

Delayed Rise of Oral Fluid Antibodies, Elevated BMI, and Absence of Early Fever Correlate With Longer Time to SARS-CoV-2 RNA Clearance in a Longitudinally Sampled Cohort of COVID-19 Outpatients

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Background. Sustained molecular detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in the upper respiratory tract (URT) in mild to moderate coronavirus disease 2019 (COVID-19) is common. We sought to identify host and immune determinants of prolonged SARS-CoV-2 RNA detection.

Methods. Ninety-five symptomatic outpatients self-collected midturbinate nasal, oropharyngeal (OP), and gingival crevicular fluid (oral fluid) samples at home and in a research clinic a median of 6 times over 1–3 months. Samples were tested for viral RNA, virus culture, and SARS-CoV-2 and other human coronavirus antibodies, and associations were estimated using Cox proportional hazards models.

Results. Viral RNA clearance, as measured by SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR), in 507 URT samples occurred a median (interquartile range) 33.5 (17–63.5) days post-symptom onset. Sixteen nasal-OP samples collected 2–11 days post-symptom onset were virus culture positive out of 183 RT-PCR-positive samples tested. All participants but 1 with positive virus culture were negative for concomitant oral fluid anti-SARS-CoV-2 antibodies. The mean time to first antibody detection in oral fluid was 8–13 days post-symptom onset. A longer time to first detection of oral fluid anti-SARS-CoV-2 S antibodies (adjusted hazard ratio [aHR], 0.96; 95% CI, 0.92–0.99; $P = .020$) and body mass index (BMI) ≥ 25 kg/m² (aHR, 0.37; 95% CI, 0.18–0.78; $P = .009$) were independently associated with a longer time to SARS-CoV-2 viral RNA clearance. Fever as 1 of first 3 COVID-19 symptoms correlated with shorter time to viral RNA clearance (aHR, 2.06; 95% CI, 1.02–4.18; $P = .044$).

Conclusions. We demonstrate that delayed rise of oral fluid SARS-CoV-2-specific antibodies, elevated BMI, and absence of early fever are independently associated with delayed URT viral RNA clearance.

Keywords. SARS-CoV-2; RT-PCR; viral RNA; COVID-19; antibody.

INTRODUCTION

Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections chiefly occur in the community, early virus and

host response kinetics have been largely described in hospitalized inpatients. The logistics of recruiting and sampling potentially infectious individuals in the outpatient setting have sustained gaps in our knowledge of infectivity and pathogenesis in mild to moderate disease. In particular, it is not known whether antibody kinetics are tightly correlated on an individual level with viral RNA decay in mild to moderate coronavirus disease 2019 (COVID-19). Several case reports of prolonged shedding of infectious virus in immune-compromised people with COVID-19 [1–4] support the hypothesis that adaptive immunity is required for clearance of infectious virus. Two studies report that prolonged viral RNA shedding is correlated with lower peak SARS-CoV-2-specific immunoglobulin G (IgG) titers and lower numbers of B and T cells in

Received 19 March 2021; editorial decision 10 April 2021; accepted 13 April 2021.

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Open Forum Infectious Diseases® 2021

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DOI: 10.1093/ofid/ofab195

blood [5, 6]. However, other studies report no difference in plasma SARS-CoV-2-specific antibody titers in patients testing positive or negative at late time points after symptom onset [7, 8]. Here we leveraged guided home sampling to longitudinally characterize viral and immune kinetics in a prospective cohort with mild to moderate COVID-19 and identified associations with duration of viral RNA shedding from the upper respiratory tract that yield insights into COVID-19 pathogenesis.

METHODS

Patient Consent Statement

Due to the timing of study enrollment and the contagious nature of COVID-19, obtaining a signed informed consent form for participants enrolled in this study was not feasible or safe for study staff. Instead, study staff obtained verbal consent using consent waiver with an alteration of informed consent. All participants provided verbal consent after documentation of understanding, as they were isolating at home due to COVID-19, according to an approved consent script in either English or Spanish. A copy of the informed consent was sent to all participants. This protocol and verbal consent were approved by the Johns Hopkins University School of Medicine Institutional Review Board. All procedures were in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association.

Study Cohort and Sampling

The Johns Hopkins University (JHU) School of Medicine Institutional Review Board approved this study. Participants

provided informed consent. The study prospectively enrolled a convenience sample of adults aged ≥ 30 years within 48 hours of a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) test from an outpatient testing site of the Johns Hopkins Health System (JHHS) between April 21, 2020, and July 23, 2020 (Figure 1A) [9, 10]. The 95 symptomatic participants with known symptom onset date and who completed 1 or more RT-PCR tests after symptom onset were included here. Self-sampled midturbinate nasal swabs and oropharyngeal (OP) swabs were obtained with telephone guidance from study staff, and swabs were combined in 3 mL of viral transport medium and frozen. Milturbinate nasal-OP RT-PCR is equivalent to clinician-collected nasopharyngeal swabs [11–15]. Gingival crevicular fluid was obtained using the Oracol device (Malvern Medical Developments Ltd., Worcestershire, UK). Saliva was collected in the tube from June 1, 2020, onwards after validation of the spit saliva sample type [1]. The sampling schedule is shown in Figure 1B. Fifty-three of 95 participants presented to a research clinic visit at 1–4 months post-symptom onset for height, weight, vital signs, blood draw, nasopharyngeal swab, and oral fluid sampling. Symptom reports were collected by telephone at enrollment and on the same days as home and sample collection and are the primary source of symptom data. Symptom reports were cross-checked by reviewing clinical notes for symptom complaints from the electronic medical record.

Laboratory Procedures

RT-PCR testing of study samples was performed on the Abbott *m2000* platform (Abbott Molecular, Des Plaines, IL, USA) in 600- μ L volumes [9]. Reported are cycle threshold (Ct) values

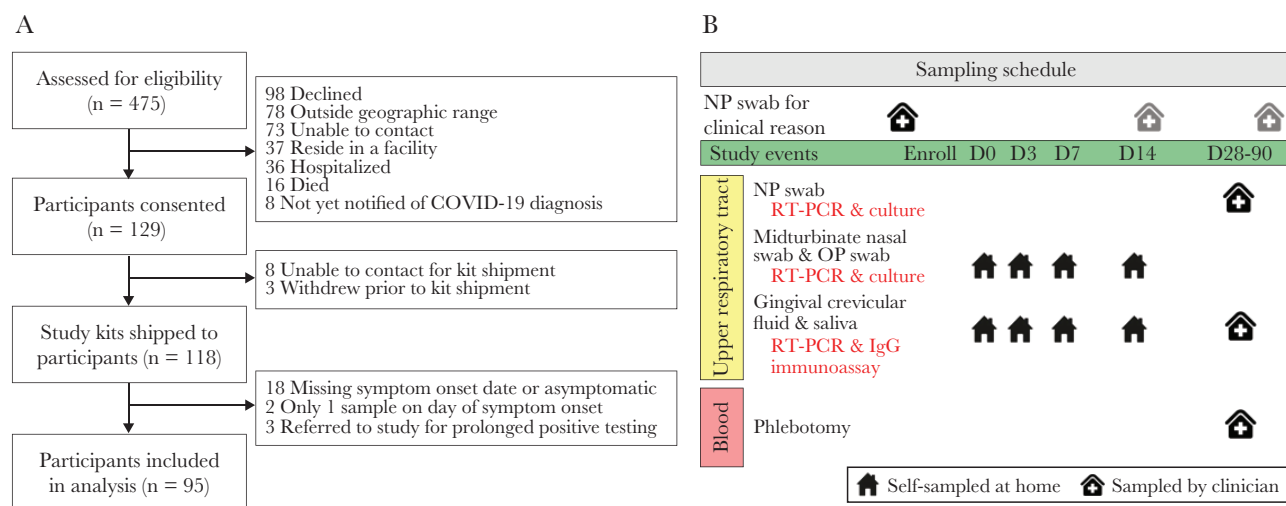


Figure 1. CONSORT flow diagram and sampling schedule. A, A convenience sample of 475 adults with recent positive SARS-CoV-2 RT-PCR tests from an outpatient testing site of the Johns Hopkins Health System was assessed for eligibility between April 21, 2020, and July 23, 2020. Preference was given to participants 40 years of age and older. Study kits were sent to 118 participants, and data and samples from 95 participants were included in the analyses presented here. B, Study sampling schedule. Clinical RT-PCR results from the medical record are included in these analyses. Samples on study days 0–14 were self-collected at home with telephone or video guidance by trained study staff. Participants presented to a research clinic for collection of samples a median (range) of 45 (27–88) days after study day 0. Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

from 1 of 2 primer/probe regions. Ct values <31.5 were positive. Two hundred-microliter volumes of oral fluid were assayed by RT-PCR. The adequacy of self-collected nasal-OP and oral fluid samples was confirmed via quantitative PCR for GAPDH human gene expression utilizing a TaqMan gene expression assay (ThermoFisher Scientific, Waltham, MA, USA) (data not shown).

Positive nasal-OP samples by RT-PCR were tested for propagation of SARS-CoV-2 in cell culture [16]. Plasma SARS-CoV-2-specific antibody titers were quantified by indirect enzyme-linked immunosorbent assay (ELISA), and neutralizing antibody titers were quantified by microneutralization assay [17, 18]. Quantification of oral fluid SARS-CoV-2-specific IgG and other human coronavirus-specific IgG was obtained from a multiplex SARS-CoV-2 immunoassay based on Luminex technology [19, 20]. The oral fluid SARS-CoV-2-specific IgG readout of this assay is highly correlated with plasma SARS-CoV-2-neutralizing antibodies and plasma S-RBD-specific IgG by ELISA [19, 20].

Statistical Methods

Kaplan-Meier plots and log-rank tests were generated using the “survival” R package [21]. Associations between covariates and clearance of upper respiratory tract SARS-CoV-2 viral RNA were determined via Cox proportional hazards models [21] after multiple imputation and model selection by least absolute shrinkage and selection operator (LASSO). A complete list of covariates for Cox model 1 may be found in [Supplementary Table 1](#). Time to RT-PCR clearance was defined as number of days from symptom onset to the midpoint between the last positive RT-PCR test and the subsequent negative test. Patterns of missing data were investigated, and logistic regression was performed on the most frequent missing patterns to confirm that the data were missing at random (Supplementary Methods, [Supplementary Tables 2–3](#)). Twenty data sets, each including all data points for all 95 participants, were imputed using predictive mean matching for univariate imputation and chained equation (MICE) for multivariate imputation using the “mice” R package [22]. Model selection was performed using LASSO on each of the imputed data sets using the “glmnet” R package [23]. Nine variables were included in the model, which used the pooled imputed data set. To determine whether the time from symptom onset to first detection of oral fluid SARS-CoV-2-specific IgG is associated with RT-PCR clearance, we performed model selection using LASSO and ran Cox regression model 2. Further details are provided in the Supplementary Methods.

RESULTS

Study Design

Ninety-five nonhospitalized, symptomatic individuals with a positive nasopharyngeal SARS-CoV-2 RT-PCR test from an

emergency room or an ambulatory testing center within the previous 48 hours were enrolled between April 21, 2020, and July 23, 2020 ([Figure 1A](#)). Study day 0 was a median (range) of 4 (2–11) days from the collection of the most recent prior positive RT-PCR test and was a median (range) of 9 (2–80) days from symptom onset. Participants presented for a single research clinic visit a median (range) of 45 (27–88) days after study day 0.

Clinical Characteristics

The median age of the participants was 56 years, and 59% were women ([Table 1](#)). A total of 39% identified as Black or African American, and 14% as Hispanic/Latinx. The median body mass index (BMI) of participants was 29.3 kg/m², similar to that of US adults, 29.4 kg/m² [24]. Eight participants (8.4%) required hospitalization after enrollment in the study [10].

Viral Kinetics in COVID-19 Outpatients

Participants’ SARS-CoV-2 RT-PCR tests from the study and from the medical record were included in these analyses, totaling 507 RT-PCR tests ([Figure 2](#)). Midturbinate nasal-OP and nasopharyngeal RT-PCR tests are the primary sample types, while oral fluid RT-PCR tests were included only if nasal samples were missing or in the 3 instances in which the nasal-OP sample was negative while the oral fluid sample was positive. Through December 15, 2020, the 95 participants had a median (interquartile range [IQR]) of 6 (4–6) RT-PCR tests during and after acute COVID-19. Longitudinal Ct values from study samples showed a rapid decay, with several instances of a negative test followed by a positive test (re-positive cases) ([Figure 3A](#), gray lines). Re-positive cases are commonly reported [8, 25, 26] and may be due to poor immune clearance of viral RNA or prolonged low-level replication of SARS-CoV-2. There was no difference in the reference gene GAPDH’s RNA cycle threshold values from samples that tested negative before a re-positive compared with samples from participants who never experienced a re-positive test ([Supplementary Figure 1](#)).

To examine Ct values as an indicator of viral load in the URT from very early time points, RT-PCR Ct values were obtained from the participant’s first positive clinical RT-PCR test, the majority of which were run on the NeuMoDx platform. The mean Ct value was lowest—meaning the viral burden was highest—before and just after symptom onset ([Figure 3B](#)). The first 8–10 cycles on the Abbott RT-PCR platform are not read, and so the Ct values of the Abbott (study samples) and NeuMoDx (clinical samples) platforms are not comparable.

Virus Culture Positivity Is Detected Through 11 Days Post-Symptom Onset

Sixteen (of 183 RT-PCR-positive) study samples cultured for SARS-CoV-2 on VeroTMPRSS2 cells [16, 27] from 14 participants were virus culture positive ([Figure 3A](#), red circles). In these 14 participants, the median time from symptom onset to

Table 1. Demographics and Clinical Characteristics of Participants

Characteristic	Participants (n = 95)
Median age at enrollment (IQR), y	56 (49–64)
Age grouping	
<65 y	74 (77.9)
≥65 y	21 (22.1)
Biological sex	
Male	39 (41.1)
Female	56 (58.9)
Race	
White	46 (48.4)
Black or African American	37 (38.9)
Asian	5 (5.3)
American Indian or Alaska Native	1 (1.1)
Native Hawaiian or other Pacific Islander	1 (1.1)
Other	5 (5.3)
Ethnicity	
Hispanic or Latinx	13 (13.8)
Median BMI (IQR), kg/m ²	29.3 (26.0–35.4)
BMI group ^a	
Normal (<25 kg/m ²)	19 (20.0)
Overweight or obese (≥25 kg/m ²)	74 (77.9)
Smoking status	
Never smoker	68 (71.6)
Ever smoker	4 (4.2)
Missing	23 (24.2)
Hypertension	39 (41.1)
Cardiovascular disease other than hypertension	15 (15.8)
COPD or asthma	16 (16.8)
Chronic kidney disease	3 (3.2)
Diabetes	15 (15.8)
Cancer not in remission	4 (4.3)
Immunocompromised ^b	9 (9.5)
Fever as 1 of first 3 COVID-19 symptoms	
Yes	30 (31.6)
No	60 (63.2)
Missing	5 (5.3)
Cycle threshold value of first RT-PCR ^c	
Median (IQR)	18.6 (15.0–23.7)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: IQR, interquartile range; BMI, body mass index; COPD, chronic obstructive pulmonary disease; RT-PCR, reverse transcription polymerase chain reaction.

^aTwo participants had missing BMI information.

^bImmunocompromised refers to participants who are solid organ or bone marrow transplant recipients, who have primary immunodeficiency or AIDS, or who were taking immune-modulating medications within 3 months of COVID-19 diagnosis such as chemotherapy, including biologics, mycophenolate mofetil, methotrexate, tumor necrosis factor inhibitors, or prednisone ≥20 mg/d.

^cThe first SARS-CoV-2 RT-PCR was performed in the clinical laboratory before enrollment. Included here are cycle threshold values from the 59 participants whose first RT-PCR was run on the NeuMoDx platform within 7 days of symptom onset.

last positive virus culture (range) was 5 (2–11) days. No sample tested positive for virus culture beyond 11 days from symptom onset, even in the case of an immunocompromised participant who had positive RT-PCRs with low Ct values 2 months post-symptom onset (Figure 3A). None of the re-positive RT-PCR samples were positive for virus culture. Virus culture was positive only in samples with Ct values <17 on the Abbott *m2000* platform. The mean Ct value of samples positive for virus

culture was significantly lower than that of samples negative for viral culture (Figure 3C).

Positive Oral Fluid SARS-CoV-2-Specific IgG Is Associated With Negative Virus Culture

To determine whether oral fluid anti-SARS-CoV-2 IgG could be used to predict which samples with low Ct values were negative for virus culture, we examined sample time points with (1) nasal Abbott RT-PCR Ct values <20, (2) nasal virus culture results, and (3) oral fluid samples with adequate total IgG for assessment (total IgG >10 µg/mL or detectable oral fluid anti-S-RBD IgG). Fourteen of 15 samples positive for oral fluid anti-S-RBD IgG were negative for virus culture (Figure 3D). The 1 culture-positive sample was collected on day 11 after symptom onset, which is at or shortly after the expected first detection of this antibody (Supplementary Table 4).

Oral Fluid SARS-CoV-2-Specific IgG Is First Detected Between 8 and 13 Days From Symptom Onset

Oral fluid SARS-CoV-2-specific IgG to multiple viral antigens was assessed (Supplementary Figures 2–4). The longitudinal kinetics of oral fluid anti-SARS-CoV-2 S IgG by day from symptom onset are shown in purple in Figure 3A. Supplementary Table 4 shows the means and 95% CIs of number of days from symptom onset to rise above cutoff for each of the 8 SARS-CoV-2 antigens (8–13 days depending on the antigen), similar to that previously described [19].

Kinetics of SARS-CoV-2 Viral RNA Clearance in Outpatients

The median time to clearance (range, IQR) was 33.5 (4.5–150, 17–63.5) days (Figure 4A), similar to hospitalized COVID-19 patients with a similar mean age [28].

Elevated BMI Is Associated With Longer Time to Viral RNA Clearance

To determine host and immune factors associated with longer time to RT-PCR clearance, we used a Cox proportional hazards model after multiple imputation and model selection by LASSO. Nine covariates were selected for inclusion in the Cox model. Shown in Figure 5 are the univariable and adjusted hazard ratios of clearance and the 95% confidence intervals. BMI ≥25 kg/m² was independently associated with longer time to viral RNA clearance in outpatients with COVID-19 in the main analysis (adjusted hazard ratio [aHR], 0.37; 95% CI, 0.18–0.78; *P* = .009) (Figures 4B and 5) and in several sensitivity analyses using different methods to estimate clearance (Supplementary Tables 5–9). This association did not appear to be explained by comorbidities such as hypertension and diabetes as inclusion of hypertension and diabetes in the model did not attenuate the BMI association. Further, hypertension was not significantly associated with time to viral RNA clearance, and diabetes was significantly positively associated with clearance, which is in the opposite direction of elevated BMI.

There was a trend toward immunocompromised hosts (*n* = 9) having a longer time to viral RNA clearance (Figure 5).

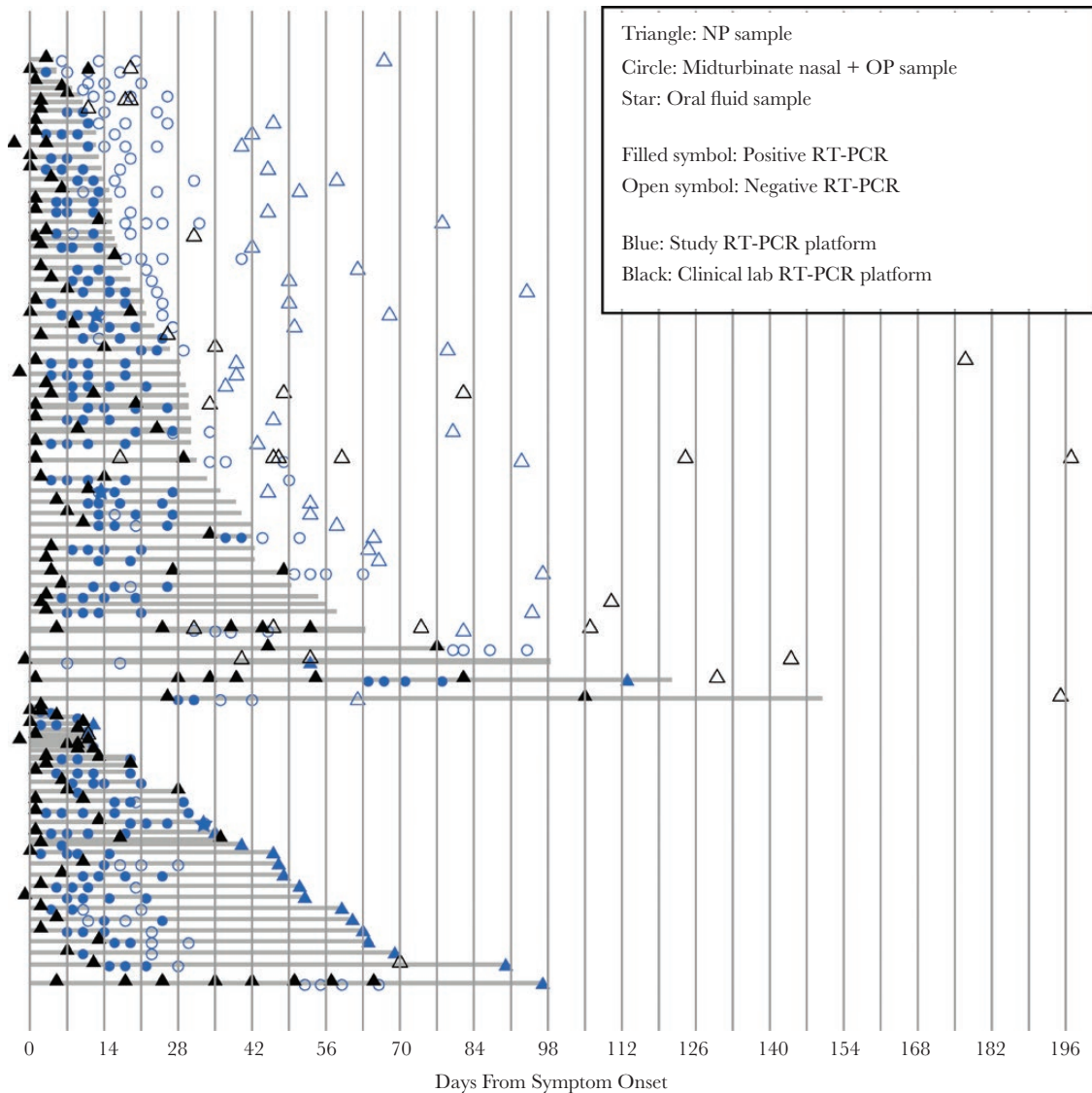


Figure 2. Longitudinal SARS-CoV-2 RT-PCR sampling in outpatients. SARS-CoV-2 RT-PCR test results by day from symptom onset. Each row represents 1 participant. Shown here are nasal samples. Stars indicate a sample time point at which the nasal sample was negative and the oral fluid sample was positive. Included in the top grouping are participants whose last RT-PCR test was negative, and in the bottom group are those whose last RT-PCR test was positive. Shaded gray lines indicate the number of days from symptom onset until the midpoint between the last positive sample and the next negative sample (top grouping) or until the last positive sample (bottom grouping). Nasopharyngeal (NP) swabs collected by a health care worker are indicated by triangles; self-collected midturbinate nasal-oro-pharyngeal (OP) swab combined in viral transport media are indicated by circles. Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

There was additionally a trend toward greater magnitude of oral fluid anti-S-RBD IgG (treated as a time-varying variable) being associated with longer time to RT-PCR clearance (Figure 5). Larger studies are needed to assess this association. The magnitude of oral fluid antihuman coronavirus IgG for the 4 circulating non-SARS human coronaviruses was not associated with time to viral RNA clearance (Figure 5; Supplementary Table 1).

COPD/Asthma, Early Fever, and Diabetes Are Associated With Faster Viral RNA Clearance

COPD/asthma, diabetes, and fever reported as 1 of the first 3 COVID-19 symptoms were each associated with faster viral

RNA clearance (Figure 5). In 4 of 5 sensitivity analyses using different methods to estimate clearance, fever as 1 of the first 3 reported COVID-19 symptoms remained associated with viral RNA clearance, whereas the association of diabetes and COPD/asthma was significant in fewer such analyses (Supplementary Tables 5–9).

Longer Time to First Detection of Oral Fluid SARS-CoV-2-Specific IgG Is Associated With Longer Time to Viral RNA Clearance

In a time-dependent covariate Cox model (Cox model 2) for viral RNA clearance, a longer time to first detection of oral fluid spike-specific IgG is associated with longer time to viral RNA

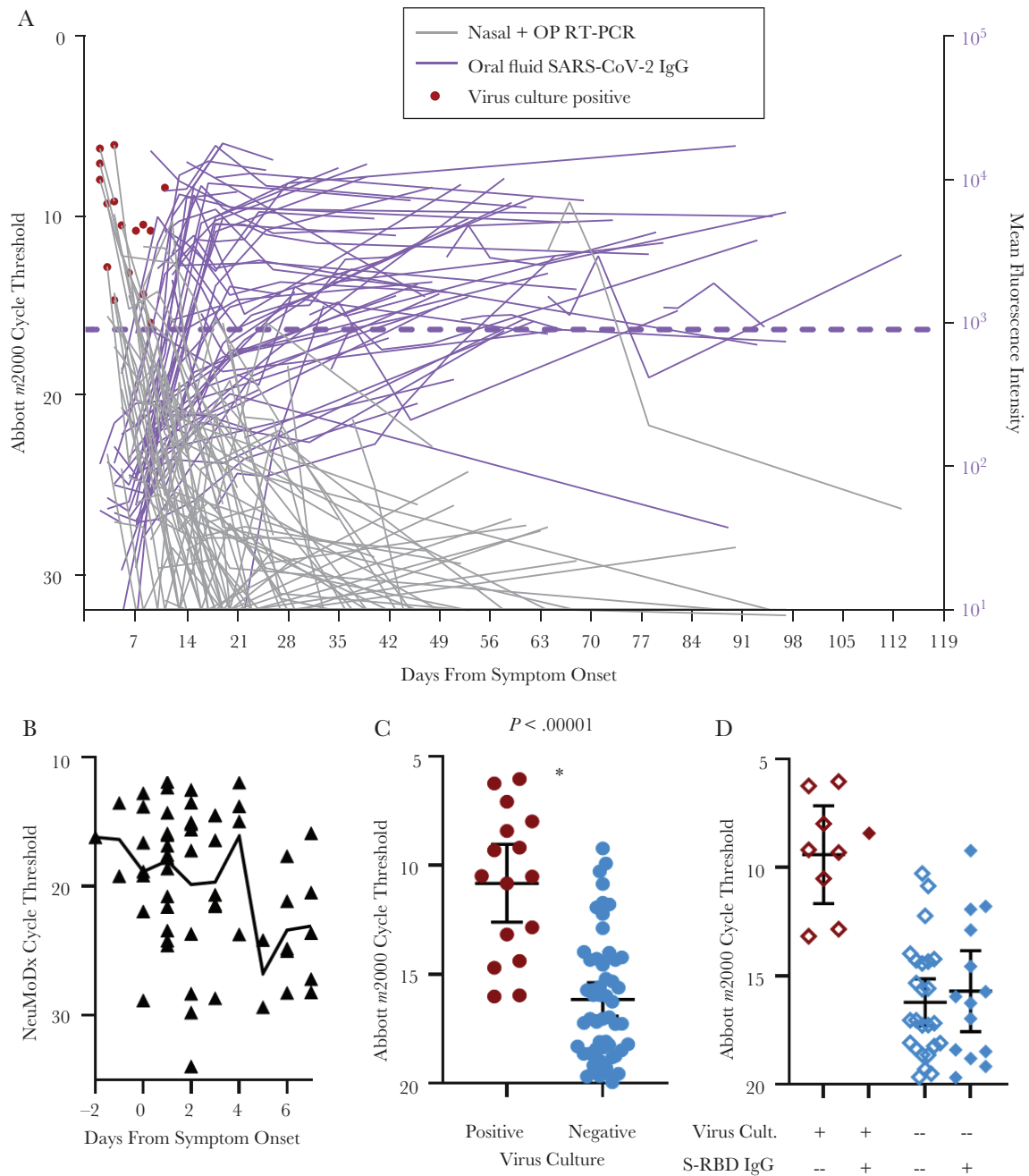


Figure 3. Kinetics of RT-PCR cycle thresholds, virus culture, and oral fluid SARS-CoV-2 antibodies in mild-moderate COVID-19. **A**, Longitudinal Abbott *m*2000 SARS-CoV-2 RT-PCR cycle threshold values from nasal-OP swab study samples (blue circles and triangles in [Figure 2](#)) by day from symptom onset are shown in gray. Longitudinal oral fluid antispike (Mt. Sinai) IgG mean fluorescence intensity shown in purple. Lines connect values from the same participant. Each sample with a positive RT-PCR result was assessed for propagation of SARS-CoV-2 on VeroE6-TMPRSS2 cells. Samples with positive virus culture are indicated by red circles. The cutoff for positive anti-S IgG is shown with a purple dotted line (see the “Methods” for cutoff calculation). **B**, The subset of 59 participants with a nasopharyngeal swab collected within 7 days of symptom onset and run on the NeuMoDx SARS-CoV-2 RT-PCR platform is shown by day from symptom onset. The line connects cycle threshold means. **C**, The subset of nasal-OP swab samples with a cycle threshold <20 is grouped according to whether virus culture of the sample was positive or negative. Means with 95% CIs are shown. $*P < .00001$ by 1-tailed Student *t* test. **D**, The subset of samples shown in (B) that had a simultaneous adequate oral fluid sample is shown here. Filled diamonds represent study time points at which oral fluid contained detectable IgG antibodies to Mt Sinai S-RBD, and open diamonds represent study time points at which oral fluid was negative for anti-S-RBD IgG. Abbreviations: Ab, antibody; COVID-19, coronavirus disease 2019; Cult, culture; IgG, immunoglobulin G; OP, oropharyngeal; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S-RBD, spike receptor binding domain.

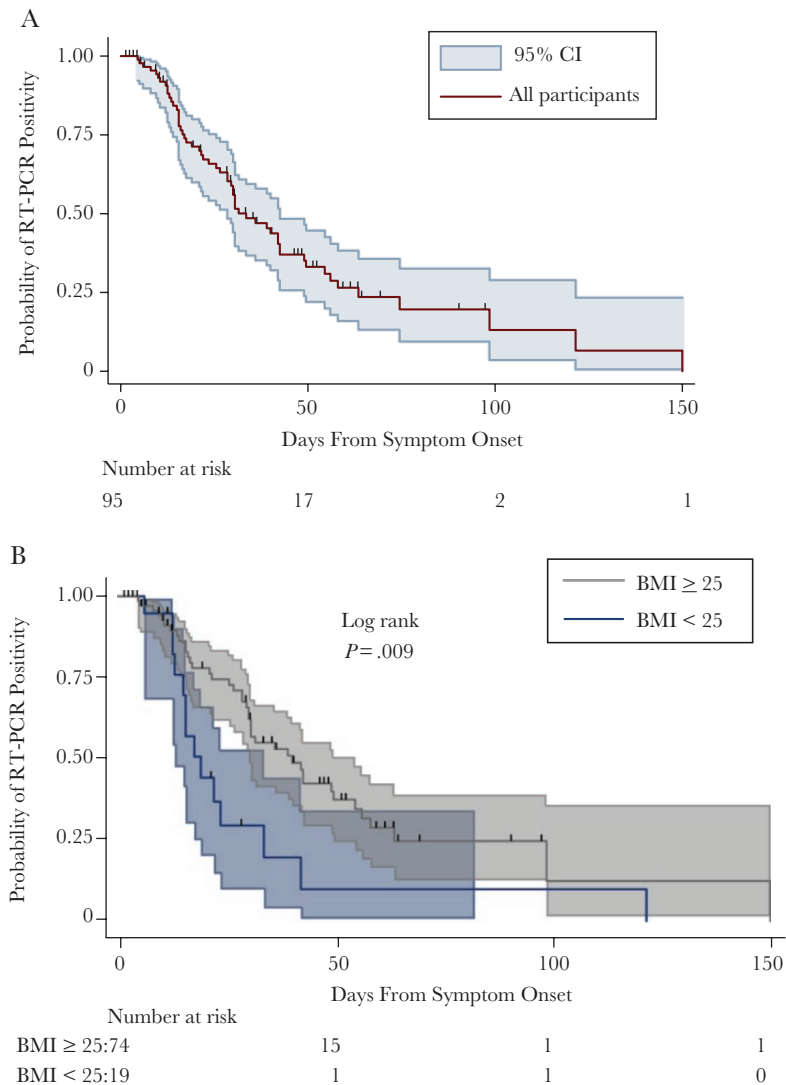


Figure 4. Kaplan-Meier plots SARS-CoV-2 RT-PCR positivity by day from symptom onset in mild–moderate COVID-19. A, Kaplan-Meier survival curve with 95% CIs for positive upper respiratory tract SARS-CoV-2 RT-PCR by day from symptom onset. B, Kaplan-Meier survival curve by BMI category. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

clearance (aHR, 0.96; 95% CI, 0.92–0.99; $P = .020$) (Table 2; Supplementary Tables 8–9).

Plasma SARS-CoV-2-Specific Antibody Titer in Convalescence Is Not Associated With Time to Viral RNA Clearance

Titers of plasma anti-S-RBD IgG, anti-S IgG, or neutralizing antibody in convalescence (measured once at 1–4 months post-symptom onset and treated as a baseline variable) were not significantly associated with viral RNA clearance in either direction. Additionally, the presence or absence of viral RNA by RT-PCR in the nasopharynx at 1–4 months post-symptom onset was not associated with plasma antibody titer (data not shown).

DISCUSSION

In a racially and ethnically diverse cohort of 95 adult outpatients with symptomatic COVID-19, we report that a longer time to

first detection of oral fluid SARS-CoV-2-specific antibodies is independently associated with a longer time to viral RNA clearance. We find that the presence of SARS-CoV-2 antibodies in oral fluid may be a predictor of negative virus culture from nasal samples, even in samples with low RT-PCR Ct values. We demonstrate that the mean time from symptom onset to first detection of oral fluid SARS-CoV-2 S-RBD-IgG is 9–11 days, and virus culture–positive samples may be detected through day 11 post-symptom onset in adults with mild to moderate COVID-19, 1 day longer than the current recommended isolation period [29].

The strengths of this cohort are its size, its prospective and intensive longitudinal sampling design beginning in the acute phase of illness, its long duration of follow-up, and its simultaneous testing of samples for SARS-CoV-2 RT-PCR, virus culture, and oral fluid antibodies. Two studies of mostly outpatients

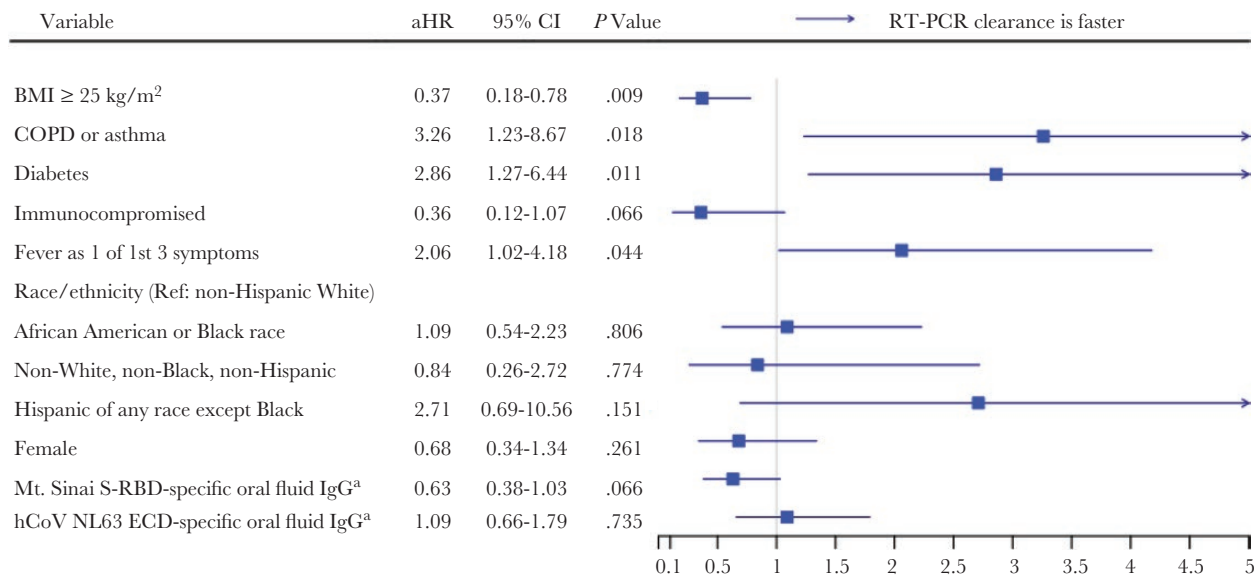


Figure 5. Cox proportional hazards model for viral RNA clearance. An aHR <1 denotes longer time to SARS-CoV-2 viral RNA clearance in the upper respiratory tract as measured by RT-PCR. To account for missing data points, 20 data sets were imputed using PPM MICE. Model selection using LASSO was performed on each of the 20 data sets. The top 9 variables of 35—selected 15 or more times out of 20 (Supplementary Table 2)—were chosen for inclusion in the Cox proportional hazards model, which was performed using the pooled imputed data set. ^aStandardized log mean fluorescence intensity and treated as a time-varying variable. Abbreviations: aHR, adjusted hazard ratio; ECD, spike ectodomain; hCoV, human coronavirus; LASSO, least absolute shrinkage and selection operator; MICE, multiple imputation by chained equation; PPM, predictive mean matching; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S-RBD, spike receptor binding domain.

included more cultured specimens but were not longitudinal [30, 31]. Our findings extend to the outpatient domain those described in 129 patients with severe COVID-19 by van Kampen and colleagues [32], who report a mean time from symptom onset to last positive virus culture of 8 days (maximum 20); in this cohort, virus cultures were more likely to be negative when plasma-neutralizing antibodies were elevated [32]. Our results suggest that outpatients testing positive for SARS-CoV-2 who have detectable oral fluid SARS-CoV-2-specific antibodies are not likely to be transmissible, but larger studies are needed for confirmation.

We demonstrate that elevated BMI is also independently associated with longer time to viral RNA clearance. This association is not likely explained by increased prevalence of comorbidities

such as hypertension or diabetes. In fact, diabetes, along with early fever and COPD/asthma, is associated with faster time to viral RNA clearance. Numerous studies report strong associations of COVID-19 severity and older age with viral RNA shedding duration [17, 33, 34]. Neither severity nor age was significantly associated with duration of viral RNA shedding in our study, likely because the vast majority of our cohort participants were in the same severity category and age range (49–64 years). Importantly, we found no effect of race or ethnicity with time to viral RNA clearance after adjustment for other demographic and comorbidities. Obesity is associated with testing positive for SARS-CoV-2, with severity of COVID-19, and with COVID-19 mortality [35–38]. Dysregulated immunity is observed in severe COVID-19, aging, and obesity, and it may be the unmeasured

Table 2. Time-Dependent Covariate Cox Proportional Hazards Model for Viral RNA Clearance (Cox Model 2)

Variable	HR	aHR	95% CI	P Value
Change from undetectable to detectable Mt. Sinai S-specific oral fluid IgG	0.96	0.96	0.92–0.99	.020
BMI ≥ 25 kg/m ² (referent: BMI <25 kg/m ²)	0.44	0.29	0.14–0.61	.001
Diabetes	2.11	2.49	1.09–5.67	.030
Immunocompromised	0.68	0.35	0.12–1.02	.055
Fever as 1 of first 3 COVID-19 symptoms	1.72	2.23	1.05–4.71	.036
Race/ethnicity (referent: non-Hispanic White)				
African American or Black race, any ethnicity	0.73	0.85	0.42–1.73	.659
Non-White, non-Black, non-Hispanic	1.20	0.56	0.14–2.23	.408
Hispanic of any race except Black	0.79	1.48	0.41–5.39	.549

Abbreviations: aHR, adjusted hazard ratio; BMI, body mass index; COVID-19, coronavirus disease 2019; HR, hazard ratio; IgG, immunoglobulin G; S, SARS-CoV-2 spike protein.

key correlate of viral RNA shedding duration. Aging drastically increases the chronic state of inflammation present in obesity [39], perhaps explaining why our study of middle-aged and older participants uncovered the strong association of elevated BMI and viral RNA shedding duration. The other risk factors that correlated with longer time to viral RNA clearance in our study—absence of early fever and longer time to detection of oral fluid antibodies—are consistent with impaired early innate and adaptive immune responses. Silva et al. report that a delay in antibody production is linked to high salivary viral loads in hospitalized patients [40]. We hypothesize that the chronic inflammatory state of aging and obesity hinders early innate and adaptive immune responses to SARS-CoV-2, leading to higher viral loads, which require higher antibody titers and more time to clear, while also presenting a cytokine milieu in which it is stochastically more likely for a patient to tip into the positive feedback loops of hyperinflammation that portend disease severity. Mechanistic studies are needed to determine how obesity prolongs viral RNA shedding.

Our study has limitations, including that it is undersampled at the time of symptom onset and at >1 month from symptom onset. We address this by including clinician-ordered samples outside of the study, but the time to viral RNA clearance may be less precisely described in participants whose study samples at 1–3 months remained positive than in others. Our analytical approaches—Kaplan-Meier estimate, log-rank test, and Cox regression analyses—were designed to properly handle and correct bias caused by censored data. We minimize bias in estimated clearance by defining clearance as the midpoint between the last positive and subsequent negative test. Several sensitivity analyses using different clearance definitions demonstrate that our conclusions are supported by the evidence. There was a nonsignificant trend toward immunocompromised host status being associated with viral RNA shedding duration, which may appear to contrast with reports of prolonged viral carriage in immunocompromised people [1–4]. This likely occurred because we grouped all immunocompromised people together, whereas there is probably a specific immunocompromised phenotype that leads to prolonged viral RNA detection. The associations of diabetes and COPD/asthma with faster time to viral RNA clearance were not found to be independent of other covariates in all sensitivity analyses (diabetes) or our second Cox model (COPD/asthma), and they conflict with 1 report of asthma being associated with longer viral RNA shedding [41]. Larger studies are needed to determine whether diabetes, COPD, and asthma are associated with time to viral RNA clearance. Finally, our study excluded people with asymptomatic SARS-CoV-2 infection, so it is not yet clear whether these results are generalizable to asymptomatic infection.

In sum, we report viral and immune kinetics in an intensively characterized cohort of 95 adult outpatients with mild

to moderate COVID-19. We demonstrate that elevated BMI, longer time to detection of oral SARS-CoV-2 IgG, and the absence of early fever are independently associated with longer time to viral RNA clearance, suggesting that functional early innate and adaptive immunity is critical for timely viral RNA clearance.

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.

Acknowledgments

We acknowledge Jeffrey Holden, Tyrone Howard, Andrew Karaba, Anastasia Lambrou, Lucy Li, Manuela Plazas Montana, Caroline Popper, and Amanda Tuchler for their assistance.

Financial support. This work was supported by the JHU COVID-19 Research and Response Program Fund and the Sherrilyn and Ken Fisher Center for Environmental Infectious Diseases Discovery Program. This research was facilitated by infrastructure and resources provided by the JHU Center for AIDS Research. This work was supported by the National Institutes of Health (grant numbers 1P30AI094189 to C.P., K08AI143391 to A.A., U54EB007958-12 to Y.M., U5411090366 to Y.M., U54HL143541-02S2 to Y.M., UM1AI068613 to Y.M., K23AI135102 to J.T., contract HHS N272201400007C to A.P., U54CA260492 to A.C. and S.K., R21AI139784 to C.H. and N.P., R01ES026973 to C.H., N.P., and K.K., R01AI130066 to C.H., U24OD023382 to C.H., K08HS025782 to S.K.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We thank the National Institute of Infectious Diseases, Japan, for providing VeroE6TMPRSS2 cells and acknowledge the Centers for Disease Control and Prevention, BEI Resources, NIAID, NIH, for SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-5228. Funding was provided by the FIA Foundation to C.H. and N.P. and by the GRACE Communications Foundation to C.D.H., N.P., and K.K. H.M. reports research collaboration and contribution of equipment and reagents from Bio-Rad Laboratories and DiaSorin Molecular.

Potential conflicts of interest. S.H.M. received speaker fees from Gilead Sciences. All other authors report no conflicts relevant to this article. 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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