Lessons from a local effort to screen for SARS-CoV-2

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It is breathtaking to consider how the response to pandemic viral pathogens has been transformed over the past century by greater knowledge of fundamental biology and technological innovations including PCR and next-generation sequencing. In striking contrast to the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the pathogen responsible for the 1918 influenza pandemic was not identified until years after the outbreak (1). The definitive text in 1927 described influenza as "an epidemiologic conception" likely caused by the bacterium *Haemophilus influenzae* (2). Six decades later, HIV-1 was discovered within a few years of the first report of AIDS, although it took another decade before HIV-1 RNA detection methods were sensitive enough to correlate viral load during clinical latency with rate of progression to AIDS (3). Four decades later, the genomic sequence of SARS-CoV-2 was publicly available on the internet within weeks of the unexplained outbreak of fatal pneumonia that is now known as COVID-19 (4–6). This critical information enabled academic researchers, vaccine manufacturers, diagnostic laboratories, and some governments to spring into action. In the midst of COVID-19 lockdown, despite collapse of reagent supply chains, independent investigators around the world shared expertise and reagents in order to establish desperately needed local screening programs for SARS-CoV-2. A paper by Yang et al. in PNAS describes the analysis of viral load data from one local screening program (7), the results of which have important implications for efforts to control the spread of SARS-CoV-2 and for understanding the pathogenesis of SARS-CoV-2 infection.

During the 2020 fall semester at the University of Colorado Boulder more than 72,500 saliva samples were tested for SARS-CoV-2 RNA using RT-PCR (7). The rate of asymptomatic infection on campus could not be

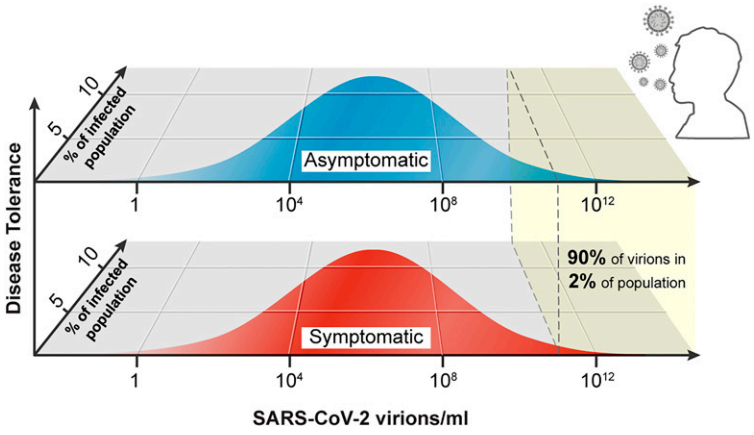

Fig. 1. SARS-CoV-2 viral load (x axis) among RT-PCR–positive individuals who were either asymptomatic (blue) or hospitalized with COVID-19 (red). The *z* axis indicates the percent of each cohort with a given viral load. Yellow shading indicates the top 2% of each cohort with the highest viral load; these individuals account for 90% of all virions in the cohorts. Lack of difference between asymptomatic and symptomatic viral load distributions indicates that these two groups differ in the ability to maintain health in the face of the same burden of SARS-CoV-2. This ability, or defense mechanism, has been called disease tolerance, and is shown along the *y* axis.

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determined from these samples since symptomatic people were excluded from testing. Previous studies have reported wide-ranging rates of asymptomatic infection in young adults, with some as high as 77% at the time of testing (8). Infection with other respiratory viruses, including rhinovirus (9) and influenza (10), can also be asymptomatic, but, in contrast to SARS-CoV-2, asymptomatic infection with SARS-CoV was uncommon during the 2002-to-2003 pandemic (11). These differences in symptomatology, as well as the shorter incubation time from exposure to peak viral load, and the earlier probability of transmission with SARS-CoV-2 (12, 13), explain in part why the SARS-CoV pandemic was shut down within 8 mo and the SARS-CoV-2 pandemic continues to rage out of control.

In most screening programs, SARS-CoV-2 RT-PCR results are reported as either positive or negative. By this simple rubric, 1,405 (2%) of the Boulder samples were positive for SARS-CoV-2 RNA. However, as demonstrated by Yang et al. (7), binary reporting of RT-PCR results discards data highly relevant to public health policy. More nuanced information can be gleaned from the cycle number during which the PCR crosses the threshold for positivity. Lower cycle numbers correlate inversely with viral load. Additionally, the authors here calibrated the RT-PCR assay so that SARS-CoV-2 genome copy number could be determined. Importantly, half of all the positive samples fell below viral load values reported by others to be required for SARS-CoV-2 propagation from oropharyngeal specimens (14). This finding suggests that half of the individuals declared positive by a binary reporting system would be unlikely to transmit SARS-CoV-2 at the time of collection.

A paper by Yang et al. in PNAS describes the analysis of viral load data from one local screening program, the results of which have important implications for efforts to control the spread of SARS-CoV-2 and for understanding the pathogenesis of SARS-CoV-2 infection.

There is more than one reason to test for SARS-CoV-2. Importantly, in the clinical setting sensitive RT-PCR tests are well-suited to diagnose SARS-CoV-2 infection and to initiate appropriate treatment within an individual. But the priority from the public health perspective is to identify those people most likely to transmit SARS-CoV-2 to others, in which case binary RT-PCR results reporting is not ideal. The findings here are consistent with other studies indicating that SARS-CoV-2–infected people only produce enough virus to transmit to other people for about 5 d but often remain RT-PCR–positive for 30 d (13, 15). In the Boulder screening program (7) subjects were tested weekly. If an individual tested positive they were removed from the screening pipeline. Thus, for any positive sample there was only a single time point of viral load data and one cannot determine whether the viral load would have increased at a later time point. If serial RT-PCR–based viral load measurements are not feasible for population screening at a given location, then high-specificity assays that are simpler and of lower sensitivity—so that SARS-CoV-2 is only detected at viral loads that are capable of transmission—are needed for repeat testing and SARS-CoV-2 pandemic control.

The viral loads in the samples that scored positive in the Boulder cohort had a log-normal distribution with a trillion-fold range (7). It follows from this distribution that a minority of infected people harbor the vast majority of virions. Specifically, 90% of all SARS-CoV-2 RNA detected in the cohort was concentrated within 2% of the population (Fig. 1). One extraordinary individual harbored more than 5% of the total RT-PCR signal in the whole cohort. Another stunning observation here is that all RT-PCR positive individuals in this study, including those with super high viral loads, were asymptomatic at the time that they were tested.

It is not possible to determine from this study whether individuals with the highest viral loads are outliers or whether most people infected with SARS-CoV-2 pass through a narrow window of high virus production. However, it is clear that, at any particular point in time, only a minority of individuals within a population of infected people are likely to have sufficiently high viral titer to transmit the virus. This asymmetry in virus distribution within the infected population accounts for why a minority of SARS-CoV-2–infected individuals are responsible for the majority of transmission events (16). Additionally, this asymmetry also explains why a higher proportion of SARS-CoV-2–infected people than influenza-infected people do not transmit virus (17).

The distribution of viral loads in this asymptomatic Boulder cohort was similar to the distribution of viral loads in 404 hospitalized patients compiled from 10 previously published studies (7). Notably, as in the Boulder cohort, just 2% of individuals harbored 90% of the virus and a single individual harbored more than 5% of the virions (Fig. 1). Lack of correlation between COVID-19 symptomatology and SARS-CoV-2 viral load has been reported before in multiple contexts throughout the pandemic (13, 18). In most people, then, the ability to control SARS-CoV-2 replication does not distinguish people with asymptomatic infection from those with severe COVID-19. Rather, what matters may be the ability to maintain normal tissue homeostasis despite high-level infection with SARS-CoV-2. This defense strategy has been called disease tolerance (19, 20). People who are asymptomatic despite SARS-CoV-2 infection would have greater disease tolerance than people who are symptomatic. This difference in disease tolerance between the symptomatic and asymptomatic groups in Yang et al. (7) is represented schematically as a quantitative trait along the *y* axis in Fig. 1.

The SARS-CoV-2 pandemic provides a unique opportunity to study mechanisms of disease tolerance in humans. Severe COVID-19 is characterized by high levels of inflammatory cytokines (21). While antiviral agents are effective when given early after infection or for moderate disease, antiinflammatory drugs have been shown to decrease mortality in severe COVID-19 (22). These observations indicate that, in addition to antiviral immunity, host intrinsic mechanisms that decrease inflammation, or promote repair of tissues damaged by inflammation, are also critical for surviving severe COVID-19. Such mechanisms are likely influenced by age and sex since risk for severe COVID-19 increases dramatically with age and is greater for males than for females (23). Risk is also increased with greater body mass index and other comorbidities (24). One potential clue to the physiological mechanisms that underlie disease tolerance comes from studies of innate lymphoid cells (ILCs), a cell type that promotes tissue homeostasis in animal models of viral infection (25). Intriguingly, blood ILCs decrease with age and are less abundant in males, and their numbers correlate inversely with multiple parameters of COVID-19 severity (26). Ultimately, better understanding of disease tolerance mechanisms may offer new approaches that complement conventional antiviral therapies to promote health and survival of pathogenic viral infections (20).

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