argentina). Demographic and clinical data were collected from all donors.

Proportions were assessed using Pearson's and Fisher's exact chi-squared test; Mann–Whitney and Kruskal–Wallis tests were used to compare continuous variables. Analysis was done with StataIC14 software (StataCorp LLC, Texas, USA).

The SARS CoV-2 collection within the BBEI was reviewed and approved by the institutional review board of the non-for-profit research organization Fundación Huésped (Comité de Bioética Humana, Fundación Huésped, Buenos Aires, Argentina).

Results

The clinical and demographic characteristics of the donors are described in Table 1. Among the 921 samples analyzed, positive ELISA Genscreen Ultra HIV Ag-Ab was detected in 15 cases. In three individuals, Determine Early Detect, RecomLine HIV1-HIV2 IgG were also positive, and HIV viral load was detectable (Table 2). Those three donors were contacted in order to confirm

Table 1. Characteristics and findings of the individuals included in the study.

Characteristic	Previous COVID with detectable IgG anti-Spike. Group 1; $n=674$	Previous COVID with negative IgG anti-Spike. Group 2; $n=200$	Vaccinated donors Group 3; $n=47$
Age, years (median, IQR)*	41 (32–54)	43 (34–56)	42 (36–57)
Sex, female (%)***	59	55	62
Days from symptoms onset to sampling, days (median, IQR)**	41 (24–62)	30 (10–49)	NA
False HIV ELISA results	12	0	0
Self-reported comorbidities (%)	Hypothyroidism (4.6%) HTN (11.7%) Asthma (2.8%) Obesity (7.8%) Diabetes (4.7%)	Hypothyroidism (3.5%) HTN (13.5%) Asthma (3%) Obesity (8.5%) Diabetes (5.5%)	Hypothyroidism (8.5%) HTN (6.4%) Asthma (2.1%)
Complications of COVID-19 (%)	Pneumonia (18.5%) Mechanical respiratory assistance (2.2%)	Pneumonia (17%)	NA

HTN, arterial hypertension; NA, not applicable. *$P=0.1024$, **$P=<0.0001$; ***$P=0.4$.

these results and they declared that they were aware of their HIV status but for different reasons did not disclose this information at the time of first donation to the BBEI.

Improper handling or mislabeling of the remaining 12 samples was first excluded by checking if samples of people living with HIV were enrolled on the same day. After excluding this option, a second ELISA and confirmatory assays were performed, and all shielded negative results (Table 2). When the samples were segregated according to the groups, none of the samples from vaccinated donors (Group 3) had positive HIV ELISA results, even though all exhibited detectable levels of specific IgG after COVID-19 vaccination. When individuals with previous COVID-19 were considered, only those who have detectable IgG antibodies against SARS-COV-2 RBD (Group1) exhibited false-positive HIV-1 ELISA results.

Globally, the false-positive HIV ELISA rate was 1.3% [95% confidence interval (95% CI) 0.66–2.22]. If this rate is compared with CDC-estimated rate of false-positive results (0.4%), it is significantly higher ($\chi^2=4.68$, $P=0.03$). If only samples from individuals with previous COVID-19 diagnosis were considered, the rate was 1.4% (95% CI 0.70–2.24), which once again is statically higher than the average rate ($\chi^2=5.16$, $P=0.02$), and specifically for those with detectable IgG SARS CoV-2 antibodies, was 1.8% (95% CI 0.91–3.06; $\chi^2=7.99$, $P=0.005$).

The median optical density (OD) of the COVIDAR assays for the detection of anti-Spike IgG was 3.55 (IQR 1.46–3.94); this value is close to the upper limit of detection of the assay (4) and correlates with a IgG titer more than 800 [16]. Once again, this could reflect a cross-reactivity of specific antibodies against epitopes of both viral proteins, which increases with higher levels of IgG. Time from symptoms onset to sampling was higher among individuals with detectable IgG SARS CoV-2, which reflects the expected dynamics of specific COVID-19 humoral response.

Considering the self-reported medical history of the 12 donors with false ELISA results, eight (67%) did not have

Table 2. Serological and virological characteristics of the samples with positive ELISA results.

ID	OD ELISA SARS	OD ELISA HIV	HIV LFA	Western blot	HIV viral load	Observation
COVID19-039	3.4063	0.415	NEG	NEG	TND	HTN
COVID19-090	4	0.258	NEG	NEG	TND	No comorbidities
COVID19-118	4	0.301	NEG	NEG	TND	Asthma
COVID19-149	3.6887	0.62	NEG	NEG	TND	No comorbidities
COVID19-177	3.7	0.948	NEG	NEG	TND	No comorbidities
COVID19-251	1.2494	0.671	NEG	NEG	TND	No comorbidities
COVID19-279	1.1003	3.454	POS	gp120, gp41, p24	< 40 cp/ml	Under ART
COVID19-293	3.9679	0.46	NEG	NEG	TND	No Comorbidities
COVID19-520	3.892	0.301	NEG	NEG	TND	HTN
COVID19-525	2.78	3.46	NEG	NEG	TND	SLE
COVID19-585	0.3993	3.468	POS	gp120, pg41, p51, p31, p24, p17	< 40 cp/ml	Under ART
COVID19-711	0.2466	3.477	POS	gp120, gp41, p51, p31, p24	35.296 cp/ml	No ART
COVID19-824	0.2582	2.329	NEG	NEG	TND	No comorbidities
COVID19-842	0.3116	3.465	NEG	NEG	TND	No comorbidities
COVID19-984	2.1038	0.266	NEG	NEG	TND	No comorbidities

ART, antiretroviral therapy; HTN, arterial hypertension; LFA, lateral flow assay; OD, optical density; SLE, systemic lupus erythematosus; TND, target not detected.

any comorbidities, two reported well controlled arterial hypertension, one mild asthma, and one systemic erythematous lupus and fibromyalgia. None of the donors was pregnant at the moment of blood sampling.

Discussion

Considering the magnitude of the COVID-19 pandemic and the still worldwide circulation of different variants of SARS CoV-2, the results of the present study should be considered when HIV screening assays are performed, particularly among low-risk individuals. The higher occurrence of these false results among individuals with detectable antibodies against Spike SARS CoV-2 proteins is in line with the previous reports of the shared homology between SARS CoV-2 and HIV proteins, and this could reflect the existence of antibody- cross-reactivity. It is also important to highlight that this cross-reactivity does not seem to appear when the antibodies against Spike protein are generated after viral vector vaccines. Also, no association was observed with the presence of previously reported causes of false HIV ELISA results. In only one case, another possible factor (autoimmune disease) associated with false serological results was found.

This study adds support to previous publications reporting an increase in HIV false-positive results during and after COVID-19 pandemic [10–14] and gives support to consider SARS CoV-2 infection among possible causes of false-positive ELISA results, as they exhibited the same rate observed in other conditions not linked to the HIV infection [5]. The reporting of this finding is crucial to inform health authorities and should be dispersed along with virology testing providers. If the same trend is found with the multiple different commercially available ELISA tests and across countries, physicians should be aware of this to advise and reassure those individuals with this serological pattern.

Acknowledgements

The authors acknowledge all the donors of the BBEI; and to Sergio Martini Novas and Laura Suarez Ornani (Dirección de Respuesta al VIH, ITS, Hepatitis virales y Tuberculosis del Ministerio de Salud de la Nación) for the provision of serological kit to conduct this study.

Y.L. and N.L. are members of the BBEI directory board. N.L. conceived the study. Y.L., L.G.A., M.P., L.C. performed laboratory work. Y.L. and N.L. and analyzed the data. Y.L., G.T., L.G.A., M.F.Q., and N.L. wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest.

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