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The Journal of Infectious Diseases

BRIEF REPORT

Upper Respiratory Tract Levels of Severe Acute Respiratory Syndrome Coronavirus 2 RNA and Duration of Viral RNA Shedding Do Not Differ Between Patients With Mild and Severe/Critical Coronavirus Disease 2019

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This study reports longitudinal viral RNA loads from the nasopharynx/throat in patients with mild and severe/critical coronavirus disease 2019 (COVID-19). We also investigated whether the duration of symptoms correlated with the duration of viral RNA shedding. A total of 56 patients were included. The highest viral loads occurred early after onset of symptoms. Neither the viral RNA loads in the upper respiratory tract nor the time to viral RNA clearance differed between patients with mild or severe/critical disease. There was a moderate correlation between number of days with symptoms and number of days with viral RNA shedding in patients with mild COVID-19.

Keywords. SARS-CoV-2, viral shedding, nasopharynx, COVID-19.

Coronavirus disease 2019 (COVID-19) is an acute respiratory tract infection caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Similar to influenza, the nasopharyngeal viral load of SARS-CoV-2 is highest at the time of presentation [1]. A previous study found that patients with severe COVID-19 have higher viral loads and shed viral RNA longer from the throat and nasopharynx than those with mild disease [2], and the authors suggested that nasopharyngeal levels of SARS-CoV-2 RNA might be used to assess disease severity and prognosis. We here report longitudinal viral loads in upper respiratory specimens from patients with mild COVID-19 and patients with severe/critical COVID-19. In addition, we have investigated whether the duration of symptoms correlates with the duration of viral RNA shedding in patients with mild COVID-19.

MATERIALS AND METHODS

Participants were recruited from the Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden, between 25 February and 23 April 2020. Severe/critical patients were defined as those requiring invasive mechanical ventilation or high-flow nasal oxygen and mild as those not requiring supplementary oxygen or hospitalization. The diagnosis of COVID-19 was made by real-time polymerase chain reaction (PCR) at the Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden. Viral load, expressed as $\log_{10}$ of viral RNA per swab, was calculated as (47 – observed cycle threshold value) / 3.4. This formula applies the average of the parameters (slope and constant) that we observe when we quantify viruses in serum using quantitative PCR with serial dilution of quantification standards (plasmid carrying target sequence) and presumes a 10-µL sample volume in the reaction and a 97% efficiency in the PCR. We collected serial upper respiratory tract samples (1 nasopharyngeal swab and 1 throat swab put in a single collection tube with 1 mL of transport medium) for real-time PCR of SARS-CoV-2 RNA for all patients. Nucleic acids were extracted from 200 µL of sample in a Magnapure instrument (Roche Molecular, Branchburg, New Jersey), and 10 µL of the purified sample was used for 1-step reverse-transcription PCR in a QuantStudio 6 real-time PCR instrument. After 30 minutes of reverse transcription at 46°C, 45 cycles of amplification with 58°C annealing temperature were performed, using RdRP_F, GTCATGTGTGGCGGTTCACT and RdRP_R, CAACACTATTAGCATAAGCAGTTGT as primers, and RdRP_P, CAGGTGGAACCTCATCAGGAGATGC as fluorescent hydrolysis probe.

The date of disease onset was defined as the day when the first symptoms of COVID-19 were observed. For individuals with mild COVID-19, we also longitudinally recorded clinical symptoms (cough, fever >37.5°C, sore throat, rhinitis, and muscle pain). Patients with severe/critical COVID-19 were not included in this part as it was not possible to determine the duration of symptoms for those in this group who were sedated and in need of mechanical ventilation.

The study protocol was approved by the Swedish Ethical Review Authority (Dnr: 2020-01771) and patients were included after informed consent.

Received 9 June 2020; editorial decision 30 September 2020; accepted 3 October 2020; published online October 5, 2020.

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The Journal of Infectious Diseases® 2021;223:15–8

DOI: 10.1093/infdis/jiaa632

RESULTS

A total of 56 adult participants were included in the study. Of those with mild disease, 24 of 39 were female (median age, 43 years [range, 19–71 years]). Among patients with severe/critical COVID-19, 16 of 17 were male (median age, 52 years [range, 46–81 years]). Three of the patients with severe/critical COVID-19 died during the study period, on days 21, 26, and 31 after onset of symptoms. A total of 328 (mean, 5.9 [range, 2–12]) nasopharyngeal and throat swabs were collected.

The highest viral loads were observed early after onset of symptoms in both groups of participants (Figure 1A and 1B). At 7 days, mean viral load among virus-positive patients was 5.8 $\log_{10}$ copies/swab for those with mild disease (n = 37) and 5.5 $\log_{10}$ copies/swab for those with severe/critical disease (n = 12) (P = .53). At 14 days, mean viral load was 4.4 $\log_{10}$ copies/swab for those with mild disease (n = 38) and 4.2 $\log_{10}$ copies/swab for those with severe/critical disease (n = 14) (P = .56). Linear interpolation was used when samples were not taken on exactly days 7 and 14, using the values measured on the closest days just before and after, to estimate the values on days 7 and 14.

Neither the viral loads in nasopharynx and throat nor the time to viral RNA clearance differed between patients with mild or severe/critical disease (Figure 1A and 1B). The median duration of viral RNA shedding was 24.0 days in patients with mild disease and 22.5 days in patients with severe/critical disease (Figure 1C).

We recorded clinical symptoms for 34 of the 39 participants with mild disease. Follow-up was done by regular phone calls. The most common presenting symptom was cough and fever, both occurring in 27 of 34 participants (79%), followed by muscle pain in 22 (65%), rhinitis in 20 (59%), and sore throat in 16 (47%). There was a moderate correlation between number of days with symptoms and number of days with viral RNA shedding (Pearson r = 0.34; P = .05) (Supplementary Figure). Most participants (26/34) continued to be positive for SARS-CoV-2 RNA in the upper respiratory tract after the resolution of symptoms; 20 (59%) were PCR positive for >2 days after symptoms had resolved, 12 (35%) for >7 days, and 10 (29%) for >14 days.

DISCUSSION

We found that the SARS-CoV-2 viral RNA loads from nasopharynx and throat were as high among individuals with mild disease as those with severe/critical disease and that there were no significant differences between the 2 groups with regard to duration of viral RNA shedding from the upper respiratory tract. These findings are both in agreement and disagreement with previous studies.

Similar to our results, previous studies, most of them small, have reported that the viral loads in the upper respiratory tract peak at the time of, or early after, onset of symptoms [1–6]. High initial viral loads in the upper respiratory tract indicate a high degree of viral RNA shedding and thereby a potential for high risk of transmission during the early stages of the disease. This pattern of viral RNA shedding is similar to influenza, but different from the Middle East respiratory syndrome and severe acute respiratory syndrome coronavirus infections, where the peak viral load usually occurs between days 7 and 10 after onset of symptoms [7, 8]. In addition, individuals with COVID-19 have been shown to shed SARS-CoV-2 viral RNA a few days prior to occurrence of symptoms [9].

There are several reports [2, 10–12] where patients with severe to critical COVID-19 have been demonstrated to have higher, in some studies much higher, viral RNA loads in the upper or lower respiratory tract compared to patients with mild to moderate COVID-19. In 1 of these studies, patients with a higher baseline viral load (at admission to hospital) were more likely to develop severe disease [11]. Based on this, it has been suggested that higher viral loads could be associated with worse clinical outcome and that the respiratory tract levels of SARS-CoV-2 RNA might be useful for estimating disease severity and prognosis. The results from our study and 2 other smaller studies [3, 7] differ from this.

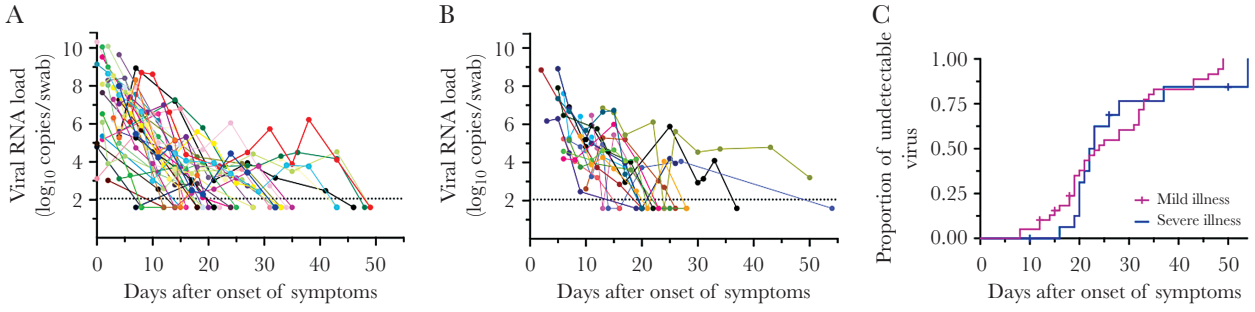

Figure 1. Viral load in nasopharyngeal and throat swabs in patients with coronavirus disease 2019 (COVID-19). Longitudinal viral loads for 39 patients with mild disease (*A*) and 17 with severe/critical disease (*B*). Dotted lines mark the detection limit of the polymerase chain reaction assay. *C*, Kaplan–Meier curve indicates time to viral RNA clearance for those with mild and severe/critical COVID-19.

We did not find any correlation between disease severity and viral RNA load; SARS-CoV-2 viral RNA loads from nasopharynx and throat in patients with mild COVID-19 were as high as in those with severe/critical disease. Our findings do not therefore support using levels of SARS-CoV-2 RNA as a prognostic marker. There are several possible explanations for the conflicting results. One is that the definitions of disease severity for participants with mild as well as severe/critical disease are not the same in all studies. There are also some differences in the study populations regarding age, sex, and/or comorbidities and the sampled material (nasopharynx, throat, sputum, or saliva). In 1 study it was not possible to determine how long after disease onset the samples were taken, since only information about number of days from hospital admission to sampling was provided [10]. Finally, various interventions such as antiviral drugs and corticosteroids were used in some studies, which could possibly have had an impact on SARS-CoV-2 viral RNA levels and duration of viral RNA shedding [10, 12]. It should be noted that samples from the lower respiratory airways, such as tracheal aspirate samples or sputum, were not analyzed in our study. SARS-CoV-2 RNA levels might be higher in the lower than the upper airways, especially later in the course of infection [1, 5, 13].

In this longitudinal study, approximately half of the patients were PCR positive for SARS-CoV-2 for >20 days after onset of symptoms and there was no significant difference in time of viral RNA clearance between those with mild and severe/critical COVID-19, which is in contrast with other studies where patients with severe COVID-19 took longer to clear the virus from the upper respiratory tract than those with mild disease [2, 14].

There was a moderate correlation between number of days with symptoms and number of days with positive PCR for SARS-CoV-2 in the nasopharynx and throat. About one-third of the participants continued to test positive for SARS-CoV-2 RNA after >14 days of resolution of symptoms. The presence of viral RNA does not always, however, correlate with viability and transmissibility of virus. Live virus has been demonstrated to be easier to isolate from sputum during the first week of symptoms as compared to later on despite continuous high viral loads; after 8 days of symptoms, virus could not be isolated from any samples in 1 study [13]. In addition, the transmissibility of SARS-CoV-2 seems to be higher when exposure occurs within the first 5 days of onset of symptoms in the index case, compared to those exposed later [15].

To conclude, in this study we found that the viral load in the upper respiratory tract did not differ between individuals with mild or severe/critical COVID-19, and neither did the duration of viral RNA shedding. Our findings therefore do not support using levels of SARS-CoV-2 RNA as a prognostic marker.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. This work was supported by Swedish State Support for Clinical Research (ALFGBG-717531); and by the SciLifeLab/KAW National COVID-19 Research Program Project (grant number V-2020-0250).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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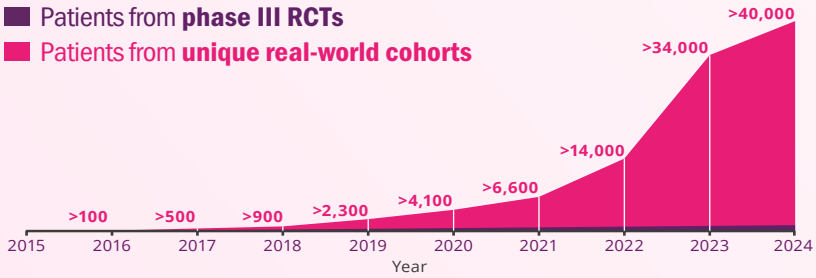

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ABBREVIATIONS

3TC, lamivudine; **CD4,** cluster of differentiation 4; **DTG,** dolutegravir; **FDA,** United States Food and Drug Administration; **FTC,** emtricitabine; **HIV,** human immunodeficiency virus; **ITT-E,** intention-to-treat exposed; **NRTI,** nucleoside/nucleotide reverse transcriptase inhibitor; **RCT,** randomised controlled trial; **RNA,** ribonucleic acid; **TAF,** tenofovir alafenamide fumarate; **TDF,** tenofovir disoproxil fumarate; **XTC,** emtricitabine.

FOOTNOTES

*Data extracted from a systematic literature review of DTG+3TC real-world evidence. Overlap between cohorts cannot be fully excluded.

**The reported rate reflects the sum-total of resistance cases calculated from GEMINI I and II (n=1/716, through 144 weeks), STAT (n=0/131, through 52 weeks), and D2ARLING (n=0/106, through 24 weeks).[5–7]

†GEMINI I and II are two identical 148-week, phase III, randomised, double-blind, multicentre, parallel-group, non-inferiority, controlled clinical trials testing the efficacy of DTG/3TC in treatment-naïve patients. Participants with screening HIV-1 RNA ≤500,000 copies/mL were randomised 1:1 to once-daily DTG/3TC (n=716, pooled) or DTG + TDF/FTC (n=717, pooled). The primary endpoint of each GEMINI study was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 48 (ITT-E population, snapshot algorithm).[13]

‡STAT is a phase IIIb, open-label, 48-week, single-arm pilot study evaluating the feasibility, efficacy, and safety of DTG/3TC in 131 newly diagnosed HIV-1 infected adults as a first line regimen. The primary endpoint was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 24.[6]

§D2ARLING is a randomised, open-label, phase IV study designed to assess the efficacy and safety of DTG/3TC in treatment-naïve people with HIV with no available baseline HIV-1 resistance testing. Participants were randomised in a 1:1 ratio to receive DTG/3TC (n=106) or DTG + TDF/XTC (n=108). The primary endpoint was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 48.[7] Results at week 24 of the study.

||The reported rate reflects the sum-total of resistance cases calculated from TANGO (n=0/369, through 196 weeks) and SALSA (n=0/246, through 48 weeks).[8,9]

¶TANGO is a randomised, open-label, trial testing the efficacy of DOVATO in virologically suppressed patients. Participants were randomised in a 1:1 ratio to receive DOVATO (n=369) or continue with TAF-containing regimens (n=372) for up to 200 weeks. At Week 148, 298 of those on TAF-based regimens switched to DOVATO. The primary efficacy endpoint was the proportion of subjects with plasma HIV-1 RNA ≥50 copies/mL (virologic non-response) as per the FDA Snapshot category at Week 48 (adjusted for randomisation stratification factor).[8,13]

#SALSA is a phase III, randomised, open-label, non-inferiority clinical trial evaluating the efficacy and safety of switching to DTG/3TC compared with continuing current antiretroviral regimens in virologically suppressed adults with HIV. Eligible participants were randomised 1:1 to switch to once-daily DTG/3TC (n=246) or continue current antiretroviral regimens (n=247). The primary endpoint was the proportion of subjects with plasma HIV-1 RNA ≥50 copies/mL at Week 48 (ITT-E population, snapshot algorithm).[9]

PM-GBL-DLL-BNNR-240004 May 2024