Title

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Journal

Clinical Infectious Diseases, 76(4)

ISSN

1058-4838

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Publication Date

2023-02-18

DOI

10.1093/cid/ciac818

Peer reviewed

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

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Running title: SARS-CoV-2 RNA and symptom duration

Abstract

Acute COVID-19 symptoms limit daily activities, but little is known about its association with SARS-CoV-2 viral burden. In this exploratory analysis of placebo recipients in the ACTIV-2/A5401 platform trial, we showed that high anterior nasal (AN) RNA levels and detectable plasma RNA were associated with delayed symptom improvement.

Key words: SARS-CoV-2; COVID-19; Symptom duration; RNA

Clinical Trial Registration: NCT04518410

Introduction

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 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 non-hospitalized adults with risk factors for severe COVID-19[2-4], but the associations between virological features and clinical outcomes remains undetermined. In this study, we aim to evaluate the association between SARS-CoV-2 viral burden and COVID-19 symptom outcomes in untreated, non-hospitalized 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 non-hospitalized adults, as previously reported[5].

Participants

Eligibility criteria were reported previously[5]. Briefly, non-hospitalized individuals ≥18 years with documented SARS-CoV-2 infection, no more than 10 days of COVID-19 symptoms, and ongoing symptoms (See Supplementary Materials) 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 three investigational agents studied in ACTIV-2 (bamlanivimab 7000 mg and bamlanivimab 700 mg, both in phase 2 (Lilly) and amubarvimab/romlusevimab 1000 mg/1000 mg in phase 2/3, Brii) between August 2020 and July 2021 when ancestral strain, alpha, and delta variants were dominant[6].

Measurement

Participants recorded 13 targeted symptoms daily from day 0 (study entry) to 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 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].

The 13 symptoms assessed for eligibility and self-assessed by participants daily days 0 to 28 were: 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: (1) time to symptom improvement, defined as the time from entry to the first of 2 consecutive days of all 13 symptoms 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<0.05 were considered significant. Statistical analyses were conducted using SAS (version 9.4, Cary, NC).

Results

This analysis included 559 participants, with a median age of 49 years, 51% female, and 7% vaccinated against COVID-19 prior to entry (Supplementary Table S1). Participants were enrolled from the United States of America (77%), South Africa (11%), Argentina (9%), Brazil (3%), Mexico (<1%) and the Philippines (<1%) (Supplementary Table S1). 479 (86%) met protocol criteria for higher risk of COVID-19 progression and median symptom duration at entry was 6 days (quartiles: 4, 7). Median symptom score at entry was 10 (quartiles 6,14); 150 (28% of 534 with available entry diary) reported at least one symptom as severe, while 3 (1%) were asymptomatic to all 13 symptoms assessed at study entry (Supplementary Table S2). 523 and 467 participants had AN and plasma SARS-CoV-2 RNA available at study entry, respectively (Supplementary Table S3). Detectable plasma RNA (19%, 89/467) but not AN RNA level was associated with more severe symptoms at entry (2.2-points higher, 95% CI 0.8-3.6, P=0.003, adjusted for symptom duration) (Supplementary Table S4).

499 participants with both available AN RNA and symptom score>0 at entry were analyzed. Participants with baseline AN RNA≥6 $\log_{10}$ copies/mL had a markedly longer time to symptom improvement compared to those with AN RNA <2 $\log_{10}$ copies/mL (median 16.0 vs 9.0 days, hazard ratio adjusted for symptom duration at entry [aHR] 0.63, 95% CI 0.47-0.84, P=0.001) (Figure 1A and Supplementary Table S5); prolonged time to symptom resolution was also observed when AN RNA≥6 $\log_{10}$ copies/mL (25.0 vs. 15.0 days; aHR 0.60, 95%CI 0.43-0.82, P=0.002) (Figure 1B and Supplementary Table S5). Among the 445 participants with plasma RNA available and symptom score>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 0.74, 95%CI 0.56-0.98, P=0.037) but not with time to symptom resolution (median 20.0 vs. 16.0 days, aHR 0.83, 95%CI 0.62-1.12, P=0.23) (Figure 1C-1D and Supplementary Table S5). Similar associations between entry RNA levels and symptom outcomes were found in models adjusted for potential confounders (Supplementary Table S5).

We next evaluated the association between SARS-CoV-2 RNA levels and resolution of selected symptoms. Compared to individuals with AN RNA<2 $\log_{10}$ copies/mL at entry, when adjusting for symptom duration, those with AN RNA≥6 $\log_{10}$ copies/mL had delayed resolution of cough (aHR 0.63, 95%CI 0.45-0.87, P=0.005) and shortness of breath (aHR 0.63, 95% CI 0.42-0.96, P=0.031) but not fatigue or body pain (Supplementary Table S6). In a similarly adjusted model, detectable plasma SARS-CoV-2 RNA was associated with delayed resolution of cough (aHR

0.67, 95% CI 0.50-0.90, P=0.008), shortness of breath (aHR 0.67, 95% CI 0.47-0.97, P=0.036) and body pain (aHR 0.74, 95% CI 0.55-0.99, P=0.042) but not fatigue (Supplementary Table S7). These associations were attenuated in models adjusted for potential confounders (Supplementary Tables S5, S6, S7).

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[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 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 non-hospitalized adults.

Additional studies are needed to determine whether accelerated declines in RNA that might be associated with vaccines or treatment will reduce symptom duration

NOTES

Acknowledgements

We thank the study participants, site staff, site investigators, and the entire ACTIV-2/A5401 study team; the AIDS Clinical Trials Group, including Lara Hosey, Jhoanna Roa, and Nilam Patel; [the UW Virology Specialty Laboratory staff, including Emily Degli-Angeli, Erin Goecker, Glenda Daza, Socorro Harb, and Joan Dragavon; the ACTG Laboratory Center, including Grace Aldrovandi and William Murtaugh; Frontier Science, including Marlene Cooper, Howard Gutzman, Kevin Knowles, and Rachel Bowman; the Harvard Center for Biostatistics in AIDS Research (CBAR) and ACTG Statistical and Data Analysis Center (SDAC); the National Institute of Allergy and Infectious Diseases (NIAID) / Division of AIDS (DAIDS); Bill Erhardt; the Foundation for the National Institutes of Health and the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) partnership, including Stacey Adams; and the PPD clinical research business of Thermo Fisher Scientific.

Funding

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1AI068634 (MDH, MG, JR, CM and LJH), UM1AI068636 (JSC, DMS, and KWC) and UM1AI106701 (RWC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. YL received NIH T32 training grant from 2021-2022 (5T32AI007387-32, PI: Daniel Kuritzkes). LJH, CM, and JR reports support from NIH/NIAID 3UM1 AI068634-15S1 (paid to institution). ESD and MDH reports support from NIH. RWC reports support from P30-AI-027757.

Conflicts of Interests

LJH: reports grants or contracts from NIH/NIAID 3 UM1 AI068636-16S1 and NIH/NIAID T32 AI007358 (paid to institution).

KWC: research funding to the institution from Merck Sharp & Dohme (paid to institution) and Amgen (research contract with institution), consultant for Pardes Biosciences, Honoraria to

author for CME presentations (not-for-profit organization) from International Antiviral Society-USA, Participation on a Data Safety Monitoring Board or Advisory Board for UCSF (served as Chair of a Safety Monitoring Committee for an investigator-initiated study where the sponsor is UCSF).

ESD: receives consulting fees from Gilead Sciences, Merck, and GSK/ViiV and research support through the institution from Gilead Sciences and GSK/ViiV and reports support from NIH; including participation on a Data Safety Monitoring Board or Advisory Board for Gilead and ViiV.

DAW has received funding to the institution to support research and honoraria for advisory boards and consulting from Gilead Sciences and grant or contracts from Lilly.

JZL has consulted for Abbvie and received research grant from Merck.

WF has received research funding to the institution from Ridgeback Biopharmaceuticals, served on adjudication committees for Janssen, Syneos, and consulted for Roche and Merck.

JJE is an ad hoc consultant to GSK/VIR, data monitoring committee (DMC) chair for Adagio Phase III studies.

JSC has consulted for Merck and Company and reports leadership or fiduciary role in other board, society, committee or advocacy group as a volunteer for the Board of Directors IAS-USA and the Foundation Board, Conference on Retroviruses and Opportunistic Infections.

DMS has consulted for the following companies Bayer Healthcare (treatment for HSV), Fluxergy, Kiadis, Linear Therapies, Matrix BioMed, VxBiosciences, Model Medicines, Bayer Pharmaceuticals, Pharma Holdings, and grants or contracts from NIH (DK131532, AI169609, DA047039, AI036214, AI131385, AI100665, AI126620: funding provided to my institution), The James B. Pendleton Charitable Trust, and The James B. Pendleton Charitable Trust John and Mary Tu Foundation; including payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events for Medscape (COVID treatment), AiCME (COVID treatment), and Kiadis (NK Cells as therapy for ID), stock or stock options for Model Medicine, Linear Therapies, and Cv Biosciences (Antiviral drug development), and receipt of equipment, materials, drugs, medical writings, gifts or other services from The James B. Pendleton Charitable Trust.

THE receives consulting fees from Tonix Pharmaceuticals.

CM reports participation on a Data Safety Monitoring Board for BONE START (unpaid participation).

Other authors declare no conflicts of interests related to this current work.

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Figure Legend

Figure 1. Association between anterior nasal (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% confidence intervals was shown.

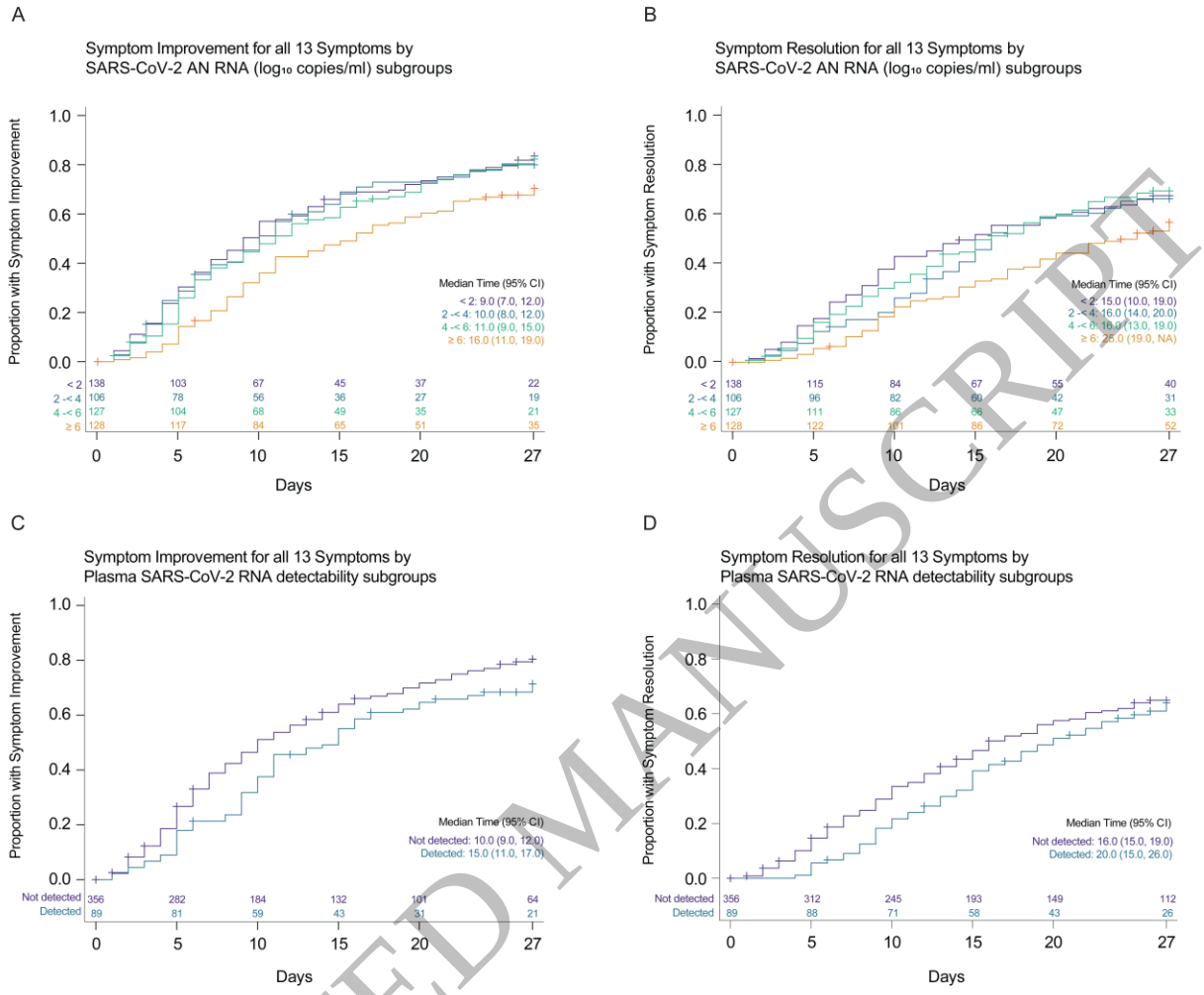

Figure 1
165x137 mm (x DPI)