Viral Load Kinetics of SARS-CoV-2 In Hospitalized Individuals with COVID-19

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ABSTRACT

SARS-CoV-2 viral kinetics remain understudied, including the impact of remdesivir. In hospitalized individuals, peak sputum viral load occurred in week 2 of symptoms while peak viremia occurred within a week of symptom-onset, suggesting early systemic seeding of SARS-CoV-2. Remdesivir treatment was associated with faster rates of viral decay.

INTRODUCTION

Understanding viral load dynamics has provided key insight on viral pathogenesis and treatment effects across the spectrum of viral infections [1]. Study of SARS-CoV-2 viral kinetics within the respiratory tract has already provided valuable information about disease course, transmission risk, and efficacy of antibody therapeutics [2-5]. During severe COVID-19 infection, SARS-CoV-2 viral 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 non-respiratory compartments [7], especially as viremia is associated with COVID-19 disease severity and mortality [6, 8].

Whereas viral decay from nasopharyngeal swab 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. While remdesivir appears to confer a clinical benefit [9], its ability to alter respiratory tract SARS-CoV-2 viral 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.

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

METHODS

Participant enrollment and sample collection

Patients hospitalized with COVID-19 were enrolled at Brigham and Women's Hospital and Massachusetts General Hospital. Longitudinal nasopharyngeal swabs, oropharyngeal swabs, sputum, and blood were collected. Each participant's medical record was reviewed to determine the oxygenation status, demographics,

comorbidities, treatment status, and clinical outcome. This study was approved by the Mass General Brigham Institutional Review Board.

SARS-CoV-2 viral load quantification and viral kinetics analysis

SARS-CoV-2 viral loads were quantified with an in-house RT-qPCR assay using the CDC 2019 nCoV-N1 primer/probe set as previously described [6].

Viral load kinetics amongst compartments were compared both with all available data and restricted to samples with follow-up at time periods spaced 7-14 days apart. To analyze the effect of remdesivir therapy on the SARS-CoV-2 decay rate, we employed mixed effects modeling [11], using Monolix Software 2018R2 (http://www.lixoft.eu). We excluded any patient with unknown treatment status, unknown symptom onset, or remdesivir treatment during a previous hospital admission. We included detectable measurements and a subsequent undetectable measurement only up to a week following the last detectable measurement, excluding any patient-compartments with a lone detectable measurement and no subsequent undetectable measurements meeting that criterion. We excluded patient-compartment data from participants for whom there were no detectable measurements and data from before viral load decay. After this post-processing, we excluded any patient compartment dataset that did not have at least two data points. We were left with data for 51 participants, with 70 distinct patient/compartment data sets.

Decaying viral loads were modeled as $V(t)=V_0e^{-rt}$, using mixed-effects modeling on the log-transformed data and treating undetectable measurements as censored at the assay limit of detection. This analysis was performed both for plasma data only and for all compartment data together.

Statistical Analysis

Levels of SARS-CoV-2 RNA were compared to 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 Fischer's exact tests.

RESULTS

Differential kinetics of SARS-CoV-2 viral loads over time

We enrolled 196 symptomatic, hospitalized participants with COVID-19. Supplemental Table 1 shows demographic information, disease severity and hospital outcomes. The proportion of samples with detectable SARS-CoV-2 RNA were highest within the first week of symptom onset 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 individuals with detectable sputum viral loads in the second week after symptom onset (week 1 vs week 2: 56% vs 100%, P=0.003) and higher median peak sputum viral loads (week 1 vs 2: 1.8 vs 5.6 $\log_{10}$ RNA copies/mL, P=0.02, Supplemental Figure 1). In the setting of delayed peak sputum viral load, the proportion of individuals with detectable sputum viral load was significantly higher in subsequent weeks compared to other compartments. For example, 4 weeks after symptom onset, 63% of participants had detectable viral load in sputum compared to 13% by nasopharyngeal swab, 25% by oropharyngeal swab, and 4% by plasma (P<0.01 for comparisons of sputum viral load against each of the other compartments).

SARS-CoV-2 viral load was significantly correlated with the number of days between symptom onset and sample collection in nasopharyngeal swabs (Spearman r = -0.36, p<0.0001), oropharyngeal swabs (r = -0.36, p=0.0001), sputum (r = -0.39, p<0.0001), and plasma (r = -0.32, p<0.0001) (Figure 1A). In the subset of participants with longitudinal samples collected between one and two weeks apart, the rate of viral decay did not vary significantly between different tissue compartments (Figure 1B). The median number of days between viral load time points was 8 days for all sample types.

Remdesivir treatment was associated with significantly faster viral decay across multiple compartments

In a model of the distribution of viral decay rates simultaneously in all anatomical compartments, we found significantly higher median viral decay rates in remdesivir treated participants (untreated vs treated: $r = 0.15$ vs 0.31, $p<0.0001$, Figure 2). Note that, in estimated the 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 to that of other sampling locations. Whereas remdesivir treatment was not associated with significantly altered SARS-CoV-2 viral load dynamics in plasma, it was associated with an increased rate of viral decay in a combined viral decay analysis across multiple compartments.

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. 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 in either the URT or LRT [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 viral kinetics. Limitations of this analysis includes the relatively limited sample size of individuals that restricted out ability to compare remdesivir-associated effects on viral decay kinetics between

different compartments. Additionally, this is an observational study, but we were able to match the treated and untreated groups based on 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.

In conclusion, we find that SARS-CoV-2 viral kinetics differed in the sputum compared to 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. Evaluations of larger clinical trials are necessary to further assess the virologic effect of remdesivir treatment.

REFERENCES

1. Perelson AS, Ke R. Mechanistic modelling of SARS-CoV-2 and other infectious diseases and the effects of therapeutics. Clin Pharmacol Ther **2021**.
2. Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature **2020; 581: 465-470**.
3. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med **2020; 26: 672-675**.
4. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. N Engl J Med **2021**; 384(3): 229-37.
5. Weinreich DM, Sivapalasingam S, Norton T, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N Engl J Med **2021**; 384(3): 238-51.
6. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun **2020**; 11(1): 5493.
7. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA **2020; 323(18):1843-1844**.
8. Hogan CA, Stevens BA, Sahoo MK, et al. High Frequency of SARS-CoV-2 RNAemia and Association With Severe Disease. Clin Infect Dis **2020**.
9. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med **2020**; 383(19): 1813-26.
10. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet **2020**; 395(10236): 1569-78.
11. Lavielle M. Mixed Effects Models for the Population Approach: Models, Tasks, Methods, and Tools. Chapman and Hall/CRC; 2014.
12. Zheng H, Li H, Guo L, et al. Virulence and pathogenesis of SARS-CoV-2 infection in rhesus macaques: A nonhuman primate model of COVID-19 progression. PLoS Pathog. 2020; 16(11):e1008949.
13. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2020; 1-16.
14. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res **2020**; 30(3): 269-71.

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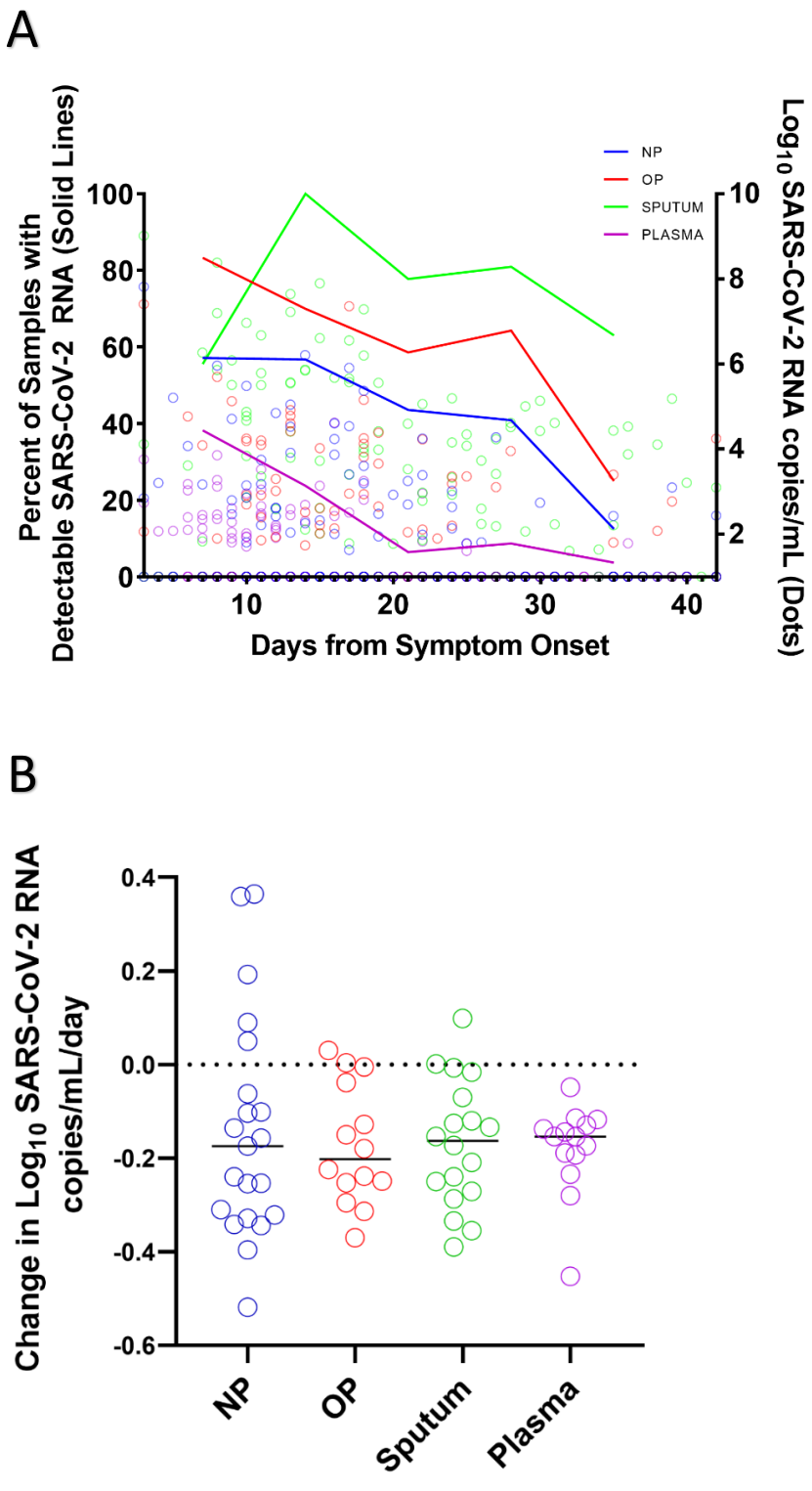


Figure 1. (A) Lines show the percentage of samples with detectable viral RNA for each compartment. Each point on the line represents the percentage of samples in the previous 7 days with detectable RNA, and the final point represents the proportion of detectable samples taken more than 28 days from symptom onset. The number of samples included for each data point are NP: 14, 37, 39, 22, 56; OP: 6, 30, 29, 14, 32; Sputum: 9, 25, 27, 21, 46; Plasma: 34, 113, 93, 46, 81. Dots show individual viral load values for all sample types on the right axis. All samples except for sputum peak in detectability during the first week after symptom onset, and sputum peaks during the second week. Sputum samples have detectable viral RNA in a significantly larger proportion of samples than NP ($P<0.0001$), OP ($P=0.001$), and plasma ($P<0.0001$) after 28 days from symptom onset. (B) Rate of change in viral load over time given as the change in $\log_{10}$ copies/mL per day for each sample compartment. 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 two groups.

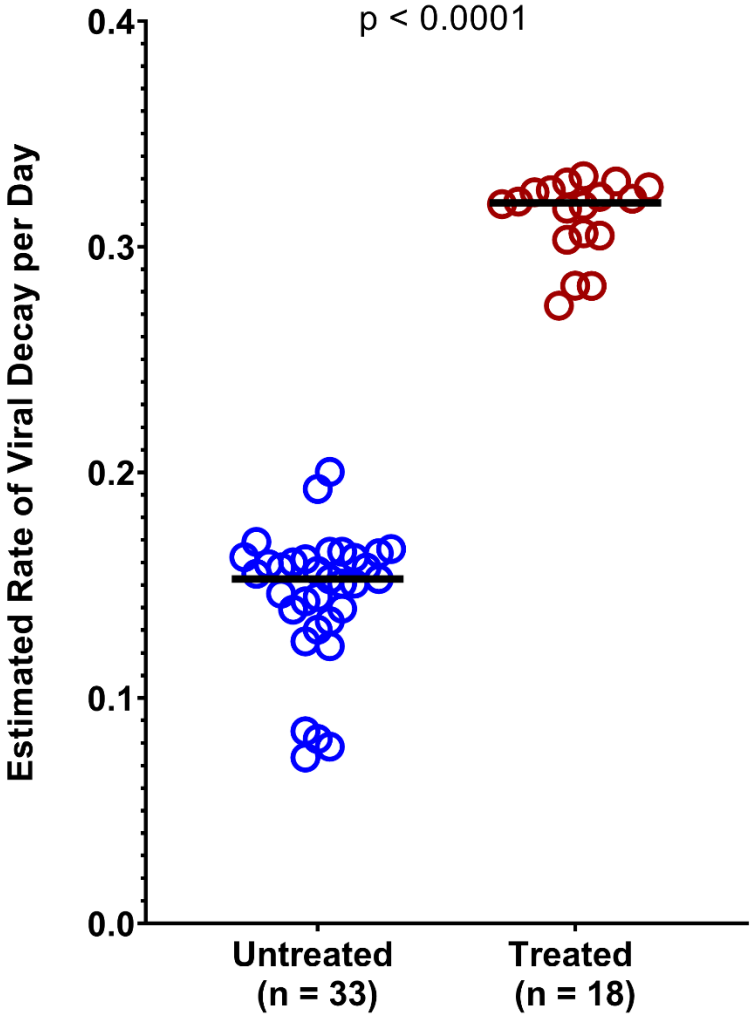
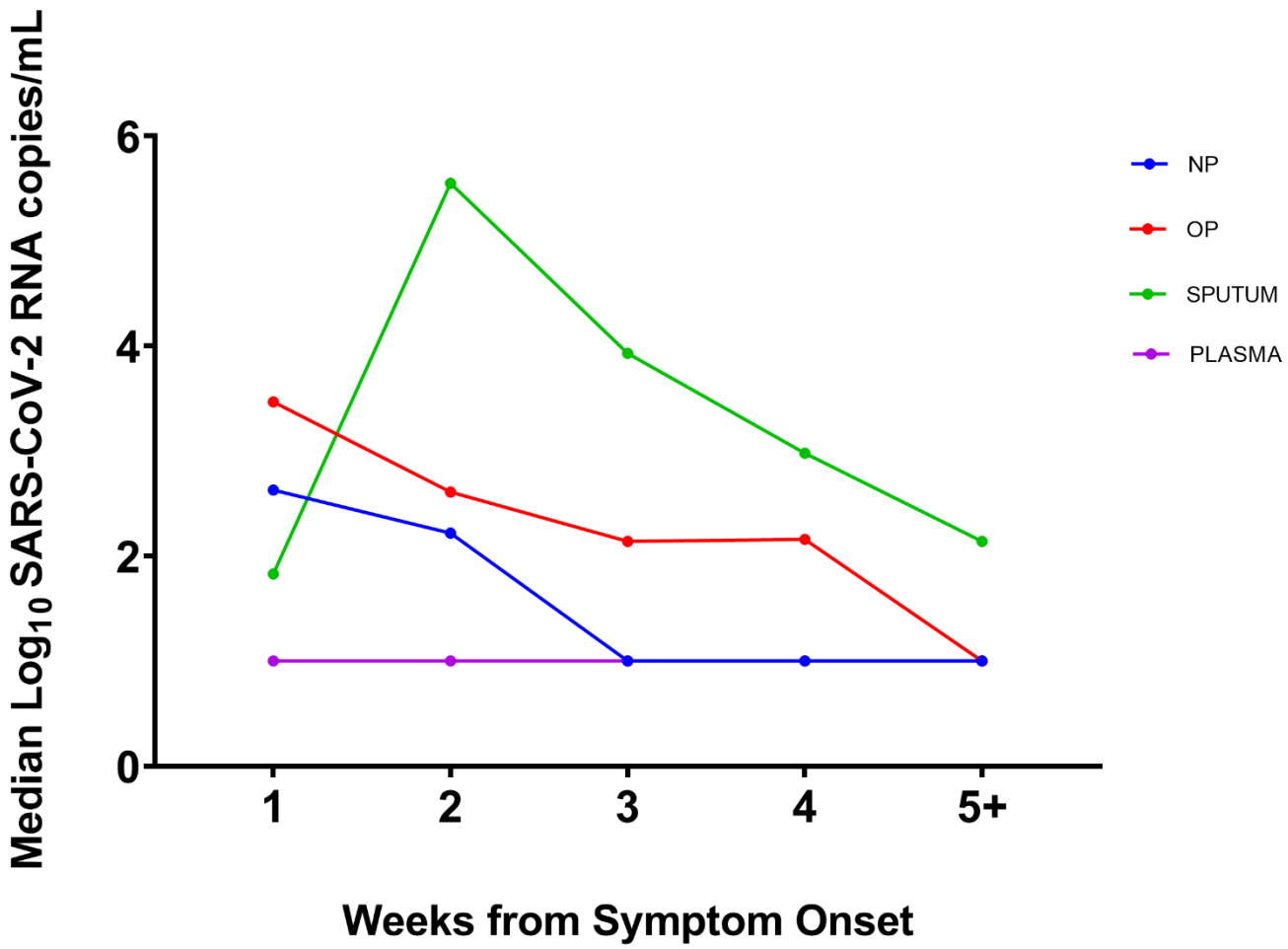


Figure 2. Estimated rate of viral decay in all anatomical compartments per day in patients who did not receive remdesivir treatment and patients who did receive remdesivir treatment. A Wilcoxon rank-sum test shows a significant difference in viral decay rates.



Supplemental Figure 1. Median viral load for all nasopharyngeal, oropharyngeal, sputum, and plasma samples during each week after symptom onset. Viral loads are aggregated based on the number of days from symptom onset with the following brackets: Week 1, days 0-7; Week 2, days 8-14; Week 3, days 15-21; Week 4, days 21-28; Week 5+, day 29 or greater.

Supplemental Table 1. Demographics and clinical characteristics of symptomatic hospitalized participants with COVID-19

Characteristic	Symptomatic Hospitalized Participants N = 196
Female sex, %	42%
Age, median years [Q1,Q3]	59 [48,66]
Ethnicity, %	
Caucasian	42%
Black/African American	20%
Hispanic/Latino	29%
Other	16%
Comorbidities, %	
Hypertension	56%
Chronic Lung Disease	19%
Diabetes	36%
BMI, median score [Q1,Q3]	29 [25,34]
Intubated at Timepoint 1, %	34%
Received Remdesivir, %	38%
Died, %	14%
Number of Timepoints, median [Q1,Q3]	2 [1,3]