Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals

Dear Editor,

We write in response to correspondence from Whitaker et al. in this Journal (1). The authors demonstrate increases in seropositivity for SARS-CoV-2 antibodies against the spike protein as the roll out of COVID-19 vaccines continues, whilst parallel assessment of nucleocapsid antibodies remained stable. This study is an example of the use of parallel assessment of spike (S) and nucleocapsid (N) antibodies to discriminate between natural infection and vaccine related seropositivity (2) (3). This approach remains attractive, but as the pandemic rolls on it is worth considering the paucity of evidence about the impact of vaccination on antibody production in response to a subsequent natural infection.

As part of a large seroprevalence study in hospital healthcare workers (PRECISE 2 (4)), we measured antibody response following vaccination in over 4000 hospital healthcare workers (HCW), coupled with a questionnaire about previous symptoms and confirmed infection. We measured anti-S and anti-N antibodies using Roche Elecsys total antibody assays to determine both serological response to vaccination and to natural infection. Twenty-three participants reported a breakthrough infection post-vaccination, defined as PCR-confirmed SARS-CoV-2 infection $\geq$14 days after completion of vaccination (5). This represented 0.6% (23/4111) of all fully vaccinated participants in the study. All had received Pfizer vaccine (which was the vaccine received by most study participants). For these 23 participants, the median number of days between second vaccine dose and positive PCR was 30 days (IQR 25–50 days). Five (22%) had symptoms at the time of the positive PCR test and 18 (78%) did not have symptoms (they were tested as close contacts or as part of hospital outbreaks). All 23 participants had detectable anti-S antibodies, as expected post vaccination (6). Notably, only 6/23 (26%, 95%CI: 11–49) had detectable anti-N antibodies in response to their infection, compared to 663/812 (82%, 95%CI: 79–84, p-value= $<$0.001 (Chi-squared) of all participants in the study with previous PCR-confirmed infection having detectable anti-N antibodies. Of the 17 that were anti-N negative, median number of days between PCR positivity and sampling mid-point was 52 (range 9–67) so it is surprising that the majority of these had not mounted an anti-N antibody response (7). This low number of seroconversions might suggest that anti-N antibodies may be insensitive as a marker of natural infection post vaccination. It is possible that early viral neutralisation, perhaps even at mucosal surfaces, might modify the natural humoral response and limit the development of anti-N antibodies.

There are a very limited number of studies evaluating the production of anti-N post infection in vaccinated individuals; Demmer et al., in a study awaiting peer-review, have commented on high sensitivity and specificity of anti-N antibody assays in detecting infection in vaccinated individuals (8), however the infection in these individuals pre-dated their vaccination. Studies such as that conducted by Whittaker et al. that we are here responding to, are continuing to rely on anti-N as a marker of seropositivity related to natural infection, including in vaccinated individuals (1). Vaccine effectiveness studies will also incorporate periodic serological testing for SARS-CoV-2 antibodies as a marker of breakthrough infection (9). To the best of our knowledge there are no published data to date that have identified a comparative reduction in anti-N seroconversion following natural infection in vaccinated individuals. Whilst our numbers are small, even in large seroprevalence studies numbers of breakthrough infections will be small (5), and further research of individuals with well-defined vaccine breakthrough infections are required. This information will be critical in informing optimal assessment of seroprevalence in vaccinated cohorts.

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