The natural history of SARS-CoV-2 infection: a composite but incomplete picture

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The Severe Acute Respiratory Syndrome coronavirus 2 (SARS CoV-2) pandemic began as an outbreak in Wuhan, China [1] and has quickly spread around the world, infecting over 25 million people globally and resulting in >800,000 deaths as of September 1, 2020 [2]. In addition, the SARS-CoV-2 pandemic has had significant impact of nearly all walks of life, stifling economies, travel, and schooling [3]. Ameliorating the pandemic will require a clear understanding of the natural history and transmission of SARS-CoV-2. The SARS-CoV-2 virus causes the collection of symptoms and signs that constitute COVID-19, which typically manifests as an acute respiratory disease with inflammatory and vascular complications. Most evidence points to transmission of SARS-CoV-2 via respiratory droplets and close contact, although airborne transmission may be possible in certain scenarios where aerosols are produced [4].

Despite the rapid advances that have been made to understand the natural history of SARS-CoV-2 infection, there are still several key questions that remain unanswered. Among the most important is the question of how long people remain infectious after acquisition, which has major implications for control of the pandemic. Epidemiologic studies indicate that there have been no transmissions from infected people after 14 days of symptom onset [5], although SARS-CoV-2 RNA can be detectable for weeks or even months. While the viral culture assay is the current gold standard for infectiousness, it is insensitive and difficult to perform; thus, there is an urgent need for an assay that can distinguish non-infectious from infectious viral shedding. One of the first groups to report viral RNA and infectious titers longitudinally in a cohort of nine people demonstrated that viral cultures were negative after 8 days of symptoms and were only positive when viral RNA levels in nasopharyngeal (NP) swabs were > ~1 million copies [6]. In this study, as in others, viral RNA was measured using quantitative real time PCR (qPCR), a platform that estimates the amount of original template present from the number of PCR doublings of the template required to cross a pre-set cycling threshold (Ct): the fewer the number of doublings, the more original template, and vice versa. In a larger study of samples from 90 persons who had assays for viral RNA and

culture performed, 26 samples were positive, none >8 days after symptom onset, and none were positive if cycle thresholds (Ct) were >= 24 [7]. Similar findings have been reported by two other groups, although notably Gniazdowski et al. report occasional positive cultures up to a Ct of 30 [8-9].

A corollary to the question of viral culture and transmissibility is whether there is a corresponding host response that appears when transmission is diminished. Several papers have reported the appearance of anti-SARS-CoV-2 antibodies. Importantly, Wolfel et al. reported that antibody seroconversion was associated with a decline in infectious titers and viral RNA. However, there appear to be differing times to seroconversion reported by the groups. In addition, the assays used differ between the studies, and there is now widespread acknowledgement that not all antibody tests are equal.

Finally, there has been a plethora of reports of the inflammatory phenotypes that appear to be associated with worse COVID-19 phenotypes. Most recently, Jacobson performed an intensive AI analysis of the host transcriptome from 9 persons with COVID-19 and 40 controls and uncovered a common and potential pathophysiologic link to bradykinin [10]. However, while several cytokines have been linked to disease severity, it has been challenging to identify which cytokines are in the causal pathway of COVID-19 disease and which are triggered by severe infection, but otherwise do not contribute to disease [11-15].

In this issue of Clinical Infectious Diseases, Young et al. attempt to address some of these open questions in a cohort of patients admitted to hospitals in Singapore with COVID-19 from January 22 to March 6, 2020 [16]. Although all individuals with documented SARS-CoV-2 infection admitted to one of seven hospitals in Singapore were eligible, 30 of the 130 (23%) were not included (29/30 declined participation) and only a portion of the 100 included patients had blood and respiratory samples collected up to 28 days after admission. Further, asymptomatic individuals and outpatients were not included. These restrictions limit the ability of this study to fully address these open questions.

Of the 100 included patients, 20% required supplemental oxygen, 12% required mechanical ventilation, and 3% died. The mean duration of viral RNA shedding, as measured by qPCR from NP swabs, was more than two weeks, with the most prolonged shedding occurring 48 days after symptom onset. Viral RNA shedding was not associated with clinical progression or disease severity. It is notable that of the 152 NP samples (from 74 patients) that were SARS-CoV-2 RNA positive, only 21 (13.8%) had a positive viral culture, speaking to its relative insensitivity. Further, there was no virus cultured when the Ct value was >30, nor if samples were obtained >14 days after symptom onset. Notably, there was no difference in the viral RNA Ct values between the clinical severity groups upon admission. Thus, this study adds to the others by demonstrating that Ct values up to 30 can be infectious and suggests that viral level is not an indicator of clinical outcome. However, it is not the end of the story since there were a large number of negative cultures when the Ct values were <30 and even <25, which highlights the fundamental difficulty of using culture as a gold standard for infectiousness.

In 30 patients with available serum specimens longitudinally, the majority of samples had detectable anti-SARS-CoV-2 IgM levels in weeks 2 and 3 after symptom onset, with similar results for IgG. The vast majority of people seroconverted by day 14, and there appeared to be a significant inverse association between the severity of illness and the time to seroconversion. Disease severity and peak titer also appeared to be correlated, which corroborates data from an earlier report [17]. Serum cytokine measurements performed longitudinally in 81 persons showed a largely pro-inflammatory milieu that was associated with disease severity and the need for oxygen, which appears to confirm many contemporaneous reports showing the same. However, details about the 23 healthy controls, such as sex or age, and the variability in the control's cytokine levels are not given; thus, it is not possible to make strong conclusions about the role of cytokines in COVID-19 disease in this cohort.

The study by Young et al. is among the largest to date to characterize longitudinal cultures, seroconversion, and cytokine profiles in persons with COVID-19. Notwithstanding its size and ambitious scope, there are still several unanswered questions. Firstly, it is important to develop methods besides culture to determine infectiousness to truly understand the duration of transmissibility. Second, it is somewhat unclear what the selection criteria were that determined which individuals underwent culture, which underwent serostatus measurements, and which underwent cytokine profiling. This is important with regards to whether the appearance of anti-SARS-CoV-2 antibodies was associated with a loss of culture positivity, as was demonstrated by Wolfel et al [6] It would also be of interest to assess whether certain cytokine profiles were associated with viral kinetics or the timing of seroconversion.

The relationship between the peak antibody titer and disease severity, in the absence of an association with viral titer or RNA, is both intriguing and confirms previous findings [17]. Taken together with the amalgamation of inflammatory cytokines present during infection, these data suggest that the significant differences in COVID-19 presentation and severity may be more the result of differences in host responses to SARS-CoV-2 than differences in viral replication. However, in the absence of a model system or a controlled perturbation, it is difficult to discern which host responses precipitate COVID-19 severity, and which are bystanders.

The past nine months have provided a wealth of data about the SARS-CoV-2 pandemic that has helped clinicians and scientists respond to this global threat. To move to the next phase of understanding will require major efforts to link the numerous disparate phenomenological threads that have been pulled from COVID-19. The present study is a step in that direction. To learn more, multi-modal longitudinal studies that include asymptomatic infected patients with samples collected systematically on everyone are needed to interrogate the distinct arms of virology and host responses, both to elucidate the pathophysiology of COVID-19 and to yield potential new therapies.

Potential Conflicts of Interest

CT reports grants from the NIH during the conduct of the study and outside the current study. All other authors have no conflicts.

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