The Journal of Infectious Diseases

MAJOR ARTICLE

Seroprofiling of Antibodies Against Endemic Human Coronaviruses and Severe Acute Respiratory Syndrome Coronavirus 2 in a Human Immunodeficiency Virus Cohort in Lesotho: Correlates of Antibody Response and Seropositivity

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Background. Serological data on endemic human coronaviruses (HCoVs) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in southern Africa are scarce. Here, we report on (1) endemic HCoV seasonality, (2) SARS-CoV-2 seroprevalence, and (3) correlates of SARS-CoV-2 seropositivity and strength of SARS-CoV-2 and endemic HCoV serological responses among adults living with human immunodeficiency virus (HIV).

Methods. Plasma samples were collected from February 2020 to July 2021 within an HIV cohort in Lesotho. We used the AntiBody CORonavirus Assay (ABCORA) multiplex immunoassay to measure antibody responses to endemic HCoV (OC43, HKU1, NL63, and 229E) and SARS-CoV-2 antigens.

Results. Results for 3173 samples from 1403 adults were included. Serological responses against endemic HCoVs increased over time and peaked in winter and spring. SARS-CoV-2 seropositivity reached >35% among samples collected in early 2021 and was associated with female sex, obesity, working outside the home, and recent tiredness or fever. Positive correlations were observed between the strength of response to endemic HCoVs and to SARS-CoV-2 and between older age or obesity and the immunoglobulin G response to SARS-CoV-2.

Conclusions. These results add to our understanding of the impact of biological, clinical, and social/behavioral factors on serological responses to coronaviruses in southern Africa.

Keywords. COVID-19; HIV; coronavirus; serology; southern Africa.

Serological analyses are a powerful tool to assess population susceptibility to infectious diseases, disease burden, and infection dynamics and risk factors [1]. Previous serological research has demonstrated that exposure to endemic human coronaviruses (HCoVs) can be protective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and improve the immunological response to SARS-CoV-2 upon infection [2]. However, data on serological cross-protection against SARS-CoV-2 in prepandemic samples from sub-Saharan Africa are conflicting [3–6].

In South Africa, SARS-CoV-2 seroprevalence increased from 13% in the third quarter of 2020% to 56% a year later; furthermore, serological analyses demonstrated vast underestimation of SARS-CoV-2 infections using cumulative incidence data of confirmed cases [7]. Comparable data on circulation of endemic HCoVs in southern Africa are scarce, although nonserological studies from specific, largely symptomatic subpopulations provide some indications of seasonality [8, 9].

Assessing potential correlates of SARS-CoV-2 seropositivity, a household survey in South Africa observed associations with

Received 15 February 2023; editorial decision 27 May 2023; accepted 27 May 2023; published online 1 June 2023

Presented in part: International Workshop on HIV and Hepatitis Observational Databases, 23–25 March 2023, Athens, Greece.

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The Journal of Infectious Diseases® 2023;228:1042–54

https://doi.org/10.1093/infdis/jiad197

age, female sex, elevated body mass index (BMI), respiratory symptoms, and lower socioeconomic status, among other factors [10]. Furthermore, both this and a separate study among pregnant women living with human immunodeficiency virus (HIV) in Mozambique observed lower SARS-CoV-2 seropositivity in people with HIV viremia [10, 11], likely owing to weaker immune response in this population [10].

Indeed, previous research has reported poorer immunological responses to SARS-CoV-2 among people living with HIV [12], but this population does not appear to have increased rates of SARs-CoV-2 infection [10, 12–14]. A large study in a South African population with a high burden of comorbid conditions demonstrated higher coronavirus disease 2019 (COVID-19) mortality rates among people living with HIV [15], though overall findings on associations of HIV with COVID-19 severity are divergent, with several studies suggesting that poorer COVID-19 outcomes may be linked to ongoing HIV viremia, low CD4 cell count, comorbid conditions, or social determinants of health rather than HIV itself [16].

To elucidate the seroepidemiology of HCoVs in the context of HIV in an understudied population in Lesotho, southern Africa, we used a previously described [2] multifactorial serological assay for seroprofiling of endemic HCoV and SARS-CoV-2 antibody responses in a longitudinal adult HIV cohort. Our study had 3 objectives. First, we describe the seasonality of endemic HCoVs and correlates of the immunological response to endemic HCoVs in this population. Second, we describe the seroprevalence of SARS-CoV-2 over time. Third, we elucidate factors associated with SARS-CoV-2 seropositivity and serological response, considering endemic HCoV serological, clinical (including HIV-related), demographic, behavioral/social, and symptomatic factors.

METHODS

Participants and Human Specimens

Plasma samples were collected within the Dolutegravir in Real Life in Lesotho (DO-REAL) cohort study [17, 18], which was nested within a larger HIV viral load monitoring cohort in Lesotho [19]. The present analyses include DO-REAL participants enrolled at Butha-Buthe Government Hospital for whom ≥1 blood plasma sample obtained between 10 February 2020 (the start of enrollment into DO-REAL) and 13 July 2021 was available. During this period, vaccination against SARS-CoV-2 was generally unavailable in this setting. In brief, all participants were in care for HIV and newly taking dolutegravir-based antiretroviral therapy (ART) after new ART initiation or transitioning from a previous ART regimen. Samples and participant data were collected on the day of starting dolutegravir-based ART, after 16 weeks, and according to routine schedules (annually if virally suppressed) thereafter. Through most of the study period, COVID-19 screening (temperature screening and questions on symptoms and recent travel) was conducted at the hospital gate, meaning that individuals did not have obvious symptoms at the time of phlebotomy.

Diagnostic Procedures

Samples previously stored at −80°C were heat inactivated for 30 minutes at 56°C and analyzed using the AntiBody CORonavirus Assay (ABCORA), version 5.4 [2]. In brief, ABCORA is a bead-based multiplex immunoassay measuring 12 SARS-CoV-2–specific antibody parameters, namely, 4 antigens (the spike [S] glyocoprotein subunits S1 and S2, the angiotensin-converting enzyme 2 receptor-binding domain in S1, and the nucleocapsid protein) across 3 immunoglobulin classes (immunoglobulin [Ig] G, IgA, IgM). In addition, antibodies against the S1 protein of HCoVs OC43, HKU1, NL63, and 229E are detected.

For each antibody parameter (ie, for each antibody class against each antigen), the strength of the readout is reported as a continuous logarithmic fold change over the empty bead control score of the median fluorescence intensity (left-censored at 0). ABCORA gives semiquantitative results and has shown high correlation with other quantitative commercial assays [2]. The ABCORA assay uses data from all 12 SARS-CoV-2–specific antibody parameters to generate a binary diagnosis of seropositivity, that is, of detectable prior SARS-CoV-2 infection, which we refer to as the ABCORA diagnosis [2].

Data Management and Statistical Analyses

Participant Characteristics

We describe demographic, clinical, and behavioral/social participant characteristics at the time of the first available sample. The CD4 cell nadir was defined as the CD4 cell count recorded at ART initiation (ie, the last measurement before ART initiation or the first measurement maximally 6 months after ART initiation). Public transport use refers to the past week; attending public gatherings of >5 people refers to the past 2 weeks. We report frequencies and percentages for categorical variables, and medians and interquartile ranges for continuous variables. Throughout analyses, we adjusted for 2 sets of variables: either age, sex, and BMI or for additional demographic, clinical, and behavioral/social variables (see Supplement 1.1).

Endemic HCoV Seasonality

We assessed the seasonality of antibody responses against endemic HCoVs using a periodic mixed-effect Tobit model accounting for left-censored data and repeated measurements among individuals (see formula in Supplement 1.2). The model was run for each immunoglobulin subtype and endemic HCoV antigen. For each individual, samples with a positive ABCORA diagnosis and any subsequent samples were excluded owing to known interdependencies between antibodies against endemic

HCoVs and SARS-CoV-2 [2]. Likelihood ratio tests were performed to test for seasonal periodic variation. We reported the calendar timing of peak responsiveness with 95% confidence interval (CI) for each immunoglobulin type/HCoV pair.

Correlates of Strength of Serological Response to Endemic HCoVs

Correlations between demographic, clinical, and social/behavioral factors with the strength of response to endemic HCoVs were determined within the above-mentioned Tobit model. We also assessed the effect of the dichotomized strength of response to each endemic HCoV at the first visit on prespecified symptoms self-reported at the second visit, using logistic regression with separate analyses for each symptom/HCoV pair. For this and all subsequent analyses using responses to endemic HCoV antigens as exposure variables, responses were dichotomized as high versus low if above versus below the median across all samples included in the respective analysis.

SARS-CoV-2 Seroprevalence Over Time

We reported the number of tests performed over time by sampling date, including SARS-CoV-2 seropositivity per ABCORA diagnosis.

Determinants of SARS-CoV-2 Seropositivity

We assessed the association of demographic, clinical, and behavioral/social factors with any SARS-CoV-2 seropositivity using a logistic regression model. In addition, we assessed the correlation of reporting having experienced prespecified symptoms within the last 2 weeks at a given visit with seropositivity at the same visit via mixed-effect logistic regression. Samples following the first positive serological result were removed from analysis. Analyses were performed separately for each symptom.

Among individuals whose first available sample was seronegative for SARS-CoV-2, we assessed the effect of the strength of response to each endemic HCoV (dichotomized as described above) in the first sample on SARS-CoV-2 seropositivity in the next available sample using logistic regression. A sensitivity analysis removing all samples within the top 20% in terms of likelihood for SARS-CoV-2 seropositivity (rather than only those testing seropositive) was conducted to address potential confounding in the case of recent SARS-CoV-2 infection at the time of the first sample, which we hypothesized may cause elevated measurements for endemic HCoV responses through cross-reactivity of the beginning anti–SARS-CoV-2 response while simultaneously yielding a false-seronegative result for SARS-CoV-2 if the anti–SARS-CoV-2 response is still below the ABCORA threshold (preventing exclusion from analysis). This could mask potential protective effects of stronger responses to endemic HCoVs against SARS-CoV-2 infection, which have been reported elsewhere [2]. Finally, we plotted the distributions and medians of the strength of endemic HCoV responses in the first samples for each individual among those whose subsequent sample was seronegative versus seropositive for SARS-CoV-2.

Correlates of Strength of Serological Response to SARS-CoV-2

We used Bayesian models to assess the correlation of demographic, clinical, and social/behavioral factors with the strength of the antibody response to SARS-CoV-2 considering the first seropositive sample of any individual. We used both a multivariate model summarizing the response of a given immunoglobulin subtype against all 4 SARS-CoV-2 antigens and a univariate model assessing each immunoglobulin subtype/SARS-CoV-2 antigen pair separately. Further information is provided in Supplement 1.3.

Finally, we assessed whether the strength of endemic HCoV responses (dichotomized as described above) measured at the first visit was associated with the strength of SARS-CoV-2 antibody responses at the subsequent visit among participants who were seronegative for SARS-CoV-2 at the first and seropositive at the second visit, using the aforementioned Bayesian models. On observing an association of sex and age with HCoV levels, we repeated these analyses with stratification by sex or age.

Statistical Tools Used

The cohort data set was prepared using Stata software, version 16.1. Subsequent analyses were performed with R software, version 4.2.1. The Bayesian analyses were done with Stan software, using the rstan package in R.

Consenting and Ethical Considerations

Both the DO-REAL cohort and the viral load monitoring cohort study in which it is nested were approved by the National Health Research Ethics Committee in Lesotho (no. ID134-2016). DO-REAL was registered with ClinicalTrials.gov on 23 January 2020 (NCT04238767). The study was conducted in accordance with the principles of the Declaration of Helsinki, and participants included in this analysis provided informed consent both for DO-REAL and for the further use of their samples.

RESULTS

Participant Characteristics

Results of 3173 samples from 1403 individuals were included in this analysis. Participant characteristics are shown in Table 1: 821 participants (59%) were female, the median age was 46 years (interquartile range, 37.5–55 years), and 1332 (95%) were already taking ART before providing the first included sample, with a median of 5.6 years (3.3–8.8) since ART initiation. The majority of participants, 1272 (91%), contributed ≥2

Table 1. Participant Characteristics at Time of First Sample

Characteristic	Participants, No. (%)[a] (N = 1403)
Sex	
Female	821 (59)
Male	582 (41)
Age group[b,c]	
<40 y	420 (31)
40–60 y	732 (54)
>60 y	193 (14)
Age, median (IQR), y[b,c]	46 (37.5–55)
BMI[b,d,e]	
<18.5	79 (6)
18.5–25	618 (44)
25–30	335 (24)
>30	367 (26)
WHO stage at ART initiation[f]	
1	598 (45)
2	410 (31)
3	294 (22)
4	18 (1)
CD4 cell count nadir, cells/μL[g]	
<200	422 (41)
200–500	458 (45)
>500	147 (14)
Time since HIV diagnosis, median (IQR), y[b,h]	6.7 (3.5–10.0)
Time since ART initiation, median (IQR), y[b]	5.6 (3.3–8.8)
Any previous VL >50 copies/mL[b,i]	407 (31)
VL >50 copies/mL at VL closest to first visit[j]	106 (8)
Work (at least partially) away from home[k]	523 (39)
Use of public transport[k]	422 (31)
Attended a gathering of >5 people[k]	486 (36)
Household size,[l]	
1 person	270 (20)
2–3 persons	500 (37)
>3 persons	586 (43)
No. of samples analyzed	
1	131 (9)
2	782 (56)
3	482 (34)
4	8 (1)
Any positive ABCORA diagnosis for SARS-CoV-2	291 (21)

Abbreviations: ABCORA, AntiBody CORonavirus Assay; ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VL, viral load; WHO, World Health Organization.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]At the time of first sample of the respective participant included in this analysis.

[c]Missing for 58 participants.

[d]Missing for 4 participants.

[e]Calculated as as weight in kilograms divided by height in meters squared.

[f]Missing for 83 participants.

[g]Missing for 376 participants.

[h]Missing for 86 participants.

[i]Missing for 85 participants.

[j]Missing for 14 participants.

[k]Missing for 61 participants.

[l]Missing for 47 participants.

samples to this analysis, and 291 (21%) had ≥1 sample that was seropositive for SARS-CoV-2.

Seasonality of Endemic HCoVs

Figure 1 shows the seasonality patterns of antibody responses to endemic HCoVs after adjustment for age, sex, and BMI. For all pairings of the 3 immunoglobulin classes with the 4 endemic HCoVs except anti-NL63 IgM, this model indicates statistically significant seasonal variations (anti-NL63 IgM, $P = .07$; all others, $P < .001$), with peaks in winter and spring (around August to November; Supplementary Figure 1). In addition, for all immunoglobulin and endemic HCoV combinations except anti-229E IgG ($P = .06$), the model indicated a statistically significant ($P < .001$ for each) increase over time. These patterns remained robust in sensitivity analyses without or with extended covariate adjustment (Supplementary Figures 1 and 2).

Correlates of Strength of Serological Response to Endemic HCoVs

The association of age, sex, and BMI with the strength of antibody response to endemic HCoVs is shown in Figure 2. Female (vs male) sex was mostly associated with a weaker antibody responses to endemic HCoVs. Older age (vs age <40 years) was associated with an increased response for some immunoglobulin type/HCoV pairs, especially for IgG against HKU1, NL63, and 229E. A corresponding analysis for additional variables is shown in Supplementary Figure 3. No robust correlation was observed between the strength of the endemic HCoV response and the symptoms reported for the previous 2 weeks at the respective visit (Supplementary Figure 4).

SARS-CoV-2 Seroprevalence Over Time

SARS-CoV-2 seroprevalence was <10% until October 2020 and then increased rapidly to >35% among samples collected in February 2021. Figure 3 shows the tests conducted, test outcomes, and seroprevalence over the study period.

Determinants of SARS-CoV-2 Seropositivity

In unadjusted analysis including demographic, clinical, and behavioral/social factors, female sex (vs male; odds ratio [OR], 2.4 [95% CI, 1.7–3.4]; unadjusted $P < .001$) and a BMI of 25–30 (vs 18.5–25 [calculated as weight in kilograms divided by height in meters squared]; OR, 1.8 [1.2–2.8]; unadjusted $P = .005$) or >30 (vs 18.5–25; OR, 3.0 [2.0–4.4]; unadjusted $P < .001$) increased the odds of SARS-CoV-2 seropositivity. In adjusted analysis, female sex (vs male; adjusted OR [aOR], 1.9 [95% CI, 1.2–2.9]; $P = .004$), a BMI >30 (vs 18.5–25; aOR, 2.3 [1.5–3.6]; $P < .001$), and working outside the home (vs not working or working from home; aOR, 1.5 [1.1–2.2]; $P = .03$) increased the odds of ever having a positive SARS-CoV-2 serological result

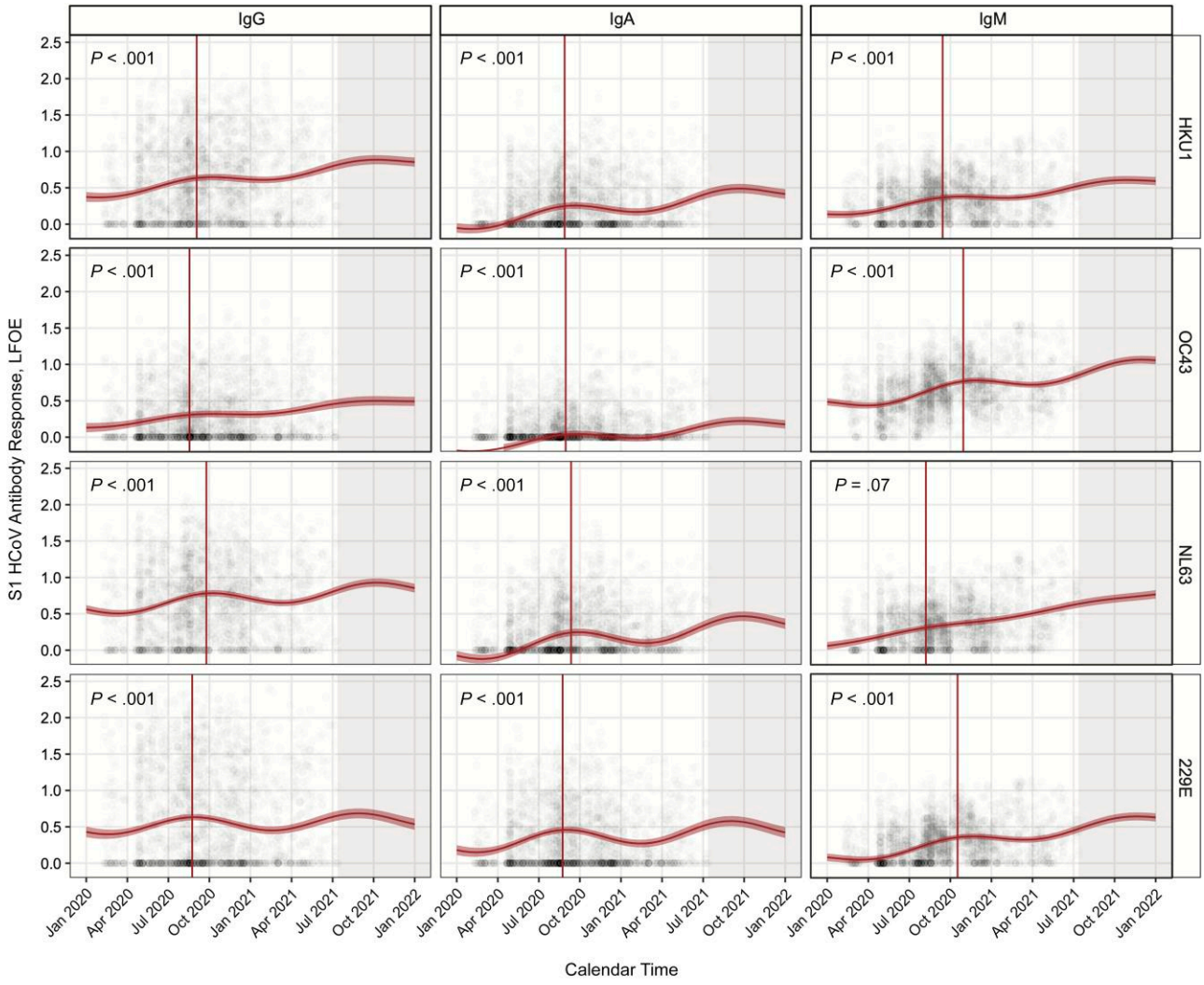

Figure 1. Seasonality of endemic human coronavirus (HCoV) seroprevalence (2364 samples from 1062 individuals). Antibody reactivity to HCoV antigen is depicted as logarithmic fold change over the empty bead control (LFOE). Results are adjusted for age, sex, and body mass index. Dark red lines along the x-axis indicate the estimated seasonality, with red ribbons indicating 95% confidence intervals for each immunoglobulin (Ig) type/HCoV pairing. Vertical red lines show the estimated peaks. *P* values refer to likelihood ratio tests assessing the statistical significance of adding a seasonality parameter, that is, of assuming periodic variation of LFOE. Abbreviation: S1, spike glycoprotein subunit 1.

(Figure 4; comparable outcomes after exclusion of commonly missing variables shown in Supplementary Figure 5).

In a separate analysis on the correlation between symptoms and seropositivity at a given visit, reporting tiredness (OR, 3.4 [95% CI, 1.3–8.6]; unadjusted $P = .01$; aOR, 4.1 [1.5–10.7]; $P = .005$) or fever (OR, 3.8 [1.2–12.1], unadjusted $P = .03$; aOR, 5.2 [1.6–17.1]; $P = .007$) during the 2 weeks preceding the visit were associated with SARS-CoV-2 seropositivity in both unadjusted and adjusted analysis (Figure 5 and Supplementary Figure 6).The strength of the serological response to endemic HCoVs did not appear to modulate the odds of SARS-CoV-2 infection; among individuals whose first available sample was seronegative for SARS-CoV-2, we did not observe any association between the strength of response to endemic HCoVs in the first sample with SARS-CoV-2 seropositivity of the subsequent sample (Supplementary Figure 7).

A sensitivity analysis to address potential masking of endemic HCoV protectiveness against SARS-CoV-2 infection by false-negative SARS-CoV-2 diagnoses for the first sample in the case of recent infection did not yield altered results (Supplementary Figure 8). Similarly, plotting the distribution of serological responses to each endemic HCoV within samples from the first visit did not indicate discernible differences between individuals whose subsequent sample was seronegative for SARS-CoV-2 and those whose subsequent sample was seropositive (Supplementary Figure 7).

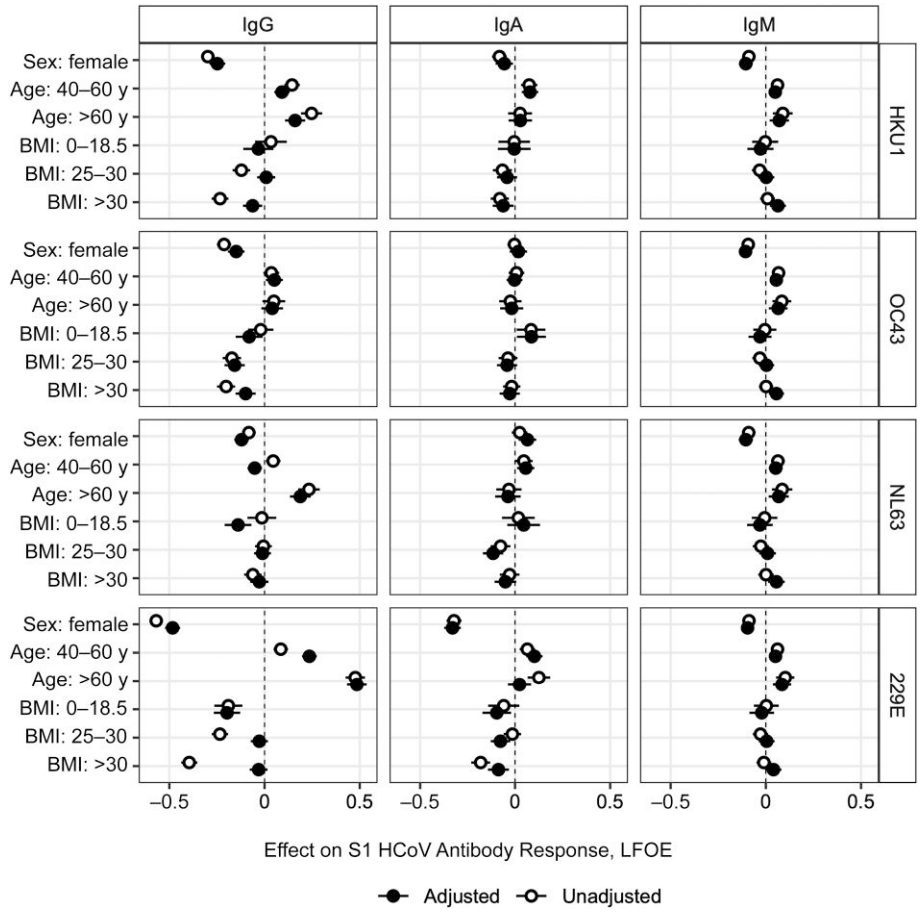

Figure 2. Correlation of sex, age, and body mass index (BMI) with serological response to endemic human coronaviruses (HCoVs) (2364 samples from 1062 individuals). Effect sizes with 95% confidence intervals are indicated. Correlations are assessed for sex (reference, male), age category (reference, age <40 years), and BMI category (reference, 18.5–25; BMI calculated as weight in kilograms divided by height in meters squared). Adjusted results consider age, sex, and BMI. Abbreviations: Ig, immunoglobulin; LFOE, logarithmic fold change over the empty bead control; S1, spike glycoprotein subunit 1.

Correlates of Strength of Serological Response to SARS-CoV-2

Among samples with a positive SARS-CoV-2 serology, BMIs ≥30 and age categories >40 years were associated with higher IgG responses to SARS-CoV-2 antigens in adjusted analysis, and female sex was minimally associated with lower IgM responses (Supplementary Figures 9–11). Stronger responses against several endemic HCoVs (specifically, higher response of IgG, IgA, or IgM to HKU1; IgM to OC43; and IgG or IgA to NL63) were each associated with stronger serological response to SARS-CoV-2 (Figures 6, Supplementary 12). This effect seems to be driven by female participants (Supplementary Figures 13 and 14), whereas we did not see any major differences in outcomes when stratifying by age (Supplementary Figures 15 and 16).

DISCUSSION

This longitudinal study in people living with HIV in Lesotho investigated antibody responses to endemic HCoVs in conjunction with anti–SARS-CoV-2 antibodies during the first 2 waves of the pandemic, aiming to determine the following: (1) endemic HCoV seasonality in southern Africa, with peaks observed in winter and spring; (2) SARS-CoV-2 seroprevalence in the first 1½ years of the pandemic, with peaks in seroprevalence in late 2020 and early 2021 mirroring major waves reported in southern Africa; (3) factors associated with SARS-CoV-2 seropositivity, notably identifying female sex and elevated BMI; and (4) factors associated with the strength of immunological response, observing a stronger response to SARS-CoV-2 with prior or stronger response to endemic HCoVs.

Prior data on HCoV seasonality in southern Africa are limited. Most previous studies focus on specific, symptomatic populations—notably young children with influenzalike illness [8, 9, 20, 21]—and few provide serological data. In the present analysis, antibody responses to endemic HCoVs appear to increase over time and peak in winter and spring. This largely corresponds to reported findings on endemic HCoV seasonality in temperate climates (such as Lesotho) [22] and matches

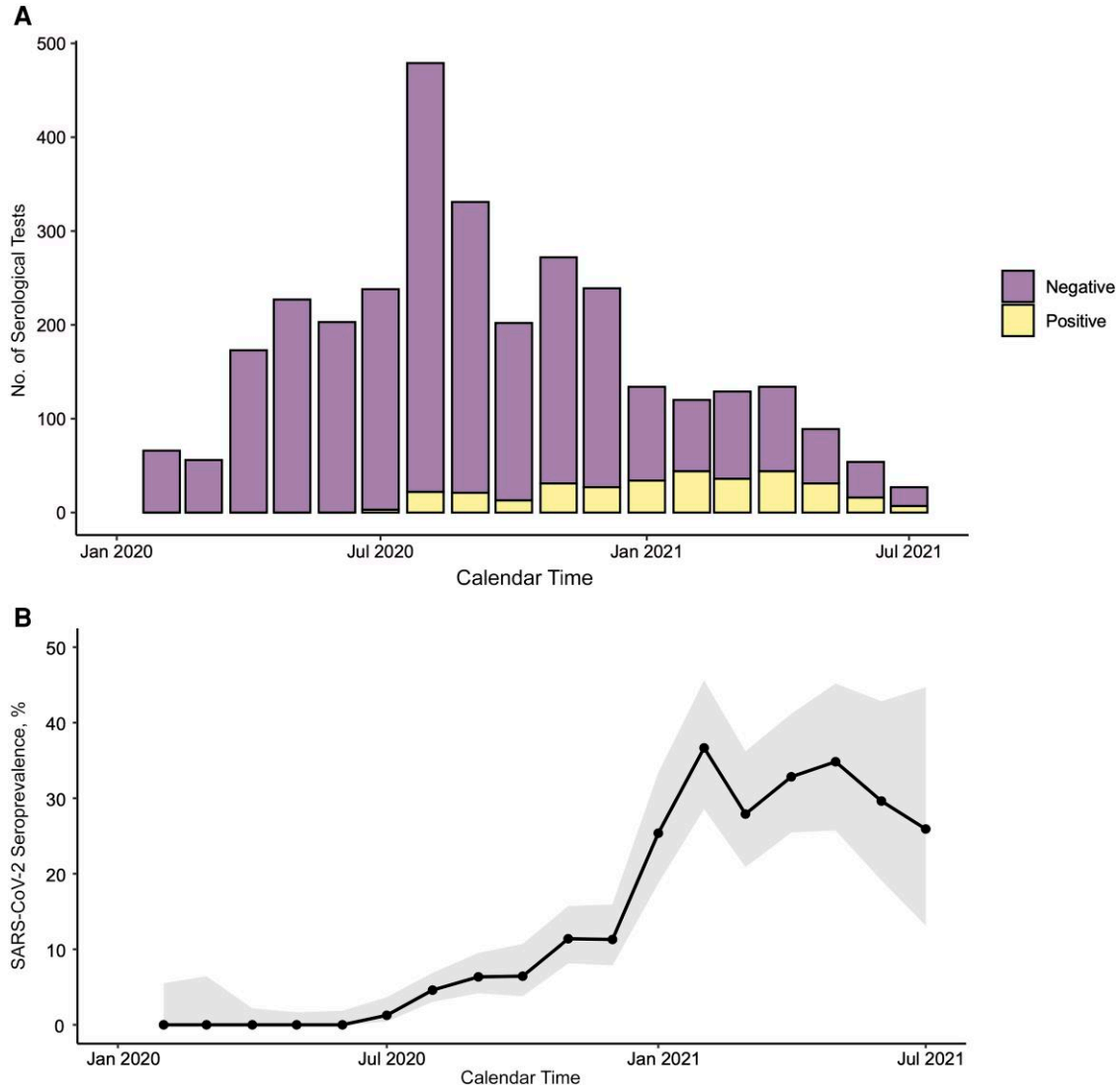

Figure 3. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence from February 2020 through July 2021 (3173 samples from 1403 individuals). *A,* Number of samples tested for SARS-CoV-2 seropositivity and AntiBody CORonavirus Assay (ABCORA) test outcome over time. *B,* SARS-CoV-2 seroprevalence among tested samples over time. Light gray area indicates the 95% confidence interval.

data from southern Africa for OC43 and HKU1, whereas peaks in warmer months have been reported for NL63 and 229E [8, 9]. For SARS-CoV-2, we observed a strong increase in seroprevalence in late 2020 and early 2021, reflecting a major wave in southern Africa and largely aligning with previous reports [7].

We observed associations of SARS-CoV-2 seropositivity with female sex, obesity, and working away from home, as well as with reports of recent tiredness or fever. Numerous studies have shown male sex [23–26] and obesity [27–29] to be associated with increased disease severity and mortality rate, whereas links to risk of SARS-CoV-2 infection are less clear. A combination of genetic, epigenetic, hormonal, and immunological sex differences alongside gender-associated social and behavioral factors may account for sex and gender imbalances relating to SARS-CoV-2, and these have been reviewed elsewhere [24–26]. However, despite divergent reports [10, 30], across Africa and globally there do not appear to be clear sex differences for risk of SARS-CoV-2 infection [7, 23]. We thus hypothesize that social and behavioral factors might underlie the higher risk of seroprevalence among female participants, though we are unable to further assess potential further confounders.

Similarly, multiple factors may link BMI to the risk of seropositivity. Despite numerous potential biological factors at play, overall obesity does not appear to be associated with a greater risk of infection or with broadly impaired antibody responses to SARS-CoV-2 [31]. Lower antibody persistence in association with obesity after vaccination has been described [32,

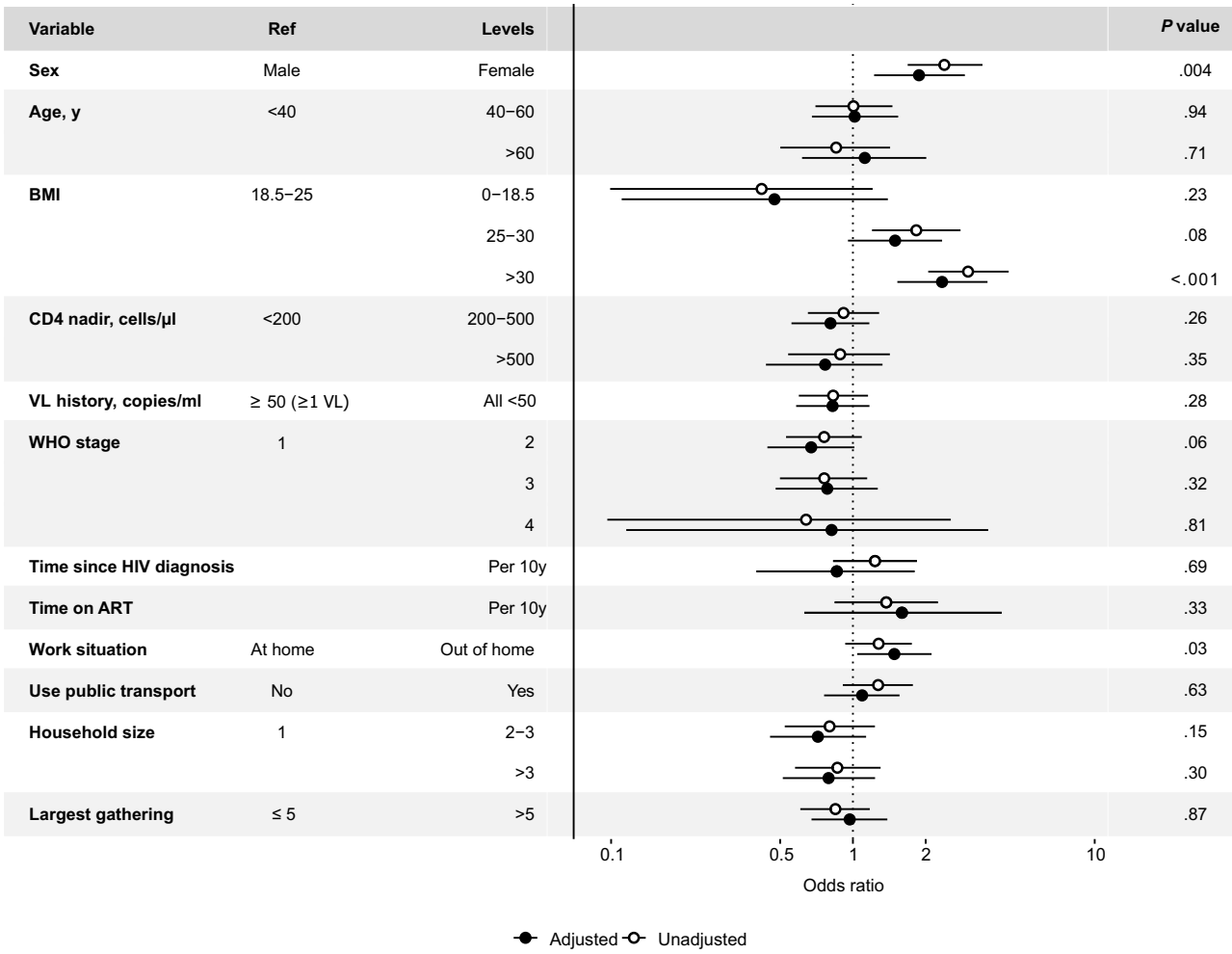

Figure 4. Association of demographic, clinical, and behavioral/social factors with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seropositivity (885 samples from 885 individuals). The association of demographic, clinical, and behavioral/social factors (assessed at the time of the first sample) with SARS-CoV-2 seropositivity across all included samples of the respective individual was assessed by logistic regression. Adjusted results use all included variables as covariates. *P* values refer to the adjusted analyses. Abbreviations: ART, antiretroviral therapy; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HIV, human immunodeficiency virus: VL, viral load; WHO, World Health Organization.

33], whereas reports on overall seropositivity or serological outcomes after infection are conflicting [10, 34–36], possibly owing to effects of greater disease severity in obese individuals [27–29], with subsequent more prominent antibody persistence [35]. Finally, underlying confounding factors such as socioeconomic status are likely to be relevant in our study setting. Overall, our findings are supported by a household survey in South Africa conducted during the second wave of the pandemic, in which female sex and elevated BMI were likewise associated with higher seropositivity [10].

We also observed stronger antibody responses to endemic HCoVs in male participants—and to a certain degree in older participants—and higher SARS-CoV-2 antibody responses, at least for some immunoglobulin type/antigen pairs with increased age, increased BMI, and male sex. Similar to our findings, stronger antibody responses to SARS-CoV-2 have previously been reported for male and older individuals, as well as for people with severe COVID-19 requiring hospitalization [37].

We observed no association of HIV-related clinical factors (including CD4 nadir, known past viremia, World Health Organization stage at ART initiation, or time since HIV diagnosis or ART initiation) with SARS-CoV-2 seropositivity. Previous studies have, perhaps surprisingly, reported similar [13] or even lower [10, 12, 14] SARS-CoV-2 incidence, assessed either by test positivity [13, 14] or seroprevalence [10–12], among people living with HIV and/or people with unsuppressed HIV. Observations of lower incidence may be related to behavioral factors, including greater cautiousness [12, 14], or, in the case of seropositivity, to weaker serological response on SARS-CoV-2 infection and subsequent negative serological results despite prior infection among people living with HIV or with unsuppressed HIV [10–12]. A study in the United States

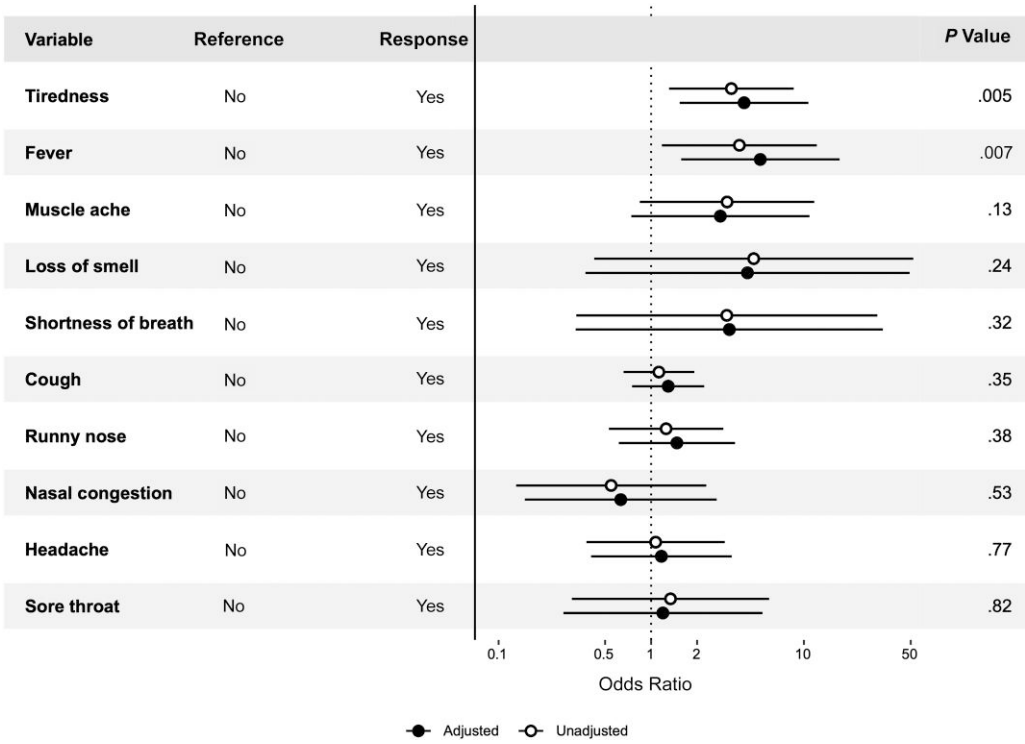

Figure 5. Association of self-reported recent symptoms with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seropositivity (3037 samples from 1345 individuals). The association of symptoms reported for the past 2 weeks at a given visit with SARS-CoV-2 seropositivity at the respective visit (removing samples taken after a positive AntiBody CORonavirus Assay (ABCORA) diagnosis) was assessed using a mixed-effect logistic regression. Separate analyses were conducted for each symptom, using all symptom responses, as well as sex, age category, and body mass index category as covariates for the adjusted odds ratio. *P* values refer to the adjusted analyses.

did not observe any association between CD4 cell counts <200/μL or unsuppressed viral loads and SARS-CoV-2 seropositivity [12], whereas low CD4 counts have been associated with adverse COVID-19 outcomes [12, 14, 15]. While behavioral considerations differ between infection and vaccine studies, the latter also provide relevant information on serological responses to SARS-CoV-2 among people living with HIV. Several studies have shown adequate response to vaccination for people living with HIV [38–43]; however, there is evidence for poorer outcomes with HIV, especially in conjunction with low CD4 cell count or viremia [39, 44–47].

As reported elsewhere [2], we found that stronger serological responses to endemic HCoVs were correlated with stronger serological response to SARS-CoV-2. However, we did not observe a protective effect of stronger serological response to endemic HCoVs against SARS-CoV-2 infection, as observed in a Swiss population–based study [2]. This discrepancy may be due to differences in the regional endemic HCoV waves, as the type and timing of endemic HCoV exposure close to SARS-CoV-2 encounter may shape protective effects. Notably, the protection conferred by cross-reacting endemic HCoV immunity is modest and may result in asymptomatic disease courses [2]. It is therefore important to note that the present study relied on retrospective serological testing for SARS-CoV-2 positivity. Polymerase chain reaction testing as performed in the Swiss study [2] has a higher chance of detecting asymptomatic infections, which often induces less pronounced antibody responses that wane more rapidly [48, 49]. Detection of SARS-CoV-2 infection in the present study may thus have been biased toward detecting more severe disease linked with more profound and sustained antibody responses.

This study has several limitations. No polymerase chain reaction or antigen testing was available to complement serological data. This means that factors decreasing the serological response might lead to false-negative inferences on infection, as it was not possible within the study to differentiate between the absence of prior infection and prior infection with a negative ABCORA diagnosis. Furthermore, the timing of past infection was not known and could thus not be included in models, and endemic HCoV responses were not measured at the time of SARS-CoV-2 exposure or infection. True prepandemic samples were not available, though sample collection began before the first case of COVID-19 was recorded in Lesotho or neighboring South Africa. As with any observational research, potential unmeasured confounding factors cannot be fully accounted for. Finally, all participants were living with HIV, meaning that we could not assess the impact of HIV infection on serological outcomes.

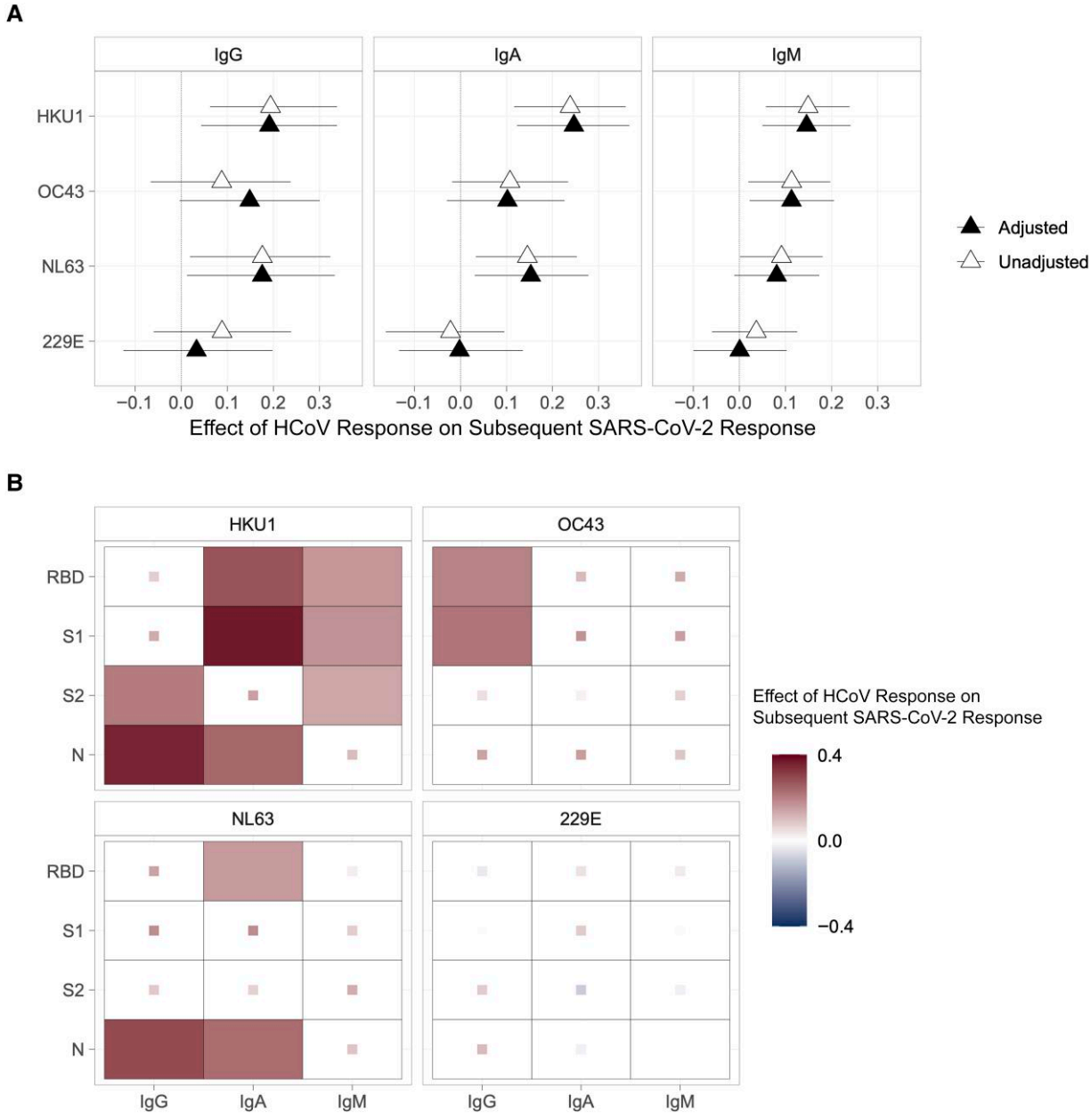

Figure 6. Association of strength of response to endemic human coronaviruses (HCoVs) at 1 visit with strength of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) response at the subsequent visit (120 samples from 120 individuals). Sample pairs were included if they tested negative for SARS-CoV-2 at the first and positive at the subsequent visit. Adjustment was made for age, sex, and body mass index. *A,* Multivariate Bayesian model. *B,* Univariate analysis with coloring indicating the effect on the antibody response to SARS-CoV-2 and full colored blocks indicating when the 95% credibility interval does not cross 0. Abbreviations: Ig, immunoglobulin; N, nucleocapsid; RBD, receptor-binding domain; S1 and S2, spike glycoprotein subunits 1 and 2.

In summary, sex, age, and BMI were correlated with several serological outcomes relating to endemic HCoVs and SARS-CoV-2 among adults living with HIV in Lesotho, with positive associations between responses to endemic HCoVs and SARS-CoV-2. These findings add to our understanding of the roles of biological, clinical, and social/behavioral factors in the epidemiology of and serological responses to coronaviruses in southern Africa.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank all contributors to the Dolutegravir in Real Life in Lesotho (DO-REAL) study as well as the staff at Butha-Buthe Government Hospital and SolidarMed in Lesotho. We gratefully acknowledge the study participants.

Author contributions. N. D. L. conceptualized the DO-REAL cohort, and J. A. B. and N. D. L. were centrally involved in its design. B. L. N. managed the cohort on site and oversaw on-site procedures, including consenting, enrollment, data collection, and sample collection. J. A. B. led the cohort off site, managed cohort data, and prepared the data set for this study. H. F. G. initiated this collaborative project between DO-REAL and the University of Zurich. I. A. A. led the development of the previously published ABCORA assay, with S. E. and A. T. providing key support. S. E. and T. M. conducted the assays for this study, and I. A. A. provided guidance. C. P. developed the bioinformatic analyses of ABCORA. A. H. performed all analyses, with C. P. and R. D. K. providing input. J. A. B., A. H., I. A. A., C. P., A. T., N. D. L., R. D. K., and H. F. G. regularly reviewed and interpreted ongoing data analyses. J. A. B. wrote the first draft of the manuscript. All authors contributed to and reviewed the manuscript.

Financial support. This work was supported by the Swiss National Science Foundation (grants PCEFP3_181355 to N. D. L. and 196906 to A. T., R. D. K., and H. F. G.); Pandemiefonds, University of Zurich Foundation (grant to A. T.); the Gilead COVID-19 RFP Research Program COMMIT (grant IN-SW-983-6078 to A. T., R. D. K., and H. F. G.); and the Promedica Foundation (research grant to I. A. A.).

Potential conflicts of interest. J. A. B. has received funds from the University of Basel Research Fund. I. A. A. has received honoraria from MSD and Sanofi, a travel grant from Gilead Sciences, and a grant from Promedica Foundation. A. T. has received honoraria from Roche Diagnostics for consultant activity; grants from the Swiss National Science Foundation (SNSF), the Swiss HIV Cohort Study, Pandemiefonds, University of Zurich Foundation; and unrestricted research grants from Gilead Sciences. N. D. L. reports receiving travel grants to attend scientific conferences from Gilead Sciences and grants from SNSF, Fondation Botnar, the Botnar Center for Child Health, the Swiss Agency for Development and Cooperation, and Moritz Straus Stiftung. R. D. K. has received grants from SNSF, the National Institutes of Health, and Gilead Sciences. H. F. G. reports having received honoraria from Gilead Sciences, Merck, ViiV, GSK, Janssen, Johnson and Johnson, and Novartis for serving on data and safety monitoring boards and/or advisory boards and has received a travel grant from Gilead Sciences. In addition, he has received grants from SNSF, the Swiss HIV Cohort Study, the Yvonne Jacob Foundation, and the National Institutes of Health and unrestricted research grants from Gilead Sciences, all paid to the institution. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Metcalf CJE, Farrar J, Cutts FT, et al. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. Lancet **2016**; 388:728–30.
2. Abela IA, Pasin C, Schwarzmüller M, et al. Multifactorial seroprofiling dissects the contribution of pre-existing human coronaviruses responses to SARS-CoV-2 immunity. Nat Commun **2021**; 12:6703.
3. Tso FY, Lidenge SJ, Peña PB, et al. High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in sub-Saharan Africa. Int J Infect Dis **2021**; 102:577–83.
4. Zar HJ, Nicol MP, MacGinty R, et al. Antibodies to seasonal coronaviruses rarely cross-react with SARS-CoV-2. Pediatr Infect Dis J **2021**; 40:e516–9.
5. Li Y, Merbah M, Wollen-Roberts S, et al. Coronavirus antibody responses before COVID-19 pandemic, Africa and Thailand. Emerg Infect Dis **2022**; 28:2214–25.
6. Stoddard CI, Sung K, Ojee E, et al. Distinct antibody responses to endemic coronaviruses pre- and post-SARS-CoV-2 infection in Kenyan infants and mothers. Viruses **2022**; 14:1517.
7. Lewis HC, Ware H, Whelan M, et al. SARS-CoV-2 infection in Africa: a systematic review and meta-analysis of standardised seroprevalence studies, from January 2020 to December 2021. BMJ Glob Health **2022**; 7:e008793.
8. Baillie VL, Moore DP, Mathunjwa A, et al. Epidemiology and seasonality of endemic human coronaviruses in South African and Zambian children: a case-control pneumonia study. Viruses **2021**; 13:1513.
9. Venter M, Lassaunière R, Kresfelder TL, Westerberg Y, Visser A. Contribution of common and recently described respiratory viruses to annual hospitalizations in children in South Africa. J Med Virol **2011**; 83:1458–68.
10. Wolter N, Tempia S, von Gottberg A, et al. Seroprevalence of SARS-CoV-2 after the second wave in South Africa in HIV-infected and uninfected persons: a cross-sectional household survey. Clin Infect Dis **2022**; 75:e57–68.
11. González R, Nhampossa T, Figueroa-Romero A, et al. SARS-CoV-2 seropositivity and HIV viral load among

Mozambican pregnant women. J Acquir Immune Defic Syndr **2023**; 92:115–21.
12. Spinelli MA, Lynch KL, Yun C, et al. SARS-CoV-2 seroprevalence, and IgG concentration and pseudovirus neutralising antibody titres after infection, compared by HIV status: a matched case-control observational study. Lancet HIV **2021**; 8:e334–41.
13. Park LS, McGinnis KA, Gordon KS, et al. SARS-CoV-2 testing and positivity among persons with and without HIV in 6 US cohorts. J Acquir Immune Defic Syndr **2022**; 90:249–55.
14. Spinelli MA, Brown LB, Glidden DV, et al. SARS-CoV-2 incidence, testing rates, and severe COVID-19 outcomes among people with and without HIV. AIDS **2021**; 35: 2545–7.
15. Western Cape Department of Health in collaboration with the National Institute for Communicable Diseases, South Africa. Risk factors for coronavirus disease 2019 (COVID-19) death in a population cohort study from the Western Cape Province, South Africa. Clin Infect Dis **2021**; 73:e2005–15.
16. Brown LB, Spinelli MA, Gandhi M. The interplay between HIV and COVID-19: summary of the data and responses to date. Curr Opin HIV AIDS **2021**; 16:63–73.
17. Brown JA, Nsakala BL, Mokhele K, et al. Viral suppression after transition from nonnucleoside reverse transcriptase inhibitor- to dolutegravir-based antiretroviral therapy: a prospective cohort study in Lesotho (DO-REAL study). HIV Med **2022**; 23:287–93.
18. Brown JA, Nsakala BL, Mokhele K, et al. Dolutegravir in real life: self-reported mental and physical health outcomes after transitioning from efavirenz- to dolutegravir-based antiretroviral therapy in a prospective cohort study in Lesotho. HIV Med **2023**; 24:153–62.
19. Glass TR, Motaboli L, Nsakala B, et al. The viral load monitoring cascade in a resource-limited setting: a prospective multicentre cohort study after introduction of routine viral load monitoring in rural Lesotho. PLoS One **2019**; 14: e0220337.
20. Nicol MP, MacGinty R, Workman L, et al. A longitudinal study of the epidemiology of seasonal coronaviruses in an African birth cohort. J Pediatric Infect Dis Soc **2021**; 10: 607–14.
21. Berkley JA, Munywoki P, Ngama M, et al. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA **2010**; 303:2051–7.
22. Berry M, Gamieldien J, Fielding BC. Identification of new respiratory viruses in the new millennium. Viruses **2015**; 7: 996–1019.
23. Peckham H, de Gruijter NM, Raine C, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. Nat Commun **2020**; 11:6317.
24. Ursin RL, Klein SL. Sex differences in respiratory viral pathogenesis and treatments. Annu Rev Virol **2021**; 8: 393–414.
25. Haitao T, Vermunt JV, Abeykoon J, et al. COVID-19 and sex differences: mechanisms and biomarkers. Mayo Clin Proc **2020**; 95:2189–203.
26. Mukherjee S, Pahan K. Is COVID-19 gender-sensitive? J Neuroimmune Pharmacol **2021**; 16:38–47.
27. Mahamat-Saleh Y, Fiolet T, Rebeaud ME, et al. Diabetes, hypertension, body mass index, smoking and COVID-19-related mortality: a systematic review and meta-analysis of observational studies. BMJ Open **2021**; 11:e052777.
28. Silverio R, Gonçalves DC, Andrade MF, Seelaender M. Coronavirus disease 2019 (COVID-19) and nutritional status: the missing link? Adv Nutr **2020**; 12:682–92.
29. Hendren NS, de Lemos JA, Ayers C, et al. Association of body mass index and age with morbidity and mortality in patients hospitalized with COVID-19: results from the American Heart Association COVID-19 cardiovascular disease registry. Circulation **2021**; 143:135–44.
30. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis **2020**; 20:1034–42.
31. Drucker DJ. Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. Cell Metab **2021**; 33:479–98.
32. Nasr M-JC, Geerling E, Pinto AK. Impact of obesity on vaccination to SARS-CoV-2. Front Endocrinol (Lausanne) **2022**; 13:898810.
33. Malavazos AE, Basilico S, Iacobellis G, et al. Antibody responses to BNT162b2 mRNA vaccine: infection-naïve individuals with abdominal obesity warrant attention. Obesity (Silver Spring) **2022**; 30:606–13.
34. Dobaño C, Ramírez-Morros A, Alonso S, et al. Sustained seropositivity up to 20.5 months after COVID-19. BMC Medicine **2022**; 20:379.
35. Karachaliou M, Moncunill G, Espinosa A, et al. Infection induced SARS-CoV-2 seroprevalence and heterogeneity of antibody responses in a general population cohort study in Catalonia Spain. Sci Rep **2021**; 11:21571.
36. Nilles EJ, Siddiqui SM, Fischinger S, et al. Epidemiological and immunological features of obesity and SARS-CoV-2. Viruses **2021**; 13:2235.
37. Klein SL, Pekosz A, Park H-S, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. J Clin Invest **2020**; 130: 6141–50.
38. Lombardi A, Butta GM, Donnici L, et al. Anti-spike antibodies and neutralising antibody activity in people living

with HIV vaccinated with COVID-19 mRNA-1273 vaccine: a prospective single-centre cohort study. Lancet Reg Health Eur **2021**; 13:100287.
39. Nault L, Marchitto L, Goyette G, et al. Covid-19 vaccine immunogenicity in people living with HIV-1. Vaccine **2022**; 40:3633–7.
40. Levy I, Wieder-Finesod A, Litchevsky V, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in people living with HIV-1. Clin Microbiol Infect **2021**; 27:1851–5.
41. Brumme ZL, Mwimanzi F, Lapointe HR, et al. Humoral immune responses to COVID-19 vaccination in people living with HIV receiving suppressive antiretroviral therapy. NPJ Vaccines **2022**; 7:28.
42. Speich B, Chammartin F, Abela IA, et al. Antibody response in immunocompromised patients after the administration of SARS-CoV-2 vaccine BNT162b2 or mRNA-1273: a randomised controlled trial. Clin Infect Dis. **2022**;75:e585–93.
43. Madhi SA, Koen AL, Izu A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in people living with and without HIV in South Africa: an interim analysis of a randomised, double-blind, placebo-controlled, phase 1B/2A trial. Lancet HIV **2021**; 8:e568–80.
44. Jedicke N, Stankov MV, Cossmann A, et al. Humoral immune response following prime and boost BNT162b2 vaccination in people living with HIV on antiretroviral therapy. HIV Med **2022**; 23:558–63.
45. Hassold N, Brichler S, Ouedraogo E, et al. Impaired antibody response to COVID-19 vaccination in advanced HIV infection. AIDS **2022**; 36:F1–5.
46. Chammartin F, Kusejko K, Pasin C, et al. Determinants of antibody response to severe acute respiratory syndrome coronavirus 2 mRNA vaccines in people with HIV. AIDS **2022**; 36:1465–8.
47. Hensley KS, Jongkees MJ, Geers D, et al. Immunogenicity and reactogenicity of SARS-CoV-2 vaccines in people living with HIV in the Netherlands: a nationwide prospective cohort study. PLoS Med **2022**; 19: e1003979.
48. Lei Q, Li Y, Hou H, et al. Antibody dynamics to SARS-CoV-2 in asymptomatic COVID-19 infections. Allergy **2021**; 76:551–61.
49. Yang Y, Yang M, Peng Y, et al. Longitudinal analysis of antibody dynamics in COVID-19 convalescents reveals neutralizing responses up to 16 months after infection. Nat Microbiol. **2022**; 7:423–33.