Postacute sequelae and adaptive immune responses in people with HIV recovering from SARS-COV-2 infection

Michael J. Peluso[a,*], Matthew A. Spinelli[a,*], Tyler-Marie Deveau[b], Carrie A. Forman[a], Sadie E. Munter[b], Sujata Mathur[c], Alex F. Tang[a], Scott Lu[c], Sarah A. Goldberg[c], Mireya I. Arreguin[a], Rebecca Hoh[a], Viva Tai[a], Jessica Y. Chen[a], Enrique O. Martinez[a], Brandon C. Yee[d], Ahmed Chenna[d], John W. Winslow[d], Christos J. Petropoulos[d], Alessandro Sette[e,f], Daniella Weiskopf[e], Nitasha Kumar[b], Kara L. Lynch[g], Peter W. Hunt[b], Matthew S. Durstenfeld[h], Priscilla Y. Hsue[h], J. Daniel Kelly[c], Jeffrey N. Martin[c], David V. Glidden[c], Monica Gandhi[a], Steven G. Deeks[a], Rachel L. Rutishauser[b,*] and Timothy J. Henrich[b,*]

Background: Limited data are available on the long-term clinical and immunologic consequences of SARS-CoV-2 infection in people with HIV (PWH).

Methods: We measured SARS-CoV-2-specific humoral and cellular responses in people with and without HIV recovering from COVID-19 ($n = 39$ and $n = 43$, respectively) using binding antibody, surrogate virus neutralization, intracellular cytokine staining, and inflammatory marker assays. We identified individuals experiencing postacute sequelae of SARS-CoV-2 infection (PASC) and evaluated immunologic parameters. We used linear regression and generalized linear models to examine differences by HIV status in the magnitude of inflammatory and virus-specific antibody and T-cell responses, as well as differences in the prevalence of PASC.

Results: Among PWH, we found broadly similar SARS-CoV-2-specific antibody and T-cell responses as compared with a well matched group of HIV-negative individuals. PWH had 70% lower relative levels of SARS-CoV-2-specific memory $CD8^+$ T cells

[a]Division of HIV, Infectious Diseases, and Global Medicine, [b]Division of Experimental Medicine, [c]Department of Epidemiology and Biostatistics, University of California, San Francisco, [d]Monogram Biosciences, South San Francisco, [e]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), [f]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego, La Jolla, [g]Division of Laboratory Medicine, and [h]Division of Cardiology, University of California, San Francisco, CA, USA.

Correspondence to Michael J. Peluso, MD, Assistant Professor of Medicine, Division of HIV, Infectious Diseases, and Global Medicine, 1001 Potrero Avenue, San Francisco, CA 94110, USA.

Tel: +1 415 476 4082 x119; e-mail: michael.peluso@ucsf.edu

[*] M.J., M.A. R.L., and T.J. contributed equally to the writing of this article.

Received: 23 May 2022; revised: 14 June 2022; accepted: 29 June 2022.

DOI:10.1097/QAD.0000000000003338

ISSN 0269-9370 Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

($P = 0.007$) and 53% higher relative levels of PD-1+ SARS-CoV-2-specific $CD4^+$ T cells ($P = 0.007$). Higher $CD4^+/CD8^+$ ratio was associated with lower PD-1 expression on SARS-CoV-2-specific $CD8^+$ T cells (0.34-fold effect, $P = 0.02$). HIV status was strongly associated with PASC (odds ratio 4.01, $P = 0.008$), and levels of certain inflammatory markers (IL-6, TNF-alpha, and IP-10) were associated with persistent symptoms.

Conclusion: We identified potentially important differences in SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in PWH and HIV-negative participants that might have implications for long-term immunity conferred by natural infection. HIV status strongly predicted the presence of PASC. Larger and more detailed studies of PASC in PWH are urgently needed.
Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

AIDS 2022, **36**:F7–F16

Keywords: coronavirus disease 2019, HIV, immune response, long coronavirus disease, postacute sequelae of SARS-CoV-2, SARS-CoV-2

Background

The intersection between the SARS-CoV-2 and HIV epidemics has gained increased attention [1]. Although early studies did not show differences in acute coronavirus disease 2019 (COVID-19) associated with HIV status [2,3], larger studies show that people with HIV (PWH) are at higher risk for adverse outcomes [4,5]. Data on SARS-CoV-2-specific adaptive immune responses in PWH remain sparse; however, with one study showing less robust immune responses among PWH [6] but another [7] suggesting similar responses. Furthermore, there is growing recognition of the clinical burden of postacute sequelae of SARS-CoV-2 infection (PASC, including 'long COVID') [8], but this condition remains poorly understood, especially in PWH.

A combination of factors might underlie PASC [9–14]. Higher prevalence of certain socioeconomic factors and comorbidities among PWH, along with differences in immune responses to SARS-CoV-2 [6,7] and persistent inflammation and immune dysregulation in the presence of antiretroviral therapy (ART) [15–18], may make PWH selectively vulnerable to developing this condition. For these reasons, examination of PASC in PWH is urgently needed.

Here, we sought to test the hypothesis that PASC would be more prevalent in PWH with a history of COVID-19 prior to vaccination and that these individuals would have reduced SARS-CoV-2-specific immune responses in comparison to HIV-negative individuals recovering from COVID-19.

Methods

Informed consent

The study was approved by the University of California, San Francisco (UCSF) Institutional Review Board. Participants provided written informed consent.

Participants

Volunteers were enrolled in the Long-term Impact of Infection with Novel Coronavirus (LIINC) study (NCT04362150) [19,20]. Briefly, participants with a positive nucleic acid amplification test for SARS-CoV-2 were enrolled 21 or more days following symptom onset. Recruitment occurred through self and clinician referrals; all PWH testing positive for SARS-CoV-2 at two UCSF-based HIV clinics were invited to participate. At each visit, participants completed an interview regarding prior and current COVID-19-attributed symptoms, medical history, and quality of life.

We selected all PWH who enrolled prior to receipt of a SARS-CoV-2 vaccine ($n = 39$) and compared them with a randomly selected group of HIV-negative individuals ($n = 43$) with a similar distribution of age, sex, COVID-19 hospitalization, and time since infection [9,21–23]. Participants were assessed at the visit closest to 16 weeks postinfection [median 112 days (IQR: 91–129)].

Clinical assessment

Our methodology for assessing PASC is described elsewhere [20]. Briefly, participants were queried regarding the presence of 32 symptoms derived from the US Centers for Disease Control (CDC) list of COVID-19 symptoms (https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html) and the Patient Health Questionnaire Somatic Symptom Scale [24] were at each visit. Symptoms were considered related to COVID-19 if they were new or worsened since SARS-CoV-2 infection; stable, chronic symptoms that predated SARS-CoV-2 infection were not included.

We defined PASC as any COVID-19-attributed symptom that was present during a visit greater than 6 weeks following SARS-CoV-2 infection [25,26]. We conducted sensitivity analyses, comparing those with three or more symptoms to those with fewer than three symptoms or with no symptoms.

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Biospecimen collection

Peripheral blood mononuclear cells (PBMCs), plasma, and serum were cryopreserved prior to testing. PBMCs were isolated using Ficol-Paque in SeptMate Tubes, frozen in heat-inactivated FBS and 10% dimethyl sulfoxide (DMSO), and stored in liquid nitrogen.

Antibody assays

Virus-specific antibody responses were measured using the Pylon COVID-19 total antibody assay (ET Health) and a validated surrogate virus neutralization test (sVNT) [27]. The lower limit of detection was a sVNT reciprocal titer less than 10 and total antibody less than 10 relative fluorescence units.

T-cell responses by intracellular cytokine staining

We performed ICS [22] with the following modifications. Two peptide megapools containing SARS-CoV-2-derived peptides experimentally determined to be recognized by $CD4^+$ or $CD8^+$ T cells were used for 18 h stimulations (1 μg/ml/peptide; CD4-E with 280 and CD8-E with 454 T-cell epitopes) [28]. Additional detail and a complete list of antibodies are listed in Supplementary Methods, http://links.lww.com/QAD/C595. All samples were acquired on a BD LSR-II analyzer and analyzed with FlowJo X. The gating strategy is shown in Supplementary Figure 1, http://links.lww.com/QAD/C597; Supplemental Table 1, http://links.lww.com/QAD/C596.

Markers of inflammation

In a subset with additional specimens, the fully automated HD-X Simoa platform was used to measure plasma biomarkers including monocyte chemoattractant protein 1 (MCP-1), Cytokine 3-PlexA (IL-6, IL-10, TNFα), interferon gamma-induced protein-10 (IP-10), and interferon-gamma (IFNγ).

Statistical methods

For comparison of humoral responses, we log-transformed sVNT and total antibody values to satisfy assumptions of a normal distribution. We used linear regression to examine differences by HIV status, adjusting for days since SARS-CoV-2 infection, age, sex, and COVID-19 hospitalization. Differences and 95% confidence intervals (CI) are presented as fold-changes in the geometric mean. $CD4^+/CD8^+$ ratio was then added as a covariate to examine if it was associated with magnitude of responses. To examine percentage differences in cellular immune responses, we used generalized linear models from the binomial distribution with bootstrapped standard errors and adjusted for the same potential confounders as above. For inflammatory markers, we fit linear regression models following log-transformation. To examine differences in PASC by HIV status, we used logistic regression adjusting for the same potential confounders.

Results

Participants

All participants were diagnosed with COVID-19 between March and December 2020 (pre-Delta and Omicron). The groups were similar in age, sex, and race/ethnicity (Table 1). The majority of PWH were men, reflecting the local HIV epidemic [29]. The vast majority of PWH were on antiretroviral therapy including integrase strand transfer inhibitors (34/39, 87%) and nucleoside reverse transcriptase inhibitors (37/39, 95%); nonnucleoside reverse transcriptase inhibitors (3/39, 8%) and protease inhibitors (2/39, 5%) were rare. PWH more commonly reported medical comorbidities including heart and lung disease. Most were managed as outpatients during acute COVID-19. All previously hospitalized participants required supplemental oxygenation and one in each group required mechanical ventilation. No participant received SARS-CoV-2-specific treatment.

The median time between COVID-19 onset and assessment was similar between groups (118 vs. 111 days, $P = 0.58$; Table 1). Initial severity was also similar (13 vs. 17% hospitalized, $P = 0.41$). Participants reported a wide array of PASC symptoms (Table 2). A larger proportion of PWH reported certain symptoms, such as fatigue, gastrointestinal and certain neurocognitive symptoms, and issues with sleep.

SARS-CoV-2-specific antibody responses

Antibody responses were similar between groups. In models incorporating days since infection, age, sex, and COVID-19 hospitalization, HIV status was not a predictor of humoral responses as measured by SARS-CoV-2-specific antibody binding (1.31-fold higher; 95% CI 0.70–2.46; $P = 0.40$; Fig. 1a) and surrogate virus neutralization (1.01-fold higher; 95% CI 0.63–1.63; $P = 0.95$; Fig. 1b). Hospitalization for COVID-19 was associated with 4.29-fold higher titers of binding antibodies (95% CI: 1.74–10.57; $P = 0.002$) and 2.57-fold higher titers of surrogate viral neutralization (95% CI 1.33–4.98; $P = 0.005$).

SARS-CoV-2-specific T-cell responses

In models incorporating days since infection, age, sex, and COVID-19 hospitalization, HIV status was not a predictor of the magnitude of interferon gamma (IFN)-producing SARS-CoV-2-specific memory $CD4^+$ T-cell responses (1.14-fold higher; 95% CI 0.76–1.71; $P = 0.55$; median: 0.070 vs. 0.068%; Fig. 1c). However, PWH had 70% lower relative levels of SARS-CoV-2-specific memory $CD8^+$ T cells (0.30-fold, 95% CI 0.13–0.72; $P = 0.007$; median: 0.016 vs. 0.034%; Fig. 1c).

PWH exhibited higher levels of PD-1 expression on SARS-CoV-2-specific memory $CD4^+$ T cells in adjusted analyses (1.18-fold higher; 95% CI 1.07–1.30; $P = 0.001$; median: 65 vs. 57.1%; Fig. 1d) but no significant

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Table 1. Characteristics of study cohort.

Characteristic	All ($n=82$)	HIV+ ($n=39$)	HIV− ($n=43$)
Demographic characteristics			
Age	52 (44–58)	54 (46–58)	51 (43–58)
Sex assigned at birth [*n* (%)]			
Female	10 (12)	2 (5)	8 (19)
Male	72 (88)	37 (95)	35 (81)
Race and ethnicity [*n* (%)]			
Hispanic/Latino	26 (32)	14 (36)	9 (21)
White	36 (43)	15 (38)	25 (60)
Black/African American	(10)	7 (18)	1 (2)
Asian	4 (5)	1 (3)	3 (7)
Pacific Islander/native Hawaiian	3 (4)	2 (5)	1 (2)
Not provided	1 (1)	0 (0)	1 (2)
Tobacco use history [*n* (%)]			
Ever smoker	28 (34)	21 (54)	7 (16)
Current smoker	11 (13)	10 (26)	1 (2)
Clinical characteristics			
Concurrent medical conditions [*n* (%)]			
Autoimmune disease	2 (2)	1 (3)	1 (2)
Cancer (with treatment received within 2 years prior to COVID-19 diagnosis)	4 (5)	3 (8)	1 (2)
Diabetes	7 (9)	4 (10)	3 (7)
Heart disease	4 (5)	4 (10)	0 (0)
Pulmonary disease	8 (10)	5 (13)	3 (7)
HIV-related laboratory parameters			
$CD4^+$ T cell count (cells/μl)	639 (486–844)	596 (404–740)	670 (594–918)
$CD4^+/CD8^+$ ratio	1.18 (0.78–1.86)	0.94 (0.51–1.10)	2.00 (1.52–2.32)
Plasma HIV RNA less than 50 copies/ml [*n* (%)]	–	37 (95)	–
BMI	28.1 (24.8–31.0)	28.8 (25.1–32.5)	27.1 (23.9–30.3)
Hospitalized during acute COVID-19 [*n* (%)]	11 (14)	4 (10)	7 (17)
Time since COVID-19 symptom onset (days)	112 (91–129)	118 (85–129)	111 (94–131)

Values reported as median (IQR) unless otherwise specified. Note: autoimmune disease reported as one case of hypothyroidism and one case of Raynaud's disease. Cancer reported as prostate cancer controlled with hormone therapy, ocular melanoma, treated Kaposi sarcoma, and resected renal cancer. Plasma HIV RNA values were greater than 50 in two participants (87 and 28 118 copies/ml). All hospitalized participants in each group required oxygen support and one hospitalized participant in each group required mechanical ventilation. None reported receiving SARS-CoV-2-targeted therapy or steroids during the hospitalization. COVID-19, coronavirus disease 2019; IQR, interquartile range.

differences in PD-1 expression on SARS-CoV-2-specific $CD8^+$ T cells (1.21-fold higher; 95% CI: 0.83–1.76; $P=0.33$; median: 25 vs. 24.1%; Fig. 1d).

Effect of $CD4^+/CD8^+$ ratio

The $CD4^+/CD8^+$ ratio was not predictive of binding antibody levels ($P=0.30$) or surrogate virus neutralization ($P=0.61$). Higher ratios were associated with 67% lower frequency of SARS-CoV-2-specific $CD4^+$ T cells (0.33-fold; 95% CI 0.19–0.97; $P=0.04$) and 36% lower SARS-CoV-2-specific PD-1 expression (0.64-fold; 95% CI 0.57–0.97; $P=0.03$). Notably, higher $CD4^+/CD8^+$ ratios were associated with 66% lower PD-1 expression on SARS-CoV-2-specific $CD8^+$ T cells (0.34-fold, 95% CI 0.13–0.87; $P=0.02$). There was a trend toward a similar finding among HIV-negative individuals (0.70-fold; 95% CI 0.47–1.04; $P=0.08$).

Relationship between antibody and T-cell immune responses

We observed strong correlations between binding ($r=0.33$, $P=0.008$) and surrogate viral neutralization responses ($r=0.33$, $P=0.007$) and between binding and surrogate viral neutralization responses and SARS-CoV-2-specific $CD4^+$ T cells ($r=0.41$, $P<0.001$ and $r=0.42$, $P<0.001$, respectively).

Markers of systemic inflammation

In PWH compared with HIV-negative individuals, mean IL-6 levels were 1.55-fold higher (95% CI 1.06–2.26; $P=0.02$), mean IP-10 levels were 1.31-fold higher (95% CI 1.06–1.62; $P=0.01$), and TNFα levels were 1.26-fold higher (95% CI 1.08–1.47; $P=0.003$).

Postacute sequelae

PWH had 4.01-fold higher odds of PASC (95% CI 1.45–11.1; $P=0.008$; 82.8 vs. 54.4%), in a model controlling for time since infection, hospitalization, and age. This was maintained when defining PASC as three or more symptoms in comparison to fewer than three symptoms [adjusted odds ratio (AOR) 2.72; 1.08–6.88; $P=0.03$; 59.8 vs. 33.6%]. PWH reported more symptoms overall [median 3 (IQR 1–6) versus median 1 (IQR 0–5), $P=0.02$], and those with HIV had a 1.91-fold higher number of PASC symptoms than those without HIV ($P=0.02$).

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Table 2. Symptoms reported at late follow-up among those with postacute sequelae of SARS-CoV-2 infection.

Symptoms reported at late follow-up	All ($n=82$)	HIV+ ($n=39$)	HIV− ($n=43$)
Constitutional			
Fatigue	26 (32)	16 (42)	10 (23)
Subjective fever	1 (1)	1 (3)	0 (0)
Chills	2 (2)	2 (5)	0 (0)
Objective fever	0 (0)	0 (0)	0 (0)
Upper respiratory			
Rhinorrhea	13 (16)	5 (13)	8 (19)
Sore throat	2 (2)	0 (0)	2 (5)
Cardiopulmonary			
Cough	7 (9)	3 (8)	4 (9)
Shortness of breath	20 (25)	11 (29)	9 (21)
Chest pain	7 (9)	3 (8)	4 (9)
Palpitations	10 (12)	4 (11)	6 (14)
Fainting	0 (0)	0 (0)	0 (0)
Gastrointestinal			
Diarrhea	7 (9)	5 (13)	2 (5)
Nausea	9 (11)	5 (13)	4 (9)
Loss of appetite	4 (5)	3 (8)	1 (2)
Abdominal pain	9 (11)	5 (13)	4 (9)
Vomiting	1 (1)	0 (0)	1 (2)
Constipation	0 (0)	0 (0)	0 (0)
Genitourinary			
Menstrual cramps	0 (0)	0 (0)	0 (0)
Dyspareunia	0 (0)	0 (0)	0 (0)
Rash	8 (10)	7 (18)	1 (2)
Musculoskeletal			
Myalgia	14 (17)	9 (24)	5 (12)
Back pain	5 (6)	5 (13)	0 (0)
Joint pain	11 (14)	8 (21)	3 (7)
Anosmia/dysgeusia	14 (17)		
Neurologic			
Headache	14 (17)	9 (24)	5 (12)
Concentration problems	24 (30)	16 (42)	8 (19)
Dizziness	11 (14)	5 (13)	6 (14)
Balance problems	9 (11)	6 (16)	3 (7)
Neuropathy	9 (11)	7 (18)	2 (5)
Vision problems	11 (14)	8 (21)	3 (7)
Parosmia	2 (2)	0 (0)	2 (5)
Trouble sleeping	20 (24)	13 (34)	7 (16)

Values reported as *n* (%). Participants were systematically asked about 32 individual symptoms at the late follow-up visit, which took place a median of 112 days from initial COVID-19 symptom onset.

Antibody and T-cell responses did not correlate with PASC (Fig. 2a–d). In models adjusting for HIV status, higher PD-1 expression on total memory $CD4^+$ T cells, but not memory $CD8^+$ T cells, was independently predictive of lower odds of PASC (Fig. 2e and f). However, this finding appeared to be driven by differences in overall PD-1 expression between the HIV-positive and HIV-negative groups. Interestingly, there appeared to be a trend toward higher PD-1 expression among those with PASC in stratified analyses (Fig. 2e and f). There was no relationship between PD-1 expression on SARS-CoV-2-specific $CD4^+$ or $CD8^+$ T cells and PASC (AOR 0.23; 95% CI 0.01–6.31; $P=0.39$ and AOR 0.20; 0.01–3.50; $P=0.27$, respectively; Fig. 2g and h).

Some inflammatory markers were associated with increased odds of PASC (Fig. 3a–e). After adjusting for HIV status, the odds of PASC in the study population increased 1.18-fold for each 10% increase in IP-10 (AOR 1.18; 95% CI 1.01–1.38; $P=0.04$); and 1.10-fold for each 10% increase in IL-6 (AOR 1.10; 95% CI 1.01–1.21; $P=0.04$); there was a trend in increased PASC with higher TNFα levels (AOR 1.19; 95% CI 0.98–1.46; $P=0.08$).

Among PWH, there were increased odds of PASC with each 10% increase in IP-10 levels (AOR 1.06; 95% CI 1.00–1.11; $P=0.05$), and a trend for increased PASC with higher TNFα levels (AOR 1.20 per 10% increase; 95% CI 0.97–1.49; $P=0.09$) but not IL-6 ($P=0.64$). This analysis was limited by the small number of individuals with HIV who reported full recovery.

Discussion

We observed that HIV status was strongly associated with PASC, raising concerns that this condition might be

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

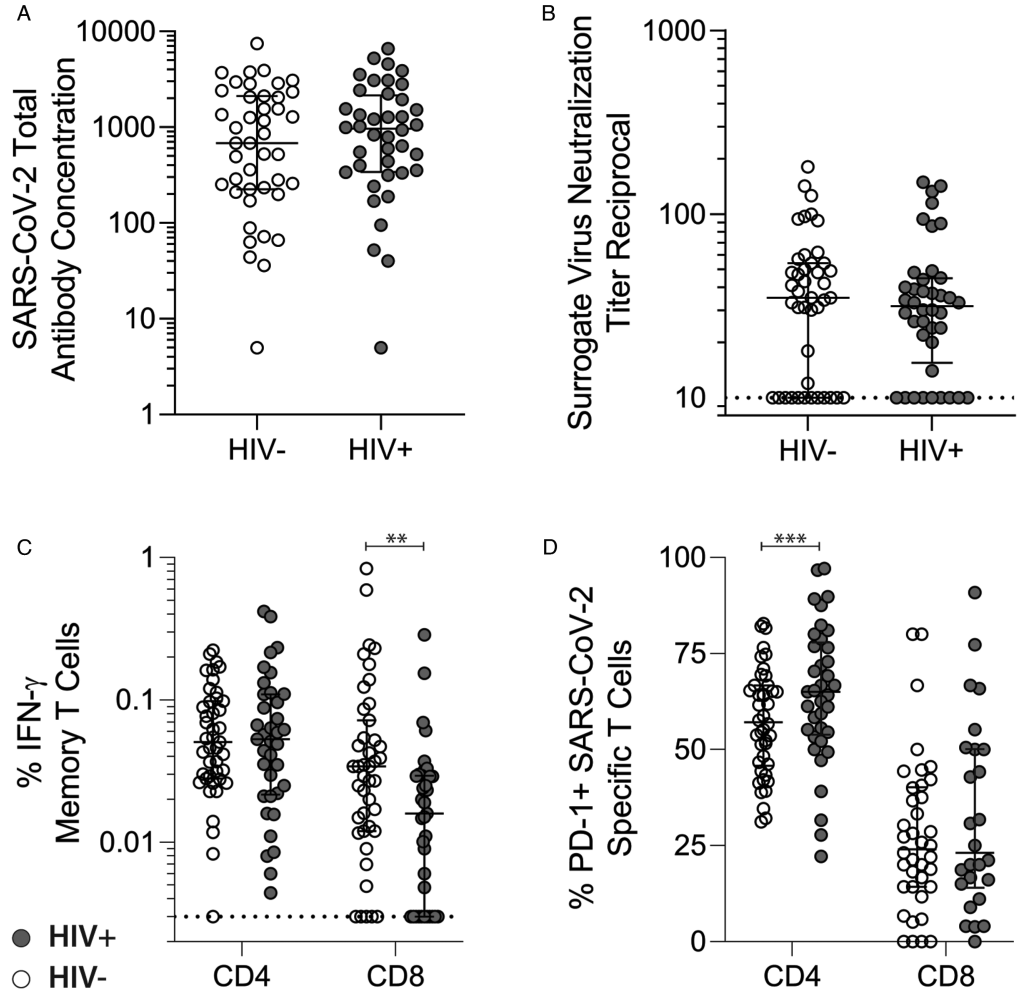

Fig. 1. SARS-CoV-2-specific immunologic parameters among people with and without HIV recovering from coronavirus disease 2019. (a) Total SARS-CoV-2 antibodies. (b) Surrogate SARS-CoV-2 neutralization titres. (c) Frequency of IFNγ+ SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cells. (d) PD-1 expression on SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells. Bars represent median and interquartile ranges. ** *P* less than 0.01; *** *P* less than 0.001 in covariate adjusted linear regression modeling.

common among PWH recovering from COVID-19. Higher levels of inflammation were associated with PASC. Finally, we observed differences in SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells that might have implications for long-term immunity conferred by natural infection. This study adds to the limited data examining SARS-CoV-2-specific immune responses in PWH and underscores the need for larger and more detailed studies of PASC in PWH.

Although there are massive efforts underway to understand the prevalence and pathophysiology of PASC, data among PWH are limited [30]. One study suggested that COVID-19 severity in PWH was associated with PASC [31]; however, it did not find an association with $CD4^+$ T-cell count, viral load, demographics or comorbidities, and did not compare PWH with HIV-negative individuals. Another study suggested that HIV was one factor associated with PASC among those requiring emergency department or hospital-based care [32], but included only 10 PWH and did not include biological measurements. Although our cohort cannot estimate the population-level prevalence of PASC, the observation that persistent SARS-CoV-2-attributed symptoms were highly prevalent in PWH and that the adjusted odds of PASC were four-fold as high as in well matched HIV-negative comparators was striking. Large-scale studies in which HIV can be examined as a predictor of PASC are urgently needed.

PASC may be driven, at least in part, by residual or ongoing inflammation following SARS-CoV-2 infection [9,10]. ART-treated HIV is a chronic inflammatory condition associated with persistent immune activation [15–18]. Further immune perturbations related to COVID-19 may, therefore, lead to a higher prevalence of PASC among PWH. Additional factors could also predispose PWH to PASC, such as autoimmunity [33],

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

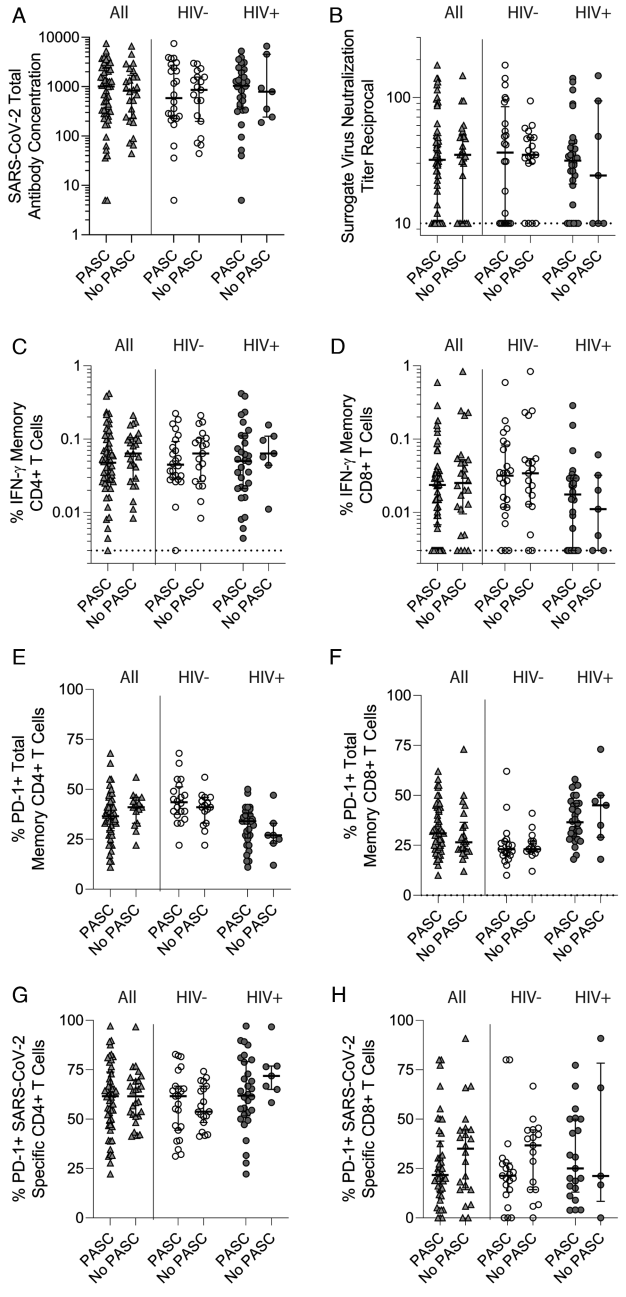

Fig. 2. SARS-CoV-2-specific immune responses among people with and without postacute sequelae of SARS-CoV-2 infection, stratified by HIV status. (a) Total SARS-CoV-2 antibodies. (b) Surrogate viral neutralization titers. (c) SARS-CoV-2-specific $CD4^+$ T cells. (d) SARS-CoV-2-specific $CD8^+$ T cells. (e) PD-1 expression on total memory $CD4^+$ T cells. (f) PD-1 expression on total memory $CD8^+$ T cells. (g) PD-1 expression on SARS-CoV-2-specific memory $CD4^+$ T cells. (h) PD-1 expression on SARS-CoV-2-specific memory $CD8^+$ T cells. Bars represent median and interquartile ranges.

localized tissue inflammation [34], human herpesvirus reactivation [13], and microvascular dysfunction [14]. Other comorbidities including substance use and metabolic disorders may further contribute. Regardless of mechanism, our observation suggests that PASC may be especially common in PWH and emphasizes the need for studies of PASC in this population.

Data comparing SARS-CoV-2-specific adaptive immune responses in people with and without HIV remain limited. Given the association between the presence of potent, durable immune responses and protection from disease upon re-exposure, it is critical to understand how HIV may modulate protective immunity. Furthermore, there is evidence that SARS-CoV-2 can cause chronic infection in certain immunocompromised individuals, including those with advanced HIV infection [35]. There is ongoing concern that PWH are less likely to develop and maintain protective immunity. Although some studies suggested lower humoral responses in PWH [6], we and others [36–38] have not observed this.

Data on cellular immune responses to SARS-CoV-2 infection in PWH are even more limited. A single high-quality study has shown similar T-cell responses between PWH on ART and HIV-negative individuals [7]. Our findings contribute three key observations regarding SARS-CoV-2-specific cellular immune responses in PWH. First, using peptide pools that include optimal SARS-CoV-2 epitopes spanning the proteome, we found that PWH had lower SARS-CoV-2-specific $CD8^+$ T cell responses. This difference was previously observed in non-Spike-specific T cell responses in PWH, and may indicate that PWH have impaired capacity to mount a protective $CD8^+$ T cell response upon re-infection, particularly with heterologous variants with immune-evading mutations in the spike protein. It is also possible that PWH have expansion of other antigen-specific $CD8^+$ T cells (e.g. CMV-specific) thereby diluting the SARS-CoV-2-specific pool as the denominator was total nonnaive $CD8^+$ T cells. Second, we found that SARS-CoV-2-specific $CD4^+$ T cells in PWH had higher expression of the co-inhibitory receptor PD-1, suggesting they may have impaired functionality upon re-encountering infection. Alternatively, PWH may have more SARS-CoV-2 antigen exposure leading to a more exhausted phenotype. Third, we found that a higher $CD4^+/CD8^+$ ratio – which can sometimes be optimized with early ART initiation [39] – was associated with lower expression of PD-1 on SARS-CoV-2-specific $CD8^+$ T cells.

This study was small and underpowered to make comparisons within PWH. Future studies could explore questions regarding differences in post-COVID cellular immunology and cytokine patterns in relation to ART regimen, potential changes in HIV gene expression or viral transcription especially in light of recent observations that viral 'blips' may be common post-COVID [40], and concomitant latent viral infections such as human herpesviruses (e.g. Epstein–Barr virus and cytomegalovirus), which are common among PWH and may

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

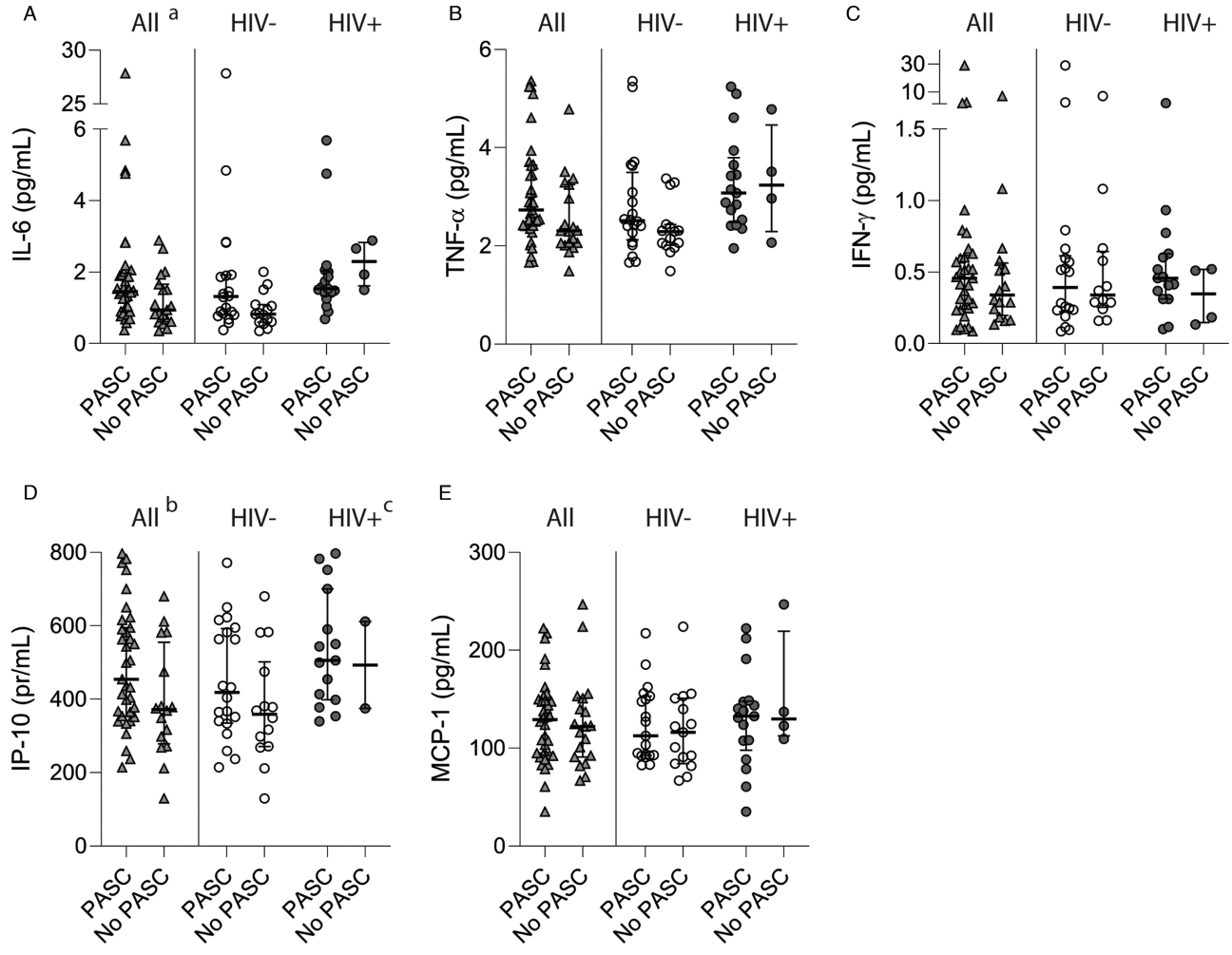

Fig. 3. Plasma markers of immune activation among people with and without postacute sequelae of SARS-CoV-2 infection, stratified by HIV status. (a) Interleukin-6 levels. (b) TNF-alpha levels. (c) Interferon-gamma levels. (d) IP-10 levels. (e) MCP-1 levels. Bars represent median and interquartile ranges. [a]The odds of PASC of the entire study population increased 1.18-fold for each 10% increase in IP-10 (AOR 1.18; 95% CI 1.01–1.38; $P = 0.04$); and [b]1.10-fold for each 10% increase in IL-6 (AOR 1.10; 95% CI 1.01–1.21; $P = 0.04$) from adjusted linear regression. [c]Among PWH, there were increased odds of PASC with each 10% increase in IP-10 levels (AOR 1.06; 95% CI 1.00–1.11; $P = 0.05$). AOR, adjusted odds ratio; CI, confidence interval.

contribute to PASC. Given the nature of recruitment, the high prevalence of PASC should not be considered to represent the population-level prevalence, which is likely lower [41]. Data on other potential confounders, such as comorbid mental health and substance use were unavailable. Inflammatory marker data was not available on all participants, although sample availability was based on the timing of collection and is not expected to bias the results; a wider array of markers including general inflammatory markers (e.g. C-reactive protein, complements, and autoantibodies) should be considered in future analyses. Finally, our population was mostly male, stable on ART, and had strong immune reconstitution, and care should be taken when extrapolating to populations with less access or adherence to ART or those with advanced HIV.

Our analysis provides compelling preliminary evidence suggesting an urgent need to better understand the epidemiology and pathophysiology of PASC within PWH. Such efforts may lead to targeted interventions to prevent or treat PASC among this special population of interest.

Acknowledgements

We are grateful to the study participants. We acknowledge current and former LIINC clinical study team members Tamara Abualhsan, Andrea Alvarez, Melissa Buitrago, Monika Deswal, Emily Fehrman, Heather Hartig, Yanel Hernandez, Marian Kerbleski, James Lombardo, Lynn

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Ngo, Antonio Rodriguez, Dylan Ryder, Ruth Diaz Sanchez, Viva Tai, Cassandra Thanh, Fatima Ticas, Leonel Torres, and Meghann Williams; and LIINC laboratory team members Amanda Buck, Joanna Donatelli, Jill Hakim, Nikita Iyer, Owen Janson, Christopher Nixon, Isaac Thomas, and Keirstinne Turcios. We thank the UCSF AIDS Specimen Bank for processing specimens and maintaining the LIINC biospecimen repository. The intracellular cytokine staining assays were performed in the UCSF Core Immunology Laboratory. We are grateful to Jon Oskarsson, Mary Shiels, Erin Pederson, Parousha Zand, and the UCSF Ward 86 and 360 Positive Health Practices for referring PWH who had COVID-19.

Author contributions: M.J.P., J.D.K., J.N.M., S.G.D., and T.J.H. designed the cohort, which was managed by R.H. and overseen by M.J.P. M.J.P., J.D.K., S.L., R.H., J. N.M., and S.G.D. designed the study instruments. M.J.P., S.E.M., A.F.T., M.I.A., V.T., and R.H. recruited participants, coordinated research visits, administered study questionnaires, and collected clinical data. C.A.F., S. M., A.F.T., S.A.G., J.Y.C., and E.O.M. performed data entry and quality control and addressed or adjudicated issues related to data integrity. S.L. cleaned the data, maintained the database, and oversaw data management. C.Y. performed the antibody measurements in the laboratory of K.L.L. N.K., R.L.R., and T.J.H. performed and/or oversaw the cellular immunology assays in the UCSF Core Immunology Laboratory. A.C., J.W.W., and C.J.P. performed and oversaw the inflammatory marker assays at Monogram Biosciences. D.W. and A.S. provided the SARS-CoV-2 peptide megapools. M.J.P., M.A.S., T. M.D., S.E.M., D.V.G., R.L.R., and T.J.H. performed the analyses. M.J.P., M.A.S., T.M.D., C.A.F., S.E.M., S.M., S. G.D., R.L.R., and T.J.H. drafted the manuscript with input from M.S.D., P.Y.H., P.W.H., J.F.K., J.N.M., D.V. G., and M.G. providing in-depth critical review. The study was funded by grants from M.A.S., M.G., S.G.D., and T.J.H. All authors reviewed, edited, and approved the manuscript.

Funding: This work was supported by the National Institutes of Health (R01 AI141003, R01 AI158013, T32 AI60530, K23 AI157875, and K23 AI135037), and the UCSF/Gladstone Center for AIDS Research (CFAR). MJP received funding from the UCSF Resource Allocation Program and the CFAR Rapid COVID grant program. This work has been supported by NIH contract 75N93019C00065 (A.S., D.W).

Posted History: This manuscript was previously posted to medRxiv: https://doi.org/10.1101/2022.02.10.22270471

Conflicts of interest

A.C., J.W.W., and C.J.P. are employees of Monogram Biosciences. A.S. is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress. and Repertoire. L. J.I. has filed for patent protection for various aspects of T-cell epitope and vaccine design work. T.J.H. reports grants from Merck and Co. and Bristol-Myers Squibb outside the submitted work. The remaining authors have no conflicts related to the current work to report.

References

1. Eisinger RW, Lerner AM, Fauci AS. **Human immunodeficiency virus/AIDS in the era of coronavirus disease 2019: a juxtaposition of 2 pandemics.** *J Infect Dis* 2021; **224**:1455–1461.
2. Ho H-E, Peluso MJ, Margus C, Matias Lopes JP, He C, Gaisa MM, *et al*. **Clinical outcomes and immunologic characteristics of coronavirus disease 2019 in people with human immunodeficiency virus.** *J Infect Dis* 2021; **223**:403–408.
3. Sigel K, Swartz T, Golden E, Paranjpe I, Somani S, Richter F, *et al*. **Coronavirus 2019 and people living with human immunodeficiency virus: outcomes for hospitalized patients in New York City.** *Clin Infect Dis* 2020; **71**:2933–2938.
4. Bertagnolio S, Thwin S, Silva R, Diaz J. Clinical characteristics and prognostic factors in people living with HIV hospitalized with COVID-19: findings from the WHO Global Clinical Platform. In: *11th International AIDS Society Conference on HIV Science*. 2021. pp. 18–21.
5. Bhaskaran K, Rentsch CT, MacKenna B, Schultze A, Mehrkar A, Bates CJ, *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.
6. Spinelli MA, Lynch KL, Yun C, Glidden DV, Peluso MJ, Henrich TJ, *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–e341.
7. Alrubayyi A, Gea-Mallorquí E, Touizer E, Hameiri-Bowen D, Kopycinski J, Charlton B, *et al*. **Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with HIV.** *Nat Commun* 2021; **12**:5839.
8. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, *et al*. **Postacute COVID-19 syndrome.** *Nat Med* 2021; **27**:601–615.
9. Peluso MJ, Lu S, Tang AF, Durstenfeld MS, Ho HE, Goldberg SA, *et al*. **Markers of immune activation and inflammation in individuals with postacute sequelae of severe acute respiratory syndrome coronavirus 2 infection.** *J Infect Dis* 2021; **224**:1839–1848.
10. Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, *et al*. **Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection.** *Nat Immunol* 2022:1–7.
11. Ong SWX, Fong S-W, Young BE, Chan YH, Lee B, Amrun SN, *et al*. **Persistent symptoms and association with inflammatory cytokine signatures in recovered coronavirus disease 2019 patients.** *Open Forum Infect Dis* 2021; **8**:ofab156.
12. Seeßle J, Waterboer T, Hippchen T, Simon J, Kirchner M, Lim A, *et al*. **Persistent symptoms in adult patients one year after COVID-19: a prospective cohort study.** *Clin Infect Dis* 2021; **74**:1191–1198.
13. Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J, *et al*. **Multiple early factors anticipate post-acute COVID-19 sequelae.** *Cell* 2022; **185**:881.e20–895.e20.
14. Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, Kell DB. **Persistent clotting protein pathology in long COVID/post-acute sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin.** *Cardiovasc Diabetol* 2021; **20**:172.
15. Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, *et al*. **HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality.** *PLoS Pathog* 2014; **10**: e1004078.

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

16. Hunt PW, Sinclair E, Rodriguez B, Shive C, Clagett B, Funderburg N, *et al*. **Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection.** *J Infect Dis* 2014; **210**:1228–1238.
17. Méndez-Lagares G, Romero-Sánchez MC, Ruiz-Mateos E, Genebat M, Ferrando-Martínez S, Muñoz-Fernández MA, *et al*. **Long-term suppressive combined antiretroviral treatment does not normalize the serum level of soluble CD14.** *J Infect Dis* 2013; **207**:1221–1225.
18. Borges ÁH, O'Connor JL, Phillips AN, Rönsholt FF, Pett S, Vjecha MJ, *et al*., INSIGHT SMART and ESPRIT Study Groups and the SILCAAT Scientific Committee. **Factors associated with plasma IL-6 levels during HIV infection.** *J Infect Dis* 2015; **212**:585–595.
19. Peluso MJ, Kelly JD, Lu S, Goldberg SA, Davidson MC, Mathur S, *et al*. **Rapid implementation of a cohort for the study of postacute sequelae of SARS-CoV-2 infection/COVID-19.** *medRxiv* 2021[Preprint].
20. Peluso MJ, Kelly JD, Lu S, Davidson MC, Mathur S, Durstenfeld MS, *et al*. **Persistence, magnitude and patterns of postacute symptoms and quality of life following onset of SARS-CoV-2 infection: cohort description and approaches for measurement.** *Open Forum Infect Dis* 2021; **9**:ofab640.
21. Peluso MJ, Takahashi S, Hakim J, Kelly JD, Torres L, Iyer NS, *et al*. **SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay.** *Sci Adv* 2021; **7**: eabh3409.
22. Peluso MJ, Deitchman AN, Torres L, Iyer NS, Munter SE, Nixon CC, *et al*. **Long-term SARS-CoV-2-specific immune and inflammatory responses in individuals recovering from COVID-19 with and without postacute symptoms.** *Cell Rep* 2021:109518.
23. Peluso MJ, Sans HM, Forman CA, Nylander AN, Ho HE, Lu S, *et al*. **Plasma markers of neurologic injury and systemic inflammation in individuals with self-reported neurologic postacute sequelae of SARS-CoV-2 infection (PASC).** *bioRxiv* 2021; **9**:e200003.
24. Kroenke K, Spitzer RL, Williams JBW. **The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms.** *Psychosom Med* 2002; **64**:258–266.
25. CDC. Healthcare workers. 2021. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/postcovid-index.html. [Accessed 25 January 2022]
26. Headquarters WHO. A clinical case definition of post COVID-19 condition by a Delphi consensus, 6 October 2021. World Health Organization, 2021. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1. [Accessed 25 January 2022]
27. Luo YR, Yun C, Chakraborty I, Wu AHB, Lynch KL. **A SARS-CoV-2 label-free surrogate virus neutralization test and a longitudinal study of antibody characteristics in COVID-19 patients.** *J Clin Microbiol* 2021; **59**:e0019321.
28. Tarke A, Sidney J, Kidd CK, Dan JM, Ramirez SI, Yu ED, *et al*. **Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases.** *Cell Rep Med* 2021; **2**:100204.
29. Department of public health. Available at: https://www.sfdph.org/dph/comupg/oprograms/HIVepiSec/HIVepiSecReports.asp. [Accessed 3 February 2022]
30. Peluso MJ, Hellmuth J, Chow FC. **Central nervous system effects of COVID-19 in people with HIV infection.** *Curr HIV/AIDS Rep* 2021; **18**:538–548.
31. Pujari S, Gaikwad S, Chitalikar A, Dabhade D, Joshi K, Bele V. **Long-coronavirus disease among people living with HIV in western India: an observational study.** *Immun Inflamm Dis* 2021; **9**:1037–1043.
32. Kingery JR, Safford MM, Martin P, Lau JD, Rajan M, Wehmeyer GT, *et al*. **Health status, persistent symptoms, and effort intolerance one year after acute COVID-19 infection.** *J Gen Intern Med* 2022; **37**:1218–1225.
33. Zandman-Goddard G, Shoenfeld Y. **HIV and autoimmunity.** *Autoimmun Rev* 2002; **1**:329–337.
34. Khoury G, Fromentin R, Solomon A, Hartogensis W, Killian M, Hoh R, *et al*. **Human immunodeficiency virus persistence and T-cell activation in blood, rectal, and lymph node tissue in human immunodeficiency virus-infected individuals receiving suppressive antiretroviral therapy.** *J Infect Dis* 2017; **215**:911–919.
35. Meiring S, Tempia S, Bhiman JN, Buys A, Kleynhans J, Makhasi M, *et al*. **Prolonged shedding of SARS-CoV-2 at high viral loads amongst hospitalised immunocompromised persons living with HIV, South Africa.** *Clin Infect Dis* 2022:ciac077.
36. Verburgh ML, Boyd A, Wit FWNM, van der Loeff MFS, van der Valk M, Bakker M, *et al*. **Similar risk of SARS-CoV-2 infection and similar nucleocapsid antibody levels in people with well controlled HIV and a comparable cohort of people without HIV.** *J Infect Dis* 2021; **225**:1937–1947.
37. Martín-Vicente M, Berenguer J, Muñoz-Gómez MJ, Díez C, Micán R, Pérez-Elías MJ, *et al*. **Similar humoral immune responses against the SARS-CoV-2 spike protein in HIV and non-HIV individuals after COVID-19.** *J Infect* 2021; **84**:418–467.
38. Snyman J, Hwa S-H, Krause R, Muema D, Reddy T, Ganga Y, *et al*. **Similar antibody responses against SARS-CoV-2 in HIV uninfected and infected individuals on antiretroviral therapy during the first South African infection wave.** *Clin Infect Dis* 2021ciab758 [Epub ahead of print].
39. Gras L, May M, Ryder LP, Trickey A, Helleberg M, Obel N, *et al*., Antiretroviral Therapy Cohort Collaboration (ART-CC). **Determinants of restoration of CD4 and CD8 cell counts and their ratio in HIV-1-positive individuals with sustained virological suppression on antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2019; **80**:292–300.
40. Peluso MJ, Bakkour S, Busch MP, Deeks SG, Henrich TJ. **A high percentage of people with human immunodeficiency virus (HIV) on antiretroviral therapy experience detectable low-level plasma HIV-1 RNA following coronavirus disease 2019 (COVID-19).** *Clin Infect Dis* 2021; **73**:e2845–e2846.
41. Ayoubkhani D, Pawelek P, Gaughan C. Technical article: Updated estimates of the prevalence of postacute symptoms among people with coronavirus (COVID-19) in the UK - Office for National Statistics Office for National Statistics. 2021. Available at: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/articles/technicalarticleupdatedestimatesoftheprevalenceofpostacutesymptomsamongpeoplewithcoronaviruscovid19intheuk/26april2020to1august2021. [Accessed 20 September 2021]

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.