Longitudinal Analysis of SARS-CoV-2 Viruses in Hospitalized Adults

Northwestern Medicine®
Feinberg School of Medicine

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Background

The rapid spread of SARS-CoV-2, the causative agent of Coronavirus disease 2019 (COVID-19), has been accompanied by the emergence of viral mutations, some of which may have distinct virological and clinical consequences. While whole genome sequencing efforts have worked to map this viral diversity at the population level, little is known about how SARS-CoV-2 may diversify within a host over time. This is particularly important for understanding the emergence of viral resistance to therapeutic interventions and immune pressure. The goal of this study was to assess the change in viral load and viral genome sequence within patients over time and determine if these changes correlate with clinical and/or demographic parameters.

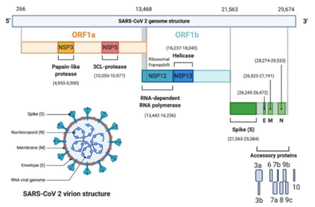

Figure 1. A representation of the structure of the SARS-CoV-2 genome; highlighting protein coding regions. A simple schematic of the virion structure is also displayed.[1]

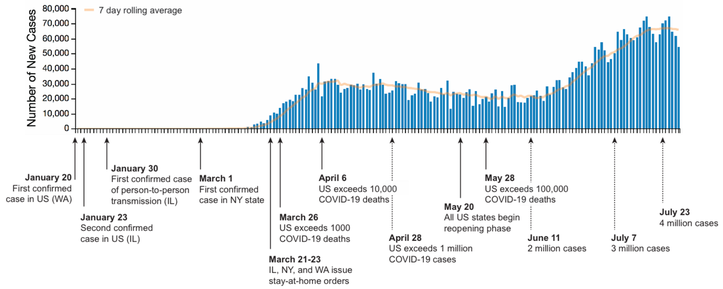

Figure 2. Reported number of cases per day in the United States from the start of the pandemic up to the end of our longitudinal collection for the study represented by blue bars. The seven day rolling average for the same time period is overlaid using a yellow line. Significant dates in the US epidemic are highlighted.

Methods

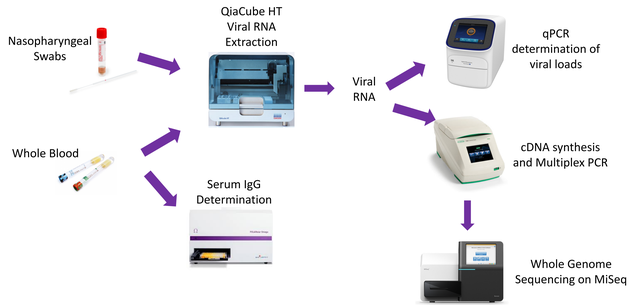

Figure 3. Nasal pharyngeal swabs were collected approximately every 4 days while study subjects remained hospitalized. Blood samples were taken approximately once per week while study subjects remained hospitalized. Nasopharyngeal swabs and serum from study participants were subjected to viral RNA extraction, qPCR quantification of the N1 gene of SARS-CoV-2 and RNase P, cDNA synthesis, multiplex PCR amplification and sequencing of the viral genome. Serum samples were also tested for IgG antibodies to the Spike protein.

Sample Collection and Testing

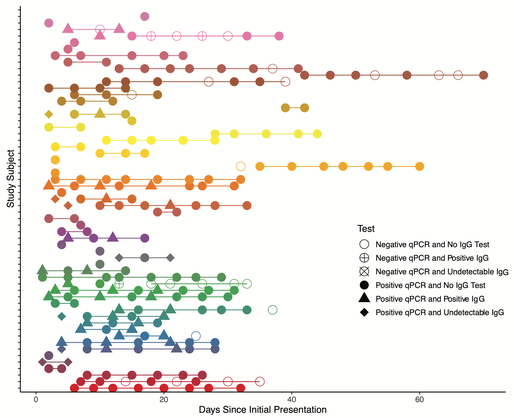

Figure 4. A graphical representation of samples taken per study subject along the course of inpatient treatment for COVID-19. All samples were tested by qPCR for SARS-CoV-2 utilizing the CDC approved assay, positive samples are indicated by solid shapes, negative samples are indicated by unfilled shapes. Serum samples were tested by ELISA for the presence of antibodies again the spike protein, samples are indicated positive or negative for IgG.

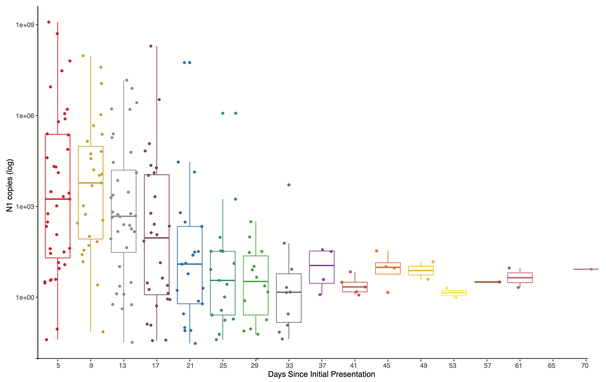

Figure 5. Viral load calculated using qPCR; copies of N1 in 5 ul of elute RNA. Each sample is plotted versus time of sample since initial presentation with COVID-19 independent of study subject. An overall decrease in viral load over time is observed.

Clinical Data

		Study Subjects
Total N (%)		63
Age in years at date of presentation		
	20-29	2 (3.2%)
	30-39	8 (12.7%)
	40-49	5 (7.9%)
	50-59	12 (19.0%)
	60-69	16 (25.4%)
	70-79	12 (19.0%)
	80-89	6 (9.5%)
	90+	2 (3.2%)
Sex		
	Female	23 (36.5%)
	Male	40 (63.5%)
Race		
	Asian	1 (1.6%)
	Black or African American	26 (41.3%)
	White	27 (42.9%)
	Other	5 (7.9%)
	Declined	4 (6.3%)
Comorbidities		
	Asthma	10 (15.9%)
	COPD	6 (9.5%)
	Cancer	15 (23.8%)
	Cardiovascular Disease	33 (52.4%)
	Chronic Liver Disease	3 (4.8%)
	Diabetes	31 (49.2%)
	HIV	5 (7.9%)
	Hypertension	48 (76.2%)
	Immunologic Disorder	4 (6.3%)
	Renal Disease	32 (50.8%)
	Solid Organ Transplant	4 (6.3%)
Smoker		
	Current	4 (6.3%)
	Former	28 (44.4%)
BMI		
	Underweight <18	0 (0.0%)
	Normal 18.5 to <25	12 (19.0%)
	Overweight 25to <30	16 (25.4%)
	Obese 30 +	33 (54.2%)
	Unknown	2 (3.2%)

Table 1. Study cohort demographics including age, race, smoker status, BMI and other comorbidities.

		Study Subjects
Total N (%)		63
Oxygen Support		
	No support	9 (14.3%)
	CPAP/BiPAP	1 (1.6%)
	High-flow Oxygen Face-mask	2 (3.2%)
	Intubation Ventilation	20 (31.7%)
	Low-flow Oxygen Nasal Canula	18 (28.6%)
	Tracheostomy Ventilation	13 (20.6%)
ICU Admission		
	No ICU care	26 (41.3%)
	ICU care	37 (58.7%)
Mortality		
	Survived	56 (88.9%)
	Deaths	7 (11.1%)
Therapeutics		
	Hydroxychloroquine	12 (19.0%)
	Remdesivir Clinical Trial	5 (7.9%)
	Remdesivir EUA	10 (15.9%)
	Tocilizumab	7 (11.1%)
	Sarilumab	6 (9.5%)
	Convalescent Plasma	5 (7.9%)
	Steroids	48 (76.2%)
	Immune modulators	10 (15.9%)
	Prone	22 (34.9%)

Table 2. Administered therapeutics and mortalities over the course of hospitalizations.

Analysis

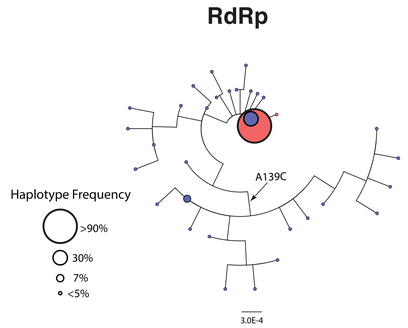

Figure 6. Temporal changes of the intra-host viral diversity. SARS-CoV2 intra-host haplotypes for the RdRp gene constructed for one study subject following longitudinal whole genome sequencing. We inferred a Maximum Likelihood phylogeny to examine the temporal dynamics of genetic variation. Sequences from two different timepoints from the same subject are represented (first timepoint in red, second timepoint in blue) as circles in the tips of the tree. The area of the circles represents the frequency of the reconstructed haplotypes within the viral population. We can observe a very significant increase in viral heterogeneity with time, as well as new mutations arising in the population. Particularly, some of the variants show the appearance of a new common mutation that has increased its frequency in the population (A139C indicated in the tree node). Some of these arising mutations are being studied to understand their effect in viral fitness and association with host responses.

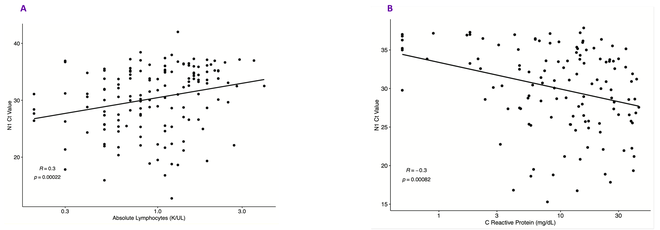

Figure 7. Multivariate Analysis of Clinical Parameters in Relationship to Viral Load. Panel A: Spearman correlation displaying the relationship between N1 cycle threshold determined by qPCR and absolute lymphocytes. Samples from subjects showing higher viral titers (low CT values) correlated with lower counts of absolute lymphocytes. Panel B: Spearman correlation displaying the relationship between N1 cycle threshold determined by qPCR and amounts of c reactive protein measured in whole blood. Samples from subjects showing lower viral titers (high CT values) correlated with lower amounts of c reactive protein.

Conclusions

- Observed SARS-CoV-2 viral load during the first two weeks post initial presentation was highly variable before trending down as time increased.
- Positive IgG against the SPIKE protein was observed as early as 1 week post initial presentation.
- Intra-host diversity can be observed within an individual study subject over time, with a significant increase in viral heterogeneity over time.
- Correlations between clinical parameters, viral titers, and outcomes are further informing our model of disease severity. Taken together with the emerging viral variants we can begin to assess viral fitness and the association with host responses.

References
1. Alanagreh L, Alzoughool F, Atoum M. The Human Coronavirus Disease COVID-19: Its Origin, Characteristics, and Insights into Potential Drugs and Its Mechanisms. Pathogens. 2020 Apr 29;9(5):331. doi: 10.3390/pathogens9050331. PMID: 32365466; PMCID: PMC7280997.