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MAJOR ARTICLE

Assessment of Virological Contributions to COVID-19 Outcomes in a Longitudinal Cohort of Hospitalized Adults

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Background. While several demographic and clinical correlates of coronavirus disease 2019 (COVID-19) outcome have been identified, their relationship to virological and immunological parameters remains poorly defined.

Methods. To address this, we performed longitudinal collection of nasopharyngeal swabs and blood samples from a cohort of 58 hospitalized adults with COVID-19. Samples were assessed for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load, viral genotype, viral diversity, and antibody titer. Demographic and clinical information, including patient blood tests and several composite measures of disease severity, was extracted from electronic health records.

Results. Several factors, including male sex, higher age, higher body mass index, higher 4C Mortality score, and elevated lactate dehydrogenase levels, were associated with intensive care unit admission. Of all measured parameters, only the retrospectively calculated median Deterioration Index score was significantly associated with death. While quantitative polymerase chain reaction cycle threshold (Ct) values and genotype of SARS-CoV-2 were not significantly associated with outcome, Ct value did correlate positively with C-reactive protein levels and negatively with D-dimer, lymphocyte count, and antibody titer. Intrahost viral genetic diversity remained constant through the disease course and resulted in changes in viral genotype in some participants.

Conclusions. Ultimately, these results suggest that worse outcomes are driven by immune dysfunction rather than by viral load and that SARS-CoV-2 evolution in hospital settings is relatively constant over time.

Keywords. COVID-19; longitudinal cohort; SARS-CoV-2; viral evolution; viral load.

A better understanding of the risk factors associated with severe coronavirus disease 2019 (COVID-19) is critical for improving risk management strategies and clinical care. Several studies early in the pandemic identified a number of demographic factors associated with severe COVID-19 including sex (male), age (65 or older), and race/ethnicity (Black, Hispanic, Native American) [1]. Comorbidities and other preexisting conditions—including diabetes, heart disease, asthma, high body mass index (BMI), and immunodeficiency—have likewise been linked with worse clinical outcomes [2–4]. Socioeconomic status, access to health care, and exposure risk also have large impacts on risk of transmission and disease [5].

A variety of composite scores have been developed to help predict clinical outcomes and inform medical management and level of care [6]. These scores are typically calculated serially over the course of illness from several clinical/demographic assessments with the goal of identifying patients who are clinically deteriorating. While several scores have been independently validated for monitoring COVID-19 patient deterioration, including the 4C Mortality (4C) score [7, 8], the Epic Deterioration Index (DI) model [9], and the Modified Early Warning Score (MEWS) [10], their relative clinical utility for predicting patient outcome remains unclear.

More direct measurements of SARS-CoV-2 infection have similarly been examined as potential biomarkers for severe disease and patient outcome. Early reports suggested that viral load in the upper respiratory tract may be associated with severe

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disease [11, 12], though this has not been observed in other studies [13]. Likewise, while several new viral lineages have evolved with enhanced transmission or fitness [14–17], few associations have been found between these new genetic variants and disease severity, presentation, or outcomes [18–21]. The host response to infection, on the other hand, has been linked to disease severity in a number of ways. Clinical markers of inflammatory responses, including lymphopenia, elevated levels of proinflammatory cytokines, and elevated C-reactive protein (CRP), have been associated with COVID-19 disease severity [22, 23]. Stronger antibody responses to SARS-CoV-2 nucleoprotein (N) as opposed to spike (S) have similarly been associated with more severe disease [24, 25].

Utilizing serial clinical data and biospecimens from an early cohort of hospitalized adults with COVID-19, we assessed the relative contribution of each of these factors to predict disease severity and participant outcome. The goal of this study was to understand how virological factors, including viral load, genotype, and viral diversity, associated with other clinical measurements of COVID-19 disease.

METHODS

Specimen Collection and Processing

Individuals over the age of 18 admitted to Northwestern Memorial Hospital with a positive polymerase chain reaction (PCR)–based COVID-19 diagnostic test, who provided informed consent themselves or through an appropriate surrogate, were enrolled in the study per institutional review board (IRB) approval STU00206850. Nasopharyngeal swabs were collected from study participants on the enrollment date and every 4 ± 1 days after enrollment for up to 30 days of hospitalization. Swabs were stored in 2–3 mL of Primestore MTM (Longhorn Vaccines & Diagnostics). The specimen was aliquoted into 2–3 vials and stored at −80°C. A total of 238 nasopharyngeal specimens were collected from 58 participants throughout the course of the study. Whole blood was collected from consenting study participants every 8 ± 1 days after enrollment for up to 30 days of hospitalization in Vacutainer CPT Mononuclear Cell Preparation tubes containing sodium heparin (Becton Dickinson). Three CPT tubes containing ~8 mL of whole blood each were collected per participant per time point. A total of 65 blood specimens were collected from 34 participants throughout the course of the study; 24 participants either declined or were unable to provide consent at the time of blood collection. CPT tubes containing whole blood were centrifuged at 1500–1800xg in a swing-bucket centrifuge for 30 minutes. Plasma from each of the 3 collection tubes was removed, pooled, and frozen in aliquots at −80°C. Peripheral blood mononuclear cells (PBMCs) were removed, washed with 1x PBS containing 0.5% BSA and 2 mM of EDTA, and frozen in cryopreservation media (1x FBS, 10% DMSO) at −80°C.

Clinical Data Extraction

All available clinical data from the Northwestern Medicine Enterprise Data Warehouse (NMEDW) and from electronic chart review were pulled for this subset of hospitalized participants per IRB approval STU00212267. These NMEDW data were utilized to determine demographics, clinical assessments, symptom onset, laboratory measurements, COVID-19 disease severity, and hospital outcomes (intensive care unit [ICU] care and death) for the study analyses.

Quantitative PCR, cDNA Synthesis, and Viral Sequencing

Viral RNA was extracted from nasopharyngeal specimens utilizing the QIAamp Viral RNA Minikit (Qiagen). Laboratory testing for SARS-CoV-2 presence was performed by quantitative reverse transcription PCR (qRT-PCR) with the Centers for Disease Control and Prevention (CDC) 2019-nCoV RT-PCR Diagnostic Panel utilizing N1 and RNase P probes as previously described [26]. cDNA synthesis was performed with the SuperScript IV First Strand Synthesis Kit (Thermo) using random hexamer primers according to the manufacturer's specifications. Direct amplification of the viral genomic cDNA was performed in multiplexed PCR reactions with 2 nonoverlapping primer pools as provided by the Artic Network (version 3). The sequencing library approach was adapted from previously published methods [27]. The pooled library was denatured and loaded onto a MiSeq, version 2, 500-cycle flow cell (Illumina). Viral genome consensus sequences were determined from sequencing reads as previously described [28].

Statistics and Modeling

All statistical analyses and modeling were performed using R, version 4.0.3. All simple correlations were performed using Spearman's rank correlation. Pairwise group comparisons were performed using the Wilcoxon rank-sum test followed by the Benjamini-Hochberg procedure to control the false discovery rate (FDR) for multiple comparisons. An FDR <0.05 was used as statistical significance cutoff. Initial modeling of outcome as a function of demographic predictor was performed by fitting a multinomial log-linear model using the nnet package followed by chi-square tests to examine the contributions of the individual factors. We included all demographic factors as well as the comorbidity score in the fitted model to examine which of these factors significantly contributed to the observed outcome.

For more detailed methods—including phylogenetic analysis, viral diversity analyses, protein purification, and enzyme-linked immunosorbent assays (ELISAs) for antibody quantification—refer to the Supplementary Methods.

RESULTS

Demographic and Clinical Characteristics of the Hospitalized Cohort

Sixty-three patients admitted to Northwestern Memorial Hospital with a positive COVID-19 diagnostic test were enrolled

between March 27, 2020, and June 9, 2020; 58 provided nasopharyngeal swabs, and 32 provided blood specimens (Figure 1A). Four participants ultimately had no samples collected, and 1 participant declined nasopharyngeal swab collection; all were excluded from these analyses. Patients were predominantly male and older (mean age, 64), similar to the overall population admitted to the hospital with COVID-19 during this time (Table 1, Figure 1B) [29].

Participants were categorized into 3 outcome groups: discharged without being admitted to the ICU (n = 23); discharged after admission to the ICU for some period (n = 29); and death due to COVID-19-related illness after ICU admission (n = 6). The time between symptom onset and hospital admission was not significantly different between different outcome groups, though total length of stay was significantly higher for participants admitted to the ICU (Figure 1C). The most frequent symptoms before admission were cough, shortness of breath, fever, fatigue, and myalgia, each of which was reported by >50% of participants (Figure 1D). Most participants had at least 1 underlying comorbid condition, with hypertension, renal disease, and cardiovascular disease being the most frequent (Figure 1E).

As these patients were enrolled early in the pandemic, most received some amount of supportive care, including supplemental oxygen (81.8% of females and 86.1% of males) (Table 2). No other treatment was prescribed to >25% of study participants, and few received therapies later proven to be effective in randomized trials. For participants who were in double-blinded clinical trials, treatment arm was not available to the study team to incorporate into this analysis [30, 31].

To assess the association between these demographic parameters and outcome in our cohort, we constructed a logistic regression model with categorical outcome (non-ICU, ICU, or death) as our dependent variable and sex, age, race, ethnicity, BMI, and total number of reported comorbidities as our independent variables (Supplementary Figure 1A). Sex ($P = .0038$) (Supplementary Figure 1B), age ($P = .0004$) (Supplementary Figure 1C), and BMI ($P = .0009$) (Supplementary Figure 1D) were significantly associated with ICU admission, while race, ethnicity, and comorbidities were not correlated with outcome group in this study.

Associations Between Composite Clinical Scores and COVID-19 Outcome

Three composite measures of disease severity were calculated for each participant at admission and/or longitudinally over their entire hospital stay: DI score [9], MEWS [10], and 4C score [7, 8]. Over the course of their hospital stay, the non-ICU group had consistently lower DI scores than the ICU and death groups, with most non-ICU participants peaking at or below a score of 50 (Figure 2A). Over their first 10 days of hospitalization, participants requiring ICU care exhibited steep increases in DI score, suggestive of rapid deterioration (Figure 2B). After the first 10 days, participants who ultimately died saw slow but steady increases in DI score, while participants who recovered from the ICU saw a lowering of the DI score after a stochastic interval (Figure 2A, B). The minimum and maximum recorded DI scores over the participant's hospital stay were significantly different between non-ICU and ICU participants, but not significantly different for ICU participants who recovered vs those who died (Supplementary Figure 2A). The first reported DI score following admission showed no significant differences by outcome group (Figure 2C).

Similarly, the non-ICU group tended to have lower MEWS scores than the ICU and death groups, though this difference was less pronounced than with DI scores (Figure 2D). Indeed, while the median and maximum MEWS values reported over a participant's hospital stay were significantly different between individuals who required ICU care and those who did not (Figure 2E; Supplementary Figure 2B), there were no significant differences in MEWS values between outcome groups at admission, and no significant differences were detected between the ICU and death groups (Figure 2E; Supplementary Figure 2B). 4C Mortality scores at admission, however, were significantly higher for both the ICU and death outcome groups compared with the non-ICU group, though they were not significantly different between the ICU and death outcome groups (Figure 2F).

Associations Between Clinical/Immunological Measures and COVID-19 Outcome

At admission, non-ICU participants had a significantly lower maximum fraction of inspired oxygen (FiO_2) and a significantly higher minimum oxygen saturation (SpO_2) than ICU and death participants, reflective of lower supplemental oxygen requirements (Supplementary Figure 3A). Lactate dehydrogenase (LDH) levels were likewise significantly lower in non-ICU participants compared with ICU participants (Supplementary Figure 3A, B). No other laboratory test result or clinical measure taken at hospital admission was significantly associated with any outcome group (Supplementary Figure 3C). Several factors were associated with outcome when considered retrospectively over a participant's entire hospital stay (Supplementary Figure 3C). Most notably, non-ICU participants had relatively low peripheral neutrophil counts that slightly decreased over the course of hospitalization, ICU participants had higher median counts that persisted throughout their stay, and participants who died had high neutrophil counts that increased over the course of hospitalization (Supplementary Figure 3D).

To assess the humoral response, plasma collected from a subset of participants (Figure 1A) was analyzed for the presence of antispike immunoglobulin G (IgG) (receptor binding domain; Supplementary Figure 3E) and antinucleocapsid IgG (N-terminal, C-terminal, and full-length protein; Supplementary Figure 3F) by ELISAs. Most participants developed an antibody response to both SARS-CoV-2 S and N

Figure 1. Cohort description and longitudinal sampling strategy. A, A graphical representation of samples taken per study participant along the clinical time course of in-patient treatment for COVID-19 (n = 58 total participants). Participants are grouped by outcome: discharge with no ICU care required (blue, n = 23), discharge with some ICU care required (purple, n = 29), and COVID-related death (red, n = 6). Sample collection is bracketed by symptom onset (asterisk) and hospital discharge or death (diamond), with the dotted line representing the time of hospital admission. Nasopharyngeal swabs (closed circles), blood (open squares), or both (closed squares) were collected from each study participant as indicated. B, Age distribution of study participants by sex, with median age depicted by the dotted line. C, Box plots comparing the time between symptom onset and hospital admission and the time between hospital admission and discharge or death by outcome (Wilcoxon rank-sum test with Benjamini-Hochberg procedure to control FDR for multiple comparisons; n.s. = FDR > 0.05; **FDR < 0.005; ****FDR < 0.00005). D, Frequency of reported symptoms among study participants during hospitalization ranked by most frequently to least frequently reported (green = present, yellow = not present, gray = not assessed). E, Frequency of reported symptoms among study participants by sex (green = present, yellow = not present). Abbreviations: COVID-19, coronavirus disease 2019; FDR, false discovery rate; ICU, intensive care unit; NP, nasopharyngeal.

Table 1. Summary of Demographics and Comorbidities of Study Participants

Variable	Descriptor	Female	Male
Total No.	-	22 (37.9)	36 (62.1)
Age	Median (IQR)	67.5 (31.0)	66.0 (17.0)
Blood type	N/A	7 (31.8)	10 (27.8)
	A negative	1 (4.5)	1 (2.8)
	A positive	5 (22.7)	4 (11.1)
	AB positive	1 (4.5)	2 (5.6)
	B positive	1 (4.5)	2 (5.6)
	O negative	0 (0.0)	2 (5.6)
	O positive	7 (31.8)	15 (41.7)
Race	Asian	0 (0.0)	1 (2.8)
	Black or African American	10 (45.5)	15 (41.7)
	Declined	1 (4.5)	3 (8.3)
	Other	3 (13.6)	2 (5.6)
	White	8 (36.4)	15 (41.7)
Ethnicity	Declined	0 (0.0)	2 (5.6)
	Hispanic or Latino	4 (18.2)	7 (19.4)
	Not Hispanic or Latino	18 (81.8)	27 (75.0)
Body mass index	Median (IQR)	36.8 (21.0)	29.9 (10.2)
Asthma		5 (22.7)	5 (13.9)
Chronic obstructive pulmonary disorder		2 (9.1)	4 (11.1)
Cancer		6 (27.3)	9 (25.0)
Cardiovascular disease		11 (50.0)	21 (58.3)
Chronic liver disease		1 (4.5)	1 (2.8)
Diabetes		10 (45.5)	19 (52.8)
HIV diagnosis		0 (0.0)	5 (13.9)
Hypertension		14 (63.6)	28 (77.8)
Immune disorder		3 (13.6)	1 (2.8)
Renal disease		7 (31.8)	23 (63.9)
Solid organ transplant		2 (9.1)	3 (8.3)
Former smoker		13 (59.1)	13 (36.1)
Current smoker		2 (9.1)	2 (5.6)

Data are represented as median (IQR) or No. (%) for continuous or categorical variables, respectively.

Abbreviation: IQR, interquartile range.

within 10–20 days of symptom onset (Supplementary Figure 3E, F). Overall, the response to each antigen was highly correlated across participants, and there was no clear difference in either the timing or intensity of the response between any of the outcome groups. Principal component analysis (PCA) on the anti-N antibody data identified 5 independent patterns of antibody response among participants, though none were associated with outcome (Supplementary Figure 3G, H).

Table 2. Summary of Treatments Administered to Study Participants During Hospitalization

Variable	Descriptor	Female	Male
Total No.	-	22 (37.9)	36 (62.1)
Hydroxychloroquine		5 (22.7)	6 (16.7)
Remdesivir	Emergency Use Authorization	4 (18.2)	4 (11.1)
	Clinical trial	1 (4.5)	5 (13.9)
Tocilizumab		1 (4.5)	5 (13.9)
Sarilumab		2 (9.1)	4 (11.1)
Steroids		18 (81.8)	24 (66.7)
Convalescent plasma		2 (9.1)	3 (8.3)
Supplemental oxygen		18 (81.2)	31 (86.1)
Intubation		10 (45.5)	20 (55.6)
Extracorporeal membrane oxygenation		0 (0.0)	4 (11.1)
Intensive care unit		11 (50.0)	24 (66.7)

Data are represented as No. (%) for all categorical variables above. Patients enrolled in the remdesivir clinical trial received either remdesivir or a placebo in a blinded manner. Note that some treatments were limited to participants in the intensive care unit, including mechanical ventilation.

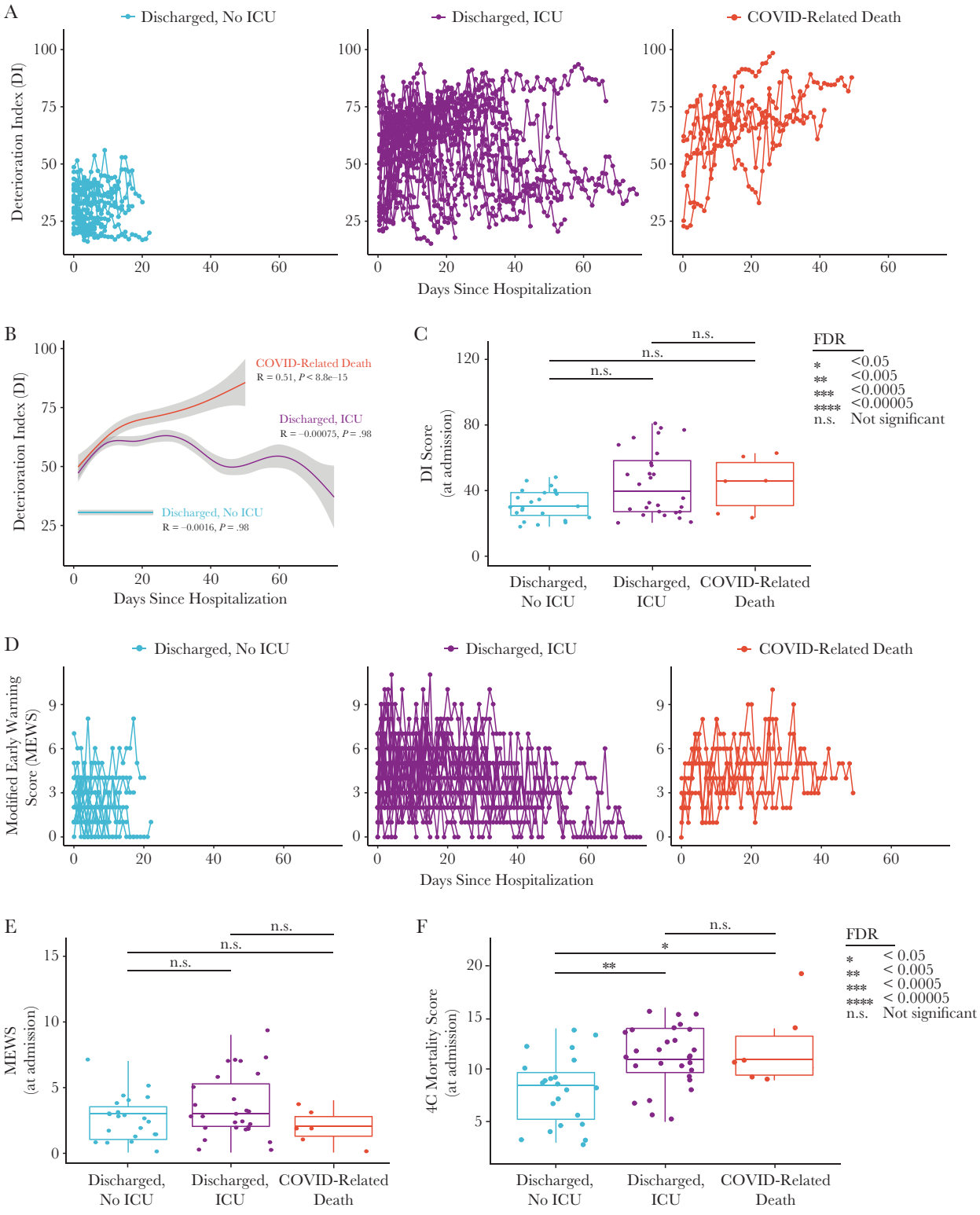

Figure 2. Analysis of correlations between clinical measures of disease severity and participant outcome. A, Plot of the DI score for each study participant over the course of hospitalization, separated by participant outcome. B, Generalized additive model fit to the average DI score of hospitalized study participants over time separated by outcome. The correlation between average DI score and time during hospitalization is provided (R) alongside the *P* value for each outcome group. C, Box plot comparing the first reported DI score following admission for each participant grouped by outcome. D, Plot of the MEWS for each study participant over the course of hospitalization, separated by participant outcome. E, Box plot comparing the first reported MEWS following admission for each participant grouped by outcome. F, Box plot comparing the 4C Mortality score measured at admission for each participant grouped by outcome. Significance between groups in all box plots was tested by Wilcoxon rank-sum test with the Benjamini-Hochberg procedure to control FDR for multiple comparisons; n.s. = FDR > 0.05; *FDR < 0.05; **FDR < 0.005; ***FDR < 0.0005; ****FDR < 0.00005. Abbreviations: DI, Epic Deterioration Index; FDR, false discovery rate; MEWS, Modified Early Warning Score; R, correlation between average DI score and time during hospitalization.

Association of Viral Cycle Threshold Values With Clinical Measures and COVID-19 Outcome

A validated quantitative PCR (qPCR) assay for SARS-CoV-2 (N1 primer set, CDC assay [26]) was used to determine cycle threshold (Ct) values as a proxy for viral load in each nasopharyngeal swab (Figure 1A). All study participants had detectable levels of virus in nasal swabs upon enrollment and generally showed an increase in Ct values (reduction in virus) over their hospital stay (Figures 3A, B). The timing of the Ct value increase was not uniform; several participants in the ICU and death groups showed transient decreases in Ct values even 2 weeks after hospitalization (Figure 3A). While Ct values measured at or within 10 days of admission showed no significant difference by outcome (Supplementary Figure 4A), all non-ICU participants had Ct values within the limit of detection at or within 10 days of discharge, while several ICU participants had at least 1 specimen with no detectable virus over the course of hospitalization (Figures 3A–C). Note that RNA was also extracted from all plasma samples in this study (n = 62) and subjected to the same qPCR assay as above; only 1 specimen (1.6%) had a Ct value within the limit of detection (data not shown).

Comparing Ct values in nasopharyngeal swabs with time-matched anti-S and anti-N antibodies in the serum, there is a modest but significant positive correlation (Supplementary Figure 4B, C). Likewise, comparing Ct values with time-matched clinical laboratory test results identified several significant correlates, including positive correlations with lymphocyte count, white cell count, and D-dimer levels and negative correlations with aspartate aminotransferase (AST) and CRP levels (Figures 3D, E). Notably, the factors most associated with disease severity (LDH level and neutrophil count; Supplementary Figure 3C) were not significantly correlated with Ct values, and vice versa.

Analysis of Viral Genotype and Intrahost Diversity

Of the 238 swabs collected, 83 had adequate viral copies for whole-genome viral sequencing (Ct value <30). Of these, 69 specimens from 34 independent participants yielded an SARS-CoV-2 sequence of sufficient quality to assemble near-complete genomes (at least 90% coverage, minimum read depth of 10 reads). Phylogenetic analysis revealed that these viruses belonged to 4 primary clades, consistent with the population structure of the epidemic in Chicago during the study (Nextstrain clades 19A, 20A, 20B, and 20C; Figure 4A). Longitudinal samples from 23 participants yielded identical consensus sequences at each time point, while 9 participants showed changes in viral consensus sequence over time (Figure 4A). Clade membership was not significantly associated with any outcome group or baseline Ct value (Figures 4B, C).

Given the appearance of mutations in some patient isolates over time, we investigated the degree of intrahost viral diversity in each sample. While the amount of diversity varied by isolate, there was no clear trend either as a function of participant outcome or as a function of time since symptom onset (Figure 4D). On the contrary, the viral population diversity of any given isolate was ~0.0001 substitutions per base pair. Diversity did vary by open-reading frame (ORF), with a majority of sequences showing some diversity in N (n = 63), membrane (M; n = 57), and nsp12 (n = 49) sequences (Figure 4E). While nsp11 and ORF7b showed the highest number of substitutions per base pair, this was in fewer isolates (n = 9 and n = 7, respectively) and was largely driven by small ORF size.

To assess selective pressure, the difference in the number of nonsynonymous substitutions per nonsynonymous site (dN) and the number of synonymous substitutions per synonymous site (dS; dN-dS) was calculated [32, 33]. Overall, dN-dS across the viral genome did not change notably by outcome or as a function of time since symptom onset (Figure 4F). dN-dS did vary as a function of ORF, with the 2 most frequently changed ORFs (M and N) showing a significant bias toward synonymous mutations, suggestive of negative selective pressure operating at the intrahost level (Figure 4G). On the other hand, Nsp11 and the immune regulatory factors ORF3a/b and OR7a/b showed some bias toward a positive dN-dS, suggestive of positive selection, though this was not apparent in all participants (Figure 4G).

Modeling of COVID-19 Outcome

A multinomial logistic model with the COVID-19 outcome as the dependent variable was constructed to assess the relative predictive value of each measured parameter at or near (within 10 days) the time of hospital admission. Based on the univariate analyses above, candidate predictors with a P value <.1 were identified for multivariable assessment, including time since symptom onset, sex, age, BMI, LDH levels, lymphocyte count, CRP levels, neutrophil count, white blood cell count, DI score, and N1 Ct value. SpO_2 and FiO_2 were excluded from these analyses as these parameters were used by clinical staff to dictate ICU admission during the course of the study. Only study participants with complete data sets for all measurements were included in the model (n = 34).

Of all examined parameters, DI score at admission was the most significant predictor ($P < .0001$), yielding an accuracy rate of 0.88, even when considered independently from all other variables. Given that DI score is proprietary and only available in hospital settings that use Epic medical record systems, we reran the model excluding DI score. After fitting the initial model (Figure 5A), we performed a stepwise selection using both forward inclusion and backward elimination of the candidate predictors. After selection, only BMI, lymphocyte count, and neutrophil count were maintained in the final model, which had an accuracy rate of 0.82 in predicting all outcome groups (Figure 5A). A detailed analysis performed on the outcome predictor effect for each variable indicated that BMI and

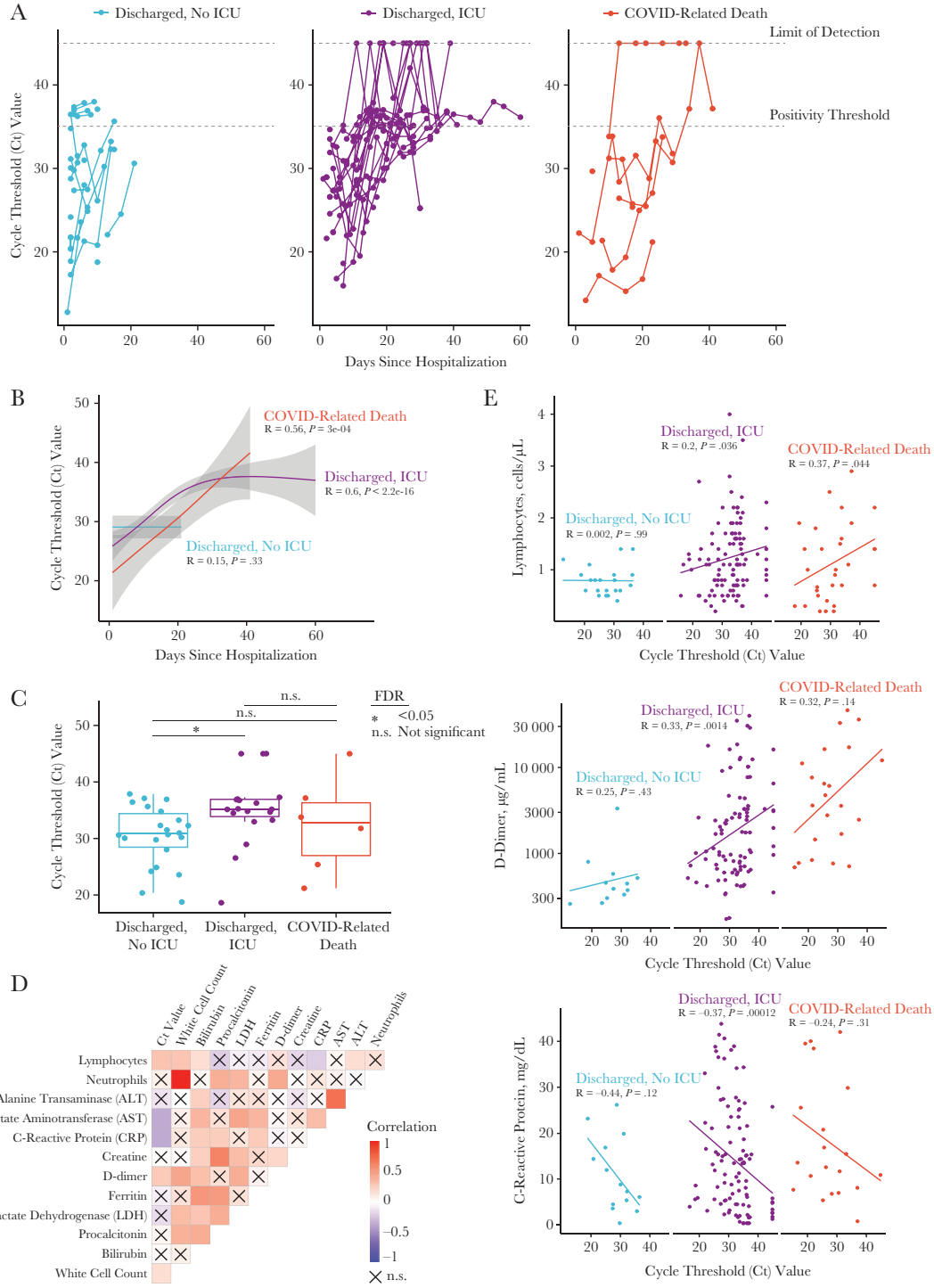

Figure 3. Analysis of correlations between Ct values, clinical laboratory tests, and participant outcome. A, Plot of the quantitative PCR Ct values for SARS-CoV-2 N1 in nasopharyngeal swabs from each study participant relative to hospitalization time, separated by participant outcome. Specimens that did not amplify were assigned a value at the limit of detection. B, Generalized additive model fit to the average Ct value of hospitalized study participants over time separated by outcome. The correlation between average DI score and time during hospitalization is provided (R) alongside the *P* value for each outcome group. C, Box plot of SARS-CoV-2 N1 Ct values from the final nasopharyngeal swab collected from each participant within 10 days of hospital discharge or death grouped by outcome (Wilcoxon rank-sum test with Benjamini-Hochberg procedure to control FDR for multiple comparisons; n.s. = FDR > 0.05; *FDR < 0.05). D, Heat map of Spearman correlation among longitudinally reported blood work variables over the entire course of hospitalization alongside Ct value. Value of the correlation coefficient is colored blue to red from more negatively to more positively correlated, respectively. Lack of significant correlation ($P > .05$) is indicated by an X. E, Scatterplot of lymphocyte count (cells/μL, top), D-dimer levels (μg/mL, middle), and C-reactive protein levels (mg/dL, bottom) in blood- vs time-matched Ct values. Study participants are separated by outcome group (no ICU care in blue, some ICU care in purple, COVID-related death in red), with the linear regression fit shown as a solid line of the same color (Spearman correlation coefficient and *P* value shown). Abbreviations: Ct, cycle threshold; DI, Epic Deterioration Index; FDR, false discovery rate; ICU, intensive care unit; PCR, polymerase chain reaction; R, correlation between average DI score and time during hospitalization; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

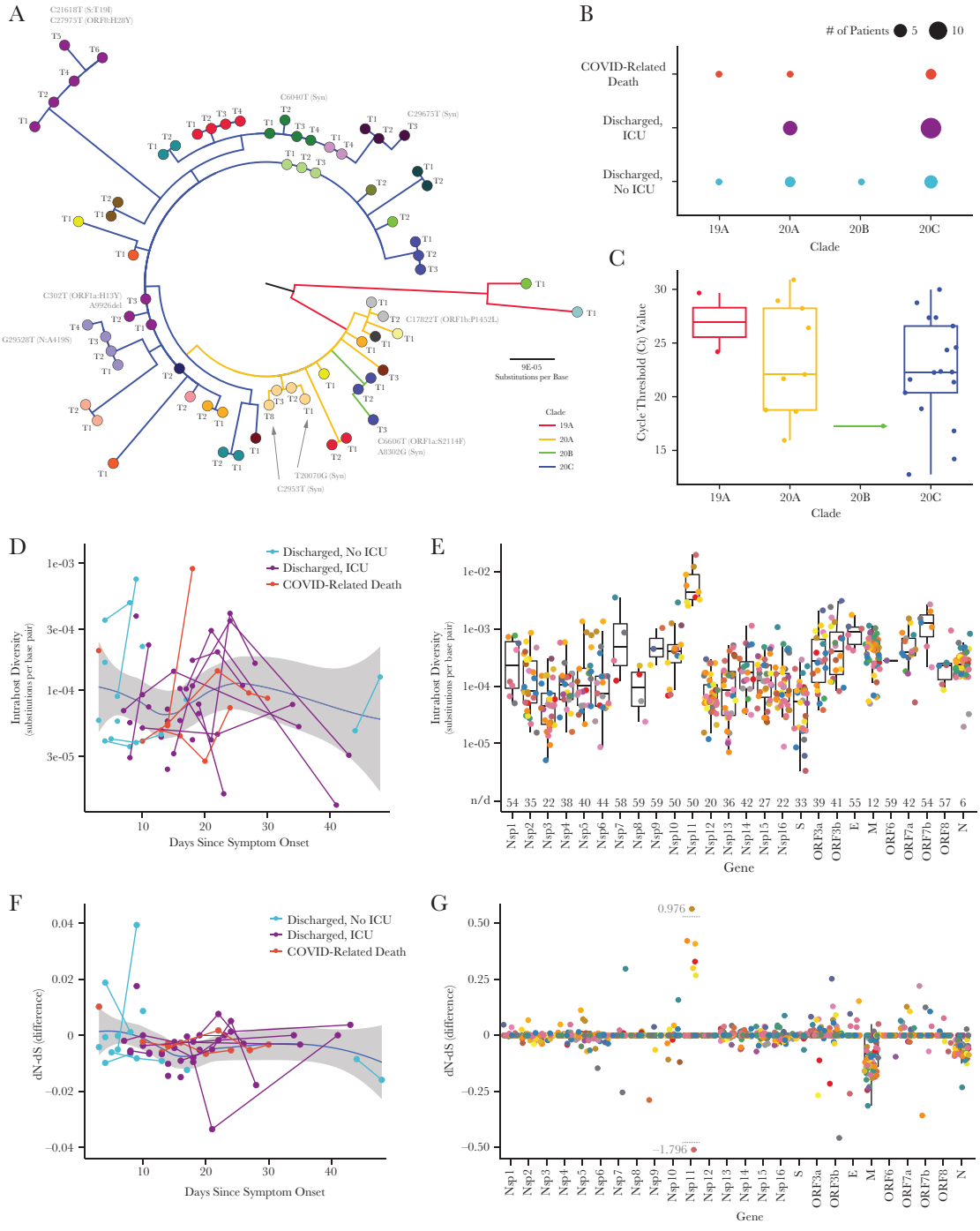

Figure 4. Analysis of correlations between viral genotype, intrahost diversity, and participant outcome. A, Phylogenetic tree of SARS-CoV-2 whole-genome sequences from each study participant and time point. Each branch is colored by corresponding viral clade, and each branch tip is colored by study participant; tips are labeled chronologically from time point 1 (T1) onward ordered clockwise. Changes in consensus viral sequence observed are labeled by nucleotide and corresponding amino acid change, when applicable. B, Dot plot depicting the clade membership of the virus isolated from each study participant by outcome group. C, Box plot comparing the SARS-CoV-2 N1 Ct value at time point 1 for each participant separated by clade. Tests for significance were performed with the Wilcoxon rank-sum test with Benjamini-Hochberg correction for multiple comparisons (all comparisons not significant, $P > .05$). D, Plot of the observed intrahost diversity (substitutions per base pair) across the entire SARS-CoV-2 genome from each study participant relative to days since symptom onset and colored by participant outcome. Samples from the same participant over time are linked by lines. A loess curve fit to the average intrahost diversity observed over time is overlaid with error. E, Box plot of the intrahost diversity observed within each indicated open reading frame in each participant specimen. Dots are colored by study participant. The number of specimens in which no intrahost diversity was observed is indicated on the bottom (n/d). F, Plot of the difference between the number of nonsynonymous substitutions per nonsynonymous site (dN) and the number of synonymous substitutions per synonymous site (dS) observed across the SARS-CoV-2 genome from each study participant relative to days since symptom onset and colored by participant outcome. Samples from the same participant over time are linked by lines. A loess curve fit to the average dN-dS observed over time is overlaid with error. G, Box plot of the dN-dS observed within each indicated open reading frame in each participant specimen. Dots are colored by study participant. Abbreviations: Ct, cycle threshold; dN, number of nonsynonymous substitutions per nonsynonymous site; dS, number of synonymous substitutions per synonymous site; n/d, number of specimens in which no intrahost diversity was observed; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

A

Initial Stepwise Model: Outcome Group ~ Days Since Onset + Sex + Age + BMI + log10(LDH) + log10(Lymphocytes) + log10(CRP) + log10(Neutrophils) + log10(White Blood Cells) + log10(N1 Ct Value)

Final Stepwise Model: Outcome Group ~ BMI + log10(Lymphocytes) + log10(Neutrophils)

Variable		No ICU	ICU	Death	PR>ChiSq
Total, No. (%)	Mean (SD)	13 (38.2)	18 (52.9)	3 (8.8)	-
BMI	Mean (SD)	28.6 (6.1)	36.6 (9.6)	32.9 (5.5)	**<0.001**
Lymphocytes, cells/μL	Mean (SD)	1.0 (0.5)	0.8 (0.5)	0.6 (0.2)	**<0.001**
Neutrophils, cells/μL	Mean (SD)	5.8 (2.2)	7.1 (5.0)	16.3 (4.4)	**<0.001**

Note: Lymphocyte and Neutrophil counts reflect the Mean (SD) of each outcome group at the time of hospital admission.

B

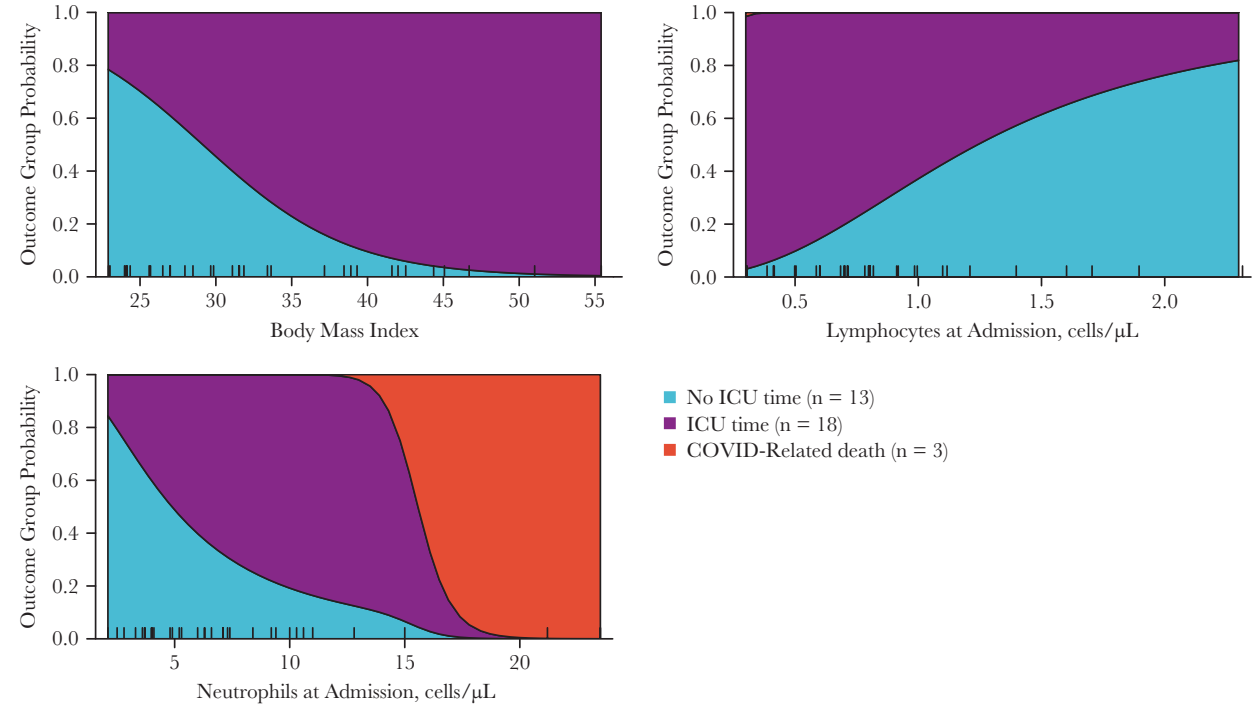

Figure 5. Stepwise regression model of participant outcome by admission data. A, Initial and final stepwise model after Akaike information criterion model selection. The distribution and statistical significance of study participant BMI, blood lymphocyte count on admission, and blood neutrophil count on admission toward predicting outcome in the final model are depicted in the table. All continuous variables are represented as mean (SD). B, The probability of each outcome was determined by BMI, lymphocyte count at admission, and neutrophil count at admission in the final model. The BMI and cell counts of each included study participant are indicated by a tick mark along the x-axis. Abbreviation: BMI, body mass index.

lymphocyte count were the primary drivers differentiating between ICU and non-ICU individuals, while neutrophil count enabled discrimination of COVID-related deaths (Figure 5B).

DISCUSSION

Several demographic and clinical factors measured at the time of hospital admission were significantly different between the non-ICU and ICU outcome groups (though not different between ICU and death). As reported by other studies, higher age, male sex, and higher body mass index (BMI) were associated with ICU admission [34, 35]. Unlike other studies, we saw no significant impact of comorbidities on outcome, though the average number of comorbidities was relatively high across groups. This study did not include a nonhospitalized control group for comparison, so it is possible that comorbidities are a better predictor of hospitalization than outcome following hospitalization. Of the clinical laboratory tests, only LDH levels showed a significant association with ICU admission. LDH is a commonly used marker of tissue damage, suggesting that these participants may have been experiencing more severe disease at the time of hospitalization. This is not a function of symptom duration before hospitalization as there were no significant differences between outcome groups in the time from symptom onset to the time of hospital admission. Of the composite scores, only the 4C Mortality score was significantly different between non-ICU and ICU participants at hospital admission. This score was originally developed by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) [8] specifically for risk stratification of COVID-19

patients. Notably, no parameter monitored at hospital admission was significantly associated with death in this cohort, emphasizing the importance of further study.

Several longitudinal measures were associated with the need for ICU care, including neutrophil count, white blood cell count, and several composite measures of patient deterioration [36–38]. Of all longitudinal measures taken, the median DI score over a patient's hospital stay was the only one that differed significantly between participants in the ICU who recovered vs those who died. The DI score is automatically calculated on a daily basis by Epic software; understanding the constituent components of the score driving this differentiation will be critical to developing simpler measures for broad implementation [9]. Regardless, this suggests that tracking cumulative measures of deterioration indices may be useful for risk stratification. Additional retrospective analyses of these measures as predictors of COVID-19 outcome need to be performed across larger and more diverse cohorts to better assess their value in risk management.

SARS-CoV-2 Ct value was not associated with outcome group in this study, consistent with prior reports [39, 40]. Likewise, the significant correlates of outcome (LDH and neutrophil count) were not significantly associated with viral Ct value. Taken together, these results suggest that inflammatory responses in response to infection and markers of lung damage are more important than viral load to predict outcomes. Interestingly, Ct value did correlate with other measured parameters in this study, including positively with AST levels and negatively with total lymphocyte count and antibody titer, none of which were associated with outcome. Examination of these markers in a larger cohort could be warranted to determine how such commonly performed lab tests could be repurposed to inform risk of transmission.

Viral genotype in the cohort was broadly reflective of the epidemiological trends in the city of Chicago at the time of sample collection and showed no significant correlation with outcome group or Ct value [41]. Sample collection was completed before the emergence of the now prevalent variants of concern (VOCs), several of which have been associated with elevated viral loads, increased transmission, and potentially worse patient outcomes [14–21]. Going forward, this cohort may prove valuable as an outgroup for more recent and future patient cohorts to determine the impact of emerging variants on disease severity and patient outcomes.

Longitudinal monitoring of viral genotype revealed 9 instances of intrahost evolution with emergence of new, predominant mutations. Most of these were rare mutations that are not detected globally at the consensus level, indicating random or host-specific adaptation events. However, 1 participant developed a mutation in the spike protein (T19I) that is shared by the Delta VOC (Clade 21A). It is unclear if these mutations were driven by humoral responses or they arose due to random chance over the course of viral replication. Viral genetic diversity remained relatively constant throughout the course of hospitalization, even up to 45 days after admission, and did not vary significantly by outcome. This suggests that the virus diversifies at a slow but constant rate over the course of disease in most individuals. That being said, all patients in this cohort were able to control viral replication within 2–3 weeks following hospital admission. Further study of viral load and viral diversity in populations that are unable to control viral replication is required to gain a better understanding of the factors that drive intrahost evolution of SARS-CoV-2 and the emergence of immune-evasive variants [42]. These results additionally emphasize the need for effective antivirals to suppress viral replication over prolonged periods of disease.

Despite its strengths, this study has several limitations. First, this is a single-center study with a population that may not fully reflect the general population. During this phase of the pandemic, Northwestern Memorial Hospital was a tertiary referral hospital that accepted both patients from its usual catchment area and referrals from other hospitals in the Chicago region, but continued exploration of these trends will be required in larger, multi-institutional cohorts. Second, there was substantial variability in participant treatments administered during the study period, though few of these were subsequently found to be clinically beneficial. As such, these data likely reflect the natural history of illness with general supportive measures. Data during later waves are needed to inform how newer therapies and variants affect these factors, for which these data may serve as a baseline. Third, the study did not include a nonhospitalized control group for comparison, so utility of the identified markers to predict hospitalization or other outcomes cannot be assessed.

In summary, this study validates previous findings that severe COVID-19 and worse patient outcomes are likely driven by immune dysfunction following infection and are not primarily due to ongoing viral replication. Further validation of a novel model based on BMI, lymphocyte count, and neutrophil count on admission may yield a useful and broadly applicable tool for predicting outcomes of hospitalized patients. Indeed, we are not the first group to report this association, with an elevated neutrophil-to-lymphocyte ratio emerging as an early hallmark of severe COVID-19 [43, 44]. For centers with access to DI score, serial measurement of this parameter may be additionally informative for predicting patient trajectory. Finally, while Ct value was not associated with outcome, it did correlate with several commonly measured inflammatory markers that in the future may serve as proxies for transmission risk in hospitalized settings.

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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References

1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol **2021**; 19:141–54.
2. Soeroto AY, Soetedjo NN, Purwiga A, et al. Effect of increased BMI and obesity on the outcome of COVID-19 adult patients: a systematic review and meta-analysis. Diabetes Metab Syndr **2020**; 14:1897–904.
3. Bansal M. Cardiovascular disease and COVID-19. Diabetes Metab Syndr **2020**; 14:247–50.
4. Skevaki C, Karsonova A, Karaulov A, et al. Asthma-associated risk for COVID-19 development. J Allergy Clin Immunol **2020**; 146:1295–301.
5. Khan M, Adil SF, Alkhathlan HZ, et al. COVID-19: a global challenge with old history, epidemiology and progress so far. Molecules **2020**; 26:39.
6. Pascarella G, Strumia A, Piliego C, et al. COVID-19 diagnosis and management: a comprehensive review. J Intern Med **2020**; 288:192–206.
7. Wellbelove Z, Walsh C, Perinpanathan T, et al. Comparing the 4C mortality score for COVID-19 to established scores (CURB65, CRB65, qSOFA, NEWS) for respiratory infection patients. J Infect **2021**; 82:414–51.
8. Gupta RK, Harrison EM, Ho A, et al; ISARIC4C Investigators. Development and validation of the ISARIC 4C deterioration model for adults hospitalised with COVID-19: a prospective cohort study. Lancet Respir Med **2021**; 9:349–59.
9. Singh K, Valley TS, Tang S, et al. Evaluating a widely implemented proprietary deterioration index model among hospitalized COVID-19 patients. Ann Am Thorac Soc **2020.** doi:10.1513/AnnalsATS.202006-698OC.
10. Barnett WR, Radhakrishnan M, Macko J, et al. Initial MEWS score to predict ICU admission or transfer of hospitalized patients with COVID-19: a retrospective study. J Infect **2021**; 82:282–327.
11. Argyropoulos KV, Serrano A, Hu J, et al. Association of initial viral load in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with outcome and symptoms. Am J Pathol **2020**; 190:1881–7.
12. Fajnzylber J, Regan J, Coxen K, et al; Massachusetts Consortium for Pathogen Readiness. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun **2020**; 11:5493.
13. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature **2020**; 581:465–9.
14. Hou YJ, Chiba S, Halfmann P, et al. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science **2020**; 370:1464–8.
15. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science **2021**; 372. doi:10.1126/science.abg3055.
16. Deng X, Garcia-Knight MA, Khalid MM, et al. Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. Cell **2021.** doi:10.1016/j.cell.2021.04.025.
17. Volz E, Hill V, McCrone JT, et al. Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity. Cell **2021**; 184:64–75.e11.
18. Zhang X, Tan Y, Ling Y, et al. Viral and host factors related to the clinical outcome of COVID-19. Nature **2020**; 583:437–40.
19. Frampton D, Rampling T, Cross A, et al. Genomic characteristics and clinical effect of the emergent SARS-CoV-2 B.1.1.7 lineage in London, UK: a whole-genome sequencing and hospital-based cohort study. Lancet Infect Dis **2021**; 21:1246–56.
20. Young BE, Fong S-W, Chan Y-H, et al. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. Lancet **2020**; 396:603–11.
21. Davies NG, Jarvis CI, Edmunds WJ, et al; CMMID COVID-19 Working Group. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature **2021**; 593:270–4.
22. Ruan Q, Yang K, Wang W, et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med **2020**; 46:846–8.
23. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. Am J Hematol **2020**; 95:834–47.
24. Legros V, Denolly S, Vogrig M, et al. A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. Cell Mol Immunol **2021**; 18:318–27.
25. Roltgen K, Powell AE, Wirz OF, et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. Sci Immunol **2020**; 5. doi:10.1126/sciimmunol.abe0240.
26. Centers for Disease Control and Prevention. Research use only 2019-novel coronavirus (2019-nCoV) real-time RT-PCR primers and probes. **2020**. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. Accessed May 5, 2020.
27. Quick J, Grubaugh ND, Pullan ST, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc **2017**; 12:1261–76.
28. Grubaugh ND, Gangavarapu K, Quick J, et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biol **2019**; 20:8.
29. Gausman J, Langer A. Sex and gender disparities in the COVID-19 pandemic. J Womens Health (Larchmt) **2020**; 29:465–6.
30. Rochwerg B, Agarwal A, Zeng L, et al. Remdesivir for severe COVID-19: a clinical practice guideline. BMJ **2020**; 370:m2924.
31. Lu CC, Chen MY, Lee WS, Chang YL. Potential therapeutic agents against COVID-19: what we know so far. J Chin Med Assoc **2020**; 83:534–6.
32. Yang Z, Bielawski JP. Statistical methods for detecting molecular adaptation. Trends Ecol Evol **2000**; 15:496–503.

33. Kimura M. Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution. Nature **1977**; 267:275–6.
34. Ejaz H, Alsrhani A, Zafar A, et al. COVID-19 and comorbidities: deleterious impact on infected patients. J Infect Public Health **2020**; 13:1833–9.
35. Gallo Marin B, Aghagoli G, Lavine K, et al. Predictors of COVID-19 severity: a literature review. Rev Med Virol **2021**; 31:1–10.
36. Xia X, Wen M, Zhan S, et al. An increased neutrophil/lymphocyte ratio is an early warning signal of severe COVID-19 [in Chinese]. Nan Fang Yi Ke Da Xue Xue Bao **2020**; 40:333–6.
37. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. J Clin Invest **2020**; 130:6151–7.
38. Bolouri H, Speake C, Skibinski D, et al. The COVID-19 immune landscape is dynamically and reversibly correlated with disease severity. J Clin Invest **2021**; 131. doi:10.1172/JCI143648.
39. Avadhanula V, Nicholson EG, Ferlic-Stark L, et al. Viral load of severe acute respiratory syndrome coronavirus 2 in adults during the first and second wave of coronavirus disease 2019 pandemic in Houston, Texas: the potential of the superspreader. J Infect Dis **2021**; 223:1528–37.
40. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ **2020**; 369:m1443.
41. Lorenzo-Redondo R, Nam HH, Roberts SC, et al. A clade of SARS-CoV-2 viruses associated with lower viral loads in patient upper airways. EBioMedicine **2020**; 62:103112.
42. Aydillo T, Gonzalez-Reiche AS, Aslam S, et al. Shedding of viable SARS-CoV-2 after immunosuppressive therapy for cancer. N Engl J Med **2020**; 383: 2586–8.
43. Guan WJ, Ni ZY, Hu Y, et al; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med **2020**; 382:1708–20.
44. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis **2020**; 71:762–8.