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Are we underestimating seroprevalence of SARS-CoV-2?

Current antibody tests fail to identify people who had mild infections

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Testing for severe acute respiratory coronavirus 2 (SARS-CoV-2), which causes covid-19, is complex and politically sensitive. Seroprevalence studies use antibodies as markers of pathogen exposure to estimate the proportion of the population that has been infected.

Considerable variation has been observed in the results of SARS-CoV-2 seroprevalence studies.[1] A recent survey in Spain suggested that a small fraction of the population was 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 those with mild or asymptomatic disease. For example, a pilot study from the Karolinska Institute found the percentage of people mounting T cell responses after mild covid-19, asymptomatic disease, or exposure to infected family members, 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.

Seroepidemiological studies may underestimate the true seroprevalence of SARS-CoV-2 for several reasons. Accuracy demands the use of an assay sensitive enough to reliably detect antibody responses to mild infection across different post-exposure scenarios. The selection of target antigen is critical, with recent data showing that the trimeric spike glycoprotein offers superior detection to the nucleocapsid in people with low level antibody responses.[4] Of the 24 serological diagnostic tests that the FDA initially authorised for emergency use, six consider only the nucleocapsid, including high throughput tests in widespread use.

The nature of the pandemic means that tests have been evaluated mostly on people who experienced severe covid-19 symptoms.[5] Recent evidence describes a clear link between the magnitude of serological responses and severity of illness.[4 6] This implies that unless assay performance is also assessed in mild and convalescent cases, the threshold for a positive result may be too high, resulting in missed community cases.

Other problems with test calibration include the effect of demographic factors such as age, sex, and ethnicity on antibody responses and hence assay results,[7] and the effect of timing, since early testing before seroconversion may result in false negative results. Preliminary reports showing rapid decline in virus specific IgG levels suggest that testing too late may also miss cases.[8]

Test performance is also influenced by the choice of antibody. Of the FDA authorised tests, most detect only IgG and IgM antibodies, the dominant components of the bloodborne antibody response. But IgA also has an important role in the immune response to respiratory tract infections and seems immunologically relevant in covid-19, particularly in asymptomatic people.[9 10]

Look for IgA

SARS-CoV-2 enters cells by interacting with host proteins expressed in the respiratory tract, cornea, and gastrointestinal tract.[11] IgA is the predominant immunoglobulin expressed at these mucosal surfaces,[12] and IgA responses with neutralising capability are described for several viral pathogens.[9 10 13] IgA antibodies specific to SARS-CoV-2 have now been detected in various biological specimens, including serum, saliva, and breast milk.[4 14 15]

Serum IgA antibody responses may be detectable earlier than IgG and IgM responses[16 17] and can persist for at least 38 days in hospital patients recovering from covid-19.[18] This is consistent with a recent Cochrane review, which found that IgA based serological testing had greater sensitivity than other methods.[5] A recent seroprevalence survey of 1473 residents (79% of the local population) in Ischgl, Austria, using a combined IgG and IgA approach found SARS-CoV-2 antibodies in 42.4% of those tested, far higher than rates in previous population based surveys of other infection hotspots.[19] Similarly, IgA antibodies were detected in 11% of 1862 people sampled from the general population in Luxembourg, whereas IgG antibodies were found in only 1.9%.[20]

Finally, mucosal and bloodborne immune responses may provide complementary information crucial for accurate assessment of viral exposure in both individuals and populations. In a cross sectional study of UK healthcare workers, combined IgG, IgA, and IgM testing for SARS-Cov-2 spike protein in saliva samples identified 15% of participants as positive despite a negative serum test result.[4]

In conclusion, current seroprevalence studies may fail to detect people who have had mild covid-19. Standardised approaches are required so seroprevalence estimates are comparable. Specific consideration should be given to the selection of the SARS-CoV-2 antigen in diagnostic assays, calibration of assay thresholds, 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 and

help inform epidemiological modelling strategies and public health policy.

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