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Are we underestimating seroprevalence in Covid-19 testing?

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Testing for severe acute respiratory coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), is a complex and politically sensitive issue. Seroprevalence studies use antibodies as surrogate biomarkers of prior pathogen exposure and provide retrospective estimates of the proportion of a population who may have been infected. Progress towards herd immunity is inferred from these proportions. Considerable variation has been observed between the results of SARS-CoV-2 seroprevalence studies.[1] A recent survey in Spain suggested that a small fraction of the population were seropositive, despite the country being severely affected by the virus.[2] However, intra-individual variation between the cellular and humoral correlates of prior viral exposure, particularly in mild or asymptomatic disease, has been observed. For example, a recent study from the Karolinska Institute found the percentage of individuals mounting anti-SARS-CoV-2 T cell responses following mild COVID-19, asymptomatic disease, or exposure to family members with COVID-19, consistently exceeded the percentage mounting detectable IgG serological responses against the virus.[3] Such discordant results could have major implications for epidemiological modelling of disease transmission and herd immunity. Additionally, the diagnostic accuracy of serological tests has been questioned.[4]

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There are several reasons why the results from existing sero-epidemiological studies may underestimate the true seroprevalence of SARS-CoV-2. An accurate seroprevalence study demands the use of an assay sensitive enough to reliably detect antibody responses to mild infection across a range of possible post-exposure timings. The selection of target antigen is critical, with recent data showing that the SARS-CoV-2 trimeric spike glycoprotein offers superior detection than the nucleocapsid in individuals with low-level antibody responses.[5] Of the 24 serological diagnostic tests for which FDA authorization for emergency use has been granted, 6 platforms consider only the nucleocapsid, including several high-throughput platforms that are in widespread use. Furthermore, the nature of the pandemic means that manufacturer and local laboratory evaluations have largely been performed on SARS-CoV-2 positive subjects who have experienced severe symptomatic disease.[6] Recent evidence describes a clear relationship between the magnitude of serological responses and severity of illness.[5,7] This implies that unless specific assessments of assay performance in mild and convalescent cases is performed, the threshold for reporting a positive result may be set too high, resulting in missed community cases. Additionally, calibration of tests is generally based on balancing the risks of providing false positive and false negative findings to individuals; this is different to the risks of false positive and false negative for serological surveys.

Test performance is also influenced by the nature of the antibody response considered. Of the FDA-authorised tests, the majority consider only IgG and IgM, and not IgA. This focus is likely to reflect guidance from national institutions such as the US Centers for Disease Control and Prevention (CDC), which advised against testing for IgA antibodies because little is known about the significance of this relative to IgG or IgM.[8] Similarly, the UK National COVID Scientific Advisory Panel assessment considered only IgG and IgM responses in their evaluation of serological tests.[9] We contend that the prominent role of IgA in the host immune response to a range of respiratory tract infections makes it immunologically relevant to consider in the context of COVID-19.[10–12] SARS-CoV-2 enters cells via interaction with the host proteins ACE2 and TMPRSS2, which are co-expressed in the respiratory tract, cornea, and gastrointestinal tract.[13] IgA is the predominant immunoglobulin class expressed at these mucosal surfaces.[14] IgA immune responses directed against viral pathogens with neutralizing capability are well described for influenza, respiratory syncytial virus and human immunodeficiency virus.[10,12,15,16] The ability to detect IgA specific to SARS-CoV-2 antigens has now been documented across various biological specimens, including serum, saliva, and breast milk.[5,17,18] Serum IgA antibody responses may be detectable earlier than IgG and IgM,[19,20] and have been shown to persist for at least 38 days in hospitalized convalescents.[21] This is consistent with a recent Cochrane Review, where IgA-based serology testing was shown to offer greater sensitivity than other methods.[6] In practice, a recent seroprevalence survey of 1473 residents (79% of the local population) in Ischgl, Austria that used a combined IgG and IgA approach found SARS-CoV-2 antibodies in 42.4% of individuals, far higher than previous population-based surveys of other infection hotspots.[22] Similarly in the CON-VINCE study, IgA antibodies were detected in 11.1% of 1862 individuals sampled from the general population in Luxembourg, whilst IgG antibodies were only found in 2.1%.[23]

Finally, mucosal and blood-borne immune responses may provide independent information critical for accurate assessment of viral exposure. The principle of compartmentalization of mucosal from blood-borne immunity has recently been demonstrated in a cross-sectional study of UK healthcare workers, where combined IgG, IgA and IgM (IgGAM) testing for SARS-Cov-2 spike protein within saliva samples revealed an additional 15% of positive results when compared to serum testing alone.[5]

In conclusion, we suggest that current seroprevalence studies may be failing to detect individuals who had mild COVID-19 infection. Specific consideration should be given to the nature of SARS-CoV-2 antigen used in diagnostic assays, calibration of assays for community-based testing, the breadth of the antibody response, and the role of mucosal antibody responses. Application of these principles in future seroprevalence surveys may offer more accurate insight into the population dynamics of COVID-19, thus informing epidemiological modelling strategies and public health policy.

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