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SHORT COMMUNICATION

IgG seroprevalence of COVID-19 among people living with HIV or at high risk of HIV in south-west Germany: A seroprevalence study

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Abstract

Objectives: Seroprevalence studies of SARS-CoV-2 have shown that there is a high number of undiagnosed missing cases. Seroprevalence of SARS-CoV-2 in people living with HIV (PLWH) is lacking. Therefore, we conducted a prospective cross-sectional study to estimate the seroprevalence of SARS-CoV-2 among PLWH without known diagnosis of COVID-19 in the south-west of Germany.

Methods: Serological testing for SARS-CoV-2 immunoglobulin G (IgG) antibodies based on two assays was performed in PLWH who visited the outpatient HIV centre of two hospitals from April to June 2020. Additionally, patients had to answer questionnaires about possible COVID-19-related symptoms and predefined risk factors. Moreover, we tested 50 non-HIV-infected patients receiving post- or pre-exposure (PEP/PrEP) HIV prophylaxis.

Results: In all, 594 (488 male, 106 female) PLWH (median age 51 years) and 50 PEP/PrEP-users were included in the study. The estimated seroprevalence of the PLWH cohort was 1.85% (11/594), with 11 positive tested cases in the cohort. Among all patients, only five had COVID-19-related symptoms. One PCR-positive patient did not show any antibody response in repeatedly carried out tests. None of the patients was hospitalized due to COVID-19. Three PrEP users were tested positive. Three patients had been previously diagnosed with SARS-COV-2 infection before inclusion. The used questionnaire did not help to detect SARS-CoV-2 positive patients.

Conclusions: Despite the limitation of being only a snapshot in time because of the ongoing pandemic, to our knowledge this is the largest study so far on seroprevalence of SARS-CoV-2 in PLWH in Germany. Our study suggests that the seroprevalence of SARS-CoV-2 in PLWH is comparable to those previously

reported for parts of the general German population and that the questionnaire used here might not be the best tool to predict COVID-19 diagnosis.

KEYWORDS
antibody, COVID-19, HIV, SARS-CoV-2, seroprevalence

INTRODUCTION

In late 2019 a newly identified coronavirus (SARS-CoV-2) was first reported in Wuhan, Hubei Province, China [1] and since then has rapidly spread worldwide reaching pandemic level. SARS-CoV-2 causes the so-called coronavirus disease 2019 (COVID-2019) which is a potentially fatal illness able to affect multiple organ systems, mainly the respiratory tract [2]. There are certain risk factors that might lead to a severe course or fatality, such as preexisting cardiovascular disease, older age, overweight or male sex [2]. Evidence exists that virus transmission is possible via asymptomatic carriers [3]. It has been shown that there is a high proportion of seropositive individuals after oligosymptomatic or asymptomatic infection. Indeed, evidence is increasing that nucleic acid amplification tests (NATs) mostly by polymerase chain reaction (PCR) do not depict the true extent of the virus spread demanding for serological tests to calculate the infection fatality rate (IFR). Hence, seroprevalence studies can be useful to determine the proportion of individuals who have antibodies against SARS-CoV-2, and can be used to estimate the actual number of individuals who have been infected. Until now, several population-based studies addressing this issue to estimate the prevalence of SARS-CoV-2 infection in Europe have been published [4,5].

Nevertheless, despite the alert of physicians worldwide there is still missing data on the risk of COVID-19 in people living with HIV (PLWH) or how it affects PLWH. At the beginning of the pandemic it was reported that PLWH receiving tenofovir/emtricitabine might have a lower risk for COVID-19 and related hospitalization [6], even though recent published data showed no direct effect of antiretroviral therapy (ART) such as protease inhibitors on the course of COVID-19 [7]. However, there is an ongoing debate as to whether PLWH might be at an increased risk of severe COVID-19. Until now, only a few case series [8] or cohort studies [9] have been published addressing this issue. With our study we aimed to contribute to the emerging data regarding the prevalence of SARS-CoV-2/COVID-19 in PLWH using serological testing.

To date, there have been many different available serological tests on the market to measure antibodies against SARS-CoV-2 and previous data suggest interassay variations with a notable impact on the subsequent seroprevalence calculation [10]. Therefore, we used two different assays for the detection of immunoglobulin G (IgG) as a specific marker for previous exposure in PLWH in the south-west of Germany to estimate the SARS-CoV-2 seroprevalence in PLWH on ART. In addition, we tested individuals who received post- or pre-exposure (PEP/PrEP) HIV prophylaxis and who are at risk of acquiring HIV. Moreover, we used a COVID-19-specific questionnaire to assess the value of paper-based questionnaires for the diagnosis of COVID-19 in this specific setting.

PATIENTS AND METHODS

Patient population and sampling

We conducted a prospective cross-sectional study using serological testing for anti-SARS-CoV-2 antibodies to assess the seroprevalence of SARS-CoV-2 infection in PLWH in the south-west of Germany in two HIV centres. From 1 April to 30 June 2020, 594 PLWH were prospectively collected to participate in the study. The study was approved by the local ethics committee and in accordance with the Declaration of Helsinki. Data on demographics, laboratory results and ART were obtained from the electronic patient files. At each HIV centre, participants were asked by a trained physician to answer a series of questions about their demographic characteristics, ART intake, recent travel returning from COVID-19 high-incidence countries, recent COVID-19-related symptoms, COVID-19-related exposures and recent hospital stay. Specific COVID-19-related symptoms included diarrhoea, dyspnoea, cough, fever, myalgia, arthralgia, anosmia and ageusia experienced during the last 12 weeks before answering the questionnaire.

All participants signed a written informed consent to participate in the study and gave their written informed consent for publication.

SARS-CoV-2 antibody testing

We used two different assays for SARS-CoV2 antibody testing. First, we used a semiquantitative and automated

enzyme-linked immunosorbent assay (ELISA) using the Euroimmun (IgG: catalogue no. EI 2606–9601 G; Lübeck, Germany) assay to detect SARS-CoV-2-specific IgG antibodies targeting the spike protein (S1 domain). This classical ELISA uses a coated SARS-CoV-2 antigen that is incubated with samples and probed with enzyme-labelled anti-human IgG antibodies. Results are provided as optical density (OD), and a ratio of OD(sample)/OD(calibrator) was calculated. Samples with a ratio above a threshold of 1.1 were considered to be positive. Second, we used an assay based on the electrochemoluminescence immunoassay (ECLIA) technique for the qualitative detection of antibodies directed against the nucleocapsid protein (NCP) of SARS-CoV-2 (Elecsys Anti-SARS-CoV-2 assay; catalogue no. 09203095190, Roche Diagnostics GmbH, Frankfurt, Germany). Thus, in contrast to the Euroimmun assay, this one detects total immunoglobulin (Ig), not solely IgG antibodies, and was therefore called the Roche-Ig test. The Roche-Ig assay is based on a sandwich principle, as previously described [10]. Results are provided as a cut-off index (COI) and are considered as positive for values > 1.0. The sensitivity (Euroimmun-IgG, 85.25; Roche-Ig, 90.16) and specificity (Euroimmun-IgG, 98.23; Roche-Ig, 100.00) were tested using convalescent and potentially cross-reactive sera of donors with confirmed endemic coronavirus infection. The seroprevalence was calculated on results retrieved with the Euroimmun-IgG and Roche-Ig assays counting sera that were positive in both assays.

Statistical analysis

Demographic and disease characteristics were summarized using descriptive statistics. Continuous variables are presented as median and interquartile range (IQR). Categorical variables are expressed as number of patients (percentage). Comparisons were assessed using the median test for continuous variables, whereas categorical variables were assessed by the χ^2 test/ANOVA test. Statistical significance was defined as a two-sided p-value < 0.05. Bonferroni adjustment for multiple tests was used. All statistical analyses were performed with SPSS v.25.0 (IBM, Ehningen, Germany).

RESULTS

In total, 594 PLWH (488 male, 106 female) aged 18–81 years (median age 51 years) and 50 PEP/PrEP users (48 male, two female, median age 42 years) were prospectively included in this study at both HIV centres. Patient baseline characteristics are shown in Table 1. Most patients had an integrase inhibitor-based ART (n = 254; Table 2), three patients were treatment-naïve or received no ART at the time of testing (all three were male, aged 29, 31 and 39 years, median CD4 count 523 cells/μL, with viral loads of 31 600, 62 600 and 360 copies/mL, respectively). SARS-CoV-2-specific IgG antibodies were detected in 11 serum samples from the HIV cohort (10 in Koblenz, one in Saarland) resulting in a seroprevalence of 1.85% (Table 1). One symptomatic patient from the Koblenz HIV cohort who tested positive by PCR during the time period did not show a positive antibody reaction despite repeated control testing. One seropositive, symptomatic patient with close contact to a PCR-confirmed SARS-CoV-2-infected person tested negative by PCR during the corresponding period. Twenty-four patients reported prior contact to a person with PCR-positive confirmed COVID-19 disease. Four of these patients had SARS-CoV-2-specific IgG antibodies (two of them were symptomatic). Regarding the PrEP/PEP cohort, a total of three people tested positive for IgG (seroprevalence 6%), and all of them were male (shown in Table S1).

TABLE 1 Baseline characteristics of the whole study cohort

	Patient population		
	PLWH (n = 594) vs. PEP/PrEP users (n = 50)		p-value[a]
Age (years) [median (IQR)]	51 (16)	42 (19)	0.001
Male [n (%)]	488 (82.1)	48 (96)	0.012
SARS-CoV−2 positive, n (%)	11 (1.85)	3 (6)	0.087

Abbreviations: IQR, interquartile range; PEP, post-exposure HIV prophylaxis; PLWH, people living with HIV; PrEP, pre-exposure HIV prophylaxis.

[a]p-values are based on median test or Pearson χ^2 test (Fischer's test).

Regarding clinical symptoms, five had prior clinical COVID-19-associated symptoms (three in the HIV cohort; two in the PrEP/PEP cohort). All courses were mild, and none of the patients required hospitalization. Among all seropositive PLWH without prior PCR testing, no patient's clinical history alone or the questionnaire was able to provide an accurate diagnosis of previous SARS-CoV-2 infection. Those PLWH who tested positive had a median CD4 count of 578 cells/μL (range 149–1601), and the HIV-1 viral load was < 50 copies/mL in only one patient (shown in Table 2).

DISCUSSION

To our knowledge, we present the largest dataset of SARS-CoV-2 antibody prevalence among PLWH in

TABLE 2 Patient characteristics of people living with HIV (PLWH) according to SARS-CoV-2 serostatus

	PLWH		
	Serostatus (SARS-CoV-2)		
	Positive ($n = 11$)	Negative ($n = 583$)	p-value[a]
Age (years) [median (IQR)]	54 (9)	50 (16)	0.135
Male [n (%)]	9 (81.8)	479 (82.2)	1.0
CD4 T-cell count (cells/μL) [median (IQR)]	578 (522)	672 (455)	0.546
CD8 T-cell count (cells/μL) [median (IQR)]	959 (535)	824 (508)	0.539
Viral load			
< 50 copies/mL [n (%)]	1 (100)[b]	133 (85.8)	1.0
ART regime			
No ART [n (%)]	0 (0)	3 (0.5)	0.81
INSTI-based [n (%)]	6 (54.5)	248 (42.5)	0.42

Abbreviations: ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; IQR, interquartile range.

[a]p-values are based on median test or Pearson χ^2 test (Fischer's test).

[b]Missing values in 10 patients.

Germany. Based on the number of confirmed positive cases ($n = 11$), the estimated seroprevalence of SARS-CoV-2 infection in our cohort of PLWH was 1.85%. However, like other seroprevalence studies before, we are only able to provide information about previous exposure, because we were not able to measure neutralizing antibodies. Nevertheless, it is still not clear whether IgG levels detected in PLWH in our study are protective and how long such protection lasts. In addition, the correlation of the titre level of SARS-CoV-2 antibodies in recovered patients and the protection of secondary SARS-CoV-2 infection in PLWH remains unclear. Therefore, it is worth noting that IgG values might only serve as an indicator of whether an individual was infected but not as evidence for existing immunity. Besides, COVID-19 questionnaires do not seem to be a very useful tool to predict COVID-19 or SARS-CoV-2 infection in PLWH as we were not able to predict recent COVID-19 infection using specific questionnaires.

Test inaccuracies, in particular due to the low pre-test probability with low incidence in the observed period and non-symptom-oriented testing with consecutively lowered positive predictive value, must be taken into account here and have been reported previously [10]. Initial uncertainties about the clinical extent of the disease, which was still very unexplored during the observed period, made clinical diagnosis difficult.

However, our prevalence data should be evaluated in the context of the pandemic development in the second quarter of 2020: cumulative numbers of PCR-confirmed SARS-CoV-2 cases of 7.022 in Rhineland-Palatinate (172 cases per 100 000 population), and 2806 in Saarland (283 cases per 100 000 population) were reported by the Robert Koch Institute by 1 July 2020. [11] An interim evaluation of the SeBluCo study at the time of our analysis revealed a nationwide seroprevalence of 1.3% (data as of 30 June 2020) among healthy blood donors [12]. Hence, studies with special cohorts with a substantial age restriction such as blood donors may underestimate the seroprevalence. Indeed, in an unpublished representative German seroprevalence study that included elderly and nursing home residents, a higher age-adjusted IFR of 2.09% was calculated [13]. The lower seroprevalence of 1.85% in the HIV cohort might thus result from a lower number of elderly and nursing home residents in this cohort. However, the majority of PLWH affected by SARS-CoV-2 were virologically suppressed and immunologically competent with a median CD4 count of 578 cells/μL which might be an explanation for the mild COVID-19 course. Recent data showed that a more pronounced immunodeficiency (current CD4 count < 350 cells/μL) might be associated with an increased risk for severe course of COVID-19 in PLWH [8] and that a CD4 count < 200 cells/μL is also associated with an increased mortality risk [8]. The putative higher seroprevalence in the PrEP/PEP cohort (6%) is in line with previously published data, which also found a higher seroprevalence of SARS-CoV-2 in PrEP users [14]. This could be due to the higher community exposure to SARS-CoV-2 among PrEP users as there is evidence that PrEP users have a higher number of condomless sexual contacts and a high presence of sexually transmitted diseases [15]. Moreover, this risk behaviour might explain the higher seroprevalence of SARS-CoV-2 in PrEP users probably due to the possibility of faecal-oral transmission of SARS-CoV-2 [16].

At the start of the COVID-19 pandemic, researchers raised the question as to whether ART might have a protective role, as protease inhibitors were initially suggested as treatment candidates for SARS-CoV-2 infection [17]. However, darunavir showed no effect on prevention or protection against worsening respiratory function because of COVID-19 in PLWH [18]. In addition, lopinavir/ritonavir showed no benefit in hospitalized adult patients with severe COVID-19 either [7]. Moreover, nucleoside reverse transcriptase inhibitors plus nonnucleoside reverse transfer inhibitors did not prevent COVID-19 infection in PLWH in China [19]. Therefore, we do not

think that ART had a direct protective effect against SARS-CoV-2 infection in our analysed cohort.

However, our study does have some limitations. First, the low pre-test probability with low incidence in the observed period and non-symptom-oriented testing with consecutively lowered positive predictive value must be taken into account here. Discrepant results between the test methods used may be due, among other things, to the different antigens used. Indeed, previous data using different tests demonstrated a dependence of the seroprevalence on the individual assay and assay performance, underlining the Roche-Ig assay as a suitable assay with which to investigate seroprevalence [10,20].

Second, due to the ongoing dynamic of weekly increasing seroprevalence rates, our study is only a snapshot in time and reflects the circumstances of the time period in which it was done. However, seroprevalence data of PLWH will help to estimate exposure rates, especially in areas with low testing capacity for acute cases. Additionally, because of available vaccines with only a small number of PLWH included in vaccine studies, ongoing seroprevalence studies might help to provide information about the extent and duration of vaccine-induced herd immunity in PLWH.

CONTRIBUTIONS

DK-M, LK, SL, JR, VW, Stephan Stilgenbauer, Sigrun Smola, LT, MB, BL, JL and AR contributed to collection, review and/or analysis of the data; VL performed statistical analysis of the data; DKM and AR wrote the manuscript.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

ETHICS APPROVAL

Approval was obtained from the ethics committee of Ärztekammer des Saarlandes. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

Data are available on reasonable request from the author (dominic.kaddu@uks.eu).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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