The Journal of Infectious Diseases

MAJOR ARTICLE

Deciphering factors linked with reduced SARS-cov-2 susceptibility in the Swiss HIV Cohort Study

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DOI: 10.1093/infdis/jiae002

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Background Factors influencing susceptibility to SARS-CoV-2 remain to be resolved. Using data of the Swiss HIV Cohort Study (SHCS) on 6,270 people with HIV (PWH) and serologic assessment for SARS-CoV-2 and circulating-human-coronavirus (HCoV) antibodies, we investigated the association of HIV-related and general parameters with SARS-CoV-2 infection.

Methods We analyzed SARS-CoV-2 PCR-tests, COVID-19 related hospitalizations, and deaths reported to the SHCS between January 1, 2020 and December 31, 2021. Antibodies to SARS-CoV-2 and HCoVs were determined in pre-pandemic (2019) and pandemic (2020) bio-banked plasma and compared to HIV-negative individuals. We applied logistic regression, conditional logistic regression, and Bayesian multivariate regression to identify determinants of SARS-CoV-2 infection and Ab responses to SARS-CoV-2 in PWH.

Results No HIV-1-related factors were associated with SARS-CoV-2 acquisition. High pre-pandemic HCoV antibodies were associated with a lower risk of subsequent SARS-CoV-2 infection and with higher SARS-CoV-2 antibody responses upon infection. We observed a robust protective effect of smoking on SARS-CoV-2-infection risk (aOR= 0.46 [0.38,0.56], $p=2.6*10^{-14}$), which occurred even in previous smokers, and was highest for heavy smokers.

Conclusions Our findings of two independent protective factors, smoking and HCoV antibodies, both affecting the respiratory environment, underscore the importance of the local immune milieu in regulating susceptibility to SARS-CoV-2.

Keywords: SARS-CoV-2, HIV, PWH, pre-existing immunity, endemic human coronaviruses, smoking

INTRODUCTION

Identifying factors that influence the acquisition, transmission, severity, prevention, and treatment of SARS-CoV-2 infection relies on comprehensive sources of health, behavioral, epidemiologic and socioeconomic data. Prospective, large-scale, longitudinal cohort studies can provide this type of data enabling critical public health research [1-3]. HIV cohorts provide a unique opportunity in resolving interactions between SARS-CoV-2, HIV-1 and other comorbidities [4-6]. COVID-19 seriously affects particularly vulnerable populations, including people with advanced age, comorbidities, or immunosuppression [7-9]. Accordingly, HIV-1 has been identified as a potential risk factor that may exacerbate COVID-19 disease, especially if HIV-1 infection is not effectively treated [10-13]. Immune responses to infections and vaccines often remain reduced in people with HIV (PWH) even under suppressive antiretroviral treatment (ART) [14-16]. This may also affect

the course of SARS-CoV-2 infection in PWH, since decreased antibody responses upon SARS-CoV-2 infection have been linked with slower clearance of SARS-CoV-2 [17]. Understanding whether and how HIV-1 positivity affects SARS-CoV-2 susceptibility and outcome is therefore important.

Here we report on a comprehensive systematic investigation of the interactions of HIV-1 and SARS-CoV-2 infection within the Swiss HIV Cohort Study (SHCS) [3] during the initial waves of SARS-CoV-2 epidemic in Switzerland. The SHCS is highly representative of PWH living in Switzerland [3]. With its longitudinal study design, plasma and blood cell biobank, and comprehensive demographic data, including HIV-related as well as general health, behavioral, and epidemiology parameters, the SHCS provides a large data resource with opportunities to address public health concerns beyond HIV infection [18, 19]. While residual immune suppression in antiretroviral treated PWH is a potential concern, most SHCS participants are fully virologically suppressed, comparatively healthy and immunologically stable [3]. Accordingly, investigation of determinants of SARS-CoV-2 infection within the SHCS may also provide insights relevant for the general population. In support, prevalence of SARS-CoV-2 infections in PWH has been found to be similar to the general population [20-23], and virologically suppressed PWH respond well to SARS-CoV-2 vaccines [24, 25].

Here, we explore the epidemiology of SARS-CoV-2 waves in Switzerland from 2020-2021 amongst PWH compared to the general Swiss population and determine parameters associated with SARS-CoV-2 infection in both populations.

METHODS

Study population and ethics

Study population with HIV: The SHCS is a prospective PWH cohort with semi-annual visits and blood collections [3]. Detailed information is available on http://www.shcs.ch. The SHCS is registered under the Swiss National Science longitudinal platform [26] and has been approved by the ethics committee of the participating institutions (1.1 Supplementary Material). Written informed consent had been obtained from all participants. In March 2020, a SARS-CoV-2 questionnaire was introduced into the SHCS, resulting in 7'073 assessments of whether participants had a PCR-test for SARS-CoV-2, whether that test was positive, and whether participants were hospitalized for COVID-19. See Flowchart Figure S1.

Study population without HIV: We included a population of 382 HIV uninfected individuals (HU) with PCR-confirmed SARS-CoV-2 infections from two prior SARS-CoV-2 studies [17, 27] with published ABCORA serology data (1.2 Supplementary Material, Table S1). Published epidemiology data from the Swiss Federal Office of Public Health (FOPH) on the number of

SARS-CoV-2 cases, hospitalizations, and deaths were used to compare these outcomes between PWH and the general Swiss population [28].

SARS-cov-2 serology

SARS-CoV-2 serology was performed on biobanked SHCS plasma samples with the ABCORA 5 assay [27]. ABCORA 5 determines antibody binding for each of the three Ig classes IgA, IgG and IgM as the median fluorescence intensity (MFI) against four SARS-CoV-2 antigens (S1 subunit, S2 subunit, NP, RBD) and the S1 subunits of the four endemic human coronaviruses (HCoVs) (HCoV-OC43, HCoV-NL63, HCoV-HKU1, HCoV-229E). MFI values were normalized relative to empty bead controls yielding binding strengths quantified as MFI-log-fold-over-empty (MFI-LFOE). We used the previously calibrated random-forest-based ABCORA 5.4 algorithm to infer SARS-CoV-2 seropositivity based on the SARS-CoV-2 MFI-LFOE values.

Hcovs testing

Routine diagnostic analyses of respiratory samples for the endemic human coronaviruses (HCoVs) (HCoV-OC43, HCoV-NL63, HCoV-HKU1, HCoV-229E) were performed with multiplex respiratory PCR panels (ePlex RP, Roche or BioFire RP2.1, BioMérieux). Repetitive tests of the same patient within 20 days of the initial positive result were excluded.

Assessing the impact of pre-pandemic hcov response in a matched PWH case-control study

We conducted a 1:2 matched nested case-control study in selected PWH from the SHCS to assess the impact of prior existing HCoV-specific antibodies on the risk of SARS-CoV-2 infection. For this we retrieved pre-pandemic plasma samples for all SARS-CoV-2 infected cases and matched controls from 2019 from the SHCS biobank and measured their HCoV antibody levels by ABCORA 5 (Figure S2AB).

Statistical analysis

To assess risk factors of acquiring SARS-CoV-2, we used logistic regression models with SARS-CoV-2 infection as an outcome (i.e., having either a positive PCR test or a positive ABCORA serology) and included a wide range of demographic, behavioral and disease variables documented in the SHCS database as explanatory variables (Figure 1, Table S2 and 1.6 Supplementary Material).

To assess the impact of pre-pandemic HCoV immunity on the risk of SARS-CoV-2 infection, we conducted a time-updated survival analysis [29] adjusted for age, sex, center, ethnicity, smoking status, and sampling time. We analyzed the data from the matched case-control study using a conditional logistic regression to estimate the risk of SARS-CoV-2 seropositivity. For each HCoV antigen, we defined the exposure variables by first summing the reactivities of IgG, IgA and IgM and then stratifying these composite reactivities by the median into two groups (low versus high reactivity). We used linear uni- and multivariate regression models to compare SARS-CoV-2

antibody responses in PWH (N=43) and HIV uninfected individuals (N= 382) with PCR-confirmed SARS-CoV-2 infection and analogously to analyze HCoV antibody responses among the 453 PWH with ABCORA 5 measurements in 2019 (1.5 Supplementary Material). Statistical analysis was conducted using R Version 4.1.2 [30].

RESULTS

Factors that influence the highly variable susceptibility to SARS-CoV-2 infection and disease severity, particularly those that result in asymptomatic disease, have not been fully elucidated [31, 32]. The SHCS [3], through its prospective, longitudinal design and systematic collection of HIV-specific and general health parameters along with behavioral and socioeconomic factors, provided us with a unique opportunity for systematic investigation of factors associated with SARS-CoV-2 acquisition. By January 2020, the SHCS database comprised over 300 parameters that were either self-reported or obtained from clinical records at the semi-annual study visits [3]. Here, we investigated the effect of 25 specific parameters on susceptibility to SARS-CoV-2 infection based on their potential significance for the general population, as well as their influence on the interaction between HIV-1 infection and SARS-CoV-2 infection (Figure 1A, Table S2).

To enable SARS-CoV-2 population studies the SHCS database included information on SARS-CoV-2 PCR testing from April 2020 onwards, and information on vaccination following its availability in Switzerland. We focused in the present analysis on the period between February 1, 2020 and December 31, 2021, to cover the initial waves of the SARS-CoV-2 epidemic in Switzerland in a SARS-CoV-2 naïve population and later on the onset of vaccination (Figure 1 B-D). During this period, 10'301 PWH were actively enrolled in the SHCS, 4'241 reported SARS-CoV-2 PCR tests, of which 1'009 were positive. During the first wave in 2020, PCR testing was not yet widely performed in Switzerland (Figure 1B, C) [33]. To improve SARS-CoV-2 prevalence assessment during this period, we performed SARS-CoV-2 multifactorial seroprofiling [27] of SHCS biobanked plasma samples from 2020 (N= 3'633) (Figure 1C). Retrospectively, 120 plasma samples were scored as SARS-CoV-2 positive. Combining SARS-CoV-2 positive PCR results (2020-2021) and seropositive serology tests (2020), we obtained information on the SARS-CoV-2 status of 6'270 SHCS participants. 1'088 (17%) were rated as SARS-CoV-2 positive (Figure 1C, D, Demographics of the study cohort in Table S1, Flowchart Figure S1). These 6'270 PWH with known SARS-CoV-2 status were used as full study cohort (Figure S1). Importantly, SARS-CoV-2 test positivity in the SHCS reflected the SARS-CoV-2 waves in the Swiss population as recorded by the Federal Office of Public Health (Figure 1D) [28].

No impact of HIV-related factors on risk of SARS-cov-2 acquisition

We explored factors influencing susceptibility to SARS-CoV-2 infection by analyzing 16 diverse parameters, including variables that apply to general population as well as HIV-related biomarkers (Figure 2A). We found that HIV-1 viral load and $CD4^+$ T cell levels, were not associated with

SARS-CoV-2 infection risk in our well-treated cohort (96% PWH with undetectable HIV RNA in plasma, Table S3). Several antiretroviral drugs, including the nucleotide inhibitor tenofovir (TDF and TAF) and the protease inhibitors lopinavir (LPV) and darunavir (DRV) have been controversially discussed to protect against SARS-CoV-2 infection [5, 34, 35]. We observed no evidence for a reduced SARS-CoV-2 infection risk through these antiretrovirals (Figures 2A).

Influence of health and demographic factors

SARS-CoV-2 vaccination had a protective effect for SARS-CoV-2 infection for the full study cohort (adjusted odds ratio aOR = 0.51, 95%-Confidence Interval [0.37, 0.7]) and when restricting to the 2021 sub-cohort (aOR = 0.49 [0.36, 0.68]) when vaccines became widely available (Figure 2A, Table S5,S6). We found no effect of age or sex on acquisition of SARS-CoV-2 (Figure 2, S3, S4), except for age > 65 years which was associated with a lower risk likely reflecting increased use of protective measures in this age group. Combined analysis of six comorbidities showed no effect on SARS-CoV-2 infection (Figure 2A), but individual analysis revealed a modest protective trend of drugs for obstructive airway diseases (Figure S5). Interestingly, the frequency of SARS-CoV-2 infection differed among ethnicities, with a significantly increased infection risk in participants of Black ethnicity (aOR = 1.4 [1.1, 1.7]) in the full study cohort. The effect was stronger in the 2020 sub-cohort but faded in 2021 (Figure 2A).

Influence of behavioral factors

HIV acquisition risk groups did not show differential SARS-CoV-2 risk (Figure 2A, Table S5). However, we observed a lower risk of infection among people living in single-person households (aOR = 0.77 [0.66, 0.9]) in 2021, but not in 2020 (Figure 2A). We attribute this to the gradual relaxation of mask-wearing and social distancing in 2021, combined with the arrival of the higher-transmissible variants of concern (VOCs) Alpha and Delta in Switzerland (Figure 1 and [28]). It is plausible that living in a single-person household during these periods of higher infection rates reduced the likelihood of transmission.

Cigarette smoking had a significant effect over all time periods studied on SARS-CoV-2 infection (Figure 2A,B) (aOR = 0.49 [0.41,0.59]) even when corrected for a range of factors including the intake of drugs for obstructive airway disease (Figure S5), an indirect indicator for underlying chronic lung disease. We therefore rated this finding and in particular the strength of the effect as highly unexpected and performed a series of sensitivity analyses. We ruled out confounding effects of a potential differential sensitivity of serology and PCR tests in smokers by stratifying the analyses per test type and test period (Figure S3, S6, S7). A negative association with SARS-CoV-2 infection among smokers persisted regardless of whether the entire study period or individual years were considered, or whether SARS-CoV-2 infection was diagnosed by PCR or serology (Figure S6, S7). The effect of smoking also remained robust when additional potential confounders were included, such as alcohol (Figure S8), marijuana use (Figure S9), or when behavioral variables were expanded to include heavy physical work, having a stable partner, or having an

occasional partner (Figure S10). None of these variables had an effect by themselves, with the exception of having occasional partners, which was associated with a higher infection risk (aOR = 1.27 [1.05, 1.55]). In support of a direct effect of smoking, stratification by the number of daily cigarettes showed a clear and striking dose-response effect, with smoking more cigarettes per day being associated with a greater reduction in infection risk (Figure 2B). Notably, even previous smokers retained some protective effect. Taken together these findings reveal a puzzling but strikingly clear protective impact of cigarette smoking on SARS-CoV-2 susceptibility that warrants further investigation.

Differential control of SARS-cov-2 infection susceptibility and severity

Over the full study period, 73 (6.7%) SARS-CoV-2 infected individuals were hospitalized and 8 (0.74%) died (Table S4). One additional participant was recorded to have likely died due to COVID-19, but could formally not be included in our study population as PCR confirmation was not available. This relatively low frequency of hospitalizations and deaths reflects the age distribution within the SHCS, with only 0.8% (82/10301) PWH aged 80 or older. When stratified by age, the SHCS exhibited slightly higher hospitalization and case-fatality ratios compared to the general population (Table S4). This modestly increased rate needs to be weighed carefully, as it may partly reflect more accurate monitoring possibilities in the SHCS than in the general population. Given the low number of hospitalized and deceased individuals, further analysis of associated parameters must be cautiously interpreted. As seen generally in COVID-19, hospitalization in the SHCS was driven by older age and the presence of comorbidities (Figure S4). We found no effect of HIV-related factors or antiretroviral medication on the risk of hospitalization. Solely, higher $CD4^{+}T$ cell values showed a trend towards a reduced risk of hospitalization (aOR =0.62 [0.36, 1.03], p=0.07). Notably, factors associated with infection risk, namely, living alone, smoking, and Black ethnicity had no impact on hospitalization rates. We observed a significantly lower hospitalization rate in 2021 compared with 2020 (aOR= 0.48 [0.22, 0.95], Figure S4) after accounting for vaccination and other potential confounders. This effect is likely due to several factors next to immunization, including increased SARS-CoV-2 immunity due to prior infection and the availability of SARS-CoV-2 therapeutics [33] for high-risk individuals in Switzerland in 2021.

Multiple factors influence the SARS-cov-2 antibody response

As the immune system of PWH, even if successfully treated, remains at least partially impaired [36], we included a control group composed of HIV-uninfected individuals (HU) with PCR-documented SARS-CoV-2 infection in 2020 (N=257) to investigate factors influencing the antibody response to SARS-CoV-2 infection. PWH with SARS-CoV-2 serology sampled after PCR-documented infection in 2020 (N=42) were compared with HU (Figure 3A). Restricting the analysis to PCR documented infections allowed to control antibody responses for the time since infection (Figure S11). Using multivariate regression models deriving the overall effect of potential determinants across all antigens and adjusting for age, sex, and time since PCR positivity,

we found that PWH had significantly lower SARS-CoV-2-specific antibody binding levels than HU (Figure 3AB, Figure S11). Reduced antibody levels in PWH were observed across all Ig-classes and antigens (Figure 3C, S12-S14). A sensitivity analysis accounting for hospitalizations in the subgroup of PWH and HU for whom this information was available corroborated the lower SARS-CoV-2 responses in PWH (Figure S15).

We observed markedly lower SARS-CoV-2 IgG antibody reactivities among smokers, as also seen in response to vaccination [24, 37] (Figure 3DE). Interestingly, PWH of Black ethnicity had higher IgG antibody binding to all three spike antigens (RBD, S1, and S2) but not to the N (nucleocapsid) antigen compared to PWH of White ethnicity (Figure 3D-E, S16-S18). This selective upregulation of spike antibodies is intriguing as it may indicate a difference in immune recognition of viral antigens depending on the ethnicity of the individual.

Pre-existing hcov immunity impacts SARS-cov-2 infection

We and others reported modest protective effects of HCoV immunity on SARS-CoV-2 infection [27, 38-41]. We sought to determine the impact of pre-pandemic HCoV antibody titers on SARS-CoV-2 infection risk. We analyzed S1 antigen reactivity of all four HCoVs in pre-pandemic plasma samples (collected in 2019 within the SHCS) from SARS-CoV-2 seropositive PWH (N=92) and PWH controls without SARS-CoV-2 infection (N=182). Controls were matched for age, sex, ethnicity, smoking status, SHCS study center, and dates of pandemic and pre-pandemic plasma collection (Figure 4AB, S18). We found that PWH with high pre-pandemic (2019) antibody levels to HCoV-229E had a significantly reduced risk of a SARS-CoV-2 infection (OR=0.58 [0.35,0.97]) whereas antibodies to the other HCoVs showed no such effect (Figure 4B). HCoV-229E was the most frequent HCoV detected in 2019 in our local virus diagnostics unit in Zurich, Switzerland (Figure 4C) suggesting that immunity to the most recent HCoV wave had the strongest impact on cross-protecting against SARS-CoV-2. We next analyzed across the full study cohort, whether HCoV antibody responses in early 2020 had an impact on subsequent SARS-CoV-2 infection in 2020 or 2021 across the full study cohort. Consistent with the observed peak of HCoV-NL63 diagnoses in early 2020 (Figure 4C), we observed a significant protective effect of HCoV-NL63 antibodies (Figure 4D).

Notably, pre-pandemic antibody titers to HCoV (in particular HCoV-NL63) were positively associated with higher SARS-CoV-2 antibody titers after a PCR-confirmed infection (Figure 5AB). These effects were robust to adjustment for age, sex, $CD4^+$T cell counts, smoking, ethnicity, and hospitalization. Thus, irrespective of disease severity (as measured by hospitalization), high pre-existing HCoV immunity promoted a higher antibody response to SARS-CoV-2 infection. Exploring factors associated with pre-pandemic HCoV antibody responses, we found that high $CD4^+$T cell values were associated with overall lower HCoV antibody responses among PWH which may indicate less frequent HCoV infections with increasing immune competence (Figure 5C, D, S19-20). Consistent with more extended exposure to HCoVs, older age was associated with higher pre-pandemic HCoV antibody responses. Intriguingly, we found that smoking was

associated with higher antibody responses to HCoV which may correlate with a potential protective mechanism considering the observed lower SARS-CoV-2 infection rates among smokers. The fact that smoker mount elevated HCoV antibody levels (Figure 5D) but decreased SARS-CoV-2 antibody responses (Figure 3E), highlights fundamental differences in HCoV and SARS-COV-2 infection biology and immune recognition that need to be resolved.

DISCUSSION

Utilizing the potential of a systematic risk-factor screen in a large prospective cohort, our study yields several key observations relevant to understanding factors influencing SARS-CoV-2 infection in PWH and the general population. It provides new insights into the interplay between HCoV and SARS-CoV-2 immunity and supports evidence of protective effects of pre-existing HCoV immune responses [27, 38-40]. Observed lower SARS-CoV-2 antibody responses in PWH are likely attributable to the known residual immune dysfunction despite long-term suppressive ART [36], but are not associated with increased infection rates or disease severity compared to the general Swiss population. Given the very high proportion of PWH with virological suppression in the SHCS, this may not apply to settings with lower treatment or suppression rates.

The observed impact of living alone in the initial phases of the pandemic underline the influence of social distancing in limiting SARS-CoV-2 spread. Higher infection rates among Black PWH may be due to socioeconomic differences that should be investigated as they may result in fewer opportunities to protect oneself at the workplace and at home. In turn, fading of the effect in 2021 may be attributable to increased herd immunity [42]. Higher (IgG) spike antibody responses in Black PWH may also have an underlying host genomic factor as we previously observed stronger and broader HIV-antibody responses among Black PWH [43, 44].

An association between smoking and reduced SARS-CoV-2 infection risk has been reported in other studies but has not found wide acceptance [45, 46]. Our results support a potential impact of smoking: The effect is strong and highly robust as demonstrated through several sensitivity analyses. The extent of smoking was associated with the effect size with heavy smokers being at the lowest risk. Future studies will be needed to unravel whether pathophysiological effects, such as stimulation of innate defenses or down-regulation of ACE-2 receptors are involved in lower susceptibility to SARS-CoV-2 [47]. While we observed a protective lower risk of SARS-CoV-2 acquisition in smokers, this should not be read that smoking will prevent against the virus or severe disease. Our study focuses on the initial SARS-CoV-2 waves in the absence of specific SARS-CoV-2 immunity. Later circulating variants are more fit than the initial strains which also may outweigh the effect of smoking. It also needs to be considered that the reduced ability of smokers to mount SARS-CoV-2 antibody responses may reduce the capacity for virus clearance [17] inflicting more severe disease courses in smokers [48-50].

This paper has several limitations and important strengths. Some analyses remain inconclusive due to limited power. This was the case for risk factors for hospitalization because of the overall small number of hospitalizations. Limited statistical power may also have hindered our ability to detect a protective effect associated with immunity to HCoVs with lower prevalence prior to the onset of the SARS-CoV-2 pandemic. As with all observational studies, confounding by unreported factors cannot be excluded. Despite careful data collection, some underreporting of SARS-CoV-2 infection remains possible and false-negative serologic tests cannot be accounted for. Despite these limitations, our study demonstrates the power of observational cohorts to achieve a comprehensive assessment of the drivers of COVID-19 in PWH and beyond. Our cohort screen confirms that SARS-CoV-2 vaccination has a clear protective effect in PWH on ART.

The depth of our analysis allowed us to identify several factors associated with SARS-CoV-2 infection risk. Overall, we found it most intriguing that two unrelated factors affecting the respiratory milieu, namely prior HCoV infection and smoking, had strong protective effects against SARS-CoV-2 acquisition. This warrants future in-depth analyses of the protective mechanisms in the respiratory mucosa to potentially implicate these factors in antiviral defense.

Author contributions

The authors confirm the following contribution to the final manuscript: study conception and design (IAA, AH, CP, RDK, AT, HFG); data collection (IAA, CP, MAS, AH); analysis and interpretation of results (IAA AH, CP, MAS, RDK, HFG, AT); draft manuscript preparation (IAA AH, CP, MAS, RDK, HFG, AT); All authors reviewed the results and approved the final version of the manuscript.

Declaration of conflict of interests

HFG reports having received honoraria from Gilead Sciences, Merck, ViiV, GSK, Janssen, Johnson and Johnson, and Novartis for serving on DSMB and/or advisory boards, and has received a travel grant from Gilead Sciences. In addition, he has received grants from the Swiss National Science Foundation (SNSF), the Swiss HIV Cohort Study, the Yvonne Jacob Foundation, the NIH and unrestricted research grants from Gilead Sciences, all paid to the institution. AT has received honoraria from Roche Diagnostics for consultant activity, and grants from the SNSF, the Swiss HIV Cohort Study, the Pandemiefonds of the UZH foundation and unrestricted research grants from Gilead Sciences. IAA has received honoraria from MSD and Sanofi, a travel grant from Gilead Sciences, and a grant from Promedica foundation. RDK has received grants from SNSF, the National Institutes of Health, and Gilead Sciences. DLB reports honoraria payed to himself for advisory boards from the companies AstraZeneca, Pfizer, Gilead, MSD and ViiV. MC'institution received research grants from Gilead, MSD and Viiv. MC institution received fees for expert opinion given to Gilead, MSD and Viiv.

Funding

This work was supported by a grant Swiss National Science Foundation 31CA30_196906 to HFG, AT, RDK, SHCS Project 870, SHCS research foundation, Gilead COVID-19 RFP Research Program COMMIT Grant #: IN-SW-983-6078 (to HFG, AT, RDK). The SHCS is supported by the Swiss National Science Foundation to HFG. IAA is supported by a research grant of the Promedica Foundation.

Acknowledgements

The authors thank all patients, physicians and nurses associated with the participating cohorts.

Data sharing

Deidentified individual-level participant data are available upon reasonable request. The data sets generated and/or analyzed during the current study are not publicly available, since they are subject to national data protection laws and restrictions imposed by the ethics committee to ensure data privacy of the study participants (http://www.shcs.ch/294-open-data-statement-shcs). The code for the analysis is archived at the University Hospital of Zurich. Requests for data sharing can be directed to the corresponding authors.

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REFERENCES

1. Aldridge RW, Yavlinsky A, Nguyen V, et al. SARS-CoV-2 antibodies and breakthrough infections in the Virus Watch cohort. Nat Commun **2022**; 13:4869.
2. Group TRS. How to RESPOND to Modern Challenges for People Living with HIV: A Profile for a New Cohort Consortium. Microorganisms **2020**; 8:1164.
3. Scherrer AU, Traytel A, Braun DL, et al. Cohort Profile Update: The Swiss HIV Cohort Study (SHCS). Int J Epidemiol **2022**; 51:33-4j.
4. Bhaskaran K, Rentsch CT, MacKenna B, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. Lancet HIV **2021**; 8:e24-e32.
5. Inciarte A, Gonzalez-Cordon A, Rojas J, et al. Clinical characteristics, risk factors, and incidence of symptomatic coronavirus disease 2019 in a large cohort of adults living with HIV: a single-center, prospective observational study. AIDS **2020**; 34:1775-80.
6. Kusejko K, Chammartin F, Smith D, et al. Developing and testing a Corona VaccinE tRiAL pLatform (COVERALL) to study Covid-19 vaccine response in immunocompromised patients. BMC Infect Dis **2022**; 22:654.
7. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol **2021**; 19:141-54.
8. Chudasama YV, Zaccardi F, Gillies CL, et al. Patterns of multimorbidity and risk of severe SARS-CoV-2 infection: an observational study in the U.K. BMC Infect Dis **2021**; 21:908.
9. Drucker DJ. Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. Cell Metab **2021**; 33:479-98.
10. Ssentongo P, Heilbrunn ES, Ssentongo AE, et al. Epidemiology and outcomes of COVID-19 in HIV-infected individuals: a systematic review and meta-analysis. Sci Rep **2021**; 11:6283.
11. Nomah DK, Reyes-Urueña J, Diaz Y, et al. Sociodemographic, clinical, and immunological factors associated with SARS-CoV-2 diagnosis and severe COVID-19 outcomes in people living with HIV: a retrospective cohort study. Lancet HIV **2021**; 8:e701-e10.
12. Joy M, Hobbs FR, Bernal JL, et al. Excess mortality in the first COVID pandemic peak: cross-sectional analyses of the impact of age, sex, ethnicity, household size, and long-term conditions in people of known SARS-CoV-2 status in England. Br J Gen Pract **2020**; 70:e890-e8.
13. Jassat W, Cohen C, Tempia S, et al. Risk factors for COVID-19-related in-hospital mortality in a high HIV and tuberculosis prevalence setting in South Africa: a cohort study. Lancet HIV **2021**; 8:e554-e67.
14. Kohler I, Kouyos R, Bianchi M, et al. The impact of vaccination on the breadth and magnitude of the antibody response to influenza A viruses in HIV-infected individuals. AIDS **2015**; 29:1803-10.
15. Tian Y, Hua W, Wu Y, et al. Immune Response to Hepatitis B Virus Vaccine Among People Living With HIV: A Meta-Analysis. Front Immunol **2021**; 12:745541.
16. Portillo V, Fedeli C, Ustero Alonso P, et al. Impact on HIV-1 RNA Levels and Antibody Responses Following SARS-CoV-2 Vaccination in HIV-Infected Individuals. Front Immunol **2021**; 12:820126.
17. Marconato M, Abela IA, Hauser A, et al. Antibodies from convalescent plasma promote SARS-CoV-2 clearance in individuals with and without endogenous antibody response. J Clin Invest **2022**; 132.

18. Ruffieux Y, Lemsalu L, Aebi-Popp K, et al. Mortality from suicide among people living with HIV and the general Swiss population: 1988-2017. J Int AIDS Soc **2019**; 22:e25339.
19. Gueler A, Moser A, Calmy A, et al. Life expectancy in HIV-positive persons in Switzerland: matched comparison with general population. AIDS **2017**; 31:427-36.
20. Lombardi F, Ricci R, Belmonti S, et al. Seroprevalence of SARS-CoV-2 Antibodies in HIV-Infected Patients in Rome, Italy during the COVID-19 Outbreak. Diagnostics (Basel) **2021**; 11.
21. Berenguer J, Diez C, Martin-Vicente M, et al. Prevalence and factors associated with SARS-CoV-2 seropositivity in the Spanish HIV Research Network Cohort. Clin Microbiol Infect **2021**; 27:1678-84.
22. Wolter N, Tempia S, von Gottberg A, et al. Seroprevalence of Severe Acute Respiratory Syndrome Coronavirus 2 After the Second Wave in South Africa in Human Immunodeficiency Virus-Infected and Uninfected Persons: A Cross-Sectional Household Survey. Clin Infect Dis **2022**; 75:e57-e68.
23. Shapiro AE, Bender Ignacio RA, Whitney BM, et al. Factors Associated With Severity of COVID-19 Disease in a Multicenter Cohort of People With HIV in the United States, March-December 2020. J Acquir Immune Defic Syndr **2022**; 90:369-76.
24. 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.
25. Chammartin F, Griessbach A, Kusejko K, et al. Bridging the Gap: identifying factors impacting mRNA SARS-CoV-2 vaccine booster response in people living with HIV-1. AIDS **2023**:10-1097.
26. Swiss National Science Foundation. Longitudinal studies. Available at: http://www.snf.ch/en/funding/programmes/longitudinal-studies/Pages/default.aspx#Currently%20supported%20longitudinal%20studies.
27. Abela IA, Pasin C, Schwarzmuller M, et al. Multifactorial seroprofiling dissects the contribution of pre-existing human coronaviruses responses to SARS-CoV-2 immunity. Nat Commun **2021**; 12:6703.
28. Swiss Federal Office of Public Health. COVID-19 Switzerland, Coronavirus Dashboard. Available at: https://www.covid19.admin.ch/en/overview. Accessed 12.08.2022 2022.
29. Therneau T, Crowson C, Atkinson E. Using Time Dependent Covariates and Time Dependent Coefficients in the Cox Model. Available at: https://cran.r-project.org/web/packages/survival/vignettes/timedep.pdf.
30. R Core Team. R: A Language and Environment for Statistical Computing. Available at: https://www.R-project.org/.
31. Ong SYQ, Flyamer IM, Bickmore WA, Biddie SC. From bedside to bench: regulation of host factors in SARS-CoV-2 infection. Exp Mol Med **2021**; 53:483-94.
32. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. Nat Rev Microbiol **2022**; 20:270-84.
33. Swiss Federal Office Of Public Health. Coronavirus: Neuigkeiten und Anpassungen. Available at: https://www.bag.admin.ch/bag/de/home/krankheiten/ausbrueche-epidemien-pandemien/aktuelle-ausbrueche-epidemien/novel-cov/information-fuer-die-aerzteschaft/neuigkeiten-und-anpassungen.html.
34. Del Amo J, Polo R, Moreno S, et al. Incidence and Severity of COVID-19 in HIV-Positive Persons Receiving Antiretroviral Therapy : A Cohort Study. Ann Intern Med **2020**; 173:536-41.

35. Labhardt ND, Smit M, Petignat I, et al. Post-exposure Lopinavir-Ritonavir Prophylaxis versus Surveillance for Individuals Exposed to SARS-CoV-2: The COPEP Pragmatic Open-Label, Cluster Randomized Trial. EClinicalMedicine **2021**; 42:101188.
36. Cai CW, Sereti I. Residual immune dysfunction under antiretroviral therapy. Semin Immunol **2021**; 51:101471.
37. Watanabe M, Balena A, Tuccinardi D, et al. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. Diabetes Metab Res Rev **2022**; 38:e3465.
38. Ortega N, Ribes M, Vidal M, et al. Seven-month kinetics of SARS-CoV-2 antibodies and role of pre-existing antibodies to human coronaviruses. Nat Commun **2021**; 12:4740.
39. Lavell AHA, Sikkens JJ, Edridge AWD, et al. Recent infection with HCoV-OC43 may be associated with protection against SARS-CoV-2 infection. iScience **2022**; 25:105105.
40. Loyal L, Braun J, Henze L, et al. Cross-reactive CD4(+) T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. Science **2021**; 374:eabh1823.
41. 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.
42. Verburgh ML, Boyd A, Wit F, et al. Similar Risk of Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Similar Nucleocapsid Antibody Levels in People With Well-Controlled Human Immunodeficiency Virus (HIV) and a Comparable Cohort of People Without HIV. J Infect Dis **2022**; 225:1937-47.
43. Kadelka C, Liechti T, Ebner H, et al. Distinct, IgG1-driven antibody response landscapes demarcate individuals with broadly HIV-1 neutralizing activity. J Exp Med **2018**; 215:1589-608.
44. Rusert P, Kouyos RD, Kadelka C, et al. Determinants of HIV-1 broadly neutralizing antibody induction. Nat Med **2016**; 22:1260-7.
45. Paleiron N, Mayet A, Marbac V, et al. Impact of Tobacco Smoking on the Risk of COVID-19: A Large Scale Retrospective Cohort Study. Nicotine Tob Res **2021**; 23:1398-404.
46. Simons D, Shahab L, Brown J, Perski O. The association of smoking status with SARS-CoV-2 infection, hospitalisation and mortality from COVID-19: A living rapid evidence review with Bayesian meta-analyses (version 10). Qeios.
47. Tanimoto K, Hirota K, Fukazawa T, et al. Inhibiting SARS-CoV-2 infection in vitro by suppressing its receptor, angiotensin-converting enzyme 2, via aryl-hydrocarbon receptor signal. Sci Rep **2021**; 11:16629.
48. Simons D, Shahab L, Brown J, Perski O. The association of smoking status with SARS-CoV-2 infection, hospitalization and mortality from COVID-19: a living rapid evidence review with Bayesian meta-analyses (version 7). Addiction **2021**; 116:1319-68.
49. Clift AK, von Ende A, Tan PS, et al. Smoking and COVID-19 outcomes: an observational and Mendelian randomisation study using the UK Biobank cohort. Thorax **2022**; 77:65-73.
50. Reddy RK, Charles WN, Sklavounos A, Dutt A, Seed PT, Khajuria A. The effect of smoking on COVID-19 severity: A systematic review and meta-analysis. J Med Virol **2021**; 93:1045-56.

FIGURE LEGENDS

Fig 1: SARS-CoV-2 surveillance in SHCS and the Swiss population A. Schematic overview of the determinants of SARS-CoV-2 infections recorded in Swiss HIV Cohort Study. B. SARS-CoV-2-cases in Switzerland as reported by the Swiss FOPH over time and waves of variant of concerns C. Number of self-reported PCR and number of serological tests performed in the SHCS; displayed by month and stratified by result) D. Test positivity in the SHCS (blue) and the Swiss population (black), by month. The dots represent the mean estimate, while the shaded area represents the 95% confidence interval assuming a binomial distribution.

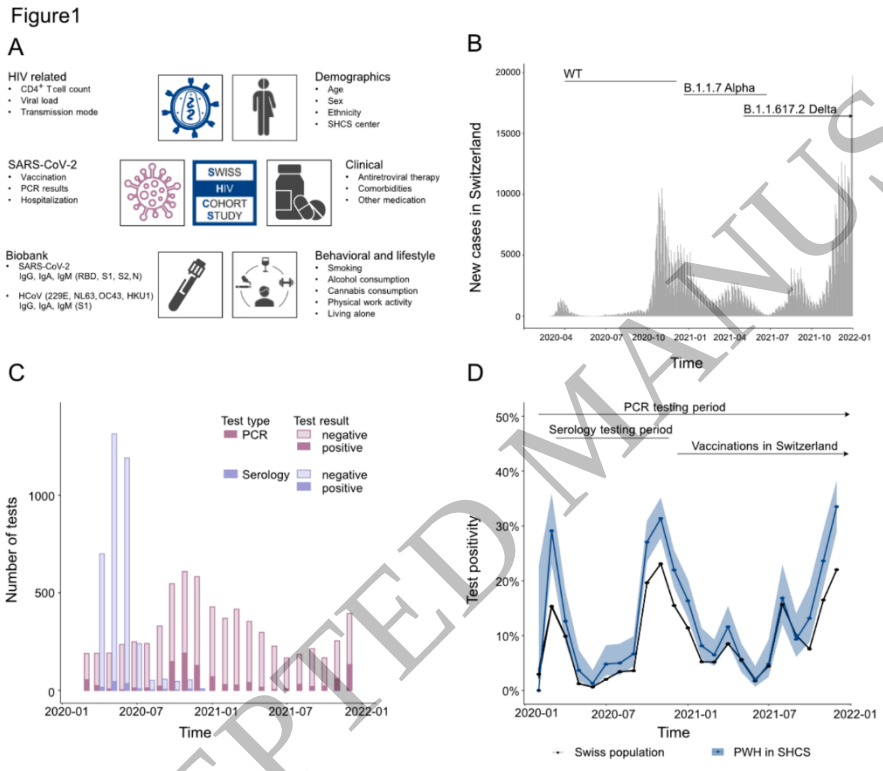
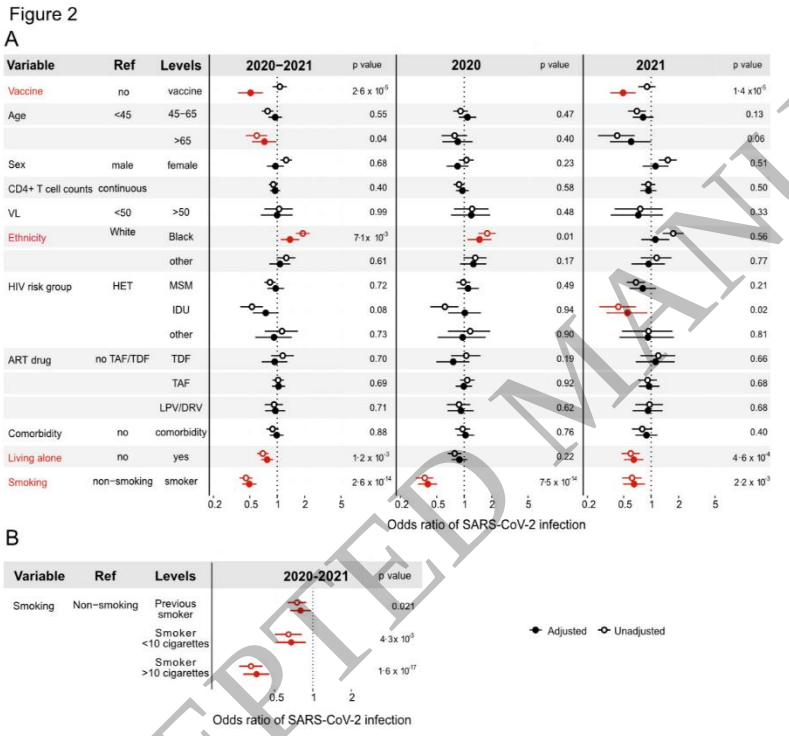


Fig 2: Risk factors of SARS-CoV-2 acquisition. A. Uni- and multivariate analysis of SARS-COV-2 infection (determined by either PCR or serology) risk factors adjusted for the depicted covariables and calendar time (included as cubic spline) and stratified by calendar time. The exposure variable comorbidities was defined as reports of either diagnoses (hypertension, diabetes), risk factors (BMI>35 kg/m2), or of the use of drugs related to comorbidities (immune-suppressive drugs, corticosteroids, or drugs for obstructive airway diseases). Numerical values of the odds ratio (Table S5) and a summary of population characteristics (Table S6) are displayed in the supplementary material. B. Uni- and multivariate analysis of the dose dependent effect of smoking on the risk SARS-CoV-2 infection (determined by either PCR or serology). Dots correspond to effect sizes and lines to 95% CIs.



DOI: 10.1093/infdis/jiae002

Fig 3: Dissecting factors that influence SARS-CoV-2 antibody response. A. Comparison of ABCORA 5 reactivity for SARS-CoV-2 in individuals who had a positive SARS-CoV-2 PCR test prior to serology, stratified by HIV status (42 PWH and 257 uninfected individuals) healthy and SARS-CoV-2 infected individuals. Horizontal lines within the violin-plots correspond to first quartile, median, and third quartile. B. Determinants of SARS-CoV-2 antibody response as analyzed by a Bayesian multivariate analyses response in the combined data set consisting of SHCS participants (N=42) and HIV uninfected individuals (N=257). The plots summarize the effects across antigen (RBD, S1, S2, N) but are stratified by Ig-class. Effects are adjusted for age, sex, HIV status, and the time since the positive SARS-COV-2 PCR (latter included as a cubic spline). C. Univariate analyses of the determinants of SARS-CoV-2 antibody response in the combined data set of SHCS participants and HIV uninfected individuals. Effect sizes are calculated using a frequentist linear model adjusted for the shown variables and for the time since the positive SARS-COV-2 PCR, which is included as a cubic spline. Colors indicate effect size and direction. The fully covered cells indicate significant effects ($p<0.05$), and small internal squares non-significant effects. D. Bayesian multivariate analyses of the determinants of SARS-CoV-2 antibody response among SHCS participants, who had a positive SARS-CoV-2 PCR test prior to serology (42 PWH). The plots summarize the effects across antigen (RBD, S1, S2, N) but are stratified by Ig-class. Effects adjusted for age, sex, CD4-cell number, smoking status, ethnicity, hospitalization, and the time since the positive SARS-COV-2 PCR, which is included as a cubic spline. E. Univariate analyses of the determinants of SARS-CoV-2 antibody responses in SHCS participants who had a positive SARS-CoV-2 PCR test prior to serology (N=42). Effect sizes are calculated using a frequentist linear model adjusting for the shown variables and for. Colors indicate effect size and direction. The fully covered cells indicate significant effects ($p<0.05$), and small internal squares non-significant effects.

Figure3

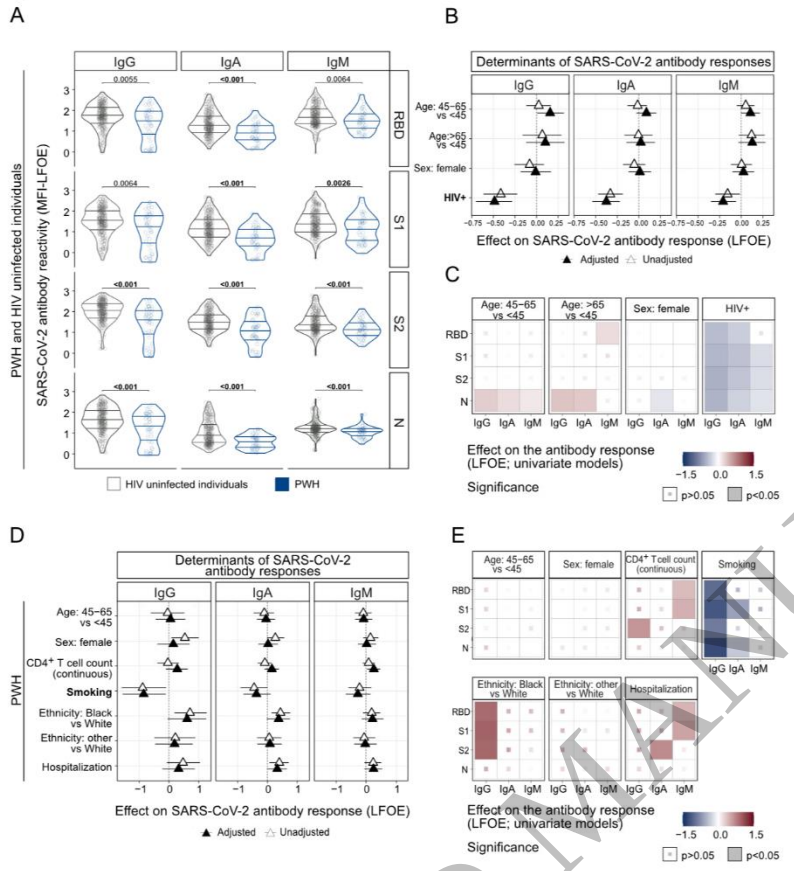


Fig 4: High pre-pandemic HCoV antibody levels are associated with a lower risk of subsequent SARS-CoV-2 infection A. Pre-pandemic HCoV S1 antibody responses among cases (PWH, PCR positive, N=92, green) and controls (PWH, PCR negative, N=182, yellow). Horizontal lines within the violin-plots correspond to first quartile, median, and third quartile. B. Test positivity of the four HCoVs (229E, NL63, OC43 and HKU1) diagnosed in local virus diagnostic unit by PCR by quarter year. C. Odds ratio (OR) of positive SARS-CoV-2 serology according to the level of pre-pandemic HCoV and SARS-CoV-2 serology calculated using conditional logistic regression on the matched data comprising cases and controls (see A and B). Only significant p-values ($p<0.05$) are displayed. D. Hazard ratios (HR) comparing the time to a positive PCR test according to the prior (pre-pandemic and/or pandemic) level HCoV and SARS-CoV-2 serology. The time-updated survival model is adjusted for age, sex, center, ethnicity, smoking status, and time of sample collection. Only significant p-values ($p<0.05$) are displayed.

DOI: 10.1093/infdis/jiae002

Figure 4

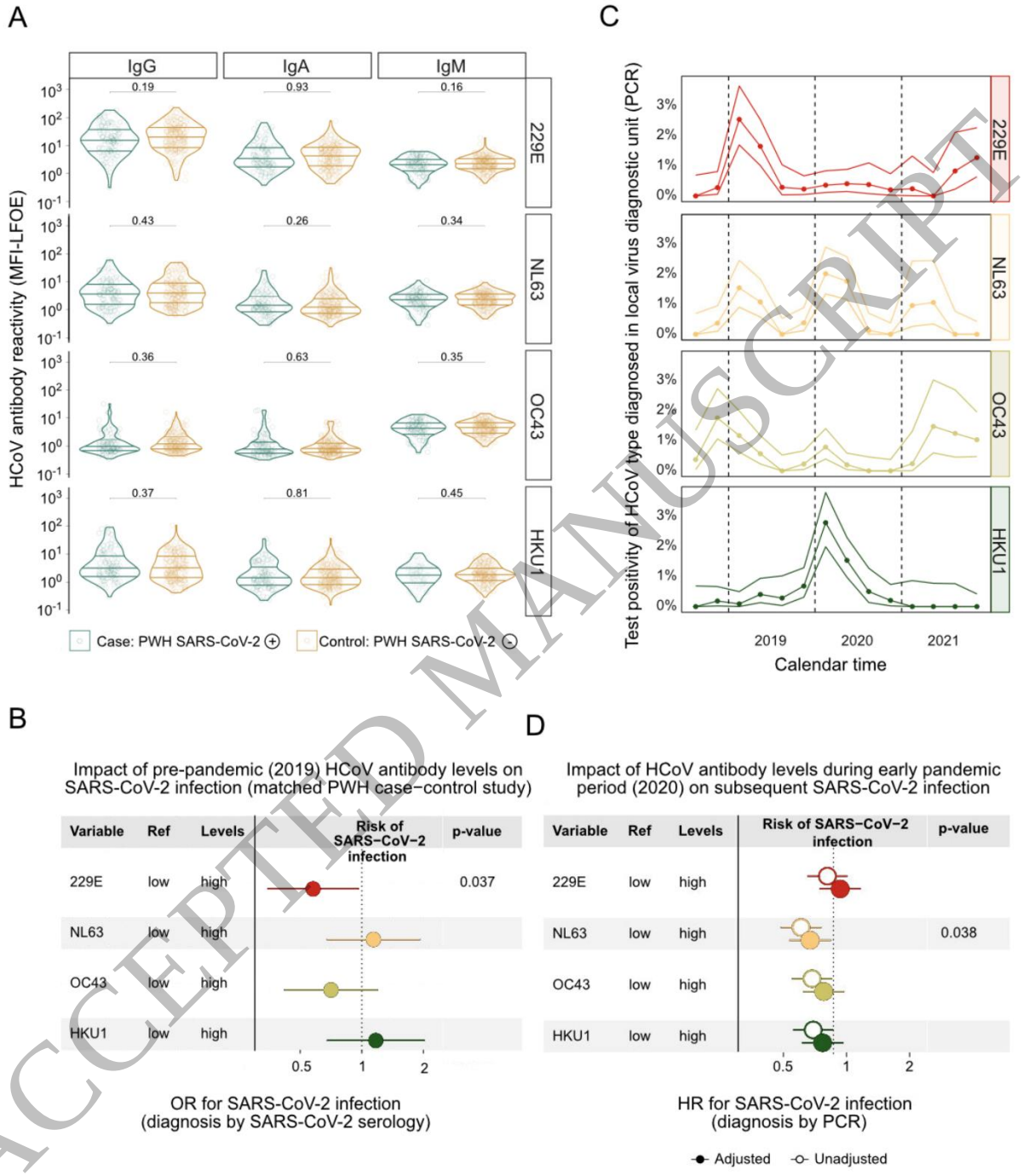


Fig 5: Dissecting the cross-talk between HCoV immunity and SARS-CoV-2 responses A. Impact of preexisting HCoV antibody response on individual SARS-CoV-2 antibody response after infection analyzed by using a Bayesian multivariate regression model. The model summarizes the effects across epitopes but is stratified by Ig-class. Effects are adjusted for age, sex, CD4-cell number, smoking status, ethnicity, hospitalization, and the time since the positive SARS-COV-2 PCR (included as a cubic spline). B. Univariate analyses of the determinants of SARS-CoV-2 antibody response in PWH with PCR-confirmed SARS-COV-2 infection and pre-pandemic serology data available (N = 32). Effect sizes are calculated using a frequentist linear model adjusted for age, sex, CD4-cell number, smoking status, ethnicity, hospitalization, and the time since the positive SARS-COV-2 PCR (included as a cubic spline). Colors indicate effect size and direction. Fully covered cells indicate significant effects ($p<0.05$), and small internal squares non-significant effects. C. Bayesian multivariate analyses of the determinants of HCoV antibody responses sampled in 2020 among SHCS participants (N=3'496). The plots summarize the effects across HCoV (HKU-1, OC43, NL63, 229E) but are stratified by Ig-class. Effects adjusted for age, sex, CD4 T cell number, smoking, and ethnicity. D. Univariate analyses of the determinants of HCoV antibody responses sampled in 2020 among SHCS participants (N=3'496). Effect sizes are calculated using a frequentist linear model. Colors indicate effect size and direction. The fully covered cells indicate significant effects ($p<0.05$), and small internal squares non-significant effects. The effects are adjusted for age, sex, CD4- T cell number, smoking, and ethnicity.

Figure 5