Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinical Microbiology and Infection 27 (2021) 802–804

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Letter to the Editor

No evidence of SARS-CoV-2 circulation in HIV-infected patients between December 2019 and February 2020 in Rome, Italy

Francesca Lombardi [1, *], Simone Belmonti [2], Rosalba Ricci [3], Alberto Borghetti [4], Massimiliano Fabbiani [5], Arturo Ciccullo [1], Roberto Cauda [1, 4], Simona Di Giambenedetto [1, 4]

[1)] *Università Cattolica del Sacro Cuore, Dipartimento di Sicurezza e Bioetica Sezione Malattie Infettive, Rome, Italy*
[2)] *Fondazione Policlinico Universitario A. Gemelli IRCCS, Area of Pathology, Department of Woman and Child Health and Public Health, Istituto di Anatomia Patologica, Rome, Italy*
[3)] *Fondazione Policlinico Universitario A. Gemelli IRCCS, Institute of Microbiology and Virology, Rome, Italy*
[4)] *Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC Malattie Infettive, Rome, Italy*
[5)] *Infectious and Tropical Diseases Unit, Azienda Ospedaliero-Universitaria Senese, Siena, Italy*

ARTICLE INFO

Article history:
Received 8 October 2020
Received in revised form
22 November 2020
Accepted 24 November 2020
Available online 2 December 2020

Editor: L. Leibovici

To the Editor,

In this monocentric cross-sectional study we evaluated the IgG seroprevalence of SARS-CoV-2 infection in HIV-infected outpatients who frequented our university hospital Fondazione Policlinico Universitario A. Gemelli IRCCS, in Rome between 1 December 2019 and 29 February 2020.

IgGs against SARS-CoV-2 were assessed on stored cryopreserved samples with an enzyme-linked immunosorbent assay (ELISA) (Dia.Pro Diagnostic Bioprobes S.r.l. Sesto San Giovanni, Milan, Italy). This assay is based on a microplate coated with a recombinant antigen of both nucleocapsid and spike proteins. At the time of the study, the reported sensitivity and specificity were ≥98% and ≥90%, respectively. The manufacturer reported that about 10% of the 'normal' population show a reactivity against the nucleocapsid, although the causes of this positive reactivity are not clarified. Therefore, we tested the reliability of the Dia.Pro with a different ELISA assay purchased from Eagle Bioscience Inc. (Amherst, NH, USA); the reported sensitivity and specificity were 100%. This test is based on a microplate coated with COVID-19 recombinant full-length nucleocapsid protein. Of note, at the time of the study both ELISA tests had already obtained CE certification.

Control samples from COVID-19-negative and -positive volunteers (confirmed with RT-PCR negative/positive nasopharyngeal swab) were assayed in each run; six controls from hospitalized ($n = 3$) and asymptomatic non-hospitalized ($n = 3$) COVID-19 patients at 14–21 days after a confirmed positive swab test and a HIV patient hospitalized with COVID-19 were used. For negative controls we ran a total of six controls.

We estimated seroprevalence as the proportion of individuals who simultaneously tested positive for anti-SARS-CoV-2 IgGs in both Dia.Pro and Eagle Bioscience assays. Seroprevalence was reported as rate and 95% CI. We analysed a single plasma sample from 451 HIV positive patients. Table 1 summarizes the main characteristics of the patients according to the date (month) of plasma samples.

Using the Dia.Pro assay, 438 (97%) patients resulted IgG negative and 13 (3%) showed the presence of IgG.

Using the Eagle Bioscience assay, we retested all 13 plasma samples from patients who were IgG positive on the Dia.Pro assay; we also retested a random subset of 164 samples that were IgG negative. Notably, all 13 'positive' samples were anti-COVID-19 IgG negative, whereas one sample out of 164 'negative' was IgG positive.

In order to test for a potential cross-reactivity in patients who had a positive result on either Dia.Pro or Eagle ELISA, we also tested a few available 'older samples' for IgGs (i.e. obtained and frozen in a period when we assumed that the virus was not circulating).

Specifically, in two patients who had detectable IgG on the Dia.Pro. assay we tested one sample obtained in 2018 and one in early 2019. Of note, these older samples confirmed a positive IgG

* Corresponding author: Francesca Lombardi, Institute of Clinical Infectious Diseases, Catholic University of the Sacred Heart, Largo Agostino Gemelli 8, 00168 Rome. Italy.
E-mail address: francesca.lombardi@gmail.com (F. Lombardi).

https://doi.org/10.1016/j.cmi.2020.11.026

Table 1
Characteristics of the 451 HIV-infected patients

	December 2019 $n = 136$	January 2020 $n = 173$	February 2020 $n = 142$	p
Gender, *n* (%)				0.267
Male	92 (68)	112 (65)	104 (73)	
Female	44 (32)	61 (35)	38 (27)	
Age, median (IQR) years	53 (43–60)	52 (44–59)	53 (38–61)	0.557
Italian, *n* (%)	104 (77)	118 (68)	105 (74)	0.167
Risk factor, *n* (%)				0.665
Homo/bisexual	40 (30)	43 (25)	41 (29)	
Heterosexual	45 (33)	57 (33)	36 (25)	
IVDU	19 (14)	25 (14)	15 (11)	
Other	6 (4)	14 (8)	8 (5)	
Unknown	26 (19)	34 (20)	42 (30)	
Time since HIV diagnosis, years, median (IQR)	15 (6–26)	16 (9–23)	14 (7–25)	0.750
Currently on ART, *n* (%)	128 (94)	161 (93)	134 (94)	0.877
Type of ARTs, n (%)				
Triple regimen	79 (62)	99 (61)	83 (62)	0.997
INSTI-based	45 (57)	61 (62)	53 (64)	
NNRTI-based	28 (35)	27 (27)	21 (25)	
PI-based	6 (8)	11 (11)	9 (11)	
Dual regimen	26 (20)	37 (23)	27 (20)	0.798
INSTI-based	18 (69)	26 (70)	17 (63)	
PI-based	8 (31)	11 (30)	10 (37)	
Other combinations	23 (18)	25 (16)	24 (18)	0.814
Time on ART, years, median (IQR)	11 (6–21)	14 (8–22)	12 (5–19)	0.255
Pre-ART HIV RNA, $\log_{10}$ copies/mL, median (IQR)	4.8 (3.9–5.5)	4.9 (4.3–5.5)	4.9 (4.3–5.4)	0.857
CD4 cell count nadir, cells/mm^3, median (IQR)	178 (56–309)	149 (44–269)	168 (52–313)	0.602
CD4 count, cells/mm^3, median (IQR)	651 (437–822)	639 (387–895)	551 (437–822)	0.072
CD8 count, median (IQR) cells/mm^3	760 (504–1096)	784 (581–1058)	686 (503–918)	0.055
CD4/CD8 ratio, median (IQR) cells/mm^3	0.77 (0.53–1.09)	0.82 (0.54–1.09)	0.8 (0.55–1.03)	
HIV-RNA <50 copies/mL, *n* (%)	103 (76)	128 (74)	96 (68)	0.271
Past AIDS-defining events (CDC stage C), *n* (%)	34 (25)	51 (30)	40 (28)	0.526
HCV co-infection, *n* (%)	24 (18)	27 (16)	20 (14)	0.859
Body mass index, median (IQR)	23 (21–25)	23 (21–26)	25 (22–28)	0.209
Hypertension	11 (8)	18 (10)	17 (12)	0.473
Reported flu-like symtoms[a], *n* (%)	9 (6.6)	18 (10)	19 (13)	0.175

ART, antiretroviral therapy; INSTI, Integrase strand transfer inhibitor; IQR, interquartile range; IVDU, intravenous drug users; NNRTI, Non-nucleoside reverse transcriptase inhibitor; PI, Protease inhibitor.
[a] Cough, cold, sore throat, myalgia and asthenia.

result on the Dia.Pro assay and a negative one on the Eagle Bio assay. For the patient who resulted IgG positive on the Eagle Bio test, we assayed three older samples, i.e. one sample obtained in 2018 and two samples in early and mid-2019; they all confirmed a positive IgG result on the Eagle Bio assay and a negative one on the Dia.Pro assay.

Overall, no patient had an IgG-positive result as per definition. Consequently, our analysis revealed a seroprevalence of 0% ($n = 0$/451; 95% CI 0.00–0.008%). Thus, in our study no evidence of SARS-CoV-2 circulation in HIV-infected patients before March 2020 in Rome was observed. This finding seems to be in contrast with those reported in some studies conducted in the general population in northern Italy which show that SARS-CoV-2 infection was already circulating in that period [1,2]. Our data suggest that SARS-CoV-2 was not circulating at all or at a very low level in central Italy among HIV-positive people before the outbreak was first recognized in our country, i.e. February 21st. Whether there were differences in the prevalence of the virus between HIV-infected individuals and the general population must be clarified in further studies.

Since the accuracy of tests for antibodies against SARS-CoV-2 remains controversial [3,4], in this study we estimated the IgG seroprevalence rate by considering as definitely positive only samples which tested positive simultaneously on both assays employed. This approach substantially increases the positive predictive value of the laboratory result (which is lower when only one assay is used when the prevalence in a population is low) and decreases the number of false positive results [5]. Despite the limitation due to the lack of availability of all the older sera for those samples which were positive using one of the two assays employed, our analysis showed that false positive results were possible in both assays. It should be noted that false positive COVID-19 serology results are possible due to cross-reaction with pre-existing antibodies against other human coronaviruses [6]. Most importantly, cautious interpretation of results based on serology is certainly warranted, given the important weaknesses in the evidence on COVID-19 serological tests.

Despite these limitations, this study provides the first important data regarding SARS-CoV-2 IgG seroprevalence in HIV-positive people in Italy. Further investigations with a large number of samples and during a subsequent outbreak are needed to fully understand the evolution of the current SARS-CoV-2 pandemic.

Transparency declaration

All authors have no conflicts of interest to disclose. No external funding was received for this work.

Ethics committee approval

The study protocol complied with Good Clinical Practice (GCP) rules and the Declaration of Helsinki. A protocol was approved by the Ethical Committee of Fondazione Policlinico Universitario A. Gemelli IRCCS, ID 3070 on 3/30/2020. Each patient signed a written

informed consents for using both the stored plasma sample and the clinical data.

Author contributions

Francesca Lombardi conceived and designed the study, collected data and performed the analysis. Francesca Lombardi and Simone Belmonti wrote the first draft. All authors provided substantial scientific input to the manuscript. All authors revised and agreed upon the final version of the manuscript.

Acknowledgements

We wish to thank Prof. Maurizio Sanguinetti (Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy) for providing the Kit elisa Dia.Pro Diagnostic Bioprobes S.r.l.

References

[1] Valenti L, Bergna A, Pelusi S, Facciotti F, Lai A, Tarkowski M, et al. SARS-coV-2 seroprevalence trends in healthy blood donors during the COVID-19 Milan outbreak. MedRxiv 2020;5:20098442. https://doi.org/10.1101/2020.05.11.20098442.

[2] Percivalle E, Cambiè G, Cassaniti I, Vecchio Nepita E, Maserati R, Ferrari A, et al. Prevalence of SARS-coV-2 specific neutralising antibodies in blood donors from the lodi Red zone in lombardy, Italy, as at 06 April 2020. Euro Surveill 2020;25:2001031.

[3] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 2020;370:m2516.

[4] Yassine HM, Al-Jighefee H, Al-Sadeq DW, Dargham SR, Younes SN, Shurrab F, et al. Performance evaluation of five ELISA kits for detecting anti-SARS-COV-2 IgG antibodies. Int J Infect Dis 2020;102:181–7.

[5] Centers for Disease Control and Prevention. Interim guidelines for COVID-19 Antibody testing in clinical and public health settings. 2020. Updated August 1, https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html.

[6] Wan WY, Lim SH, Seng EH. Cross-reaction of sera from COVID-19 patients with SARS-CoV assays. Ann Acad Med Singap 2020;49:523–6.