

A COVID-19 OBSERVATIONAL STUDY: EVALUATION OF CHANGES IN VIRAL LOAD OVER TIME IN OTHERWISE HEALTHY, ASYMPTOMATIC PARTICIPANTS WITH DETECTABLE LEVELS OF SARS-COV-2 VIRUS

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BACKGROUND

- Coronavirus disease-2019 (COVID-19) was first declared a pandemic by the WHO in March 2020 [1], and remains as such while new variants emerge and the disease continues to spread.
- Rapid viral proliferation of SARS-CoV-2, the virus that causes COVID-19, is typically observed 5 to 6 days prior to the onset of symptoms [2-4].
- As the majority of published studies report viral levels since onset of first symptom and rely on qualitative Ct values [2, 4], there is limited quantitative data on the viral kinetics for asymptomatic infected individuals.
- It is believed that asymptomatic carriers of the virus represent an estimated 20-75% of all positive cases [5].
- Therefore, we conducted an observational study to examine the time course of changes in viral load in otherwise healthy adult male and female subjects, using an in-house ultrasensitive quantitative polymerase chain reaction (PCR) method.

METHODS

- As part of risk mitigation procedures, routine PCR SARS-CoV-2 virus screening was performed on all employees, contractors, study participants and visitors entering Celerion's clinical research unit (CRU). Anyone detected positive for the virus (between September 2020 and April 2021) was invited to take part in an IRB-approved observational study.
- Eligible participants returned to the CRU's designated COVID-19 Screening Area every 2-3 days for the first 10 visits, and again on study Day 28, 42, and 56. Participants were queried about symptoms at each visit.
- Nasopharyngeal swab samples were collected on Visits 1-10. Briefly, samples were collected in a viral transport medium (Hardy Diagnostics, CA, USA). An ultrasensitive, extraction-free PCR method was developed and validated fit-for purpose using CDC recommended primer/probe sets for 2019-nCoV selected from the nucleocapsid gene (N1) and human RNase P gene (RP) (Integrated DNA Technologies, NC, USA). The standard curve was prepared using Heat-Inactivated 2019-nCoV (Integrated DNA Technologies).
- Screening PCR Ct values were extrapolated to genome copies/uL from an average standard curve.
- Serum samples were collected over 8 weeks to measure the immune response mounted using antibody assays against the SARS-CoV-2 spike protein (COBAS Elecsys Anti-SARS-CoV-2 S, Roche, Switzerland).

TABLE 1. SUMMARY OF DEMOGRAPHIC CHARACTERISTICS

Demographic Characteristic	Study Population (n=30)
Age (years)	30.8±8.77 (20 - 49)
Gender (Male/Female)	11 / 19
Self-Reported Race (%)	
• Asian	2 (7%)
• Black or African American	1 (3%)
• White	26 (87%)
• Not reported	1 (3%)
Self-Reported Ethnicity (%)	
• Filipino	1 (3%)
• Hispanic or Latino	17 (57%)
• Non-Hispanic or Non-Latino	10 (33%)
• Not reported	2 (7%)
Weight (kg)	166.13±38.83 (105 - 246)
BMI (kg/m ²)	27.15±4.78 (18.60 - 36.32)
History of Smoking	
• Non-smoker	28 (93%)
• Smoker	2 (7%)
Number of Days from 1st Positive Test to Visit 1	9.8±7.1 (1 - 28)

Data presented as mean±SD with range in parenthesis.

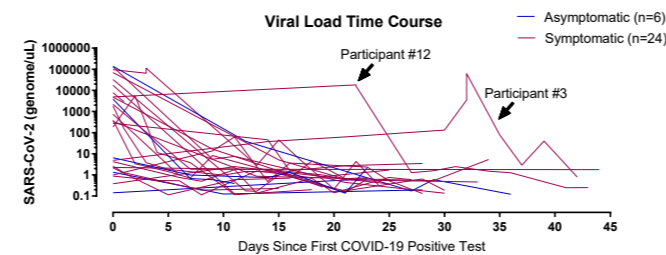
TABLE 2. SYMPTOM SUMMARY

COVID-19 Symptoms	Number (%)
Total Number of Subjects	30 (100%)
Number of Asymptomatic Subjects	6 (20%)
Number of Subjects reporting 1-2 Symptoms	11 (37%)
Number of Subjects reporting 3-5 Symptoms	9 (30%)
Number of Subjects reporting >5 Symptoms	4 (13%)
Symptoms:	
Fever	2 (7%)
Chills	6 (20%)
Persistent Cough	7 (23%)
New Loss of Taste or Smell	7 (23%)
Shortness of Breath or Difficulty Breathing	4 (13%)
Fatigue	9 (30%)
Muscle or Body Aches	4 (13%)
Headache	11 (37%)
Sore Throat	3 (10%)
Congestion or Runny Nose	17 (57%)
Nausea or Vomiting	6 (20%)
Diarrhea	4 (13%)
Newly Identified COVID-19 Symptoms (Brain Fog, Hair Loss, Toe Coloration, etc.) or other	11 (37%)

RESULTS

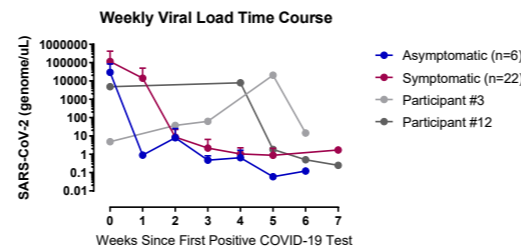
- As anticipated, nearly all participants demonstrate a dramatic reduction in viral load 5-10 days post first positive test. However, participants #3 and #12 maintained extremely high viral levels >20 days post first positive test.
- Participants that remained asymptomatic throughout the entire study had an initial screening viral load 2.2x lower than symptomatic subjects.
- For symptomatic participants, the most common symptoms reported include headache, fatigue, persistent cough, congestion or runny nose, and loss of taste and/or smell. All symptoms were mild in nature.
- Kaplan-Meier plot reveals that asymptomatic subjects tend to clear the virus faster than symptomatic counterparts.
- Asymptomatic subjects started with antibody titer levels 20x higher than symptomatic subjects after 1 week of testing positive for COVID-19. Two weeks and on, antibody titer continued to increase and was similar between both groups. Participants #3 and #12 reached max antibody titer levels by weeks 5 and 9 respectively.

FIGURE 1. SARS-COV-2 NASOPHARYNGEAL VIRAL LOAD



Viral levels when detected. Not detected treated as a missing value. Participants with elevated virus levels > 20 days following a positive COVID-19 test are indicated with arrows.

FIGURE 2. ASYMPTOMATIC PARTICIPANTS DISPLAY LOWER MEAN VIRAL LOAD



Data presented as mean±SD for viral levels when detected. Not detected treated as a missing value. Symptomatic participants with high viral load > 20 days post first positive test are shown individually.

FIGURE 3. ASYMPTOMATIC PARTICIPANTS REACH NOT DETECTED STATUS FASTER THAN SYMPTOMATIC SUBJECTS

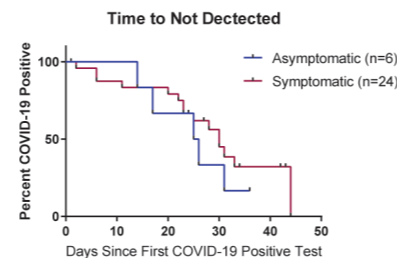
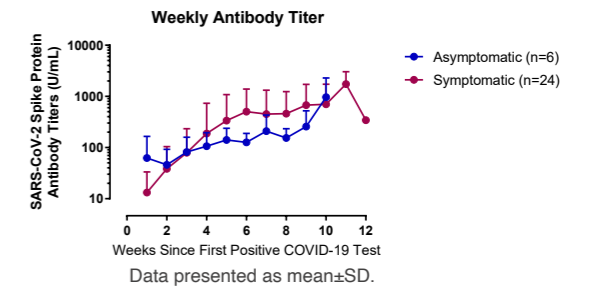


FIGURE 4. HIGHER INITIAL SARS-COV-2 SPIKE ANTIBODY TITERS IN ASYMPTOMATIC SUBJECTS



DISCUSSION & CONCLUSIONS

- It is still unknown why some otherwise healthy individuals develop symptoms to COVID-19 infection while others do not. Our theory is a threshold of viral load is needed to induce symptoms, and indeed symptomatic individuals present with a 2.2x higher initial viral load.
- It is unknown whether residual virus was artifact or replicate-competent for participants #3 and #12 >20 days post a positive COVID-19 test. Meanwhile, symptoms finally resolved by day 30 and 22 for participants #3 and #12 respectively.
- Interestingly, asymptomatic carriers tend to clear the virus faster and displayed higher levels SARS-CoV-2 spike protein antibodies after the first week of COVID-19 detection compared to subjects that reported symptoms over the course of the study.
- As more variants of the virus emerge, there is a pressing need to understand the viral kinetics of this coronavirus as well as the relationship between systemic immune biomarker concentration including response to infection and the potential for disease progression to symptomatic status.
- To that end, the Delta variant which represents approximately >80% of cases in the US [6], is estimated to have a 21-fold increase in viral load compared to other variants such as the Alpha strain, initially detected in the UK [7].

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