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BRIEF REPORT

Nasal and Plasma Severe Acute Respiratory Syndrome Coronavirus 2 RNA Levels Are Associated With Timing of Symptom Resolution in the ACTIV-2 Trial of Nonhospitalized Adults With Coronavirus Disease 2019

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Acute Coronavirus Disease 2019 symptoms limit daily activities, but little is known about its association with severe acute respiratory syndrome coronavirus 2 viral burden. In this exploratory analysis of placebo recipients in the ACTIV-2/A5401 platform trial, we showed that high anterior nasal RNA levels and detectable plasma RNA were associated with delayed symptom improvement.

Clinical Trials Registration. https://clinicaltrials.gov/ct2/show/NCT04518410.

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Coronavirus disease 2019 (COVID-19) has a spectrum of symptomatology with variability of severity [1]. Acute symptoms last from days to weeks, and delayed recovery limits daily activities and hinders return to work and school. The virological determinants for acute symptom duration remain poorly understood. Identifying these determinants will further our understanding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis and identify key viral compartments as targets for antiviral interventions. In randomized clinical trials, different therapeutic agents have shortened the duration of symptoms in nonhospitalized adults with risk factors for severe COVID-19 [2–4], but the associations between virological features and clinical outcomes remain undetermined. In this study, we aimed to evaluate the association between SARS-CoV-2 viral burden and COVID-19 symptom outcomes in untreated, nonhospitalized individuals.

METHODS

Study Design

The Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV)-2/A5401 Study is a multicenter phase 2/3 adaptive platform randomized controlled trial for the evaluation of therapeutics for COVID-19 in nonhospitalized adults, as previously reported [5].

Participants

Eligibility criteria were reported previously [5]. Briefly, nonhospitalized individuals aged 18 years or older with documented SARS-CoV-2 infection, no more than 10 days of COVID-19 symptoms, and ongoing symptoms within 48 hours before enrollment were eligible. Participants with certain comorbidities (chronic lung disease or moderate to severe asthma, body mass index >35 kg/m^2, hypertension, cardiovascular disease, diabetes, or chronic kidney or liver disease) and/or older than 55 years were categorized as the high-risk group.

As our focus is on evaluating associations of symptom outcomes and virologic status in the natural history setting, we only included participants randomized to and who received placebo (saline) by infusion for the first 3 investigational agents studied in ACTIV-2 (bamlanivimab 7000 mg and bamlanivimab 700 mg, both in phase 2 [Eli Lilly and Company] and amubarvimab/romlusevimab 1000 mg/1000 mg in phase 2/3 [Brii Biosciences]) between August 2020 and July 2021 when ancestral strain, Alpha, and Delta variants were dominant [6].

Measurements

Participants recorded 13 targeted symptoms daily from day 0 (study entry) to day 28 as absent (assigned score 0), mild (1), moderate (2), or severe (3) in a symptom diary [5]. For each day, a symptom score was calculated as the sum of scores for the 13 symptoms (range: 0–39). Anterior nasal (AN) and plasma SARS-CoV-2 RNA at entry were measured with quantitative polymerase chain reaction (PCR) with a lower limit of quantification of 2.0 $\log_{10}$ copies/mL and a limit of detection of 1.4 $\log_{10}$ copies/mL [5].

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The 13 symptoms assessed for eligibility and self-assessed by participants daily on days 0 to 28 were as follows: fever or feeling feverish, cough, shortness of breath or difficulty breathing at rest or with activity, sore throat, body pain or muscle pain/aches, fatigue (low energy), headache, chills, nasal obstruction or congestion (stuffy nose), nasal discharge (runny nose), nausea, vomiting, and diarrhea [5].

Outcomes

The primary outcomes for this study included the following: (1) time to symptom improvement, defined as the time from entry to the first of 2 consecutive days of all 13 symptoms being improved (with lower severity score) from entry, and (2) time to symptom resolution, defined as the time from entry to the first of 2 consecutive days of all 13 symptoms recorded as absent. We also examined time to resolution for each of shortness of breath, cough, fatigue, and body ache symptoms, selected as the potentially most disabling.

Statistical Methods

The association between RNA levels and symptom scores at entry was evaluated using linear regression. Associations of time to symptom improvement or resolution with virologic variables were evaluated using proportional hazards regression. The primary model adjusted for duration of symptoms at entry. In secondary models, we additionally adjusted for age, comorbidities, country of enrollment, ethnicity, race, and sex. *P* values less than .05 were considered significant. Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA).

RESULTS

This analysis included 559 participants, with a median age of 49 years, 51% of whom were female, and 7% of whom were vaccinated against COVID-19 prior to entry (Supplementary Table 1). Participants were enrolled from the United States (77%), South Africa (11%), Argentina (9%), Brazil (3%), Mexico (<1%), and the Philippines (<1%) (Supplementary Table 1). A total of 479 (86%) met protocol criteria for higher risk of COVID-19 progression and median symptom duration at entry was 6 days (interquartile range: 4, 7). Median symptom score at entry was 10 (interquartile range: 6, 14); 150 participants (28% of 534 with available entry diary) reported at least 1 symptom as severe, while 3 (1%) were asymptomatic to all 13 symptoms assessed at study entry (Supplementary Table 2). A total of 523 and 467 participants had AN and plasma SARS-CoV-2 RNA available at study entry, respectively (Supplementary Table 3). Detectable plasma RNA (19%; 89/467) but not AN RNA level was associated with more severe symptoms at entry (2.2-points higher; 95% confidence interval [CI]: .8–3.6; $P = .003$, adjusted for symptom duration) (Supplementary Table 4).

A total of 499 participants with both available AN RNA and symptom score more than 0 at entry were analyzed. Participants with baseline AN RNA of 6 $\log_{10}$ copies/mL or greater had a markedly longer time to symptom improvement compared with those with AN RNA of less than 2 $\log_{10}$ copies/mL (median: 16.0 vs 9.0 days; hazard ratio adjusted for symptom duration at entry [aHR]: .63; 95% CI: .47–.84; $P = .001$) (Figure 1*A* and Supplementary Table 5); and a prolonged time to symptom resolution was also observed when AN RNA was greater than or equal to 6 $\log_{10}$ copies/mL (25.0 vs 15.0 days; aHR: .60; 95% CI: .43–.82; $P = .002$) (Figure 1*B* and Supplementary Table 5). Among the 445 participants with plasma RNA available and symptom score greater than 0 at entry, when adjusted for symptom duration at entry, detectable plasma RNA was associated with longer time to symptom improvement (median: 15.0 vs 10.0 days; aHR: .74; 95% CI: .56–.98; $P = .037$) but not with time to symptom resolution (median: 20.0 vs 16.0 days; aHR: .83; 95% CI: .62–1.12; $P = .23$) (Figure 1*C* and 1*D* and Supplementary Table 5). Similar associations between entry RNA levels and symptom outcomes were found in models adjusted for potential confounders (Supplementary Table 5).

We next evaluated the association between SARS-CoV-2 RNA levels and resolution of selected symptoms. Compared with individuals with AN RNA of less than 2 $\log_{10}$ copies/mL at entry, when adjusting for symptom duration, those with AN RNA of 6 $\log_{10}$ copies/mL or greater had delayed resolution of cough (aHR: .63; 95% CI: .45–.87; $P = .005$) and shortness of breath (aHR: .63; 95% CI: .42–.96; $P = .031$) but not fatigue or body pain (Supplementary Table 6). In a similarly adjusted model, detectable plasma SARS-CoV-2 RNA was associated with delayed resolution of cough (aHR: .67; 95% CI: .50–.90; $P = .008$), shortness of breath (aHR: .67; 95% CI: .47–.97; $P = .036$), and body pain (aHR: .74; 95% CI: .55–.99; $P = .042$) but not fatigue (Supplementary Table 7). These associations were attenuated in models adjusted for potential confounders (Supplementary Tables 5–7).

DISCUSSION

In this study in largely unvaccinated participants with COVID-19 during the Delta and pre-Delta variant period of the pandemic, higher AN and plasma SARS-CoV-2 RNA levels in the first 10 days of symptoms were associated with longer time to resolution of acute COVID-19 symptoms. Most previous studies have focused on SARS-CoV-2 viral burden or shedding and hospitalization/death [7–10] and have not examined symptom duration, which can significantly impact daily life and are important patient-reported outcomes in evaluations of antiviral therapeutics. Our findings contrast with results from the only published human challenge trial in 36 young adults that found no correlation between viral burden and symptom severity

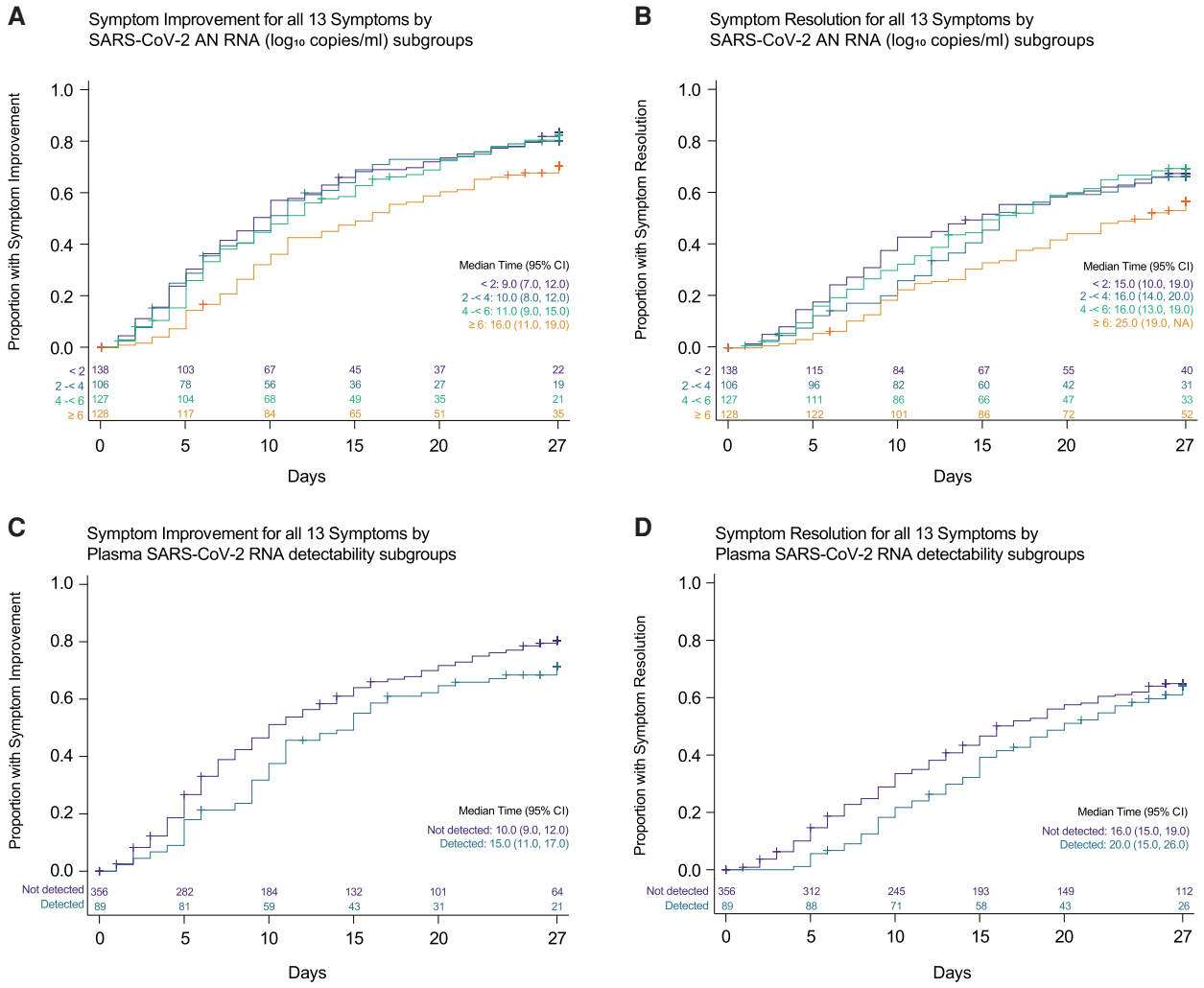

Figure 1. Association between AN or plasma SARS-CoV-2 RNA levels and symptom improvement or resolution. Kaplan-Meier curves demonstrating the time from entry of the study to the observation endpoints. *A*, AN SARS-CoV-2 RNA (log_{10} copies/mL) and time to symptom improvement. *B*, AN SARS-CoV-2 RNA (log_{10} copies/mL) and time to symptom resolution. *C*, Plasma SARS-CoV-2 RNA detectability and time to symptom improvement. *D*, Plasma SARS-CoV-2 RNA detectability and time to symptom resolution. "+" indicates censored. Median time to events with 95% CIs are shown. Abbreviations: AN, anterior nasal; CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[11]. We also demonstrate that SARS-CoV-2 viremia is associated with delayed symptom improvement, especially cough, shortness of breath, and body pain. This association could be due to higher levels of inflammation and tissue injury with SARS-CoV-2 viremia [12]. Our findings implicate the use of nasal and plasma SARS-CoV-2 RNA levels in the outpatient setting, especially to prognosticate acute symptom duration, although this is limited by the availability of plasma SARS-CoV-2 RNA testing, which is currently primarily available in the research setting.

This study is limited by the few participants vaccinated against COVID-19 or with Omicron infection, as it is possible that associations will be different with COVID-19 following prior vaccination or with current variants. We also examined acute symptom outcomes only; additional studies will be needed to evaluate associations with post-acute sequalae of COVID-19. Furthermore, sputum sampling was not obtained in this study and, thus, we were unable to evaluate lower respiratory RNA burden and symptom evolution. Finally, we focused on the available nasal and plasma viral RNA results at study entry, which can vary depending on the timing of enrollment from the onset of disease [13], and thus we adjusted for symptom duration in the primary model (model 1).

In summary, we demonstrate that SARS-CoV-2 RNA burden in the upper respiratory tract and in plasma is associated with COVID-19 acute symptom duration in nonhospitalized adults. Additional studies are needed to determine whether accelerated declines in RNA that might be associated with vaccines or treatment will reduce symptom duration.

Supplementary Data

Supplementary materials are available at *Clinical 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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