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SUPPLEMENT ARTICLE

Immune Status and SARS-CoV-2 Viral Dynamics

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Immunocompromised individuals are disproportionately affected by severe coronavirus disease 2019, but immune compromise is heterogenous, and viral dynamics may vary by the degree of immunosuppression. In this study, we categorized ACTIV-2/A5401 participants based on the extent of immunocompromise into none, mild, moderate, and severe immunocompromise. Moderate/severe immunocompromise was associated with higher nasal viral load at enrollment (adjusted difference in means: 0.47 95% confidence interval, .12–.83 $\log_{10}$ copies/mL) and showed a trend toward higher cumulative nasal RNA levels and plasma viremia compared to nonimmunocompromised individuals. Immunosuppression leads to greater viral shedding and altered severe acute respiratory syndrome coronavirus 2 viral decay kinetics.

Clinical Trials Registration. NCT04518410.

Keywords. COVID-19; RNA; SARS-CoV-2; immunocompromise.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disproportionately affects immunocompromised individuals, leading to prolonged symptoms and higher rates of hospitalization and death [1]. Multiple case reports and case series have demonstrated prolonged viral shedding and evolution in severely immunocompromised individuals [2–4]. However, immunocompromised persons have heterogeneous disease processes and broad-ranging degrees of immunosuppression. There is a knowledge gap in the relationship between different types and degree of immunocompromise and SARS-CoV-2 virological features, as there have been few larger-scale studies with systematic categorization of immunocompromised conditions and comprehensive virological evaluation. To this end, we leveraged accelerating COVID-19 therapeutic interventions and vaccines-2 (ACTIV-2)/A5401, a platform trial studying different coronavirus disease 2019 (COVID-19) therapeutics in the outpatient setting, to answer these questions.

METHODS

Study Design

ACTIV-2/A5401 is a multicenter phase 2/3 adaptive platform trial to evaluate COVID-19 therapeutics in nonhospitalized adults (ClinicalTrials.gov identifier NCT04518410) [5]. Nonhospitalized individuals ≥18 years were eligible if they had documented SARS-CoV-2 infection, ≤10 days of COVID-19 symptoms, and ongoing symptoms within 24–48 hours before enrollment [5]. Participants were randomized to 1 of a number of investigational agents or blinded placebo.

Immunocompromise Categorization

Based on a recent publication describing humoral response to COVID-19 vaccination for different immunocompromising conditions [6], we categorized participants as immunocompetent (no immunocompromising conditions impacting humoral response) or mildly, moderately, or severely immunocompromised using medical history and medications reported at study entry. Mild immunocompromising conditions included diabetes mellitus, kidney and liver diseases, autoimmune diseases not receiving immunosuppressants, and human immunodeficiency virus (HIV) infection. Moderate immunocompromising conditions included autoimmune diseases receiving immunosuppressants and solid malignant tumor. Severe immunocompromising conditions included solid organ or stem cell transplant, B-cell deficiency, lymphoma/leukemia, or receiving ≥3 types of concurrent immunosuppressants. Detailed criteria are listed in Supplementary Table 1 and moderate/severe

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immunocompromising conditions are listed in Supplementary Table 2.

Virologic and Serologic Measures

Anterior nasal and plasma SARS-CoV-2 RNA was measured with quantitative polymerase chain reaction with a lower limit of quantification (LLOQ) of 2.0 $\log_{10}$ copies/mL and a limit of detection of 1.4 $\log_{10}$ copies/mL, as reported in our previous studies [5, 7]. Nasal RNA was measured at day 0 (enrollment) and at days 3, 7, 14, and 28. Plasma RNA was measured at days 0 and 7. SARS-CoV-2 serostatus was assessed by Bio-Plex platform for binding immunoglobulin G (IgG) antibodies to nucleocapsid (N), receptor-binding domain, and spike S1 domain for participants enrolled to the first agent in the trial (n = 309) and subsequently by Roche platform (anti-N and anti-S binding IgG) for all other participants, except that no serology testing was performed for participants enrolled to the open-label uncontrolled arm of bamlanivimab. Seropositivity was defined as any detectable IgG to any of the antigens tested.

Statistical Analysis

SARS-CoV-2 RNA levels (copies/mL) were transformed to the $\log_{10}$ scale for all analyses. At day 0, associations between immunocompromised status and SARS-CoV-2 RNA levels from nasal swabs were evaluated using linear regression models for censored data (RNA values below the LLOQ were left censored), and associations with SARS-CoV-2 RNA from plasma (detectable vs undetectable) were evaluated using Poisson regression with robust variance. Longitudinal analyses of SARS-CoV-2 RNA levels from nasal swabs, defined as the $\log_{10}$ area under the curve (AUC) above the LLOQ through day 28, were compared between immunocompromised groups using linear regression, restricted to those who received placebo and who were above the LLOQ at day 0. Longitudinal summaries of plasma RNA were descriptive. Regression models adjusted for potential confounders including age, sex, race/ethnicity, body mass index, smoking, symptom duration at entry, and COVID-19 vaccination status were examined. Given the small number of severely immunocompromised participants, the moderate and severe immunocompromise groups were combined for analyses. Statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

Participants included in this analysis were enrolled between 27 August 2020 and 17 August 2021 and included 577 immunocompromised (383 mild, 159 moderate, 35 severe) and 1956 immunocompetent participants. Symptom duration (median, 6 days) and vaccination status (95% unvaccinated) at entry were similar across immunocompromised groups (Supplementary Tables 1 and 2). Median age was 48 years and 52% of participants were female. In the subset of

Table 1. Summary of Association Between Immunocompromised Status (None, Mild, Moderate/Severe) and Severe Acute Respiratory Syndrome Coronavirus 2 RNA Levels From Nasal Swabs and Plasma

Immunocompromised status and day 0 nasal swab SARS-CoV-2 RNA levels ($\log_{10}$ copies/mL)				
Model[a]		Difference in Means	(95% CI)	*P* Value
Unadjusted (n = 2533)	Mild vs none	0.18	(−.10 to .47)	.21
	Moderate/severe vs none	0.73	(.35–1.11)	<.001
Adjusted[b] (n = 2438)	Mild vs none	0.16	(−.10 to .43)	.23
	Moderate/severe vs none	0.47	(.12–.83)	.009
Immunocompromised status and day 0 plasma SARS-CoV-2 RNA (detectable vs undetectable)				
Model[c]		Risk Ratio	(95% CI)	*P* Value
Unadjusted (n = 2351)	Mild vs none	1.20	(1.00–1.45)	.054
	Moderate/severe vs none	1.29	(1.02–1.63)	.036
Adjusted[b] (n = 2256)	Mild vs none	1.15	(.95–1.39)	.14
	Moderate/severe vs none	1.18	(.93–1.51)	.18
Immunocompromised status and nasal swab SARS-CoV-2 RNA $\log_{10}$ AUC (day 0 to 28)				
Model[d]		Difference in Means	(95% CI)	*P* Value
Adjusted for day 0 nasal RNA (n = 476)	Mild vs none	−0.009	(−.117 to .009)	.87
	Moderate/severe vs none	0.142	(−.022 to .305)	.09
Fully adjusted[b] (n = 453)	Mild vs none	−0.022	(−.131 to .087)	.69
	Moderate/severe vs none	0.135	(−.038 to .308)	.13

Abbreviations: AUC, area under the curve; CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Linear regression for censored data with RNA values below the lower limit of quantification (LLOQ) left censored.

[b]Adjusted for day 0 nasal RNA, age (years), sex (female vs male), body mass index (≤35 vs >35 kg/m^2), smoking (current/former vs never), symptom duration at entry (days), and coronavirus disease 2019 vaccination status (any doses vs none).

[c]Modified Poisson regression with robust variance.

[d]Linear regression, restricted to those on placebo with day 0 RNA above the LLOQ.

participants with available day 0 SARS-CoV-2 serology results (n = 1475), a smaller percentage of the moderate/severe immunocompromise group was seropositive at baseline (33% [30/90]), compared to 54% (121/225) in the mild group and 44% (510/1160) in the nonimmunocompromised group ($P = .002$; Supplementary Table 3).

At enrollment, median nasal RNA level was 4.57 $\log_{10}$ copies/mL (quartiles 2.31, 6.33), and 25% (581/2351) had detectable plasma RNA. Nasal RNA levels were higher in the moderate/severe immunocompromise group compared to the immunocompetent group in both unadjusted and adjusted models (adjusted difference in means, 0.47 [95% confidence interval {CI}, .12–.83] $\log_{10}$ copies/mL; Table 1). Mild immunocompromise participants had nasal RNA level comparable to immunocompetent participants at day 0 (adjusted difference in means, 0.16 [95% CI, −.10 to .43] $\log_{10}$ copies/mL; Table 1). The moderate/severe immunocompromise group tended to have higher nasal RNA levels, regardless of symptom duration at entry (Supplementary Figure 1). Plasma RNA was detectable in 23.5% of immunocompetent participants and in 28.2% and 30.2% of participants with mild and moderate/severe immunocompromise, respectively. In unadjusted analysis, the moderate/severe group was associated with higher risk of having detectable plasma RNA compared to the immunocompetent group (risk ratio [RR], 1.29 [95% CI, 1.02–1.63]; Table 1), but the RR was attenuated with adjustment for potential confounders (adjusted RR, 1.18 [95% CI, .93–1.51]; Table 1).

In the longitudinal analysis, which included 685 participants who received placebo, 538 were immunocompetent, 106 had mild immunocompromise, and 41 had moderate/severe immunocompromise. At day 28, 12.9% (4/31) of participants in the moderate/severe immunocompromise group had detectable nasal RNA, compared to 5.3% (5/94) in the mild and 7.8% (36/460) in the immunocompetent group. Longitudinal SARS-CoV-2 RNA levels from participants with day 0 SARS-CoV-2 RNA levels above the LLOQ are shown in Figure 1, Supplementary Figure 2, and Supplementary Table 4. There was a trend toward higher cumulative nasal RNA level across the 28-day follow-up in the moderate/severe group compared to the immunocompetent group (adjusted mean difference in RNA $\log_{10}$ AUC, 0.135 [95% CI, −0.038 to 0.308], Table 1; corresponding to a 1.36 [95% CI, .92–2.03] fold difference). In contrast, the mild immunocompromise group showed no differences in AUC compared to the immunocompetent group (Table 1). There was a numerically higher proportion of immunocompromised participants with detectable plasma viral RNA at day 7 (moderate/severe 9%, mild 11%) compared to the immunocompetent group (3%) (Supplementary Table 5).

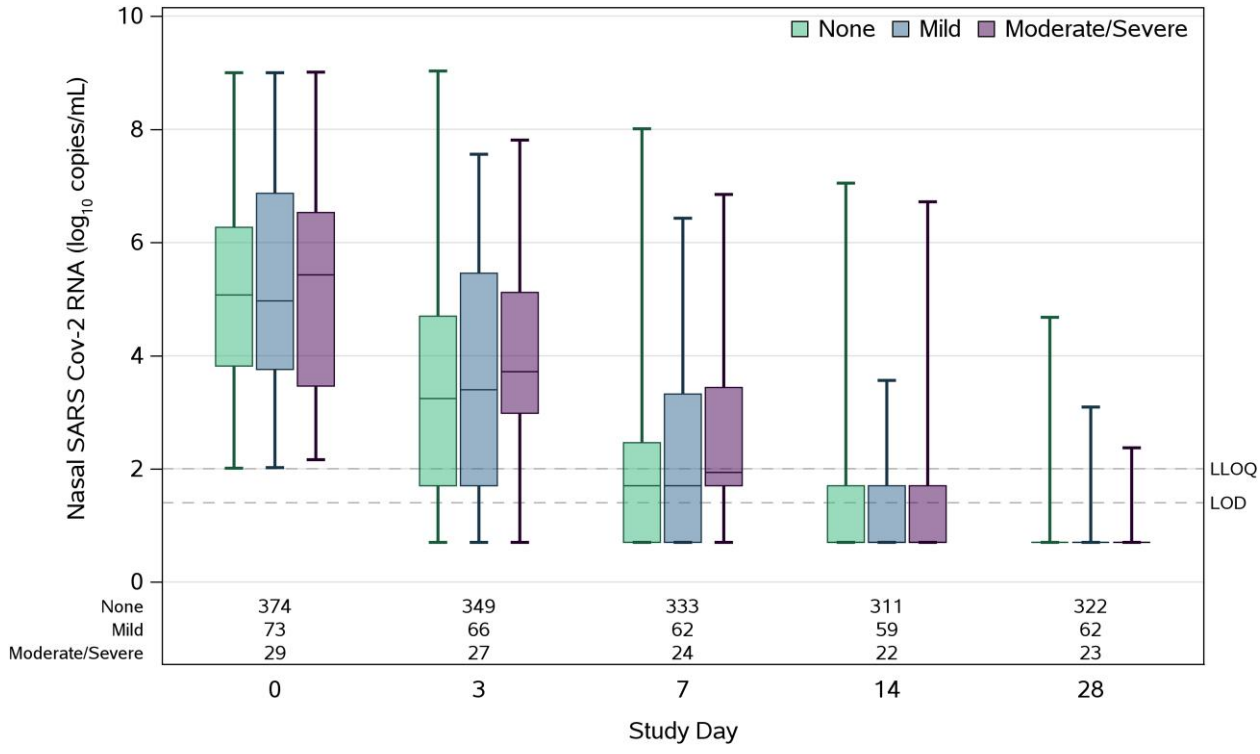

Figure 1. Nasal RNA over time by immunocompromised status among participants on placebo. Only participants with day 0 severe acute respiratory syndrome coronavirus 2 RNA levels above the lower limit of quantification are shown. Abbreviations: LLOQ, lower limit of quantification; LOD, limit of detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

DISCUSSION

In this study, we evaluated SARS-CoV-2 viral dynamics over a range of severity of immunocompromise in outpatient clinical trial participants. We demonstrated that, in a largely unvaccinated study population, moderate/severe immunocompromise (mainly malignancy, autoimmune conditions treated with immunosuppression, B-cell deficiency, hematological malignancy, and stem cell or solid organ transplant) was associated with higher nasal viral RNA levels at the time of study enrollment and a trend toward higher cumulative nasal viral RNA levels longitudinally. In addition, there was a signal that systemic/disseminated virus (plasma RNA detection) may be more prolonged in moderate/severely immunocompromised persons.

Previous studies have shown that severe immunocompromise is associated with prolonged viral shedding and replication that could last weeks to months [2, 4, 6]. In a cohort study, Maltezou et al demonstrated that immunosuppression is associated with higher upper respiratory tract viral load in a cross-sectional analysis [8]. Our study took a further step by stratifying the degree of immunocompromise. Mildly immunocompromising conditions (especially diabetes and kidney disease), though associated with lower levels of antibody response in previous studies [9–11], were not associated with lower seropositivity rates or higher viral burden at entry. Longitudinally, most participants cleared the SARS-CoV-2 nasal RNA by day 28, although the moderate/severe immunocompromise group tended to have higher cumulative nasal viral RNA, which is consistent with previous cases reports and case series [1, 2, 6].

Plasma SARS-CoV-2 RNA, an important biomarker associated with COVID-19 outcomes [12–14], did not differ among immunocompromise groups at enrollment. However, we note that both the mild and moderate/severe immunocompromise groups had delayed plasma SARS-CoV-2 RNA clearance at day 7. This finding is reminiscent of our previous findings in an emergency department cohort, which showed that delayed SARS-CoV-2 plasma RNA clearance was linked to impaired antibody development and predicted higher mortality [15], likely due to increased viral dissemination and end-organ tissue damage [13].

There are some limitations to this study. All participants were enrolled before the introduction of the Omicron variant. Due to limited availability of vaccinations during the study period, most were unvaccinated. Future studies are needed to understand the impact of both SARS-CoV-2 vaccination and current variants on viral dynamics. Furthermore, the longitudinal analyses in this study are limited by small sample size due to being restricted to the placebo recipients. Due to limited number of participants with severe immunocompromising conditions, we were unable to further dissect viral dynamics in participants with T-cell, B-cell, and mixed T-cell/B-cell immunodeficiency.

In conclusion, in primarily unvaccinated persons with acute COVID-19, nasal SARS-CoV-2 burden is greater in those with moderate/severe immunocompromise, and risk of prolonged plasma viremia may be increased with all degrees of immunocompromise. These findings shed light on potential mechanisms of increased risk of progression to severe disease in persons across the clinical spectrum of conditions with impaired immune responses.

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

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References

1. Dioverti V, Salto-Alejandre S, Haidar G. Immunocompromised patients with protracted COVID-19: a review of "long persisters." Curr Transplant Rep **2022**; 9:209–18.
2. Choi B, Choudhary MC, Regan J, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med **2020**; 383:2291–3.
3. Gandhi S, Klein J, Robertson AJ, et al. De novo emergence of a remdesivir resistance mutation during treatment of persistent SARS-CoV-2 infection in an immunocompromised patient: a case report. Nat Commun **2022**; 13:1547.
4. Hensley MK, Bain WG, Jacobs J, et al. Intractable coronavirus disease 2019 (COVID-19) and prolonged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in a chimeric antigen receptor-modified T-cell therapy recipient: a case study. Clin Infect Dis **2021**:73: e815–21.
5. Li Y, Harrison LJ, Chew KW, et al. Nasal and plasma SARS-CoV-2 RNA levels are associated with timing of symptom resolution in the ACTIV-2 trial of non-hospitalized adults with COVID-19. Clin Infect Dis **2023**; 76:734–7.
6. Haidar G, Agha M, Bilderback A, et al. Prospective evaluation of coronavirus disease 2019 (COVID-19) vaccine responses across a broad spectrum of immunocompromising conditions: the COVID-19 Vaccination in the Immunocompromised Study (COVICS). Clin Infect Dis **2022**; 75:e630–44.
7. Chew KW, Moser C, Daar ES, et al. Antiviral and clinical activity of bamlanivimab in a randomized trial of non-hospitalized adults with COVID-19. Nat Commun **2022**; 13:4931.
8. Maltezou HC, Raftopoulos V, Vorou R, et al. Association between upper respiratory tract viral load, comorbidities, disease severity, and outcome of patients with SARS-CoV-2 infection. J Infect Dis **2021**; 223:1132–8.
9. Zhao M, Slotkin R, Sheth AH, et al. Serum neutralizing antibody titers 12 months after COVID-19 mRNA vaccination: correlation to clinical variables in an adult, US-population. Clin Infect Dis **2023**; 76:e391–9.
10. Kolb T, Fischer S, Müller L, et al. Impaired immune response to SARS-CoV-2 vaccination in dialysis patients and in kidney transplant recipients. Kidney360 **2021**; 2: 1491–8.

11. Piotrowska M, Zieliński M, Tylicki L, et al. Local and systemic immunity are impaired in end-stage-renal-disease patients treated with hemodialysis, peritoneal dialysis and kidney transplant recipients immunized with BNT162b2 Pfizer-BioNTech SARS-CoV-2 vaccine. Front Immunol **2022**; 13:832924.
12. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun **2020**; 11:5493.
13. Li Y, Schneider AM, Mehta A, et al. SARS-CoV-2 viremia is associated with distinct proteomic pathways and predicts COVID-19 outcomes. J Clin Invest **2021**; 131:e148635.
14. Su Y, Yuan D, Chen DG, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. Cell **2022**; 185: 881–95.e20.
15. Wang C, Li Y, Kaplonek P, et al. The kinetics of SARS-CoV-2 antibody development is associated with clearance of RNAemia. mBio **2022**; 13:e0157722.