***BMJ Editorial***

**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 markers of prior pathogen exposure to estimate of the proportion of a population who have been infected.

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, within-individual variation has been observed in immune responses to viral exposure, particularly in mild or asymptomatic disease cases. For example, a pilot study from the Karolinska Institute found the percentage of individuals mounting anti-SARS-CoV-2-specific 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

There are several reasons why the results from existing sero-epidemiological studies may underestimate the true seroprevalence of SARS-CoV-2. Accuracy demands the use of an assay sensitive enough to reliably detect antibody responses to mild infection across a range of possible post-exposure scenarios. 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 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 are performed, the threshold for reporting a positive result may be set too high, resulting in missed community cases. A further issue is timing: early testing before seroconversion is complete will result in false negatives. Similarly, preliminary reports showing rapid decline in virus-specific IgG levels in individuals suggest testing too late may also lead to missed cases.7

Test performance is also influenced by the nature of the antibody response considered. Of the FDA-authorised tests, the majority consider only IgM and IgG, as the dominant components of the blood-borne antibody response. 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, particularly in asymptomatic individuals for whom viral infection may not reach the bloodstream.8-10 SARS-CoV-2 enters cells via interaction with proteins expressed in the respiratory tract, cornea, and gastrointestinal tract.11 IgA is the predominant immunoglobulin class expressed at these mucosal surfaces12 and demonstrates neutralizing capability to range of viral pathogens .8,10,13,14 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.5,15,16 Serum IgA antibody responses may be detectable earlier than IgG and IgM,17,18 and have been shown to persist for at least 38 days in hospitalized convalescents.19 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.20 Similarly in the CON-VINCE study, IgA antibodies were detected in 11.0% of 1862 individuals sampled from the general population in Luxembourg, whilst IgG antibodies were only found in 1.9%.21

Finally, mucosal and blood-borne immune responses may provide independent information critical for accurate assessment of viral exposure. The principle of compartmentalization of immunity (i.e. an immune response may be present in one tissue but not in another) has recently been demonstrated in a cross-sectional study of UK healthcare workers, where combined IgG, IgA and IgM testing for SARS-Cov-2 spike protein within saliva samples increased diagnostic yield by 15% compared to serum testing alone.5

In conclusion, we suggest that current seroprevalence studies may fail 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. Standardized procedures are required for comparability of seroprevalence estimates. 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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