

Predictors of SARS-CoV-2 RNA From Nasopharyngeal Swabs and Concordance With Other Compartments in Nonhospitalized Adults With Mild to Moderate COVID-19

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Background. Identifying characteristics associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA shedding may be useful to understand viral compartmentalization, disease pathogenesis, and risks for viral transmission.

Methods. Participants were enrolled August 2020 to February 2021 in ACTIV-2/A5401, a placebo-controlled platform trial evaluating investigational therapies for mild-to-moderate coronavirus disease 2019 (COVID-19), and underwent quantitative SARS-CoV-2 RNA testing on nasopharyngeal and anterior nasal swabs, oral wash/saliva, and plasma at entry (day 0, pretreatment) and days 3, 7, 14, and 28. Concordance of RNA levels (copies/mL) across compartments and predictors of nasopharyngeal RNA levels were assessed at entry (n = 537). Predictors of changes over time were evaluated among placebo recipients (n = 265) with censored linear regression models.

Results. Nasopharyngeal and anterior nasal RNA levels at study entry were highly correlated ($r = 0.84$); higher levels of both were associated with greater detection of RNA in plasma and oral wash/saliva. Older age, White non-Hispanic race/ethnicity, lower body mass index (BMI), SARS-CoV-2 immunoglobulin G seronegativity, and shorter prior symptom duration were associated with higher nasopharyngeal RNA at entry. In adjusted models, body mass index and race/ethnicity associations were attenuated, but the association with age remained (for every 10 years older, mean nasopharyngeal RNA was 0.27 log₁₀ copies/mL higher; $P < .001$). Examining longitudinal viral RNA levels among placebo recipients, women had faster declines in nasopharyngeal RNA than men (mean change, -2.0 vs -1.3 log₁₀ copies/mL, entry to day 3; $P < .001$).

Conclusions. SARS-CoV-2 RNA shedding was concordant across compartments. Age was strongly associated with viral shedding, and men had slower viral clearance than women, which could explain sex differences in acute COVID-19 outcomes.

Keywords. SARS-CoV-2 RNA; COVID-19; nasal swabs; nasopharyngeal swabs; predictors; serostatus; sex differences.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19), is

primarily transmitted through viral shedding from the upper respiratory tract. SARS-CoV-2 RNA can be found across upper respiratory tract compartments that can be readily sampled, such as the anterior nose, nasopharynx, and oropharynx [1]. High levels of viral RNA sampled from the respiratory tract have been linked to an increased risk of disease severity, detection of culturable virus, and increased risk of viral transmission [2–4]. The diagnosis and monitoring of SARS-CoV-2 infection has thus relied on viral RNA or antigen detection through upper respiratory tract sampling, though the detection of SARS-CoV-2 is not limited to the respiratory tract as viral RNA can also be detected in plasma RNAemia, and levels of plasma RNAemia are associated with risk of disease progression [5, 6].

There remains uncertainty over differences in the levels and duration of viral shedding across different respiratory and blood compartments. Rigorous characterization across compartments

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has been limited by the logistical challenges of performing intensive, longitudinal sampling from multiple sites at standardized time points. In addition, although the vast majority of studies to date have relied on qPCR cycle threshold (Ct) values as a surrogate for viral load, it is well known that Ct value interpretation and comparison can be unreliable due to variation between runs, instruments, and methodologies [7].

In this study, we analyzed quantitative SARS-CoV-2 RNA results obtained from longitudinal nasopharyngeal (NP), anterior nasal (AN), oral wash/saliva, and plasma samples collected from outpatients with mild-to-moderate COVID-19 in the ACTIV-2/A5401 study, enrolled during the pre-Omicron era. We assessed the relationship between RNA levels in these compartments at study entry and across follow-up in those receiving placebo. We also evaluated the impact of symptom duration and other baseline factors on SARS-CoV-2 RNA kinetics. Identifying characteristics that are associated with levels of upper respiratory tract viral shedding may be useful in understanding viral compartmentalization, disease pathogenesis, and viral transmission [8–10].

METHODS

Study Design and Population

ACTIV-2/A5401 is a phase II/III randomized controlled platform trial designed to evaluate the safety and efficacy of investigational agents for treatment of nonhospitalized adults with mild or moderate COVID-19 (NCT04518410). The population for this report included participants enrolled in the placebo-controlled phase II evaluations of the first 2 agents studied in ACTIV-2, bamlanivimab and amubarvimab + romlusevimab [11, 12]. Participants were randomized to bamlanivimab or placebo between August and November 2020 and to amubarvimab + romlusevimab or placebo between January and February 2021. Enrollment to amubarvimab + romlusevimab or placebo was restricted to persons at higher risk for severe COVID-19 (see the [Supplementary Data](#) for protocol definition of higher risk). All participants were enrolled in the United States within 10 days of symptom onset, had documented SARS-CoV-2 infection by viral RNA or antigen testing, and had symptoms present within 24 (protocol version 2.0) or 48 (protocol version 1.0) hours of study entry.

Patient Consent

The protocol was approved by a central institutional review board (IRB), Advarra (Pro00045266), with additional local IRB review and approval as required by participating sites. All participants provided written informed consent.

Virology

Dry NP swabs, dry AN swabs, oral wash/saliva (on a subset), and plasma samples were collected using standardized

procedures at study entry/day 0 (before initiating treatment) and days 3, 7, 14, and 28. NP swabs, oral wash/saliva, and plasma were collected by site staff, and AN swabs were self-collected by the participants. The collection methods are described in the [Supplementary Data](#).

All samples were frozen and stored at -80°C (-65°C to -95°C) and shipped on dry ice to the central laboratory (University of Washington) for quantitative SARS-CoV-2 RNA testing using the qPCR Abbott m2000sp/rt platform with a validated internal standard. The collection, storage, processing, and assay methods have previously been validated [13].

The SARS-CoV-2 RNA qPCR assay has a limit of detection (LoD) of 25 copies/mL ($1.4 \log_{10}$ copies/mL), a lower limit of quantification (LLoQ) of 100 copies/mL ($2 \log_{10}$ copies/mL), and an upper limit of quantification (ULoQ) of 10 million copies/mL ($7 \log_{10}$ copies/mL). Assays were rerun with dilution for results above the ULoQ to obtain a quantifiable result that was rescaled by the dilution factor.

Serology

Serum binding antibody assays were performed at day 0 to evaluate immunoglobulin (Ig)G responses to SARS-CoV-2 nucleocapsid (N), spike S1 and S2 domains, and receptor binding domain (RBD) using the Bio-Plex multiplex assay, per the manufacturer's protocols (Bio-Rad Laboratories, Inc, Hercules, CA, USA). IgG seropositivity was defined as detectable IgG to any of N, RBD, S1, and S2 antigens, where "detectable" was defined using manufacturer-specified thresholds or determined in-house following manufacturer recommendations for values above the limit of detection [14].

Statistical Analyses

SARS-CoV-2 RNA values (copies/mL) were transformed to \log_{10} scale for all analyses. Baseline associations were evaluated among all participants. For samples that resulted in unquantifiable RNA levels above the ULoQ (4 of the 537 participants), an imputed value of $8 \log_{10}$ copies/mL was used, which was close to the mean NP RNA value ($7.8 \log_{10}$ copies/mL) among the 87 samples that had initial results $>$ ULoQ and underwent dilution and retesting. Concordance of SARS-CoV-2 RNA from AN vs NP swabs at day 0 was evaluated using descriptive plots and Spearman's correlation. Concordance of SARS-CoV-2 RNA from plasma and oral wash/saliva with NP swabs was limited to descriptive summaries due to a large proportion of plasma and oral wash/saliva results being below the LLoQ. To assess whether AN swabs, which are potentially easier to obtain in clinical trials, might be an alternative to the gold standard NP swab for quantifying RNA, regression analysis was conducted. For participants with quantifiable NP RNA levels at day 0, the magnitude of the difference between AN and NP RNA was evaluated using linear regression models for censored data; AN RNA values below the LLoQ were

censored. Associations between day 0 SARS-CoV-2 RNA and baseline clinical/sociodemographic characteristics were evaluated with linear regression models for censored data (RNA values < LLoQ were left-censored), with and without adjustment for prior symptom duration and serostatus (seropositive vs seronegative) at day 0. Effect modification of associations between clinical/sociodemographic characteristics and serostatus was also considered in the models.

To evaluate predictors of viral RNA decay over time in the absence of therapeutic intervention, the study population was further restricted to participants who received placebo. The mean change from day 0 to day 3 was modeled using linear regression models for censored data, with and without adjustment for duration of symptoms, serostatus, and NP RNA at day 0. This analysis was restricted to those who had NP SARS-CoV-2 RNA above the LLoQ at day 0 as the large majority (80%, 36/45) of participants with RNA values below the LLoQ at day 0 remained below the LLoQ at day 3, so changes could not be reliably quantified. Reference groups for categorical variables in regression models were specified as the largest subgroup, or as “no” for variables categorized as “yes” vs “no.” All analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

The analysis of baseline (pretreatment) measures included 537 participants, including 265 who subsequently received placebo and contributed to the analysis of untreated changes in RNA from day 0 to day 3. The median (interquartile range [IQR]) age was 48 (37–57) years, 49% were female sex, and 2% self-identified as Black Hispanic, 7% as Black non-Hispanic, 24% as White Hispanic, 59% as White non-Hispanic, and 9% as other races/ethnicities (Table 1). Two participants had a history of SARS-CoV-2 vaccination, and 50% were IgG seropositive at enrollment. The median (IQR) symptom duration at study entry was 6 (4–8) days, with 39% enrolling within 5 days of symptom onset. The majority (67%) were considered at higher risk for severe COVID-19. See Supplementary Table 1 for prevalence of high-risk comorbidities as defined by the protocol.

Concordance Across Compartments

At day 0, the median (IQR) NP RNA and AN RNA were 5.21 (3.24–6.69) and 4.25 (2.11–6.09) log₁₀ copies/mL, respectively, with 17% and 23% below the LLoQ, including 9% and 15% undetectable (Figure 1). NP and AN RNA were highly correlated (Spearman $r = 0.84$; $P < .001$). The mean AN RNA was about 1 log₁₀ copies/mL lower than NP RNA across the range of quantifiable NP RNA values estimated from regression analysis (Figure 2). Most oral wash/saliva (75%) and plasma (99%) samples had RNA < LLoQ (Figure 1). NP RNA increased with

Table 1. Baseline Characteristics of Study Participants

	Total (n = 537)
Age, median (IQR), y	48 (37–57)
Sex, No. (%)	
Female	264 (49)
Male	273 (51)
Gender identity, No. (%)	
Cis-gender	535 (100)
Transgender	1 (0)
Not reported	1 (0)
Race, No. (%)	
American Indian or Alaska Native	1 (0)
Asian	18 (3)
Black or African American	49 (9)
Multiple	9 (2)
Native Hawaiian or other Pacific Islander	1 (0)
Other	17 (3)
White	444 (83)
Not reported	1
Race/ethnicity, No. (%)	
Black Hispanic/Latino	9 (2)
Black not Hispanic/Latino	37 (7)
Hispanic/Latino other race ^a	16 (3)
Not Hispanic/Latino other race ^a	30 (6)
White Hispanic/Latino	129 (24)
White not Hispanic/Latino	313 (59)
Not reported	3
Country, No. (%)	
United States	537 (100)
Body mass index, median (IQR), kg/m ²	28.7 (25.4–33.5)
>35 kg/m ² , No. (%)	107 (21)
≤35 kg/m ² , No. (%)	401 (79)
Not reported	50
Diabetes, No. (%)	
Yes	61 (12)
No	447 (88)
Not reported	29
Hypertension, No. (%)	
Yes	174 (34)
No	334 (66)
Not reported	29
High risk, No. (%)	
Yes	359 (67)
No	178 (33)
Symptom duration at study entry, median (IQR), d	6 (4–8)
History of SARS-CoV-2 vaccination, No. (%)	
Yes	2 (0)
No	535 (100)
SARS-CoV-2 IgG serostatus, No. (%)	
Positive	259 (50)
Negative	259 (50)
Not reported	19

Abbreviations: IgG, immunoglobulin G; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aOther race defined as self-reporting a race as not Black or White.

higher ordered categories of oral wash/saliva and plasma RNA. For oral wash/saliva, the median (IQR) NP RNA

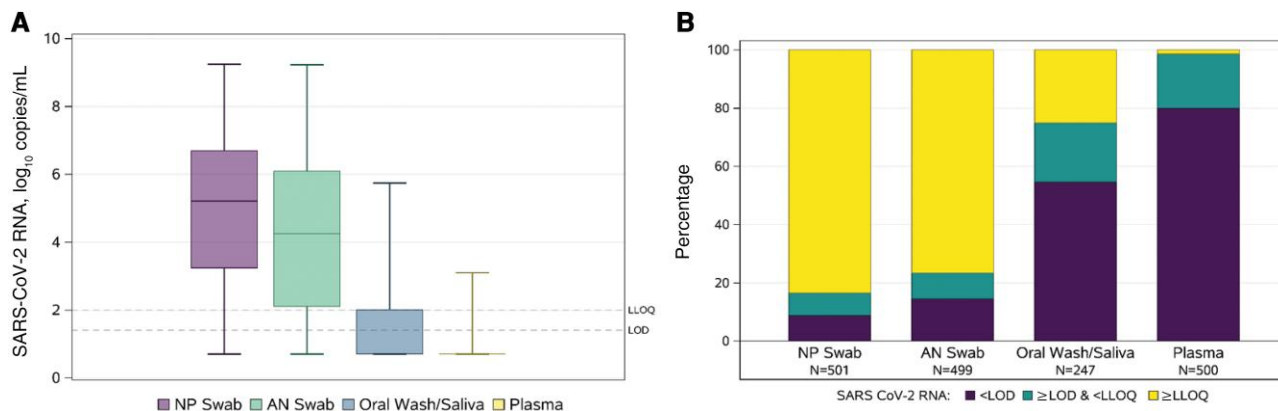


Figure 1. Distribution of RNA by compartment at day 0. *A*, Levels of RNA (log₁₀ copies/mL), with horizontal line = median, box = interquartile range, whiskers = minimum/maximum. *B*, Proportion with quantitative RNA, detectable but not quantifiable RNA, and undetectable. For (*A*), results below the LoD were imputed as 0.7 log₁₀ copies/mL (half the distance from 0 to the LoD), results above the LoD but below the LLoQ were imputed as 1.7 log₁₀ copies/mL (half the distance between the LoD and LLoQ), and values above the ULoQ that were not able to be quantified with dilution were imputed as 8 log₁₀ copies/mL. Abbreviations: LLoQ, lower limit of quantification; LoD, limit of detection; NP, nasopharyngeal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ULoQ, upper limit of quantification.

was 4.27 (2.74–5.87) log₁₀ copies/mL for undetectable oral wash/saliva RNA, 5.80 (4.88–6.74) log₁₀ copies/mL for detectable <LLOQ, and 6.90 (5.94–7.79) log₁₀ copies/mL for ≥LLOQ ($P < .001$); for plasma, median (IQR) NP RNA values were 5.00 (2.91–6.48) log₁₀ copies/mL for undetectable plasma RNA and 6.15 (4.84–7.18) log₁₀ copies/mL for detectable RNA (either <LLOQ or ≥LLOQ; $P < .001$). Similar associations were observed comparing AN RNA with ordered categories of oral wash/saliva and plasma (Supplementary Table 2). Among those with NP RNA <LLOQ at day 0, all plasma RNA and all but 1 oral wash/saliva RNA were <LLOQ.

Baseline Associations

Across all compartments, the median day 0 RNA was lower for those who were seropositive (vs seronegative) and for those with >5 days of prior symptoms (vs ≤5 days) (Supplementary Tables 3 and 4, Supplementary Figure 1). Univariable analysis also identified that older age and lower BMI were associated with higher day 0 NP RNA, and Black non-Hispanic race/ethnicity (vs White non-Hispanic) but not other race/ethnicity groups was associated with lower day 0 NP RNA (Supplementary Table 5). Examining these factors together in multivariable models with and without adjustment for prior symptom duration and serostatus (Table 2), the associations between age and day 0 NP RNA were stronger than in the univariable models (for every 10 years older age, the adjusted mean NP RNA was 0.27 log₁₀ copies/mL higher; $P < .001$). Black non-Hispanic race/ethnicity remained associated with lower NP RNA levels, though the effects were attenuated (the mean NP RNA was 0.85 log₁₀ copies/mL lower for Black non-Hispanic than White non-Hispanic participants; $P = .036$). Associations between BMI and NP RNA were no longer significant.

Longitudinal Changes in RNA

Among 265 participants who received placebo, RNA decreased over time in all compartments (Supplementary Figure 2 and 3). At both day 3 and day 7, NP RNA levels were higher for participants who were seronegative vs seropositive at entry, but by day 14 >80% of participants in both serostatus groups had NP RNA <LLOQ (Figure 3A and B). Similar differences by serostatus were observed for AN swabs, oral wash/saliva, and plasma (Supplementary Figures 4 and 5). However, among participants with NP RNA >LLOQ at day 0, the change in NP RNA from day 0 to day 3 did not differ by serostatus (mean change, −1.67 log₁₀ copies/mL for seropositive and −1.65 log₁₀ copies/mL for seronegative; difference, −0.02; 95% CI, −0.45 to 0.42; $P = .94$) or by symptom duration (mean change, −1.70 for >5 days and −1.57 for ≤5 days; difference, −0.14; 95% CI, −0.28 to 0.56; $P = .52$).

Women had faster declines in NP RNA from day 0 to day 3 compared with men (mean change, −2.00 log₁₀ copies/mL for women and −1.32 log₁₀ copies/mL for men; difference, −0.68; 95% CI, −1.08 to −0.28; $P < .001$) (Figure 3C and D). This result persisted with adjustment for day 0 RNA level, symptom duration at day 0, and serostatus at day 0, none of which differed by sex. This association did not depend on serostatus ($P_{\text{interaction}} = .55$). Change in NP RNA levels was not associated with other participant characteristics, including age, race/ethnicity, risk for severe COVID-19, BMI, diabetes status, obesity, or hypertension (Supplementary Table 6).

DISCUSSION

This study analyzed SARS-CoV-2 RNA, measured in multiple types of upper respiratory and plasma samples that were collected as part of a large randomized trial to evaluate

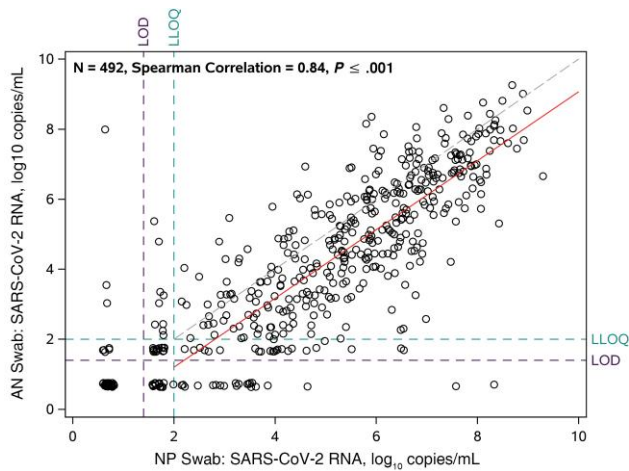


Figure 2. Scatterplot of nasopharyngeal RNA vs anterior nasal RNA at day 0. Dashed line represents perfect concordance, where NP RNA = AN RNA; solid line represents association from linear regression model among participants with NP RNA >LLOQ (the model was fit using methods for censored data with AN RNA values <LLOQ considered left-censored at the LLOQ). For graphical presentation, results below the LoD were imputed as 0.7 log₁₀ copies/mL (half the distance from 0 to the LoD), results above the LoD but below the LLOQ were imputed as 1.7 log₁₀ copies/mL (half the distance between the LoD and LLOQ), and values above the ULoQ that were not able to be quantified with dilution were imputed as 8 log₁₀ copies/mL. Participants had their values jittered for presentation when AN RNA was equal to NP RNA. Abbreviations: AN, anterior nasal; LLoQ, lower limit of quantification; LoD, limit of detection; NP, nasopharyngeal; ULoQ, upper limit of quantification.

concordance of viral levels across anatomic compartments and predictors of measured viral levels. Concerning concordance of viral levels across compartments, SARS-CoV-2 RNA levels from NP swabs and AN swabs were highly correlated ($r=0.84$), consistent with other reports [15, 16]. Although NP swabs are viewed as the “gold standard,” many studies have demonstrated good diagnostic performance of AN swabs (or combined oropharynx/AN) compared with NP swabs for SARS-CoV-2 RT-PCR testing [17–19]. Thus, self-collected AN swabs can be considered to assess early SARS-CoV-2 infection status and response to interventions in research and clinical settings as AN swabs are easier to collect and are better tolerated than NP swabs and show concordant results with NP swabs. However, as RNA levels from AN swabs were consistently 10-fold lower than RNA levels from NP swabs, their utility for assessing response to antiviral medications is likely most valuable in early SARS-CoV-2 infection, when nasal RNA levels are highest [11]. This study also found that when NP swabs demonstrated high levels of SARS-CoV-2 RNA, oral wash/saliva and plasma samples were more likely to show detectable viral RNA. While only a subset of oral wash/saliva and plasma samples had RNA levels above the lower limit of quantification, these results highlight that while SARS-CoV-2 RNA levels are higher in certain anatomic compartments, higher overall viral burden is reflected across multiple anatomic compartments.

Table 2. Multivariable Models Evaluating Associations of Nasopharyngeal RNA at Day 0 With Age, BMI, and Race/Ethnicity

Model	Variable	Estimate	95% CI	P Value
Model 1	Age (per 10 y)	0.329	0.18, 0.48	<.001
	BMI (per 5 kg/m ²)	−0.128	−0.29, 0.03	.12
	Race/ethnicity ^a	
	(Black not Hispanic/Latino)	−1.662	−2.58, −0.75	<.001
	(Hispanic/Latino, any race)	−0.254	−0.74, 0.23	.30
	(Multiracial/others)	−0.318	−1.27, 0.63	.51
Model 2: adjusted also for symptom duration and serostatus	Age (per 10 y)	0.274	0.15, 0.40	<.001
	BMI (per 5 kg/m ²)	−0.125	−0.26, 0.01	.07
	Race/ethnicity ^a	
	(Black not Hispanic/Latino)	−0.848	−1.64, −0.06	.036
	(Hispanic/Latino, any race)	−0.353	−0.77, 0.06	.09
	(Multiracial/others)	−0.290	−1.08, 0.50	.47

Estimates, 95% CIs, and P values were obtained from linear regression models for censored data.

Abbreviation: BMI, body mass index.

^aWhite not Hispanic/Latino is the reference group for race/ethnicity comparisons.

Concerning predictors of viral RNA levels, we found in this population enrolled in the first year of the pandemic (ie, prior to widespread exposure to SARS-CoV-2 or vaccination), shorter prior symptom duration and seronegativity at the time of RNA measurement and older age were all associated with higher NP RNA levels. The association of prior symptom duration and serostatus with NP RNA levels has been observed in other large COVID-19 therapeutics trials conducted early in the pandemic [20–22], but analyses of associations with age have shown conflicting results [21, 23–26]. The association of older age with higher NP RNA even among seropositive participants may reflect unmeasured immune deficiencies with aging such as decreased mucosal interferon responses [27–30] or less robust or functional humoral responses not captured by examining serostatus as a blunt qualitative measure.

Of interest, we found that Black non-Hispanic participants had lower NP RNA at day 0 compared with White non-Hispanic participants, though there was some attenuation of this difference when adjusting for prior symptom duration and serostatus. Black non-Hispanic participants (compared with White non-Hispanic participants) were more likely to be seropositive (71% vs 46%) and to have longer symptom duration at study entry (median, 8 days vs 6 days), suggesting that these individuals were more likely to enter the study later in

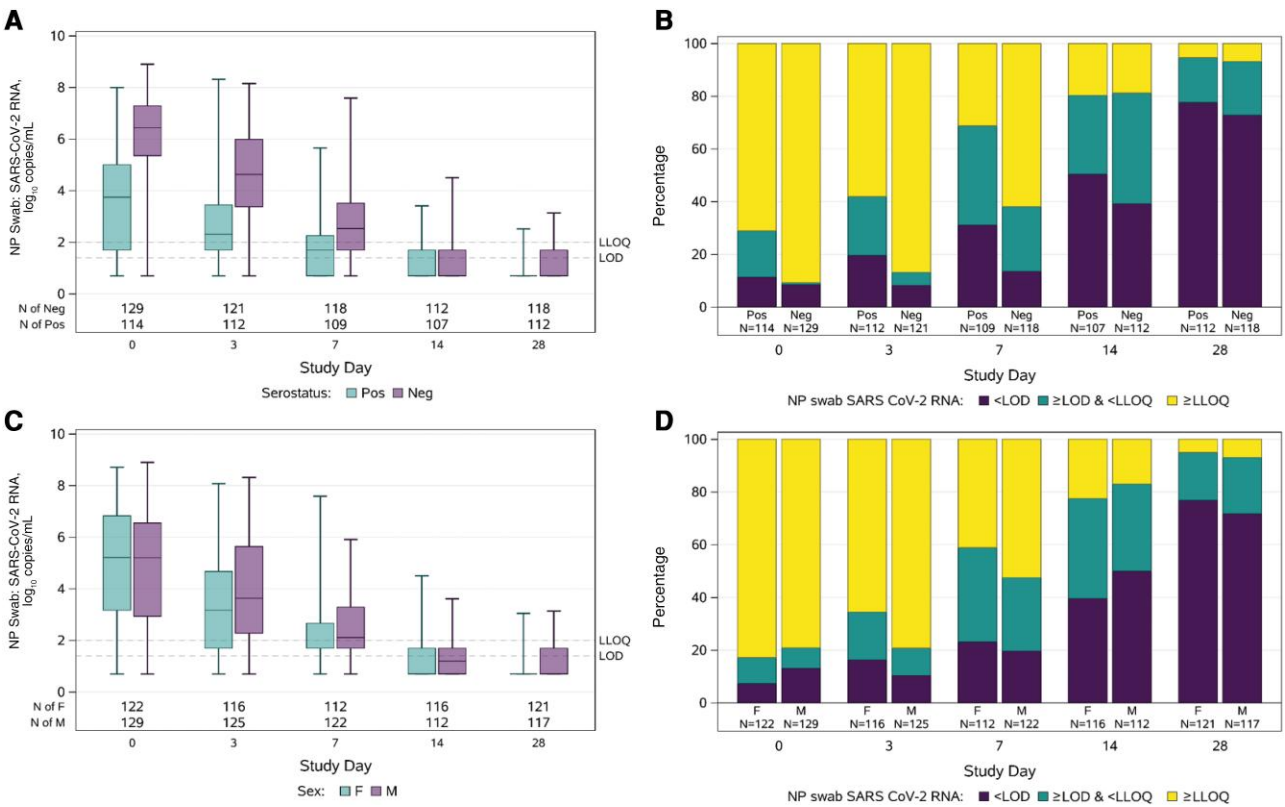


Figure 3. Distributions of nasopharyngeal RNA over time by serostatus (A and B) and sex (C and D) among the 265 participants who received placebo. Levels of RNA (\log_{10} copies/mL), with horizontal line = median, box = interquartile range, whiskers = minimum/maximum (A and C). Proportion with quantitative RNA, detectable but not quantifiable RNA, and undetectable (B and D). Results below the LoD were imputed as $0.7 \log_{10}$ copies/mL (half the distance from 0 to the LoD), results above the LoD but below the LLoQ were imputed as $1.7 \log_{10}$ copies/mL (half the distance between the LoD and LLoQ), and values above the ULoQ that were not able to be quantified with dilution were imputed as $8 \log_{10}$ copies/mL. Abbreviations: AN, anterior nasal; LLoQ, lower limit of quantification; LoD, limit of detection; neg, seronegative; NP, nasopharyngeal; pos, seropositive; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ULoQ, upper limit of quantification.

the course of their infections. Our findings may reflect differences in access to testing and trial research sites for minoritized groups rather than true differences in viral levels by race/ethnicity, as we do not suspect that SARS-CoV-2 RNA kinetics differ biologically by race/ethnic identity [31, 32].

We also found that women had faster declines in NP RNA between day 0 and day 3 compared with men, which persisted in analyses adjusting for serostatus, prior symptom duration, and NP RNA level at day 0. This is a novel finding in the outpatient setting, but is consistent with reports of hospitalized patients with COVID-19 that found male sex to be associated with prolonged duration of SARS-CoV-2 RNA shedding [33–36] as well as a population surveillance study in Italy, which reported higher proportions of women achieving early viral clearance [37]. These data are consistent with a large body of literature that suggests that females are able to clear pathogens faster than males, potentially due to greater innate and adaptive immune responses to infections, including SARS-CoV-2 [38–42]. Whether these differences in SARS-CoV-2 clearance rates contribute to sex-based differences in COVID-19 outcomes that have been observed, including increased risk for

COVID-19 hospitalization and mortality among men, is not known [43, 44].

This study has limitations, including the small number of participants in some racial/ethnic subgroups, which limited our ability to make inferences for these populations. Another important limitation is that this study enrolled earlier in the pandemic, so it does not allow us to evaluate associations among participants infected with later variants, like Omicron, or persons who were vaccinated. Additionally, serology data were limited to day 0, preventing us from making inferences about changes in viral load and prospective seroconversion. Although duration of symptoms is often used as a surrogate for duration of infection, we cannot be certain when infection occurred, which could also influence serostatus. In addition, there was low sensitivity of oral wash–collected saliva for SARS-CoV-2 RNA detection, which was likely due to the method of specimen collection, as other approaches to saliva sampling have had higher yields [1, 45, 46].

In summary, in this analysis of viral levels in plasma and multiple upper airway compartments early in symptomatic SARS-CoV-2 infection, we observed concordant viral loads

across compartments and that older age, seronegativity, and shorter prior symptom duration at the time of measurement were all associated with higher viral RNA levels from NP and AN swabs. Faster declines in RNA levels were seen for women compared with men, but not by serostatus or other clinical or demographic factors. These findings should be explored further to understand the potential mediators of sex-based differences in SARS-CoV-2 clearance and their relationship to important clinical outcomes.

Supplementary Data

Supplementary materials are available at *Open Forum 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.

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References

- Mohammadi A, Esmailzadeh E, Li Y, Bosch RJ, Li JZ. SARS-CoV-2 detection in different respiratory sites: a systematic review and meta-analysis. *eBioMedicine* **2020**; 59:102903.

- 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.
- Singanayagam A, Patel M, Charlett A, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveill* **2020**; 25:2001483.
- Marks M, Millat-Martinez P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. *Lancet Infect Dis* **2021**; 21:629–36.
- Jacobs JL, Bain W, Naqvi A, et al. Severe acute respiratory syndrome coronavirus 2 viremia is associated with coronavirus disease 2019 severity and predicts clinical outcomes. *Clin Infect Dis* **2022**; 74:1525–33.
- 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**; 131:148635.
- Rhoads D, Peaper DR, She RC, et al. College of American Pathologists (CAP) Microbiology Committee perspective: caution must be used in interpreting the cycle threshold (Ct) value. *Clin Infect Dis* **2021**; 72:e685–6.
- Weinreich DM, Sivapalasingam S, Norton T, et al. REGEN-COV antibody combination and outcomes in outpatients with COVID-19. *N Engl J Med* **2021**; 385:e81.
- Magleby R, Westblade LF, Trzebecki A, et al. Impact of severe acute respiratory syndrome coronavirus 2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis* **2021**; 73:e4197–205.
- Dougan M, Azizad M, Mocherla B, et al. A randomized, placebo-controlled clinical trial of bamlanivimab and etesevimab together in high-risk ambulatory patients with COVID-19 and validation of the prognostic value of persistently high viral load. *Clin Infect Dis* **2022**; 75:e440–9.
- 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.
- Evering TH, Giganti M, Chew KW, et al. LB2. Safety and efficacy of combination SARS-CoV-2 monoclonal neutralizing antibodies (mAb) BR11-196 and BR11-198 in non-hospitalized COVID-19 patients. *Open Forum Infect Dis* **2021**; 8:S807–8.
- Berg MG, Zhen W, Lucic D, et al. Development of the RealTime SARS-CoV-2 quantitative laboratory developed test and correlation with viral culture as a measure of infectivity. *J Clin Virol* **2021**; 143:104945.
- Bio-Rad Laboratories Inc. Bio-Plex Pro Human IgG SARS-CoV-2 Serology Assays product data sheet. Accessed 24 October 2022.
- McCulloch DJ, Kim AE, Wilcox NC, et al. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection. *JAMA Netw Open* **2020**; 3:e2016382.
- Aleman A, Millat-Martinez P, Ouchi D, et al. Self-collected mid-nasal swabs and saliva specimens, compared with nasopharyngeal swabs, for SARS-CoV-2 detection in mild COVID-19 patients. *J Infect Elsevier* **2021**; 83:709–37.
- Tsang NNY, So HC, Ng KY, Cowling BJ, Leung GM, Ip DKM. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis. *Lancet Infect Dis* **2021**; 21:1233–45.
- Tsujimoto Y, Terada J, Kimura M, et al. Diagnostic accuracy of nasopharyngeal swab, nasal swab and saliva swab samples for the detection of SARS-CoV-2 using RT-PCR. *Infect Dis* **2021**; 53:581–9.
- LeBlanc JJ, Pettipas J, Di Quinzio M, Hachette TF, Patriquin G. Reliable detection of SARS-CoV-2 with patient-collected swabs and saline gargles: a three-headed comparison on multiple molecular platforms. *J Virol Methods* **2021**; 295:114184.
- Weinreich DM, Sivapalasingam S, Norton T, et al. REGEN-COV antibody combination and outcomes in outpatients with COVID-19. *N Engl J Med* **2021**; 385:e81.
- Mollan KR, Eron JJ, Krajewski TJ, et al. Infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus in symptomatic coronavirus disease 2019 (COVID-19) outpatients: host, disease, and viral correlates. *Clin Infect Dis* **2022**; 75:e1028–36.
- Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with COVID-19. *N Engl J Med* **2021**; 384:238–51.
- Knudtzen FC, Jensen TG, Lindvig SO, et al. SARS-CoV-2 viral load as a predictor for disease severity in outpatients and hospitalised patients with COVID-19: a prospective cohort study. *PLoS One* **2021**; 16:e0258421.
- Liao T, Yin Z, Xu J, et al. The correlation between clinical features and viral RNA shedding in outpatients with COVID-19. *Open Forum Infect Dis* **2020**; 7:ofaa331.
- Leeds JS, Raviprakash V, Jacques T, Scanlon N, Cundall J, Leeds CM. Risk factors for detection of SARS-CoV-2 in healthcare workers during April 2020 in a UK hospital testing programme. *EClinicalMedicine* **2020**; 26:100513.
- To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during

- infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* **2020**; 20:565–74.
27. Molony RD, Nguyen JT, Kong Y, Montgomery RR, Shaw AC, Iwasaki A. Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. *Sci Signal* **2017**; 10:eaan2392.
 28. Shodell M, Siegal FP. Circulating, interferon-producing plasmacytoid dendritic cells decline during human ageing. *Scand J Immunol* **2002**; 56:518–21.
 29. Pérez-Cabezas B, Naranjo-Gómez M, Fernández MA, Grifols JR, Pujol-Borrell R, Borràs FE. Reduced numbers of plasmacytoid dendritic cells in aged blood donors. *Exp Gerontol* **2007**; 42:1033–8.
 30. Feng E, Balint E, Poznanski SM, Ashkar AA, Loeb M. Aging and interferons: impacts on inflammation and viral disease outcomes. *Cells* **2021**; 10:708.
 31. Khazanchi R, Evans CT, Racism MJ, Race N. Drives inequity across the COVID-19 continuum. *JAMA Netw Open* **2020**; 3:e2019933.
 32. Kullar R, Marcelin JR, Swartz TH, et al. Racial disparity of coronavirus disease 2019 in African American communities. *J Infect Dis* **2020**; 222:890–3.
 33. Shi D, Wu W, Wang Q, et al. Clinical characteristics and factors associated with long-term viral excretion in patients with severe acute respiratory syndrome coronavirus 2 infection: a single-center 28-day study. *J Infect Dis* **2020**; 222:910–8.
 34. Dezza F C, Oliva A, Cancelli F, et al. Determinants of prolonged viral RNA shedding in hospitalized patients with SARS-CoV-2 infection. *Diagn Microbiol Infect Dis* **2021**; 100:115347.
 35. Xu K, Chen Y, Yuan J, et al. Factors associated with prolonged viral RNA shedding in patients with coronavirus disease 2019 (COVID-19). *Clin Infect Dis* **2020**; 71: 799–806.
 36. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. *BMJ* **2020**; 369:m1443.
 37. Fortunato F, Martinelli D, Lo Caputo S, et al. Sex and gender differences in COVID-19: an Italian local register-based study. *BMJ Open* **2021**; 11:e051506.
 38. Takahashi T, Ellingson MK, Wong P, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* **2020**; 588:315–20.
 39. Klein SL. Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. *Bioessays* **2012**; 34:1050–9.
 40. Ghosh S, Klein RS. Sex drives dimorphic immune responses to viral infections. *J Immunol* **2017**; 198:1782–90.
 41. van Lunzen J, Altfeld M. Sex differences in infectious diseases—common but neglected. *J Infect Dis* **2014**; 209:S79–80.
 42. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol* **2008**; 8:737–44.
 43. Scully EP, Schumock G, Fu M, et al. Sex and gender differences in testing, hospital admission, clinical presentation, and drivers of severe outcomes from COVID-19. *Open Forum Infect Dis* **2021**; 8:XXX–XX.
 44. de Miguel-Diez J, Lopez-de-Andres A, Jimenez-Garcia R, et al. Sex differences in COVID-19 hospitalization and hospital mortality among patients with COPD in Spain: a retrospective cohort study. *Viruses* **2022**; 14:1238.
 45. Beyene GT, Alemu F, Kebede ES, et al. Saliva is superior over nasopharyngeal swab for detecting SARS-CoV2 in COVID-19 patients. *Sci Rep* **2021**; 11:22640.
 46. Butler-Laporte G, Lawandi A, Schiller I, et al. Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for detection of SARS-CoV-2: a systematic review and meta-analysis. *JAMA Intern Med* **2021**; 181:353–60.