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Association Between Anterior Nasal and Plasma SARS-CoV-2 RNA Levels and Hospitalization or Death in Nonhospitalized Adults With Mild-to-Moderate COVID-19

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Background. There is little information regarding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA as a predictor for clinical outcomes in outpatients with mild-to-moderate coronavirus disease 2019 (COVID-19).

Methods. Anterior nasal (AN) and plasma SARS-CoV-2 RNA data from 2115 nonhospitalized adults who received monoclonal antibodies (mAbs) or placebo in the ACTIV-2/A5401 trial were analyzed for associations with hospitalization or death.

Results. One hundred two participants were hospitalized or died through 28 days of follow-up. Higher day 0 (pretreatment) AN RNA was associated with increasing risk of hospitalization/death (risk ratio [RR], 1.24 per $\log_{10}$ copies/mL [95% confidence interval {CI}, 1.04–1.49]) among placebo recipients, ranging from 3% to 16% for <2 to ≥6 $\log_{10}$ copies/mL. Although only 1% had quantifiable levels, there was a similar trend across day 0 plasma RNA categories. Higher day 3 AN RNA was associated with subsequent hospitalization/death among placebo recipients (RR, 1.42 per $\log_{10}$ copies/mL [95% CI, 1.00–2.03]), but not mAb recipients (RR, 1.02 per $\log_{10}$ copies/mL [95% CI, 0.68–1.56]). The proportion of treatment effect (reduction in hospitalizations/deaths after day 3 for mAb vs placebo) explained by day 3 AN RNA was 8%.

Conclusions. SARS-CoV-2 RNA levels are predictive of hospitalization/death in the natural history setting, but AN RNA levels may not be a reliable surrogate marker of mAb treatment effect in COVID-19 trials.

Clinical Trials Registration. NCT04518410.

Keywords. COVID-19; hospitalization; outpatient; SARS-CoV-2 RNA; surrogate marker.

There is a critical need to identify markers that can predict disease progression of coronavirus disease 2019 (COVID-19) and serve as surrogate endpoints in clinical trials for evaluating potential treatments. Since the onset of the COVID-19 pandemic, levels of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA from nasal or nasopharyngeal (NP) swabs have been considered promising candidate markers. This optimism was originally based on historical precedent, as RNA levels have been used to monitor treatment response for other viral infections such as human immunodeficiency virus type 1 [1].

Recent studies among hospitalized or emergency department patients with COVID-19 have shown that SARS-CoV-2 serum or plasma viremia is associated with poor clinical outcomes, including intubation [2], intensive care unit admission [3], and mortality [4–10]. Findings from the Blocking Viral Attachment and Cell Entry with SARS-CoV-2 Neutralizing Antibodies (BLAZE-1) trial suggest that persistently high NP viral load in patients with mild-to-moderate COVID-19 is associated with hospitalization or death [11]. The supplementary material presented in reports of 3 randomized trials of outpatient treatments for COVID-19 suggests increased risk of hospitalization or death in higher versus lower categories of baseline RNA in NP samples among participants receiving placebo [12–14]. There is less information, however, regarding nasal and plasma RNA as surrogate markers for outcomes in outpatients.

In this study, we investigated whether SARS-CoV-2 RNA is predictive of clinical outcomes in outpatients with mild-to-moderate COVID-19 and whether RNA levels may be a reliable

surrogate marker of monoclonal antibody (mAb) treatment effect for use in COVID-19 therapeutical trials. We evaluated associations between plasma and self-collected anterior nasal (AN) SARS-CoV-2 RNA levels and subsequent hospitalization or death using data from the evaluation of 2 anti-SARS-CoV-2 mAb regimens for the treatment of nonhospitalized adults with mild-to-moderate COVID-19 in the phase 2/3 platform clinical trial Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV)-2/AIDS Clinical Trials Group (ACTG) A5401. Notably, both mAb regimens (bamlanivimab monotherapy and amubarvimab plus romlusevimab combination therapy) have been shown in randomized controlled trials to reduce hospitalizations and death in nonhospitalized adults [15, 16].

METHODS

Study Design

ACTIV-2/A5401 (NCT04518410) is a platform trial to evaluate the safety and efficacy of investigational agents to treat symptomatic nonhospitalized adults with COVID-19 [17]. The study population consists of adults (aged ≥18 years) with documented positive SARS-CoV-2 antigen or nucleic acid test within 7 days prior to study entry, no more than 10 days of symptoms of COVID-19 at study entry, and ongoing symptoms within 48 hours prior to study entry.

An exploratory objective of ACTIV-2/A5401 is to evaluate whether baseline and follow-up virologic measures are associated with clinical outcomes. In this report, analyses were restricted to participants who contributed to the evaluation of 2 mAb regimens, bamlanivimab monotherapy and amubarvimab plus romlusevimab combination therapy. Participants were enrolled in either placebo (saline)–controlled evaluations of bamlanivimab given at a dose of 7000 mg or 700 mg or the combination of amubarvimab 1000 mg plus romlusevimab 1000 mg, or an uncontrolled evaluation of bamlanivimab 700 mg (Supplementary Table 1).

Samples for measurement of SARS-CoV-2 RNA included ethylenediaminetetraacetic acid plasma samples obtained on day 0 prior to infusion of mAb or placebo and in participant self-collected AN swabs at days 0 and 3. Quantitative SARS-CoV-2 RNA testing was performed at a central laboratory using the Abbott m2000sp/rt platform. The assay limit of detection (LoD) was 1.4 $\log_{10}$ copies/mL, the lower limit of quantification (LLoQ) was 2 $\log_{10}$ copies/mL, and the upper limit of quantification (ULoQ) was 7 or 8 $\log_{10}$ copies/mL. For samples with RNA >ULoQ, the assay was rerun with dilutions to obtain a quantitative value. If a sample was not retested or the retested value was >ULoQ, then an imputed value of 1 $\log_{10}$ copies/mL higher than the ULoQ was used in analysis. In total, there were 21 samples at day 0 and none at day 3 with unquantifiable RNA levels >ULoQ after dilution and retesting that were assigned an imputed value.

The protocol was approved by a central institutional review board (IRB), Advarra (Pro00045266), for sites in the United States (US) (with additional local IRB review and approval as required by the site), and by local ethics committees for sites outside the US. All participants provided written informed consent prior to undergoing study procedures.

Statistical Analyses

Analyses were restricted to participants who initiated study intervention. Participants were summarized according to the treatment received.

SARS-CoV-2 RNA levels were summarized for AN and plasma samples at day 0 (ie, baseline, immediately prior to infusion of study agent) and AN samples at day 3. For day 3 AN swabs, the visit window was ±1 day. AN RNA levels were categorized into 1 of 4 levels (unquantifiable, 2.0–3.99 $\log_{10}$ copies/mL, 4.0–5.99 $\log_{10}$ copies/mL, or ≥6.0 $\log_{10}$ copies/mL), and plasma RNA levels were categorized into 1 of 3 levels (not detected, detectable but not quantifiable, or 2.0–3.99 $\log_{10}$ copies/mL). No participants had plasma RNA levels ≥4.0 $\log_{10}$ copies/mL.

The primary clinical outcome was hospitalization for any reason or death from any cause through day 28. Hospitalization was defined as ≥24 hours of acute care. Associations between RNA and risk of hospitalization or death through day 28 were assessed using log-binomial regression models (or Poisson regression [18] if convergence issues occurred) with robust variance estimates. Each treatment group was modeled separately. Risk ratios, robust 95% confidence intervals (CIs), and 2-sided P values from a Wald test are reported. Differences in associations of risk with RNA level between mAb-treated and placebo were assessed in models for the combined population with main effects for RNA, treatment (mAb or placebo), and their interaction.

The unadjusted risk of hospitalization/death between day 0 and 28 was compared per 1 $\log_{10}$ copies/mL increase (among participants with RNA ≥LLoQ at day 0) and between categories of day 0 AN and plasma RNA levels. Unadjusted and mutually adjusted associations between AN RNA levels at days 0 and 3 and the risk of hospitalization/death between days 4 and 28 were also assessed, with RNA levels for a given day described by 2 covariates: an indicator variable for whether the RNA level was ≥LLoQ or <LLoQ and a continuous variable equal to $\log_{10}$(RNA) LLoQ for those ≥LLoQ, and equal to 0 otherwise. Analyses involving the risk of hospitalization/death between days 4 and 28 were restricted to participants who met all 3 criteria of alive and in follow-up on day 3, not hospitalized by day 3, and with both day 0 and day 3 AN RNA results available.

Two models were also fit to estimate the association between treatment (mAb vs placebo) and subsequent risk of hospitalization/death, adjusting for $\log_{10}$ RNA at day 0 only or in combination with $\log_{10}$ RNA at day 3. The proportion

of treatment effect explained (PTE) was calculated as the proportionate change between the 2 models in the regression coefficient for the log risk ratio (RR) for hospitalizations/deaths for mAb-treated versus placebo [19]. Recognizing that the comparison of mAb to placebo in the overall study population is not a pure randomized comparison because it includes the uncontrolled cohort that received bamlanivimab, we repeated this analysis restricting to participants receiving amubarvimab plus romlusevimab or its placebo. The PTE was also calculated for an analysis population restricted to participants with ≤5 days from symptom onset to day 0. Statistical analyses were performed using SAS version 9.4 software (SAS Institute).

RESULTS

A total of 2115 participants were enrolled between August 2020 and July 2021 and received bamlanivimab 700 mg (n = 111) or 7000 mg (n = 48) or their placebos (n = 158) in placebo-controlled evaluation, bamlanivimab 700 mg in uncontrolled evaluation (n = 990), or amubarvimab plus romlusevimab (n = 405) or its placebo (n = 403) (Table 1). Participants were enrolled at 137 sites in 6 countries, including the United States (87%), South Africa (6%), Argentina (5%), Brazil (2%), Mexico (<1%), and the Philippines (<1%). The median age was 49 years, 51% were female sex at birth, >99% were cisgender, 39% identified as Hispanic/Latino, and 80% identified as White. The median time between symptom onset and enrollment was 6 days, 75% met criteria for being at "higher" risk of progression to severe COVID-19 based on the protocol definition at time of enrollment as defined in Supplementary Table 1, and 3% had a history of SARS-CoV-2 vaccination.

A total of 102 (5%) participants were hospitalized or died through day 28 (Supplementary Table 2), including 14 who were hospitalized and then died by day 28, 1 who died without a hospitalization, and 87 who were hospitalized but did not die by day 28. The majority of events occurred early in follow-up, including 57 (56%) through day 3 and 82 (80%) through day 7. Among participants not hospitalized or dead through day 28, only 3% had <28 days of follow-up.

Day 0 AN SARS-CoV-2 RNA as Predictors of Hospitalization/Death

There were 2015 (95%) participants with AN RNA measurement at day 0. The median day 0 AN RNA was higher among participants who were hospitalized or died through day 28 compared to those without a hospitalization/death event for placebo-treated participants (5.9 vs 3.9 $\log_{10}$ copies/mL) but not mAb-treated participants (5.0 vs 4.9 $\log_{10}$ copies/mL) (Table 2). Similar results were also observed in the subgroup who enrolled within 5 days of symptom onset (Supplementary Table 3).

Among participants who received placebo, there was a trend of increasing proportion who were hospitalized or died by increasing day 0 AN RNA level: 2.7% (4/147) for participants

Table 1. Baseline Characteristics

Characteristic	Placebo (n = 561)	mAb-Treated (n = 1554)	Total (N = 2115)
Study treatment/placebo			
Amubarvimab + romlusevimab	0 (0)	405 (26)	405 (19)
Bamlanivimab 7000 mg	0 (0)	48 (3)	48 (2)
Bamlanivimab 700 mg	0 (0)	111 (7)	111 (5)
Bamlanivimab 700 mg (uncontrolled cohort)	0 (0)	990 (64)	990 (47)
Placebo for amubarvimab + romlusevimab	403 (72)	0 (0)	403 (19)
Placebo for bamlanivimab 7000 mg	46 (8)	0 (0)	46 (2)
Placebo for bamlanivimab 700 mg	112 (20)	0 (0)	112 (5)
Age, y			
Median (Q1, Q3)	49 (38, 57)	50 (39, 60)	49 (39, 59)
≥60	118 (21)	408 (26)	526 (25)
Sex, female	287 (51)	797 (51)	1084 (51)
Gender identity, cisgender	559 (>99)	1546 (99)	2105 (>99)
Race[a]			
White	430 (77)	1263 (81)	1693 (80)
Black/African American	74 (13)	159 (10)	233 (11)
Asian	30 (5)	75 (5)	105 (5)
Ethnicity[a]			
Hispanic/Latino	249 (45)	564 (36)	813 (39)
Country			
Argentina	49 (9)	49 (3)	98 (5)
Brazil	18 (3)	15 (1)	33 (2)
Mexico	2 (<1)	3 (<1)	5 (<1)
Philippines	1 (<1)	0 (0)	1 (<1)
South Africa	59 (11)	72 (5)	131 (6)
United States	432 (77)	1415 (91)	1847 (87)
History of SARS-CoV-2 vaccination (yes)	39 (7)	32 (2)	71 (3)
Higher risk of severe COVID-19 progression[b] (yes)	477 (85)	1112 (72)	1589 (75)
Comorbidities			
Hypertension[a]	200 (36)	529 (34)	729 (35)
Obesity[a]	137 (24)	290 (19)	427 (20)
Diabetes[a]	78 (14)	214 (14)	292 (14)
Days from symptom onset to study day 0			
Median (Q1, Q3)	6 (4, 7)	5 (4, 7)	6 (4, 7)
≤5	259 (46)	790 (51)	1049 (50)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: COVID-19, coronavirus disease 2019; mAb, monoclonal antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Data were missing for the following variables: race (n = 2), ethnicity (n = 5), hypertension (n = 3), obesity (n = 3), diabetes (n = 3).

[b]"Higher" risk is based on the protocol definition at time of enrollment as defined in Supplementary Table 1.

with AN RNA <LLoQ, 7.3% (8/110) for 2–3.99 $\log_{10}$ copies/mL, 9.0% (12/134) for 4–5.99 $\log_{10}$ copies/mL, and 15.8% (21/133) for ≥6 $\log_{10}$ copies/mL (Table 3). Among participants with AN RNA ≥LLoQ, this corresponded to an RR of hospitalization/death of 1.24 per 1 $\log_{10}$ copies/mL higher day 0 AN

		Placebo			mAb-Treated		
		Hospitalized/Died Between Day 0 and Day 28			Hospitalized/Died Between Day 0 and Day 28		
Collection Type and Study Visit	SARS-CoV-2 RNA ($\log_{10}$ copies/mL)	Yes (n = 51)	No (n = 510)	Total (n = 561)	Yes (n = 51)	No (n = 1503)	Total (n = 1554)
Anterior nasal, day 0	No.	45	479	524	49	1442	1491
	Median (Q1, Q3)	5.9 (3.8, 6.9)	3.9 (<LLoQ, 5.8)	4.1 (<LLoQ, 6.0)	5.0 (3.8, 6.4)	4.9 (2.7, 6.7)	4.9 (2.7, 6.7)
	Not detected	3 (7%)	102 (21%)	105 (20%)	2 (4%)	167 (12%)	169 (11%)
	Detected, <LLoQ	1 (2%)	41 (9%)	42 (8%)	2 (4%)	99 (7%)	101 (7%)
	≥LLoQ	41 (91%)	336 (70%)	377 (72%)	45 (92%)	1176 (82%)	1221 (82%)
Plasma, day 0	No.	35	433	468	48	1413	1461
	Not detected	18 (51%)	361 (83%)	379 (81%)	18 (38%)	1055 (75%)	1073 (73%)
	Detected, <LLoQ	14 (40%)	71 (16%)	85 (18%)	24 (50%)	345 (24%)	369 (25%)
	≥LLoQ	3 (9%)	1 (<1%)	4 (1%)	6 (13%)	13 (1%)	19 (1%)
		Hospitalized/Died Between Day 4 and Day 28			Hospitalized/Died Between Day 4 and Day 28		
Collection Type and Study Visit	SARS-CoV-2 RNA ($\log_{10}$ copies/mL)	Yes (n = 23)	No (n = 505)	Total (n = 528)	Yes (n = 22)	No (n = 1500)	Total (n = 1522)
Anterior nasal, day 0	No.	21	475	496	21	1439	1460
	Median (Q1, Q3)	6.0 (3.8, 7.6)	3.9 (<LLoQ, 5.8)	4.0 (<LLoQ, 6.0)	6.3 (6.0, 7.0)	4.9 (2.7, 6.7)	5.0 (2.7, 6.7)
	Not detected	2 (10%)	101 (21%)	103 (21%)	0 (0%)	167 (12%)	167 (11%)
	Detected, <LLoQ	0 (0%)	41 (9%)	41 (8%)	1 (5%)	99 (7%)	100 (7%)
	≥LLoQ	19 (90%)	333 (70%)	352 (71%)	20 (95%)	1173 (82%)	1193 (82%)
Anterior nasal, day 3	No.	20	484	504	19	1386	1405
	Median (Q1, Q3)	4.9 (<LLoQ, 6.0)	2.5 (<LoD, 4.3)	2.5 (<LoD, 4.4)	3.5 (2.5, 4.7)	2.3 (<LoD, 3.7)	2.3 (<LoD, 3.7)
	Not detected	1 (5%)	147 (30%)	148 (29%)	4 (21%)	359 (26%)	363 (26%)
	Detected, <LLoQ	5 (25%)	66 (14%)	71 (14%)	0 (0%)	271 (20%)	271 (19%)
	≥LLoQ	14 (70%)	271 (56%)	285 (57%)	15 (79%)	756 (55%)	771 (55%)

Data are presented as No. (%) unless otherwise indicated. "Not detected" and "<LoD" represent results <1.4 $\log_{10}$ copies/mL; "Detected, <LLoQ" represents results 1.4–1.99 $\log_{10}$ copies/mL; "≥LLoQ" represents results ≥2 $\log_{10}$ copies/mL.

Abbreviations: LLoQ, lower limit of quantification; LoD, limit of detection; mAb, monoclonal antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

RNA (95% CI, 1.04–1.49). Minimal change in the association was observed after adjusting for key baseline characteristics (age ≥60 years, sex, ethnicity, race) or the 3 most common co-morbidities (hypertension, obesity, diabetes), with adjusted RR estimates ranging from 1.21 to 1.25.

Increasing hospitalization/death across day 0 AN RNA categories was also observed among participants who received mAbs, but proportions were lower in each category: 1.5% (4/270) for AN RNA <LLoQ, 3.1% (9/291) for 2–3.99 $\log_{10}$ copies/mL, 3.8% (16/416) for 4–5.99 $\log_{10}$ copies/mL, and 3.9% (20/514) for ≥6 $\log_{10}$ copies/mL. Compared to participants who received mAbs with AN RNA <LLoQ on day 0, the RR of hospitalization/death was 2.09 (95% CI, 0.65–6.70) among those with AN RNA 2–3.99 $\log_{10}$ copies/mL, 2.60 (95% CI, 0.88–7.68) among those with AN RNA of 4–5.99 $\log_{10}$ copies/mL, and 2.63 (95% CI, 0.91–7.61) among those with AN RNA ≥6 $\log_{10}$ copies/mL. There was also no apparent association between quantitative level of day 0 AN RNA and risk of hospitalization/death (RR, 0.97 per 1 $\log_{10}$ copies/mL higher AN RNA [95% CI, 0.84–1.13]). There was evidence of a difference in RR associated with RNA level >LLoQ between participants who received placebo versus mAb treatment (RR, 1.24 vs 0.97 per 1 $\log_{10}$ copies/mL higher RNA, respectively; test for interaction, $P = .04$).

Day 0 Plasma SARS-CoV-2 RNA as Predictors of Hospitalization/Death

There were 1929 (91%) participants with plasma RNA results at day 0, but most (n = 1452 [75%]) had undetectable levels; only 1% (n = 23) had levels ≥LLoQ (all values were in the 2–3.99 $\log_{10}$ copies/mL range). The proportion with detectable plasma RNA was higher among participants who were hospitalized/died through day 28 compared to those without a hospitalization/death event in both the placebo (49% vs 17%) and mAb-treated (63% vs 25%) participants (Table 2).

Among participants who received placebo, there was a trend of increasing proportion who were hospitalized or died by increasing category of plasma RNA: 4.7% (18/379) for plasma RNA <LoD, 16.5% (14/85) for values in the detectable but not quantifiable range, and 75% (3/4) for ≥LLoQ (Table 3). A trend of increasing risk with increasing plasma RNA was also observed among participants who received mAbs; however, proportions were lower in each category: 1.7% (18/1073) for plasma RNA <LoD, 6.5% (24/369) for detectable but not

Table 3. Association Between Day 0 Severe Acute Respiratory Syndrome Coronavirus 2 RNA Results and Subsequent Hospitalization/Death Through Day 28

Covariate and Comparison	Placebo				mAb-Treated			
	No.	Event Frequency (%)	Risk Ratio (95% CI)	P Value	No.	Event Frequency (%)	Risk Ratio (95% CI)	P Value
Day 0 anterior nasal RNA	524				1491			
<LLoQ		4/147 (2.7%)	Ref			4/270 (1.5%)	Ref	
2–3.99 $\log_{10}$ copies/mL		8/110 (7.3%)	2.67 (0.83–8.65)	.10		9/291 (3.1%)	2.09 (0.65–6.70)	.22
4–5.99 $\log_{10}$ copies/mL		12/134 (9.0%)	3.29 (1.09–9.96)	.0350		16/416 (3.8%)	2.60 (0.88–7.68)	.0848
≥6 $\log_{10}$ copies/mL		21/133 (15.8%)	5.80 (2.04–16.47)	<.001		20/514 (3.9%)	2.63 (0.91–7.61)	.0751
Per 1 $\log_{10}$ copies/mL above LLoQ higher		…	1.24 (1.04–1.49)	.0167		…	0.97 (0.84–1.13)	.72
Day 0 blood plasma RNA	468				1461			
Not detected		18/379 (4.7%)	Ref			18/1073 (1.7%)	Ref	
Detected, <LLoQ		14/85 (16.5%)	3.47 (1.80–6.69)	<.001		24/369 (6.5%)	3.88 (2.13–7.06)	<.001
2–3.99 $\log_{10}$ copies/mL		3/4 (75.0%)	15.79 (7.66–32.56)	<.001		6/19 (31.6%)	18.82 (8.42–42.10)	<.001

"Not detected" represents results <1.4 $\log_{10}$ copies/mL; "Detected, <LLoQ" represents results 1.4–1.99 $\log_{10}$ copies/mL.

Event outcome was hospitalization or death through day 28. For the quantitative day 0 plasma RNA model involving placebo recipients, the 95% CIs and 2-sided Wald test P values are from Poisson regression due to convergence issues with log-binomial regression. All other 95% CIs and 2-sided Wald test P values are from log-binomial regression.

Abbreviations: CI, confidence interval; LLoQ, lower limit of quantification; mAb, monoclonal antibody.

quantifiable values, and 31.6% (6/19) for ≥LLoQ. There was insufficient evidence that the association between plasma RNA categories and hospitalization/death was different between treatment groups (test for interaction, $P = .94$).

Day 0 and Day 3 AN SARS-CoV-2 RNA as Predictors of Subsequent Hospitalization/Death

Among the 2050 participants in follow-up without hospitalization/death through day 3, 1956 (95%) had an RNA result at day 0 and 1909 (93%) had an RNA result at day 3 (Table 2 and Supplementary Figure 1). Median AN RNA levels were higher at day 0 compared to day 3, with a larger difference for mAb-treated participants (5.0 vs 2.3 $\log_{10}$ copies/mL) compared to participants who received placebo (4.0 vs 2.5 copies/mL). Median day 3 AN RNA was higher among participants who were hospitalized or died from days 4 through 28 compared to those without an event for both placebo (4.9 vs 2.5 $\log_{10}$ copies/mL) and mAb-treated (3.5 vs 2.3 $\log_{10}$ copies/mL) participants.

There were 1831 (478 placebo, 1353 mAb-treated) participants who were in follow-up with no event through day 3 and who had AN results at both day 0 and day 3. Among participants who received placebo, the risk of subsequent hospitalization/death was highest among those who had AN RNA levels ≥6 $\log_{10}$ copies/mL at both day 0 and day 3 (14.7% [5/34]) and was lowest among participants who had RNA <LLoQ at both days (0% [0/123]) (Table 4). Findings were similar in the subgroup who enrolled within 5 days of symptom onset (Supplementary Table 4).

When quantitative AN RNA values at both days were included in the same regression model, the association was stronger for AN RNA at day 3 (RR, 1.42 per 1 $\log_{10}$ copies/mL higher [95% CI, 1.00–2.03]) than for AN RNA at day 0 (RR, 1.28 [95% CI, 0.82–1.98]) (Table 5).

Among participants who received mAbs, a different pattern of risk associations was found (Table 5). Quantitative AN RNA at day 3 showed no notable relationship in predicting subsequent hospitalization/death among participants who received mAbs in regression models with adjustment for day 0 AN RNA (RR, 1.02 [95% CI, 0.68–1.56]) or without adjustment (RR, 1.17 [95% CI, 0.81–1.67]). However, there was insufficient evidence to demonstrate a difference in association between quantitative RNA above the LLoQ at day 3 and risk of hospitalization/death for participants who received placebo versus mAbs ($P = .23$).

Effect of mAb Treatment on Hospitalization/Death Explained by AN SARS-CoV-2 RNA

The estimated RR of hospitalization/death between days 4 and 28 for participants receiving mAb treatment compared to placebo was 0.29 (95% CI, 0.15–0.55), adjusted for AN RNA at day 0 (Supplementary Table 5). This RR changed little when also adjusted for AN RNA at day 3 (RR, 0.32 [95% CI, 0.16–0.62]). The corresponding PTE by AN RNA at day 3 in the regression models was 8%.

The PTE by AN RNA at day 3 increased but remained low when the analysis population was restricted to participants who received amubarvimab plus romlusevimab or its corresponding placebo (16%; Supplementary Table 6) or to participants who enrolled within 5 days of symptom onset (14%; Supplementary Table 7).

DISCUSSION

Findings from this exploratory analysis of >2000 nonhospitalized participants with COVID-19 provide considerable insights

Table 4. Proportion Hospitalized or Died Between Day 4 and Day 28 by Anterior Nasal Severe Acute Respiratory Syndrome Coronavirus 2 RNA Results at Day 0 and Day 3

Treatment Group	Day 0 AN SARS-CoV-2 RNA	Day 3 AN SARS-CoV-2 RNA			
		<LLoQ	2–5.99 log$_{10}$ Copies/mL	≥6 log$_{10}$ Copies/mL	All
Placebo only	<LLoQ	0/123 (0.0%)	1/8 (12.5%)	0/5 (0.0%)	1/136 (0.7%)
	2–5.99 log$_{10}$ copies/mL	4/83 (4.8%)	3/132 (2.3%)	1/10 (10.0%)	8/225 (3.6%)
	≥6 log$_{10}$ copies/mL	1/3 (33.3%)	3/80 (3.8%)	5/34 (14.7%)	9/117 (7.7%)
	All	5/209 (2.4%)	7/220 (3.2%)	6/49 (12.2%)	18/478 (3.8%)
mAb-treated only	<LLoQ	1/231 (0.4%)	0/12 (0.0%)	0/2 (0.0%)	1/245 (0.4%)
	2–5.99 log$_{10}$ copies/mL	2/331 (0.6%)	1/307 (0.3%)	0/6 (0.0%)	3/644 (0.5%)
	≥6 log$_{10}$ copies/mL	1/51 (2.0%)	12/378 (3.2%)	1/35 (2.9%)	14/464 (3.0%)
	All	4/613 (0.7%)	13/697 (1.9%)	1/43 (2.3%)	18/1353 (1.3%)
All participants	<LLoQ	1/354 (0.3%)	1/20 (5.0%)	0/7 (0.0%)	2/381 (0.5%)
	2–5.99 log$_{10}$ copies/mL	6/414 (1.4%)	4/439 (0.9%)	1/16 (6.3%)	11/869 (1.3%)
	≥6 log$_{10}$ copies/mL	2/54 (3.7%)	15/458 (3.3%)	6/69 (8.7%)	23/581 (4.0%)
	All	9/822 (1.1%)	20/917 (2.2%)	7/92 (7.6%)	36/1831 (2.0%)

Data are presented as proportion (%). "<LLoQ" represents results below the lower limit of quantification (2 log$_{10}$ copies/mL).

Abbreviations: AN, anterior nasal; LLoQ, lower limit of quantification; mAb, monoclonal antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 5. Association Between Severe Acute Respiratory Syndrome Coronavirus 2 RNA Results at Days 0 and 3 and Subsequent Hospitalization/Death Through Day 28

Regression Model	Covariate	Comparison	Placebo (n = 478)		mAb-Treated (n = 1353)	
			Risk Ratio (95% CI)	P Value	Risk Ratio (95% CI)	P Value
Unadjusted model 1	Day 0 AN RNA	2 log$_{10}$ copies/mL vs <LLoQ	2.29 (0.20–26.51)	.51	1.03 (0.11–9.95)	.98
		Per 1 log$_{10}$ copies/mL above LLoQ higher	1.35 (0.96–1.91)	.0840	**1.39 (1.09–1.76)**	**.0071**
Unadjusted model 2	Day 3 AN RNA	2 log$_{10}$ copies/mL vs <LLoQ	0.54 (0.12–2.37)	.41	2.17 (0.58–8.15)	.25
		Per 1 log$_{10}$ copies/mL above LLoQ higher	**1.59 (1.15–2.19)**	**.0047**	1.17 (0.81–1.67)	.40
Mutually adjusted model	Day 0 AN RNA	2 log$_{10}$ copies/mL vs <LLoQ	4.06 (0.29–56.66)	.30	0.98 (0.09–10.35)	.99
		Per 1 log$_{10}$ copies/mL above LLoQ higher	1.28 (0.82–1.98)	.28	**1.33 (1.01–1.76)**	**.0456**
	Day 3 AN RNA	2 log$_{10}$ copies/mL vs <LLoQ	0.22 (0.03–1.58)	.13	1.29 (0.33–5.15)	.71
		Per 1 log$_{10}$ copies/mL above LLoQ higher	1.42 (1.00–2.03)	.0526	1.02 (0.68–1.56)	.91

"<LLoQ" represents results below the lower limit of quantification (2 log$_{10}$ copies/mL). Event outcome was hospitalization or death between days 4 and 28. Analyses were restricted to participants with no event before or on day 3.

Unadjusted models 1 and 2 include covariates corresponding to day 0 AN RNA only and day 3 AN RNA only, respectively. The mutually adjusted model includes covariates corresponding to both day 0 and day 3 AN RNA. Separate models were fit for each arm (mAb-treated and placebo). For the day 3 AN RNA only model involving placebo recipients, the 95% CIs and 2-sided Wald test P values are from Poisson regression due to convergence issues with log-binomial regression. All other 95% CIs and 2-sided Wald test P values are from log-binomial regression. Risk ratios with 95% CIs that do not include 1 are bolded.

Abbreviations: AN, anterior nasal; CI, confidence interval; LLoQ, lower limit of quantification; mAb, monoclonal antibody.

into the value of nasal and plasma SARS-CoV-2 RNA levels as predictors of subsequent hospitalization/death both in the natural history setting (ie, among placebo recipients) and among individuals treated with mAbs. The study also provides important insights into the value of nasal SARS-CoV-2 RNA level measured at day 3 as a possible surrogate outcome in clinical trials.

We observed an increased risk of hospitalization/death with increasing AN RNA at day 0 with no apparent threshold of RNA above which risk increased, ranging from 2.7% for levels <2 log$_{10}$ copies/mL to 15.8% for levels ≥6 log$_{10}$ copies/mL among placebo recipients. We also observed that the proportion of participants who were hospitalized or died was 14% higher for persistently high RNA levels (≥6 log$_{10}$ copies/mL)

compared to participants with unquantifiable RNA levels (<2 log$_{10}$ copies/mL) at both day 0 and day 3. These findings are consistent with those from the BLAZE-1 trial, which identified an association of persistently high RNA levels in NP samples with risk of hospitalization or death in a combined population of mAb and placebo recipients, though participants who were hospitalized or died were predominantly placebo recipients [11]. This, along with findings showing that AN and NP RNA levels were highly correlated [20], suggests that self-collected AN samples may provide similar performance as provider-collected samples in assessing risk of hospitalization or death among nonhospitalized adults.

When we considered the utility of RNA levels at days 0 and 3 jointly for predicting risk of hospitalization or death between

days 4 and 28, there was no association with RNA level at day 3 among mAb-treated participants, in contrast to a stronger association among placebo recipients. Although this difference in association did not achieve conventional levels of statistical significance, if real, the predictive value of RNA level achieved following mAb treatment may be limited compared to the predictive value for untreated individuals. This finding needs further evaluation in studies with larger numbers of hospitalizations and deaths.

We also found that the substantial efficacy of mAb treatment versus placebo that continued after day 3 was minimally explained by RNA levels at day 3. Thus, the continued effect of mAb treatment after day 3 may be mediated considerably through mechanisms not associated with the effect of mAb treatment on RNA shedding measured in AN samples. For example, there may be strong direct antiviral effects of mAb treatment in the lungs that are only weakly reflected in the effects of mAb treatment on RNA shedding in AN samples or effects on inflammation that may be mediated by antiviral effects [17]. The large difference in risk of hospitalization or death between participants with detectable versus undetectable RNA in plasma at day 0 suggests exploring whether plasma RNA levels might explain more of the effect of treatment on risk of hospitalization/death, particularly if more sensitive assays become available that can quantify lower RNA levels in plasma.

Our findings, particularly the large effect of mAb treatment after day 3 not explained by AN RNA levels at day 3, as well as the possibility of different associations between mAb and placebo recipients, might raise concerns about relying on RNA levels as a surrogate outcome in randomized clinical trials [21]. A meta-regression analysis across randomized trials suggested that differences in risk of hospitalization/death over 28 days between treated and placebo recipients were associated with corresponding differences in mean change in NP RNA from baseline to 5–7 days [22]. Although associations within different classes of treatment (mAbs, convalescent plasma therapy, direct antiviral agents) were not evaluated, there did not appear to be an association across the 7 randomized trials of mAb treatments in their graphical presentation of results, and hence our results involving 2 mAb treatments may be consistent with their collation of trial-level results. This also emphasizes the need to repeat the evaluation of associations that we have studied in other classes of treatment, particularly for direct antiviral drugs.

Our study has several limitations. SARS-CoV-2 RNA levels were evaluated in participant self-collected AN samples rather than samples collected by research staff. However, standardized instructions were provided, day 0 collections were observed by staff for all participants, and we have shown strong correlation between RNA levels in these 2 collection types [20]. Many hospitalizations occurred prior to when posttreatment RNA levels were first measured, potentially limiting precision in assessing

predictive associations. While evaluations in studies with earlier RNA measurements would be valuable, the feasibility of earlier RNA measurements is limited. With 25% classified as lower risk of COVID-19 progression and some participants enrolling when mAb treatment was either unavailable or difficult to obtain, there is some uncertainty regarding the comparability of our analysis population to those receiving mAb treatment in clinical care. However, the consistency of results within the subgroup who received amubarvimab plus romlusevimab at a time when enrollment was restricted to higher risk participants suggests that our overall findings are applicable. To increase precision, we combined data from evaluations of 2 different mAb treatments within the ACTIV-2 trial. However, it is possible that associations might differ between the treatments or might be confounded by the lack of a control group for the open-label cohort. Finally, given that only 3% of participants had a history of SARS-CoV-2 vaccination, we cannot be certain that these findings are generalizable in a population of individuals who are highly vaccinated or have hybrid immunity.

This study is the first large-scale evaluation of the predictive value of nasal and plasma SARS-CoV-2 RNA levels for hospitalizations and deaths in people with mild-to-moderate COVID-19, both treated and untreated. Our results demonstrate that a single quantitative AN or plasma SARS-CoV-2 RNA measurement, whether subsequently mAb-treated or not, and persistently high AN RNA levels over 3 days, if untreated, predict risk of subsequent hospitalization or death in people with mild-to-moderate COVID-19. Our results also suggest the possibility that associations of AN RNA level with hospitalizations and deaths are different in people who have received mAb treatment, possibly limiting the value of AN RNA levels as a potential surrogate outcome measure in trials evaluating mAb treatments. Plasma RNA as a potential surrogate marker should be further evaluated.

Supplementary Data

Notes

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