Detection of SARS-CoV-2 RNA in Blood of Patients with COVID-19: What Does It Mean?

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The SARS-CoV-2/COVID-19 pandemic has severely impacted global societies and taken hundreds of thousands of lives prematurely. A striking paradox about SARS-CoV-2 infection is the broad range of clinical manifestations – ranging from asymptomatic infection, to more severe disease requiring hospitalization, to death despite intensive care. Such a broad range of illness is characteristic of other infectious diseases including poliovirus infection for which only 1 in 100 to 1 in 1000 infections result in paralytic disease with the remainder being asymptomatic or minimally symptomatic [1]. Initial studies of prognosis in SARS-CoV-2/COVID-19 have focused on host risk factors for severe disease, which have identified older age, male sex, non-white race, and comorbidities including obesity, among others [2]. Most studies of SARS-CoV-2 pathogenesis have focused on cellular and serologic responses, immunopathology, and qualitative or semi-quantitative measurements of SARS-CoV-2 RNA in the nasopharynx. The contribution of immunopathology to COVID-19 disease severity has been established. Host response to infection is generally a well-regulated process containing complex checks and balances to avoid host injury from overactive innate or adaptive immune responses. In COVID-19, however, there can be immune dysregulation and excessive inflammatory responses that can tip the balance toward severe disease [3,4]. As for most virus infections, some combination of virus- and immune-mediated damage is likely responsible for severe disease.

Analyses of SARS-CoV-2 RNA in the nasopharynx, often inappropriately termed “viral load”, have not shown consistent associations with asymptomatic versus symptomatic disease or with severity of symptomatic disease [5]. This result is not surprising given that measuring viral RNA at the local site of initial infection may not provide an accurate assessment of viral replication in the lower respiratory track or dissemination of virus through blood to other organs. For many infections, including HIV and Ebola as examples, the quantity of the pathogen in blood is a strong indicator of prognosis [6,7].

SARS-CoV-2 has been consistently shown to primarily infect and replicate in type 2 pneumocytes of the lung, but reports of expanded receptor expression coupled with presence of virus in peripheral tissues such as the GI tract beg the question of whether disseminated viral infection could contribute to severe disease. Although current data do not suggest that virus replicates to a significant degree in peripheral blood cells, virus could disseminate to other organs through the blood supply. Virus particles or infected cells in blood could also be an important indicator of lung tissue breakdown with leakage of virions, viral components, or infected cells into the bloodstream. Along these lines, a few groups, including Veyer et al. in the current issue of *Clinical Infectious Diseases*, have reported the detection of SARS-CoV-2 RNA in plasma [8,9]. Veyer et al. used a droplet digital PCR-based assay to interrogate plasma samples from SARS-CoV-2 nasopharyngeal-positive patients for SARS-CoV-2 RNA. They detected SARS-CoV-2 RNA in 75% of plasma samples, and level of plasma viral RNA (termed RNAaemia) was associated with severity of COVID-19. Anecdotally, the patient with the highest level of “RNAaemia” in the study succumbed to COVID-19 quickly. An expansion of these preliminary observations to include longitudinal samples from patients across the spectrum of disease severity, including those with progressive disease during the observation period, would provide additional insight into the usefulness of SARS-CoV-2 as a prognostic marker.

Veyer et al. used ddPCR for detection and quantification of SARS-CoV-2 RNA in plasma, a PCR technology that relies on partitioning nucleic acids into droplets containing a single target for quantification without the need for a standard curve. It is widely touted as superior to traditional PCR in sensitivity and ease of use since a standard curve is not necessary. ddPCR is a natural choice for detection of a novel pathogen early in the course of an outbreak since materials required for constructing a viral RNA standard curve are not yet widely available. Nevertheless, it is important to note that the sensitivity of any PCR assay is dependent upon a variety of factors, many of which are not intrinsic to the PCR technology used, such as the integrity and amount of source material assayed, the efficiency of recovery

of nucleic acid from the sample, and the proportion of total sample assayed, along with added effects of primer and probe design on PCR efficiency and product detection. Indeed, many highly sensitive assays based on traditional quantitative PCR have been reported in the literature, including a RT-qPCR assay with single copy sensitivity for detection of HIV-1 RNA in plasma [10]. It is likely that application of such single copy assays to blood samples from patients with COVID-19 will increase the frequency of SARS-CoV-2 RNA detection in severe disease and possibly less severe disease.

Finding nucleic acid of any viral pathogen in blood leads to several important questions: Does the viral nucleic acid indicate the presence viral particles and/or infected cells? If virions are present are they infectious; and if infected cells are present, are they producing infectious virus? Centrifugation and antibody-mediated pull-down experiments of SARS-CoV-2 RNA positive blood samples will help answer whether viral particles are present, and virus culture in a BSL-3 facility can determine whether infectious virus is present.

Ultimately, the clinical significance of SARS-CoV-2 “RNAaemia” needs to be defined. Could the detection of viral RNA in plasma signal uncontrolled infection and risk for complications requiring earlier intervention? Similarly, could therapies that prevent or reduce viremia be associated with improvement in outcome? The latter result could accelerate the screening and subsequent development of effective therapies of COVID-19, as was certainly the case for antiretroviral therapy of HIV-1 infection [11,12].

As important studies on the presence of SARS-CoV-2 in pulmonary and extrapulmonary compartments accumulate, there will be a better understanding of the relative contributions of viral replication and/or dissemination and excessive immune response to COVID-19 outcome. These insights will improve the precision of antiviral and anti-inflammatory therapeutic approaches and their clinical effectiveness in preventing acute and chronic morbidity or death from COVID-19. Such advances will help restore global societies and their health toward pre-pandemic times.

Conflicts of Interest:

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