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BRIEF REPORT

Viral Load Kinetics of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospitalized Individuals With Coronavirus Disease 2019

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) kinetics remain understudied, including the impact of remdesivir. In hospitalized individuals, peak sputum viral load occurred in week 2 of symptoms, whereas viremia peaked within 1 week of symptom-onset, suggesting early systemic seeding of SARS-CoV-2. Remdesivir treatment was associated with faster viral decay.

Keywords. COVID-19; remdesivir; SARS-CoV-2; viral kinetics; viral load.

Understanding viral load dynamics has provided key insight on viral pathogenesis and treatment effects across the spectrum of viral infections [1]. Study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) kinetics within the respiratory tract has already provided valuable information about disease course, transmission risk, and efficacy of antibody therapeutics [2–5]. During severe coronavirus disease 2019 (COVID-19) infection, SARS-CoV-2 viral ribonucleic acid (RNA) can be detected not only in the upper (URT) and lower respiratory tracts (LRT), but also systemically in plasma [6]. Viral decay kinetics can be influenced by multiple factors, including replication dynamics, host cell turnover, and focal intensity of immune responses. However, little is known about the differences in viral decay between respiratory and nonrespiratory compartments [7], especially because viremia is associated with COVID-19 disease severity and mortality [6, 8].

Whereas viral decay from nasopharyngeal sampling has been valuable in evaluating the efficacy of monoclonal antibody treatments against SARS-CoV-2 [4, 5], the effect of remdesivir on viral dynamics remains unclear. Although remdesivir seems to confer a clinical benefit [9], its ability to alter respiratory tract SARS-CoV-2 kinetics has not been demonstrated [10]. It is unknown whether remdesivir treatment effects may be more accurately observed by evaluating a range of specimen types.

We present an observational study of viral kinetics in patients hospitalized for COVID-19. We quantify SARS-CoV-2 viral load in longitudinal samples from the respiratory tract and plasma, and we evaluate the effect of remdesivir on viral load decay.

METHODS

Participant Enrollment and Sample Collection

We enrolled patients hospitalized with COVID-19 . Longitudinal nasopharyngeal swabs, oropharyngeal swabs, sputum, and blood were collected from some patients. Each participant's medical record was reviewed to determine their oxygenation status, demographics, comorbidities, treatment status, and clinical outcome.

Patient Consent Statement

Informed written consent was obtained from all patients except for 10 patients who received waivers of informed consent. This study was approved by the Mass General Brigham Institutional Review Board.

Severe Acute Respiratory Syndrome Coronavirus 2 Kinetics Analysis

Severe acute respiratory syndrome coronavirus 2 viral loads were quantified with an in-house reverse-transcription quantitative polymerase chain reaction assay as previously described [6]. Viral load kinetics among compartments were compared using all data from 196 participants and also compared using only longitudinal data from participants with samples collected 7–14 days apart. To analyze the effect of remdesivir on SARS-CoV-2 decay rate, we used mixed-effects modeling on a subset of data comprising participants who were sampled longitudinally [11], using Monolix Software 2018R2 (http://www.lixoft.eu). We modeled viral decay using patient-compartment datasets, which consist of the viral load data for a single anatomical compartment in a single patient. Starting from our original 196-patient dataset, patients without longitudinal

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sampling were excluded along with any patient with unknown treatment status, unknown symptom onset, or remdesivir treatment during a previous hospital admission. We included detectable measurements and 1 subsequent undetectable measurement if it was within 1 week of the last detectable measurement. We excluded patient-compartment data from participants for whom there were no detectable measurements and data from before viral load decay. After this postprocessing, we excluded any patient-compartment dataset that did not have at least 2 data points. We were left with data for 51 participants, with 70 distinct patient-compartment datasets. Of these 70 datasets, 18.6%, 15.7%, 45.7%, and 20% belonged to the nasopharyngeal, oropharyngeal, plasma, and sputum compartments, respectively.

Decaying viral loads were modeled as $V(t) = V_0 e^{-rt}$, using mixed-effects modeling on the $\log_{10}$-transformed data and treating undetectable measurements as censored at the assay limit of detection. This analysis was performed for all compartment data together.

Statistical Analysis

Levels of SARS-CoV-2 RNA were compared with the duration of time between symptom onset and sample collection. All correlation analysis was performed using Spearman rank-based testing. Changes in SARS-CoV-2 viral load were calculated as the change in $\log_{10}$ copies of RNA per day between sample collections and were treated as a continuous variable. Estimated decay rate r was treated as a continuous variable. All continuous variables were analyzed with non-parametric rank-based testing. Comparison of viral loads (detectable vs undetectable) were treated as categorical variables and analyzed using Fisher's exact tests.

RESULTS

Differential Kinetics of Severe Acute Respiratory Syndrome Coronavirus 2 Viral Loads Over Time

We enrolled 196 symptomatic, hospitalized participants with COVID-19 (Supplemental Table 1). The proportion of samples with detectable SARS-CoV-2 RNA were highest within the first week of symptoms for samples collected from the nasopharyngeal (57%), oropharyngeal (83%), and plasma (38%) compartments (Figure 1A). In contrast, there was a delay in viral seeding of the LRT with significant increases in the proportion of samples with detectable sputum viral loads in the second week after symptom onset (week 1 vs 2: 56% vs 100%, $P = .003$) and higher median peak sputum viral loads (week 1 vs 2: 1.8 vs 5.6 $\log_{10}$ RNA copies/mL, $P = .02$) (Supplemental Figure 1). In the setting of delayed peak sputum viral load, the proportion of samples with detectable sputum viral load was significantly higher in subsequent weeks compared with other compartments. Four weeks after symptom onset, 63% of participants had detectable viral load in sputum compared with 13% by nasopharyngeal swab, 25% by oropharyngeal swab, and 4% by plasma ($P < .01$ for comparisons of sputum viral load against each of the other compartments).

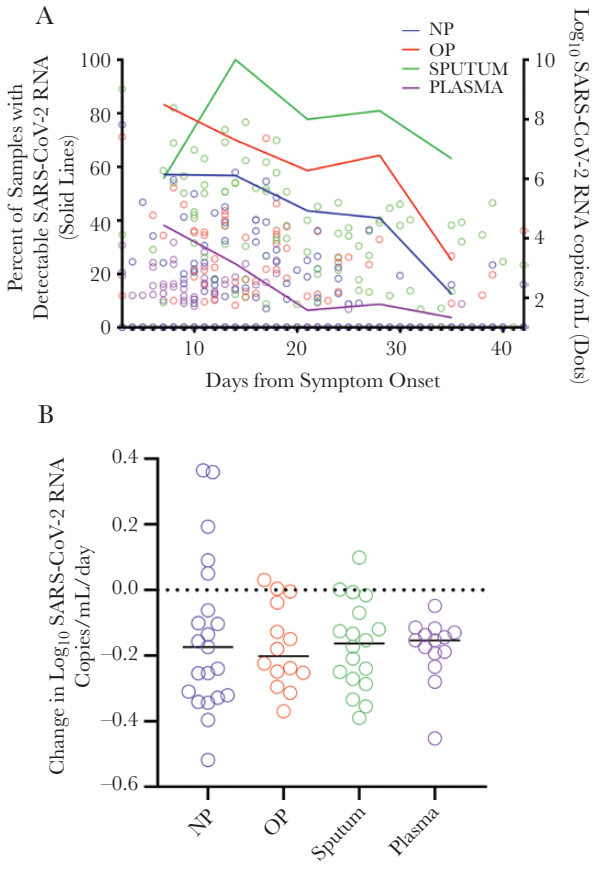

Figure 1. (A) Lines show the percentage of samples with detectable viral ribonucleic acid (RNA) for each compartment. Each point on the line represents the percentage of samples in the previous 7 days with detectable RNA, and the final point represents the proportion of detectable samples taken more than 28 days from symptom onset. Dots show individual viral load values for all sample types on the right axis. (B) The average rate of change in viral load over time given as the change in $\log_{10}$ copies/mL per day for each sample compartment. Thirty-eight participants had 2 data points spaced between 7 and 14 days apart in at least 1 compartment and were included in this analysis. Sputum analysis included samples collected after 1 week of symptoms given the delayed peak in viral load. Wilcoxon rank-sum tests show no significant differences between any 2 groups. NP, nasopharyngeal; OP, oropharyngeal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Severe acute respiratory syndrome coronavirus 2 viral load was correlated with the number of days between symptom onset and sample collection in nasopharyngeal swabs (Spearman $r = -.36$, $P < .0001$), oropharyngeal swabs ($r = -.36$, $P = .0001$), sputum ($r = -.39$, $P < .0001$), and plasma ($r = -.32$, $P < .0001$) (Figure 1A). In the subset of participants with longitudinal samples collected 7–14 days apart, the rate of viral decay did not vary significantly between different compartments (Figure 1B). The median number of days between viral load timepoints was 8 days for all compartments.

Remdesivir Treatment Was Associated With Faster Viral Decay Across Multiple Compartments

We analyzed a subset of participants with longitudinal sample collections to model the distribution of viral decay rates simultaneously in all anatomical compartments (Supplemental Figure 2). This subset of participants had more severe illness and longer hospitalizations when compared with all participants (Supplemental Table 1). In this model, we found higher median viral decay rates in remdesivir-treated participants (untreated vs treated: $r = .15$ vs $.31$, $P < .0001$) (Figure 2). Note that, in the estimated decay rate r, we found no statistical support for the anatomical compartment being a covariate. Remdesivir-treated and untreated participants in this analysis had comparable estimated initial viral loads across all compartments (treated vs untreated, 5.9 vs 6.0 $\log_{10}$ RNA copies/mL).

DISCUSSION

In this study, we evaluated SARS-CoV-2 viral load dynamics across multiple anatomic compartments in hospitalized individuals with COVID-19 and assessed the effect of remdesivir treatment on viral kinetics. Our results demonstrate that viral loads in the blood and URT were highest within 1 week of symptom onset, while suggesting that both viral peak and clearance in sputum were delayed compared with that of other sampling locations. Remdesivir treatment was associated with an increased rate of viral decay in a combined viral decay analysis across multiple compartments.

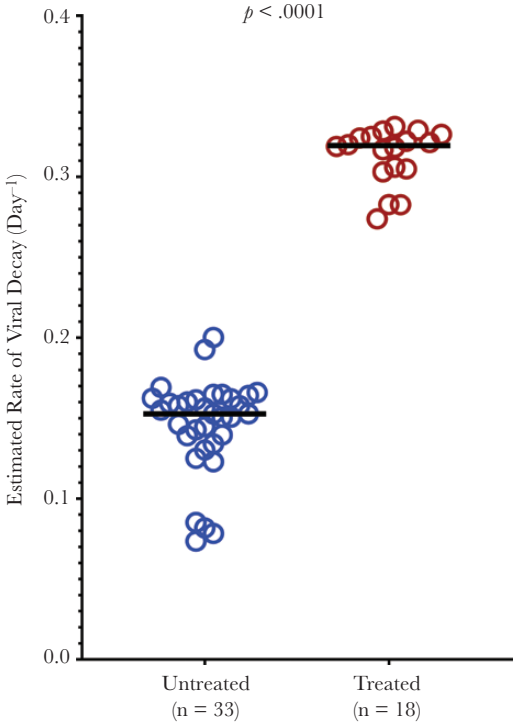

Figure 2. Estimated rate of viral decay in all anatomical compartments per day in patients who did not receive remdesivir treatment and patients who did receive remdesivir treatment. The decay rate coefficient r has units of Day^{-1} (ie, per day) to satisfy the implicitly nondimensional $\log_{10}$-transformed regression equation: $\log_{10}(V) = \log_{10}(V_0) - rt$. A Wilcoxon rank-sum test shows a significant difference in viral decay rates.

Our observation of a delayed viral peak in sputum samples is consistent with the viral dynamics observed in animal models [12]. A study of SARS-CoV-2-infected rhesus macaques suggests that viral load peaks earlier in the URT than in the LRT, and that virus disseminates from the URT to the rest of the body. Our data also show that viral RNA is detected in the LRT for a longer period than other anatomical compartments, suggesting the importance of LRT testing for the diagnosis and monitoring of patients with severe infection. The detection of SARS-CoV-2 viremia has been attributed to viral extravasation from the pulmonary tract. Unexpectedly, our results show that viral load kinetics may be asynchronous between the LRT and plasma. The early viremia peak suggests that systemic seeding and disseminated infection may be occurring sooner than previously recognized. Efficient viral replication is a major factor in these early SARS-CoV-2 dynamics [13], but viral decay is also affected by immune responses and cell turnover. Additional studies are needed to determine whether the relatively similar viral decay rates suggest uniform impact of these factors across compartments.

Whereas there is in vitro data that remdesivir inhibits SARS-CoV-2 replication [14], to date no evidence has been published that remdesivir has significant effects on viral load [10]. In our model across sampling compartments, we observed a significant increase in viral decay rate for participants treated with remdesivir. To the best of our knowledge, this is the first in vivo data to suggest that remdesivir affects SARS-CoV-2 kinetics. Limitations of this analysis include the relatively limited sample size of individuals that restricted our ability to compare remdesivir-associated effects on viral decay kinetics between different compartments. In addition, this is an observational study, but we were able to match the treated and untreated groups based on baseline virological data. Finally, our analysis is based solely on hospitalized individuals and the findings may not be generalizable to patients with asymptomatic or mild disease.

CONCLUSIONS

In conclusion, we find that SARS-CoV-2 kinetics differed in the sputum compared with other compartments, and that systemic spread of SARS-CoV-2 into the circulatory system occurs early in the disease course. Remdesivir-treated individuals had significantly faster rates of viral decay in a combined analysis across multiple compartments. Larger clinical trials are necessary to further assess the virologic effect of remdesivir treatment.

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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Potential conflicts of interest. J. Z. L. has consulted for Abbvie and Jan Biotech. 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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