SARS-CoV-2 RNA and Nucleocapsid Antigen Are Blood Biomarkers Associated With Severe Disease Outcomes That Improve in Response to Remdesivir

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Background. Although antivirals remain important for the treatment COVID-19, methods to assess treatment efficacy are lacking. Here, we investigated the impact of remdesivir on viral dynamics and their contribution to understanding antiviral efficacy in the multicenter Adaptive COVID-19 Treatment Trial 1, which randomized patients to remdesivir or placebo.

Methods. Longitudinal specimens collected during hospitalization from a substudy of 642 patients with COVID-19 were measured for viral RNA (upper respiratory tract and plasma), viral nucleocapsid antigen (serum), and host immunologic markers. Associations with clinical outcomes and response to therapy were assessed.

Results. Higher baseline plasma viral loads were associated with poorer clinical outcomes, and decreases in viral RNA and antigen in blood but not the upper respiratory tract correlated with enhanced benefit from remdesivir. The treatment effect of remdesivir was most pronounced in patients with elevated baseline nucleocapsid antigen levels: the recovery rate ratio was 1.95 (95% CI, 1.40–2.71) for levels >245 pg/mL vs 1.04 (95% CI, .76–1.42) for levels <245 pg/mL. Remdesivir also accelerated the rate of viral RNA and antigen clearance in blood, and patients whose blood levels decreased were more likely to recover and survive.

Conclusions. Reductions in SARS-CoV-2 RNA and antigen levels in blood correlated with clinical benefit from antiviral therapy.

Clinical Trial Registration. NCT04280705 (ClinicalTrials.gov).

Keywords. antiviral efficacy; clinical trial; COVID-19; remdesivir; SARS-CoV-2.

The Adaptive COVID-19 Treatment Trial 1 (ACTT-1) was one of the earliest randomized placebo-controlled trials conducted during the COVID-19 pandemic and the first to demonstrate the clinical efficacy of the antiviral remdesivir [1]. In that study, remdesivir shortened the time to recovery in patients hospitalized with COVID-19 and lowered risk of disease progression and death among those requiring oxygen supplementation. Today, remdesivir remains one of few safe and effective antiviral treatment options for COVID-19 that retains activity against all known variants of concern [2]. However, fundamental questions that could better inform its clinical use remain unanswered. In particular, our understanding of remdesivir's impact on viral dynamics and our strategies to identify patients who would benefit from treatment are unclear. One approach to improve these gaps in knowledge is through the development of prognostic laboratory markers to guide therapy. Yet, the specific biomarkers that may aid in these goals remain unknown.

We analyzed viral and immunologic biomarkers from ACTT-1 to identify subsets of patients who derive greater benefit from remdesivir. In doing so, we compared 3 viral assays (1) to determine if any could measure the antiviral effects of remdesivir and (2) to assess if reductions in viral load early in the course of treatment affected severe disease outcomes. To optimize the precision and accuracy of these measurements, we utilized sensitive assays that quantitated viral RNA and antigen in blood (plasma RNA and serum nucleocapsid antigen) [3–5] and the upper respiratory tract (RNA), as well as

SARS-CoV-2–specific antibody responses. We also evaluated interleukin 6 (IL-6) and C-reactive protein (CRP): 2 inflammatory biomarkers associated with worse disease [6–8].

METHODS

Study Design and Participants

The original ACTT-1 protocol and study design have been described [1]. This analysis was conducted with data and biospecimens from ACTT-1 participants who received at least 1 day of treatment with remdesivir or placebo and from whom biospecimens were available for measurement of plasma viral RNA, upper respiratory tract viral RNA, serum viral nucleocapsid, IL-6, and CRP—all collected at baseline and at least 1 other time point within the 6 days following randomization (Supplementary Figure 1). Written informed consent was obtained from each patient or legally authorized representative. Patients were grouped into 2 categories based on disease severity and ordinal scale group per the National Institute of Allergy and Infectious Diseases:

Moderate/severe disease: patients were hospitalized but not critically ill and consisted of those in group 4 (not requiring supplemental oxygen) or group 5 (requiring low-flow supplemental oxygen)

Critical disease: patients required care in the intensive care unit and consisted of those in group 6 (requiring high-flow oxygen or noninvasive ventilation) or group 7 (requiring invasive mechanical ventilation) [1]

These groupings were based on an a priori hypothesis that antiviral treatment would demonstrate better efficacy earlier in the course of illness, when viral replication is suspected to be higher. Symptom duration was dichotomized at 5 days based on our hypothesis that this time point could differentiate changes in viral load given prior reports of improved efficacy with early remdesivir use [1].

Details for our a priori hypotheses regarding viral and inflammatory biomarkers were defined in a statistical analysis plan (supplementary material). Serum, plasma, and upper respiratory tract specimens were collected at designated visits during hospitalization, including study days 1 (baseline), 3, and 5 (ie, prior to and at 2 and 4 days after treatment initiation, respectively). All laboratory assays were performed centrally.

Measurement of SARS-CoV-2 RNA and Nucleocapsid

Oropharyngeal swabs were preferentially collected over nasopharyngeal swabs when available; both were collectively categorized as *upper respiratory* specimens. For the primary analysis, samples collected from baseline through study day 5 were included. Upper respiratory and plasma viral RNA was measured with the Roche cobas SARS-CoV-2 RT-PCR assay on a Roche cobas 6800 instrument via the SeraCare AccuPlex SARS-CoV-2 Verification Panel as the exogenous standards and calibrators. Validation of the quantitative viral load assay in plasma was performed with spike-in of clinical materials, standards, and calibrators into reconstituted commercial plasma (Lyphochek 2211-08; Bio-Rad). For quantitative calculations, similar to prior studies [9], values below the assay limit of quantification (LOQ) of 62 copies/mL ($1.79 \log_{10}$ copies/mL) were imputed as half the LOQ (31 copies/mL or $1.49 \log_{10}$ copies/mL) when RNA was detected but not quantifiable and one-quarter the LOQ (15.5 copies/mL or $1.19 \log_{10}$ copies/mL) when RNA was not detected.

SARS-CoV-2 nucleocapsid levels were determined in 90 μL of serum in duplicate with a quantitative Simoa assay (Quanterix) at baseline through study day 5. Simoa (single-molecule array) is a novel and ultrasensitive microparticle-based enzyme-linked immunosorbent assay platform capable of precise quantification of specific protein and/or antibody targets within the femtomolar range of concentrations [10]. The lower level of quantification for the SARS-CoV-2 nucleocapsid assay is 3 pg/mL. For quantitative calculations, values below that level were imputed as half this value (1.5 pg/mL).

Measurement of Immunologic Biomarkers

Baseline anti–SARS-CoV-2 spike IgG antibody levels were measured with 10 μL of serum in duplicate via a semiquantitative Simoa assay (Quanterix); seropositivity was defined as patients having measurements above the assay cutoff of 770 ng/mL. IL-6 and CRP were measured from serum in duplicate via electrochemiluminescence (Meso Scale Discovery) per the manufacturer's instructions.

Clinical and Virologic End Points

The primary clinical outcome of ACTT-1 was time to recovery, defined as the point at which a patient met criteria for improvement to ordinal scale 1 (not hospitalized, without limitation of activities), 2 (not hospitalized, with limitation of activities but not requiring new supplemental oxygen), or 3 (hospitalized but no longer requiring ongoing medical care; ie, still hospitalized for infection control or placement reasons). Patients who did not recover failed to progress to ordinal score 3, 2, or 1 over the course of the study. Mortality through day 28 was a secondary clinical end point. Virologic end points were evaluated as the daily rate of change in viral load and as changes in viral load trajectories.

Statistical Analyses

A statistical analysis plan (supplementary material) was written prior to analysis of the unblinded data, and it was amended once to add prespecified analyses for plasma viral RNA and anti–SARS-CoV-2 antibody measurements. Clinical and demographic characteristics of study participants were summarized with descriptive statistics. Differences in distributions of viral

assays were compared with the Mann-Whitney U test. Correlations were estimated by Pearson correlation coefficient. Log-rank tests assessed differences in survival and recovery times through methods applied in the original study [1]. Kaplan-Meier curves are shown. Time-to-event analyses were supplemented by proportional hazards models and the Fine-Gray method for competing risk for recovery, with death as a competing risk [11]. To evaluate longitudinal changes in biomarkers, linear regression models were fit to all available values for each patient to estimate the patient-specific daily rate of decline as a *derived variable*; sensitivity analyses per linear mixed effects models were estimated when appropriate. Additionally, slopes were dichotomized to determine viral load trajectories, defined as *increasing* (persistently positive) for positive slopes (or zero slopes when a baseline value was above the LOQ) or *decreasing* (persistently negative) for negative slopes (or zero slopes when a baseline value was below the LOQ). Primary analyses adjusted for potential baseline imbalances in measurements of viral load (ie, prior to receipt of remdesivir or placebo) by inclusion of baseline viral load as a covariate in a linear regression model. Tests of proportions were conducted with the chi-square test. Wald confidence intervals were used for all primary analyses. All *P* values were 2-sided. All analyses were conducted in R statistical software and confirmed independently by 2 biostatisticians. Figures were produced with R software (*ggplot2* and *forester* packages [12]) and Prism (GraphPad).

RESULTS

Study Design and Participants

We tested a substudy cohort of 642 ACTT-1 participants who provided specimens at baseline and at least 1 subsequent time point within the first 6 days following randomization (Supplementary Figure 1). Demographic and clinical characteristics were generally well balanced between the participants in the placebo arm (n = 319) and remdesivir arm (n = 323), as well as between the overall cohort and original ACTT-1 study population (Table 1, Supplementary Table 1). The median age was 59 years; the median time from symptom onset was 9 days; and mortality was 9% vs 11% in the remdesivir and placebo arms, respectively. Biomarker analyses were stratified by disease severity, comparing patients with moderate/severe disease (ie, patients considered noncritically ill who required no or minimal oxygen supplementation) with those who presented with critical disease (ie, patients considered critically ill who required high-flow oxygen, mechanical ventilation, or extracorporeal membranous oxygenation).

Comparison of Baseline Measures of Viral Load and Inflammatory Biomarkers

The majority of participants had quantifiable viral loads at study entry irrespective of assay used: 87.9% (564/642) for serum viral nucleocapsid, 85.2% (547/642) for upper respiratory

Table 1. Demographic and Clinical Characteristics of the Study Population at Baseline

|	Placebo		Remdesivir	
	Included (n = 319)	Total (n = 516)	Included (n = 323)	Total (n = 532)
Age, y, median (IQR)	59 (47-68)	60 (49-70)	59 (48-69)	59 (49-69)
Male sex	205 (64.26)	328 (63.57)	210 (65.02)	347 (65.23)
Race and ethnicity				
Asian	30 (9.40)	56 (10.85)	42 (13.00)	79 (14.85)
Black or African American	84 (26.33)	114 (22.09)	73 (22.60)	105 (19.74)
White	167 (52.35)	286 (55.43)	168 (52.01)	273 (51.32)
Ethnicity: Hispanic or Latino	69 (21.63)	114 (22.09)	89 (27.55)	132 (24.81)
Clinical characteristics				
Any comorbidities	255 (79.94)	417 (80.81)	271 (83.90)	435 (81.77)
Days of symptoms, median (IQR)	9 (7-13)	9 (7-13)	9 (6-12)	9 (6-12)
Baseline ordinal scale [a]				
4	38 (11.91)	63 (12.21)	39 (12.07)	75 (14.10)
5	114 (35.74)	202 (39.15)	148 (45.82)	231 (43.42)
6	56 (17.55)	98 (18.99)	42 (13.00)	94 (17.67)
7	111 (34.80)	153 (29.65)	94 (29.10)	132 (24.81)
Disease severity subgroups [b]				
Moderate/severe	152 (47.65)	265 (51.36)	187 (57.89)	306 (57.52)
Critical	167 (52.35)	251 (48.64)	136 (42.11)	226 (42.48)

Patients in our primary biomarker analysis cohort (*Included*) vs all patients from the original Adaptive COVID-19 Treatment Trial 1 (*Total*). Data are presented as No. (%) unless noted otherwise.

[a]National Institute of Allergy and Infectious Diseases categorization of COVID-19 disease severity.

[b]Moderate/severe disease is a combined subgroup of ordinal scales 4 and 5. Critical disease is a combined subgroup of ordinal scales 6 and 7.

tract viral RNA, and 56.9% (365/642) for plasma viral RNA. Patients with critical disease had higher baseline plasma and upper respiratory tract viral RNA levels as compared with patients with moderate/severe disease: plasma viral RNA median, 2.40 vs 1.49 $\log_{10}$ copies/mL, $P < .001$; upper respiratory tract viral RNA median, 3.64 vs 3.26 $\log_{10}$ copies/mL, $P = .002$ (Figure 1A, Supplementary Tables 2 and 3). Patients with critical disease also presented later in illness with modestly protracted symptoms (mean days, 10.5 vs 9.3; $P = .002$). Viral loads were lower among patients reporting longer durations of symptoms prior to enrollment and, on average, decreased over the early period of observation among placebo recipients (Supplementary Figure 2).

Plasma viral RNA and serum nucleocapsid levels correlated with inflammatory biomarkers (Figure 1B, Supplementary Table 4); modest but stronger correlations were seen between these blood viral biomarkers and CRP (Pearson correlation, $r = 0.237$ for plasma RNA and $r = 0.189$ for nucleocapsid; both $P < .001$) and IL-6 ($r = 0.361$ for plasma RNA and
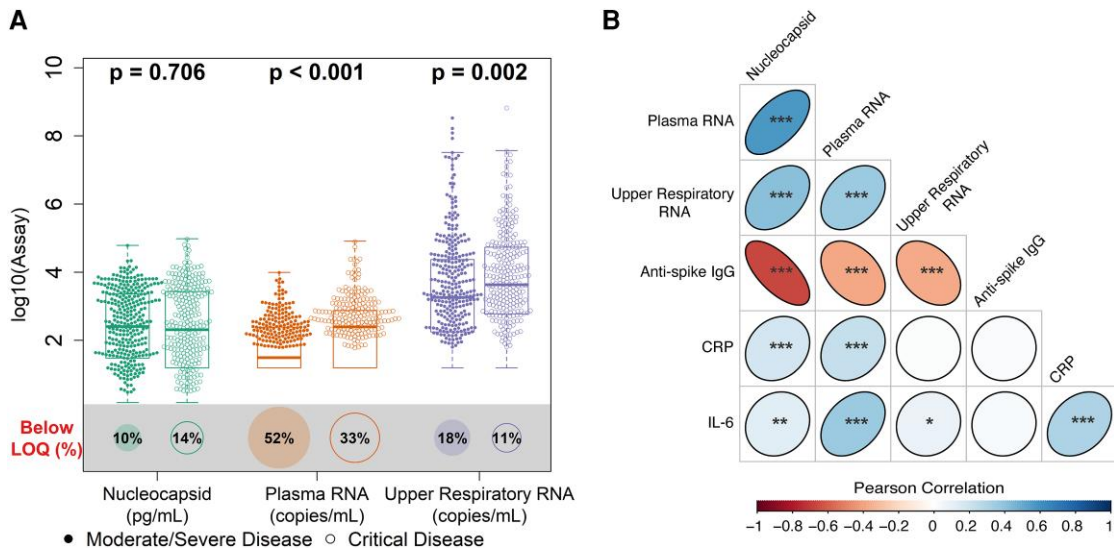
Figure 1. Distribution of baseline viral loads by disease severity and correlation with other biomarkers. *A*, Distribution of baseline viral loads by assay categorized by disease severity. Box plots indicate the median (line) and IQR (box). Wilcoxon *P* values assess differences in medians between disease severity groups, and proportions of samples are shown below each assay's limit of quantification (LOQ; illustrated by size of bottom circle). RNA values below the assay LOQ of 1.79 $\log_{10}$ copies/mL were set as half the LOQ (1.49 $\log_{10}$ copies/mL) when measurements were above the lower limit of detection and one-quarter the LOQ (1.19 $\log_{10}$ copies/mL) when RNA was not detected; nucleocapsid antigen values below the assay LOQ of 3 pg/mL were set as half the LOQ (1.5 pg/mL). *B*, Matrix heat map of Pearson correlations between baseline biomarker levels among all patients in the primary cohort. *$P < .05$. **$P < .01$. ***$P < .001$. Coefficient estimates are provided in Supplementary Table 4. CRP, C-reactive protein; IL-6, interleukin 6.
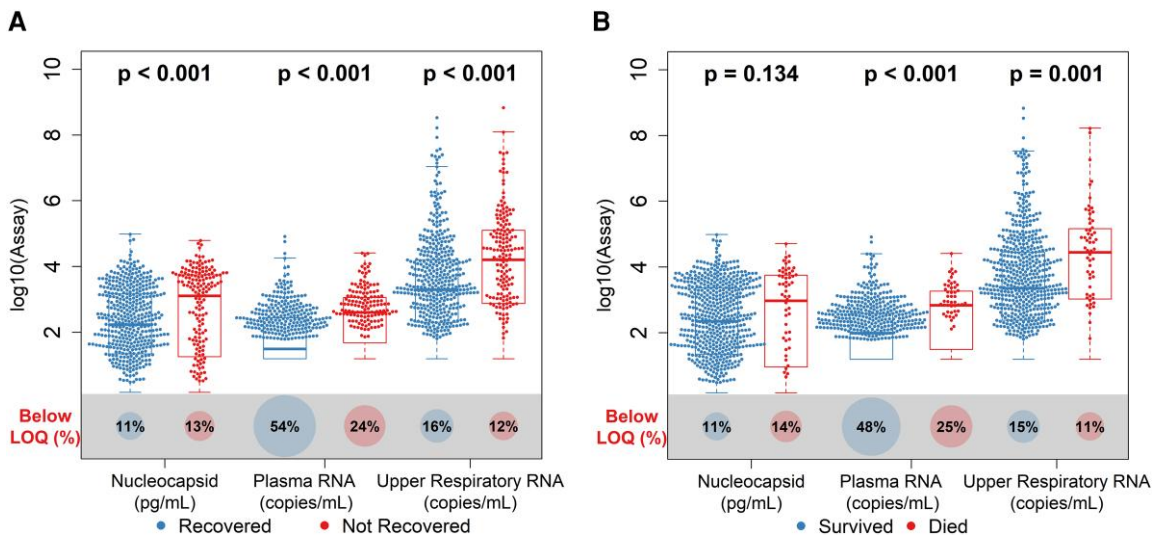
Figure 2. Distribution of baseline viral loads by assay according to the clinical outcome: *A*, recovery; *B*, mortality. Box plots indicate the median (line) and IQR (box). Wilcoxon *P* values assess differences in medians between disease severity groups, and proportions of samples are shown below each assay's limit of quantification (LOQ; illustrated by size of bottom circle). RNA values below the assay LOQ of 1.79 $\log_{10}$ copies/mL were set as half the LOQ (1.49 $\log_{10}$ copies/mL) when measurements were above the lower limit of detection and one-quarter the LOQ (1.19 $\log_{10}$ copies/mL) when RNA was not detected; nucleocapsid antigen values below the assay LOQ of 3 pg/mL were set as half the LOQ (1.5 pg/mL).

$r = 0.122$ for nucleocapsid; $P < .001$ and $P = .002$, respectively), in contrast to upper respiratory tract viral RNA. Anti–SARS-CoV-2 spike antibodies were detected in 57.3% patients, and antibody levels negatively correlated with all 3 measures of viral load.

Higher Baseline Viral Loads Were Associated With Worse Clinical Outcomes

Patients with higher baseline viral loads were less likely to recover or survive (Figure 2, Supplementary Table 5). Cox proportional hazard models showed that higher baseline viral

Table 2. Likelihood of Recovery or Death Based on Baseline Viral Load or Inflammatory Biomarker

Disease Severity: Biomarker	Recovery			Mortality		
	RRR	95% CI	*P* Value	HR	95% CI	*P* Value
Moderate/severe						
Nucleocapsid, $\log_{10}$ pg/mL	0.53	.42–.66	<.001	3.92	1.11–13.86	.034
RNA, $\log_{10}$ copies/mL						
Plasma	0.41	.33–.53	<.001	4.44	1.41–13.96	.011
Upper respiratory	0.50	.40–.64	<.001	7.80	1.70–35.82	.008
Inflammatory Biomarkers, $\log_{10}$ pg/mL						
CRP	0.83	.67–1.04	.106	1.79	.65–4.95	.259
IL-6	0.53	.42–.67	<.001	3.68	1.32–10.29	.013
Critical						
Nucleocapsid, $\log_{10}$ pg/mL	0.77	.58–1.03	.078	1.07	.61–1.87	.805
RNA, $\log_{10}$ copies/mL						
Plasma	0.58	.44–.78	<.001	1.53	.83–2.85	.175
Upper respiratory	0.84	.63–1.12	.239	1.12	.64–1.98	.687
Inflammatory Biomarkers, $\log_{10}$ pg/mL						
CRP	0.74	.55–1.00	.050	1.75	.93–3.29	.085
IL-6	0.39	.28–.53	<.001	3.55	1.41–8.96	.007

Cox model estimates of HR for mortality and RRR based on baseline elevation of viral load or inflammatory biomarker (dichotomized at baseline median) when adjusted for treatment arm.
Abbreviations: CRP, C-reactive protein; HR, hazard ratio; IL-6, interleukin 6; RRR, recovery rate ratio

loads were associated with an increased risk of mortality (plasma viral RNA: hazard ratio (HR), 4.44 [95% CI, 1.41–13.96]; upper respiratory tract viral RNA: HR, 7.80 [95% CI, 1.70–35.82]; serum viral nucleocapsid: HR, 3.92 [95% CI, 1.11–13.86]) and lower likelihood of recovery (plasma viral RNA: recovery rate ratio [RRR], 0.42 [95% CI, .33–.53]; upper respiratory tract viral RNA: RRR, 0.51 [95% CI, .40–.64]; serum viral nucleocapsid: RRR, 0.53 [95% CI, .42–.66]) among patients with moderate/severe disease (Table 2). Higher baseline plasma viral RNA levels also correlated with a lower likelihood of recovery among patients who were critically ill (RRR, 0.582 [95% CI, .44–.78]), a finding not observed with other measures of viral load. Elevated IL-6 levels were similarly associated with a lower likelihood of survival and recovery across all categories of disease severity.
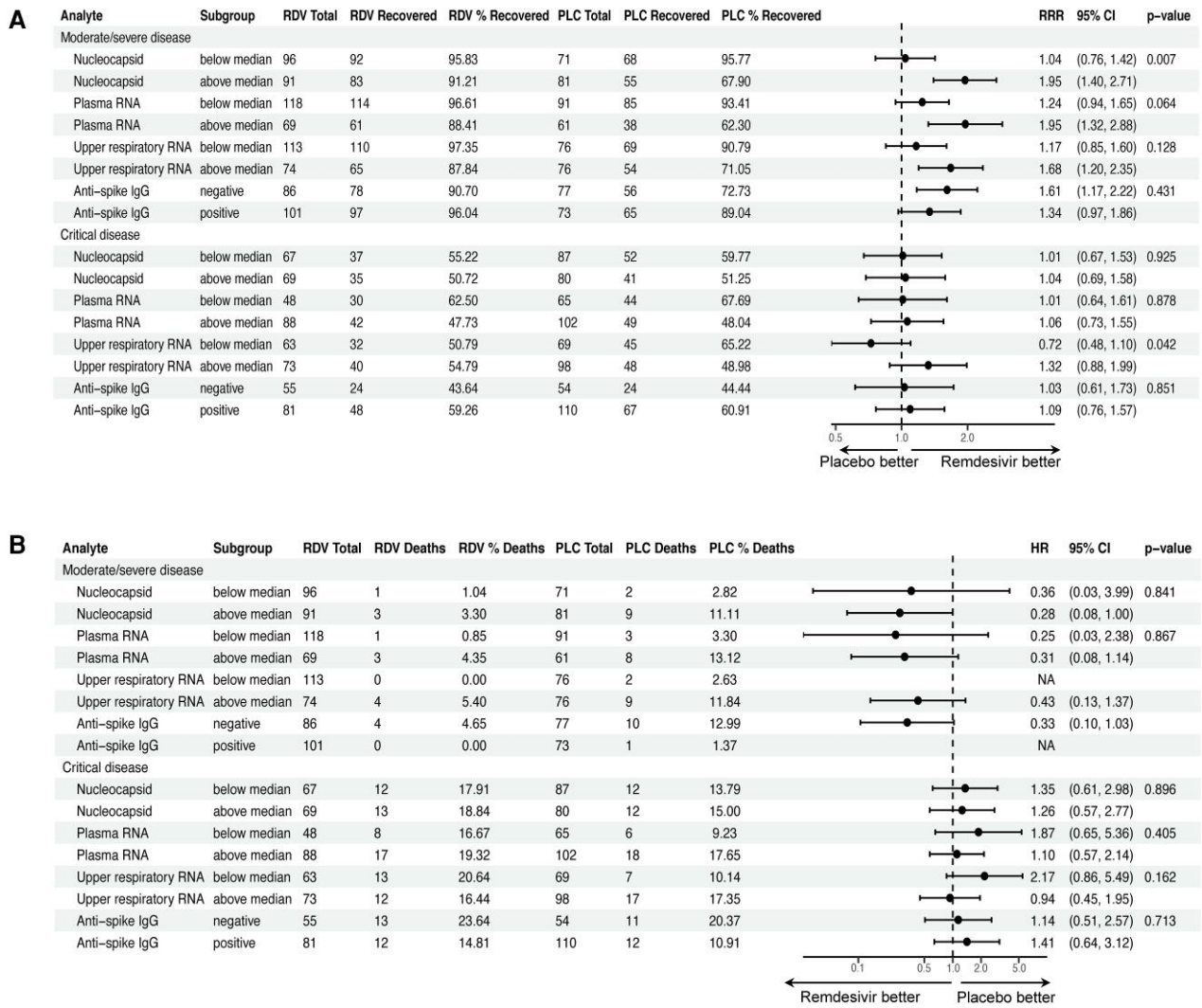
Patients With Elevated Blood Viral Loads at Baseline Recovered Faster With Remdesivir

Among participants with moderate/severe disease, those with higher baseline serum viral nucleocapsid levels recovered faster when treated with remdesivir vs those who received placebo (RRR, 1.95 vs 1.04 for levels above and below the cohort median of 2.39 $\log_{10}$ pg/mL; for interaction, *P* = .01; Figure 3A). A similar trend was seen for patients with higher baseline plasma viral RNA levels (RRR, 1.95 vs 1.24 for levels above and below the cohort median of 2.09 $\log_{10}$ copies/mL; for interaction, *P* = .06). Remdesivir also shortened the time to recovery when given earlier in illness. Among patients with moderate/severe disease who were treated within 5 days of initial symptoms, 93% vs 54% recovered by day 28 in the remdesivir and placebo arms, respectively (RRR, 3.0; 95% CI 1.70–5.28), as

opposed to 94% vs 88% among those treated 5 days after symptom onset (RRR, 1.27; 95% CI, .99–1.63; for interaction, *P* = .006; Supplementary Table 6). Similar findings were observed if symptom duration was analyzed as a continuous variable (Supplementary Table 7). There was no differential treatment effect for remdesivir on mortality across subgroups of viral load (Figure 3B) or symptom duration. Additionally, although patients with antispike antibodies had improved clinical outcomes when compared with individuals who were seronegative, clinical benefit from remdesivir did not vary by baseline antibody status.

Remdesivir Accelerated the Reduction of Viral Load in Blood

Relative to placebo, participants with moderate/severe disease who received remdesivir had larger averaged daily declines in levels of serum viral nucleocapsid (difference, −0.062 $\log_{10}$ pg/mL/d; *P* = .003) and plasma viral RNA (difference, −0.040 $\log_{10}$ copies/mL/d; *P* = .004; Figure 4A, Supplementary Table 8); sensitivity analyses based on linear mixed effects models demonstrated comparable results (Supplementary Table 9). Reductions in blood viral RNA and antigen were greater among patients treated within 5 days of initial symptoms (Supplementary Table 10). Remdesivir similarly reduced the likelihood of an increasing viral load trajectory in blood during the first 5 days of treatment among patients with moderate/severe disease: the relative risk of an increasing viral load comparing remdesivir against placebo was 0.068 for serum viral nucleocapsid (*P* < .001) and 0.366 for plasma viral RNA (*P* = .006; Figure 4B). In contrast, no effect of remdesivir was observed on the upper respiratory tract viral RNA levels among

A

Analyte	Subgroup	RDV Total	RDV Recovered	RDV % Recovered	PLC Total	PLC Recovered	PLC % Recovered	RRR	95% CI	p-value
Moderate/severe disease										
Nucleocapsid	below median	96	92	95.83	71	68	95.77	1.04	(0.76, 1.42)	0.007
Nucleocapsid	above median	91	83	91.21	81	55	67.90	1.95	(1.40, 2.71)	
Plasma RNA	below median	118	114	96.61	91	85	93.41	1.24	(0.94, 1.65)	0.064
Plasma RNA	above median	69	61	88.41	61	38	62.30	1.95	(1.32, 2.88)	
Upper respiratory RNA	below median	113	110	97.35	76	69	90.79	1.17	(0.85, 1.60)	0.128
Upper respiratory RNA	above median	74	65	87.84	76	54	71.05	1.68	(1.20, 2.35)	
Anti–spike IgG	negative	86	78	90.70	77	56	72.73	1.61	(1.17, 2.22)	0.431
Anti–spike IgG	positive	101	97	96.04	73	65	89.04	1.34	(0.97, 1.86)	
Critical disease										
Nucleocapsid	below median	67	37	55.22	87	52	59.77	1.01	(0.67, 1.53)	0.925
Nucleocapsid	above median	69	35	50.72	80	41	51.25	1.04	(0.69, 1.58)	
Plasma RNA	below median	48	30	62.50	65	44	67.69	1.01	(0.64, 1.61)	0.878
Plasma RNA	above median	88	42	47.73	102	49	48.04	1.06	(0.73, 1.55)	
Upper respiratory RNA	below median	63	32	50.79	69	45	65.22	0.72	(0.48, 1.10)	0.042
Upper respiratory RNA	above median	73	40	54.79	98	48	48.98	1.32	(0.88, 1.99)	
Anti–spike IgG	negative	55	24	43.64	54	24	44.44	1.03	(0.61, 1.73)	0.851
Anti–spike IgG	positive	81	48	59.26	110	67	60.91	1.09	(0.76, 1.57)	

0.5 1.0 2.0
← Placebo better   Remdesivir better →

B

Analyte	Subgroup	RDV Total	RDV Deaths	RDV % Deaths	PLC Total	PLC Deaths	PLC % Deaths	HR	95% CI	p-value
Moderate/severe disease										
Nucleocapsid	below median	96	1	1.04	71	2	2.82	0.36	(0.03, 3.99)	0.841
Nucleocapsid	above median	91	3	3.30	81	9	11.11	0.28	(0.08, 1.00)	
Plasma RNA	below median	118	1	0.85	91	3	3.30	0.25	(0.03, 2.38)	0.867
Plasma RNA	above median	69	3	4.35	61	8	13.12	0.31	(0.08, 1.14)	
Upper respiratory RNA	below median	113	0	0.00	76	2	2.63	NA		
Upper respiratory RNA	above median	74	4	5.40	76	9	11.84	0.43	(0.13, 1.37)	
Anti–spike IgG	negative	86	4	4.65	77	10	12.99	0.33	(0.10, 1.03)	
Anti–spike IgG	positive	101	0	0.00	73	1	1.37	NA		
Critical disease										
Nucleocapsid	below median	67	12	17.91	87	12	13.79	1.35	(0.61, 2.98)	0.896
Nucleocapsid	above median	69	13	18.84	80	12	15.00	1.26	(0.57, 2.77)	
Plasma RNA	below median	48	8	16.67	65	6	9.23	1.87	(0.65, 5.36)	0.405
Plasma RNA	above median	88	17	19.32	102	18	17.65	1.10	(0.57, 2.14)	
Upper respiratory RNA	below median	63	13	20.64	69	7	10.14	2.17	(0.86, 5.49)	0.162
Upper respiratory RNA	above median	73	12	16.44	98	17	17.35	0.94	(0.45, 1.95)	
Anti–spike IgG	negative	55	13	23.64	54	11	20.37	1.14	(0.51, 2.57)	0.713
Anti–spike IgG	positive	81	12	14.81	110	12	10.91	1.41	(0.64, 3.12)	

0.1 0.5 1.0 2.0 5.0
← Remdesivir better   Placebo better →

Figure 3. Impact of remdesivir on clinical outcomes according to viral load subgroups. Forest plots illustrate subgroup analyses of Cox models to evaluate for a differential effect of remdesivir on (*A*) recovery and (*B*) mortality based on baseline viral load or presence of antispike antibodies. Viral load measurements were dichotomized at median values per assay: 2.39 $\log_{10}$ pg/mL (245 pg/mL) for serum nucleocapsid, 2.09 $\log_{10}$ copies/mL (123 copies/mL) for plasma RNA, and 3.41 $\log_{10}$ copies/mL (2570 copies/mL) for upper respiratory RNA. *P* values for treatment interaction are shown. HR, hazard ratio; PLC, placebo; RDV, remdesivir; RRR, recovery rate ratio.

patients with moderate/severe disease. No significant changes in viral loads were observed in patients who were critically ill.

Reductions in Viral Load Were Associated With Improved Clinical Outcomes

Patients with declines in plasma viral RNA or serum viral nucleocapsid over the first 5 days had improved times to clinical recovery as compared with individuals without declines (Figure 5, Supplementary Table 11). This association was seen in patients with moderate/severe disease ($P = .001$ for plasma viral RNA, $P = .018$ for serum viral nucleocapsid) and those with critical disease ($P = .004$ for plasma viral RNA, $P = .013$ for serum viral nucleocapsid). Notably, no patient who was critically ill and exhibited increasing serum levels of viral nucleocapsid recovered. Decreasing trajectories of plasma

viral RNA and serum viral nucleocapsid were similarly associated with reduced mortality across both disease severity subgroups (Supplementary Figure 3, Supplementary Table 12). In contrast, the trajectories of upper respiratory viral RNA had weaker associations with clinical outcomes in patients with moderate/severe disease and failed to show any association with outcomes among patients with critical illness.

DISCUSSION

In this analysis of a large-scale, phase 3, randomized clinical trial, we hypothesized that accelerated reductions in peripheral SARS-CoV-2 viral load would correlate with improved patient outcomes. By assessing 3 methods to quantify viral load, we demonstrated that the 2 blood measurements (viral RNA
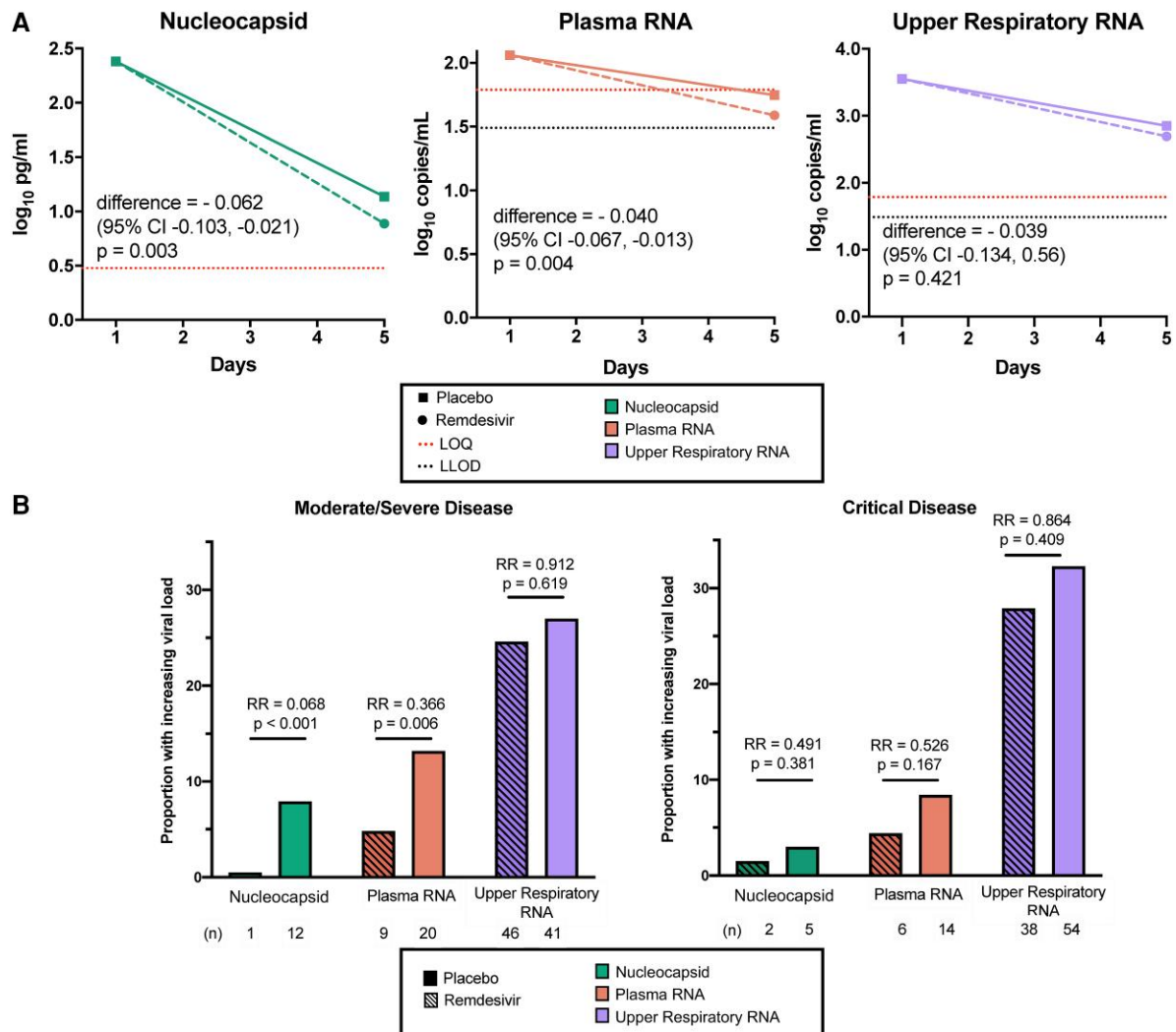
Figure 4. Virologic efficacy of remdesivir vs placebo. *A*, Differences in mean daily rate of change of viral load among patients with moderate/severe disease by treatment arm. *P* values are shown for differences in adjusted daily rate of change when normalized to baseline level. For calculations, RNA values below the assay limit of quantification (LOQ) of 1.79 $\log_{10}$ copies/mL were set as half the LOQ (1.49 $\log_{10}$ copies/mL) when measurements were above the lower limit of detection (LLOD) and one-quarter the LOQ (1.19 $\log_{10}$ copies/mL) when RNA was not detected. *B*, Bar plot of patients with increasing or persistently elevated viral loads measured through 5 days of treatment. Relative risk (RR) of an increasing viral load comparing remdesivir and placebo recipients are shown with corresponding *P* values.

and nucleocapsid antigen) more consistently associated with the clinical risk of severe disease outcomes and the benefits of antiviral therapy when compared with upper respiratory RNA.

ACTT-1 was the first randomized placebo-controlled trial to demonstrate the efficacy of remdesivir in improving recovery in patients hospitalized with COVID-19. Since its completion, several other trials have corroborated the clinical efficacy of remdesivir in similar and disparate populations [13, 14]. However, because identifying the specific patients within this broader population who are likely to benefit from treatment has continued to prove challenging, we hypothesized that augmenting commonly used categorizations of disease severity with laboratory assessments might offer new insights. We previously showed that simple hematologic parameters in

combination with baseline disease severity better predicted risk of disease progression [15]. Here, we report that SARS-CoV-2 biomarkers in the peripheral blood may also offer new insights. In particular, the associated benefits of remdesivir were most pronounced among patients who were not critically ill with elevated serum nucleocapsid antigen levels (>2.39 $\log_{10}$ pg/mL) or shorter durations of symptoms, with a similar favorable trend seen in patients with higher levels of plasma viral RNA (>2.09 $\log_{10}$ copies/mL). Moreover, early trajectories of both these viral analytes correlated with clinical outcomes. These findings support the hypothesis that antivirals are more effective when used earlier in illness and that unmitigated viral replication (or an inability to decrease peripheral viral loads) is associated with worse prognoses.
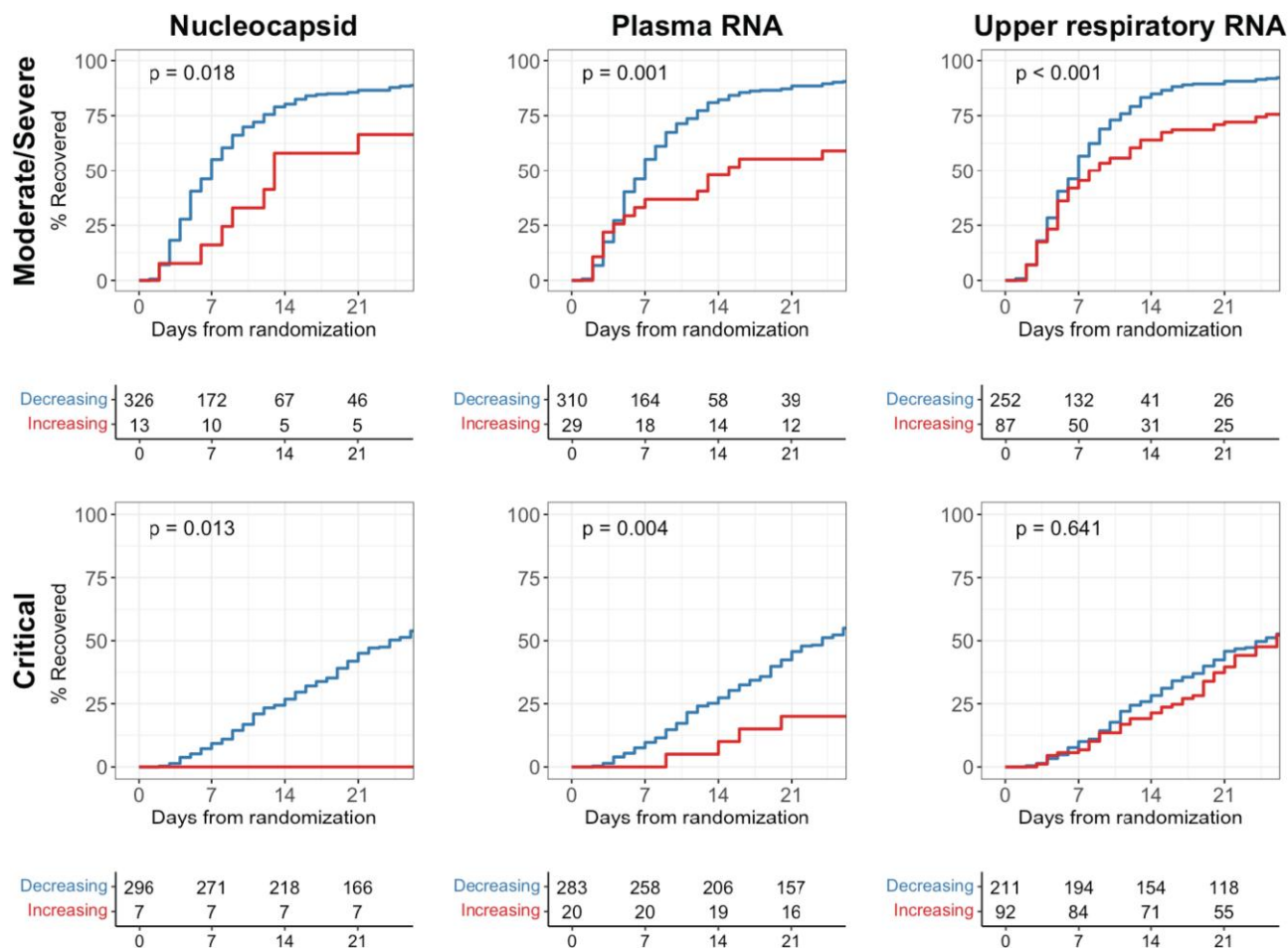
Figure 5. Time to recovery comparing patients with increasing vs decreasing viral loads. Kaplan-Meier curve analyses compare patients with decreasing viral loads (blue) and those with increasing or persistently elevated viral loads (red) through the first 5 days of treatment. *P* values represent test of the log-rank statistic.

Although upper respiratory specimens are commonly used to measure viral loads in clinical and research settings, measurements in peripheral blood may offer several advantages. In our study, plasma viral RNA and serum nucleocapsid assays more consistently associated with mortality and recovery across a wider range of disease severity when compared with upper respiratory RNA measurements. Plasma RNAemia as a correlate of worse disease has been reported with other respiratory viral infections [16, 17], and several studies have similarly found it to be predictive of critical illness and poorer outcomes in COVID-19 [18–22]. Here, we demonstrate that SARS-CoV-2 RNAemia may serve as a useful prognostic biomarker among patients without critical illness, although sensitivity may decline in mild or early illness where plasma RNA levels may be low or absent [23]. In contrast, nucleocapsid antigen may be a more sensitive marker earlier in infection [3, 24–29], and in our study it was present in a considerably higher proportion of patients when compared with plasma viral RNA (87.9% vs 56.9%), potentially pointing to the added value of measuring circulating antigen levels concurrently with plasma viral RNA. In a similar cohort of patients, plasma antigenemia in ranges comparable to those that we observed correlated with poorer outcomes, including worse pulmonary disease and prolonged hospitalizations [5]. Peripheral blood biomarkers may provide new insights into COVID-19 pathogenesis: IL-6 and CRP correlated better with viral RNA and antigen measurements in blood than with viral RNA levels in the upper respiratory tract, suggesting that the presence and magnitude of viremia better reflect the immune dysregulation associated with worse patient outcomes [30–32].

To the best of our knowledge, ours is one of the first studies to demonstrate the systemic antiviral efficacy of remdesivir as compared with placebo in the setting of a randomized controlled trial. We observed that 5 days of remdesivir accelerated declines and improved trajectories of viral biomarkers in blood but not in the upper respiratory tract. With rare exception [33], multiple prospective clinical trials of remdesivir similarly did not find any antiviral impact of remdesivir on upper respiratory

tract viral RNA levels [34–37], supporting the assertion that this specimen type may be less sensitive for in vivo monitoring of antiviral therapies and/or that the antiviral effect of remdesivir may be relatively modest in hospitalized patients, particularly those treated later in illness. Nonetheless, the concordant reductions in peripheral viral RNA and antigen levels that we observed suggest possible detection of an in vivo inhibition of viral replication [27, 38, 39]. These findings may hint at an underlying rationale, such as reduced concentrations of remdesivir in lung tissue [40] and/or spillover from primary sites of replication, wherein the peripheral blood offers a better compartment in which to measure treatment responses in real-world settings. Note that ACTT-1 was conducted prior to recommendations for the use of immunomodulating therapies to temper hyperinflammatory responses in severe COVID-19 disease. As such, it remains to be seen if the antiviral effects of remdesivir may even be greater among patients receiving immunosuppressive therapies (eg, corticosteroids), which may prolong the duration of viral replication and shedding [41].

Our study has some limitations. First, our analyses were predefined but exploratory and not adjusted for multiple comparisons. Second, some patients did not contribute samples, which may have been missing at random due in part to the overwhelming burden placed on the medical system at the time when ACTT-1 was conducted. However, the proportion of recoveries and ventilator-free survivals captured in our study were comparable to those observed in the original cohort (Supplementary Table 1), suggesting that any missingness was not largely driven by outcomes. Additionally, sensitivity analyses that included all patients with a baseline viral load (Supplementary Tables 3 and 5) or assessed for cohort selection bias (Supplementary Tables 13 and 14) supported our primary findings. Third, restricting enrollment to hospitalized patients may have selected for overall lower baseline viral loads, potentially underestimating the strength of associations with clinical outcomes, particularly among patients who presented later in illness (Supplementary Figure 2A). Fourth, quantitative polymerase chain reaction and Simoa assays may not be available in all settings and would benefit from further study to validate diagnostic cutoffs and better determine their routine clinical applicability. Last, as ACTT-1 enrolled unvaccinated patients during the first wave of the pandemic, our findings may vary by factors such as immune status and SARS-CoV-2 variant. Yet, growing evidence suggests that plasma viral RNA and nucleocapsid antigen correlate with clinical parameters across more recent viral variants [5, 42].

In summary, in this analysis of a randomized clinical trial, we report that SARS-CoV-2 plasma RNA and nucleocapsid antigen have potential as surrogate biomarkers for evaluating the benefit of antiviral therapy and warrant further study. As remdesivir is one of the few available treatments that retains activity against circulating variants of concern [43–45], an

understanding of its real-world antiviral effects and their ability to inform clinical outcomes remains important. Our study may also offer insights into strategies that could be applied to the evaluation of other antiviral drug candidates.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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