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Commentary

Persistent viral RNA shedding in COVID-19: Caution, not fear

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With the continued community transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) worldwide, the efficacy of laboratory testing in mitigating and supressing viral spread has received increasing interest and concern. SARS-CoV-2 can be transmitted by a carrier who is unaware of the infection, and a body of evidence has demonstrated that viral transmission from asymptomatic and pre-symptomatic subjects has underpinned the current pandemic [1]. Nucleic acid amplification tests (NAATs), particularly real time reverse transcription polymerase chain reaction (rRT-PCR) assays, used since the beginning of the pandemic in order to accurately detect the virus, are considered the operational gold standard for diagnosing and confirming Coronavirus 2019 disease (COVID-19) [2]. However, in addition to the false negative rates ranging from 2 to 29% as reported in studies finding that negative rRT-PCR tests were positive at repeat testing [3], the continuing PCR positivity compared with the risk of transmission remains a major concern. Persistently or intermittently PCR positive individuals raise the question of the true risk of disease transmission, and the safe duration of self-isolation. This in turn, is a major problem not only for individual patients and relatives, but also for the population at large in the preventing community transmission and enabling a safe return to the workplace and the resumption of social activities. The new insights gained in the paper by Line K Vibholm and colleagues, published recently in *EbioMedicine* allow a greater understanding of the prolonged RNA detection in pharyngeal swabs from COVID-19 patients, the risk associated with this finding, and the immunological responses in convalescent individuals with a history of rRT-PCR verified SARS-CoV-2 infection [4]. Two hundred and three patients with documented SARS-CoV-2 PCR positivity 12 weeks prior to the study who had fully recovered from COVID-19 were monitored at two time points (time point 1: 14 days-12 weeks after positive PCR; time point 2: 6-10 weeks after time point 1) using digital PCR (ddPCR) to detect viral RNA on pharyngeal swabs. ddPCR assures maximal sensitivity (100%) in detecting SARS-CoV-2, allowing for the absolute quantitation of copy numbers [5]. Twenty-six individuals (12.8%) were positive for SARS-CoV-2 at time 1 while, at time point 2, five (5.3%) were found to be positive (up to 105 days after recovery), and a subset of participants had remarkably high viral loads (10^3 copies per swab). This value is comparable to that found in acutely affected COVID-19 patients, even if sampling was performed more than four weeks after symptom onset. Therefore, overall in enrolled participants, SARS-CoV-2 PCR positivity declined over time, but viral RNA was detected in some individuals up to 109 days after symptom onset, and COVID-19 patients with mild or asymptomatic disease (group 1) were more likely to be persistently PCR positive than participants with a more severe illness (groups 2 and 3). Two hundred and two participants (99.5%) seroconverted at time point 1, at a median of 45 days after symptom onset, and no difference was found between the PCR-positive and PCR-negative groups for specific antibodies level. However, higher total antibodies levels were found to be associated with a lower likelihood of persistent viral RNA shedding (fewer SARS-CoV-2 copies per swab). Unfortunately, the authors did not measure neutralizing antibodies even if the serological assays used have been reported to assure results well related to neutralization activity [6]. Participants with a higher pharyngeal viral load presented an increased breadth and magnitude of SARS-specific CD8 T-cell responses, the CD8 T-cell response magnitude being significantly correlated with SARS-CoV-2 copies per swab.

Importantly, the findings made in the study show that persistently PCR positive individuals present elevated SARS-CoV-2-specific CD8 immune responses, thus providing new insight on the fundamental role played by T-cell immune responses in protecting against and eventually eradicating this new coronavirus [7]. Moreover, the data pave the way for further studies aiming to ascertain whether persistent viral RNA shedding should lead to a more durable immunity. Furthermore, the authors found that persistent viral RNA shedding did not increase the risk of transmission: among 757 close contacts, no infection was identified in a median time interval of 23 days (median) after recovery, thus reflecting a very low, if not absent, transmission risk (ranging from 0-0.13%) of developing symptomatic COVID-19 disease. The paper by Vibholm and colleagues is therefore welcome, since it increases our understanding of the immune response in COVID-19 and paves the way for further research. From a clinical viewpoint, fully recovered individuals with persistent viral RNA shedding are unlikely to be a significant source of SARS-CoV-2 transmission and seem to have a more durable immunity, strongly reducing the risk of re-infection [8]. In addition, the

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article highlights the need for additional laboratory tests integrating traditional rRT-PCR methods to evaluate infectivity risk of transmission in individuals with persistent viral RNA shedding [9]. From a research viewpoint, this study prompts us to further evaluate the role of immune responses, particularly in relation to the role of T-cells (e.g. CD8), and interrelationships with B-cell immune responses. In addition, further research might confirm the authors' intriguing opinion that while the virus from persistently PCR positive individuals remains viable, other factors such as mucosal IgA prevent transmission.

Declaration of Competing Interest

No conflict of interest to be declared.

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