Short Communication

Using SARS-CoV-2 anti-S IgG levels as a marker of previous infection: example from an Israeli healthcare worker cohort.

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A B S T R A C T

Objectives: Determining COVID-19 status is important for global epidemiology and individual-level vaccination decision-making. SARS-CoV-2 infection can generally only be detected during a 7–10-day period using polymerase chain reaction or rapid antigen testing, and infection-specific antinucleocapsid IgG assays are not universally available. We determined whether SARS-CoV-2 antispike (anti-S) IgG levels could discriminate between vaccination and previous infection when interpreted alongside vaccination timing.

Methods: We measured SARS-CoV-2 anti-S-IgG level in 535 vaccinated Israeli healthcare workers with known previous infection status 6–8 months after the second dose.

Results: Anti-S IgG levels above 1000 AU/ml at that time point was 93.3% predictive of infection in the previous 3 months, whereas the negative predictive value for infection in the past 3 months of a level below that threshold was 99.5%.

Conclusion: When interpreted alongside vaccination timing, anti-S serological assays can confirm or exclude previous infections within the previous 3 months.

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Detecting current or previous SARS-CoV-2 infection is an essential component of pandemic management. Beyond case ascertainment and contact tracing, previous infection knowledge determines reinfection risk, the number and timing of vaccine doses required (Abu Jabal et al., 2021; Hansen et al., 2021), and can serve as evidence to attribute postviral symptoms to infection. Determining acute infection status relies on detecting viral DNA through polymerase chain reaction (PCR) or viral proteins through rapid antigen testing which has limited sensitivity (Wölfl-Duchek et al., 2022). Another major limitation of these methods is the short 7–10 day window of opportunity (from shortly before symptom onset to a few days after) to detect infection (Murad et al., 2021), beyond which the opportunity to detect infection through these modalities is lost in most cases, although viral RNA remains detectable for longer period of time in some cases (Sethuraman et al., 2020). This issue is compounded by the large proportion of asymptomatic infections (Sah et al., 2021), especially in highly vaccinated populations, for which there is no

trigger to getting tested. Measuring circulating IgG immunoglobulins is another approach to determining immunity. Antinuclocapsid (N) IgG antibodies are natural infection–specific but can wane as quickly as 12 weeks (Shrotri et al., 2021), whereas antispike (S) antibodies last longer (Levin et al., 2021) but are generated either as a result of natural infection or vaccination. Anti-S assays are more commonly available and used than anti-N assays. We analyzed serological data from vaccinated healthcare workers cohort from Ziv Medical Centre, Israel to determine whether it was possible to discriminate between natural infection and vaccination using anti-S IgG, taking circulating antibody levels and time since vaccination into consideration, using the time period between the second and third vaccine doses as an example (ie, before boosting). The cohort has been described elsewhere (Abu Jabal et al., 2021). Briefly, we measured circulating anti-S IgG levels approximately every 2 months among all consenting healthcare workers at the hospital using a LIASON Diasorin S1/S2 assay. Of 998 enrolled participants, 222 were identified as infected, either through N IgG detection before initiating vaccination (n = 119) or through PCR at various points following the initiation of vaccination (n = 103). PCR tests were conducted upon COVID-19 clinical suspicion. To identify asymptomatic infections, individuals with no documented infection and unexpected rises in anti-S IgG were tested using

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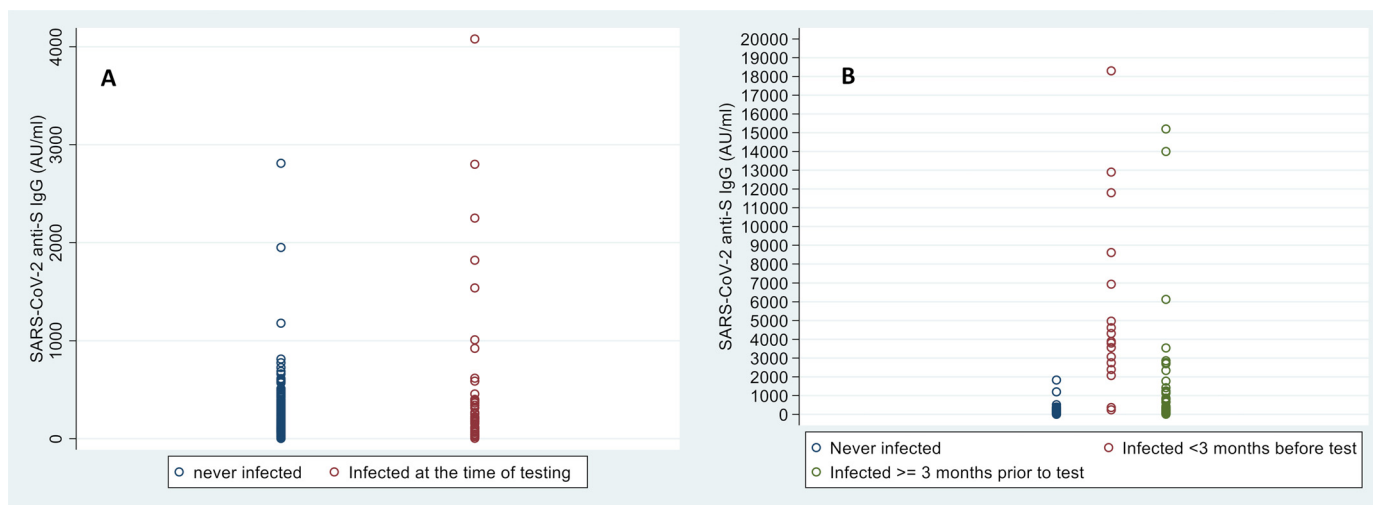
Fig. 1. IgG levels 4-6 months (A) and 6-8 months (B) post dose 2 vaccine for each two-dose vaccinated participant.

Table 1
Antibody levels among 2-dose vaccinated individuals 6-8 months post second dose (n=535).

Infection status	Number of participants	
	With IgG levels Below 1000 AU/ml	With IgG levels Above 1000 AU/ml
No record of previous infection	428	2
Positive PCR in the previous 3 months	2	15
Positive PCR >3 months from serological test	75	13

P value for difference in proportions between groups (chi-square): <0.0001
PCR = polymerase chain reaction.

an additional N-antibody assay shortly after the detected anti-S IgG increase to exclude previous infection. Our longitudinal data suggest that in the first 6 months following the second dose, it is difficult to discriminate between vaccine-induced and infection-induced immunity (Figure 1A). However, as time passes, 2 phenomena occur: (i) vaccine-induced IgG antibody wane faster than infection induced and (ii) breakthrough infections cause a boost-like anamnestic response, leading to very high IgG levels that is in excess of what can be induced by vaccination in most cases (Figure 1A). Focusing on participants who were serologically tested 190–250 days after dose 2 and before dose 3 (n = 535), 2/430 (0.46%) uninfected individuals had IgG levels above 1000 AU/ml (chosen as an arbitrary threshold), whereas 15/17 (88%) individuals who were infected in the previous 3 months had IgG levels above the threshold (Table 1, Figure 1B). Among those infected earlier, 75/88 (85%) had IgG levels below 1000 AU/ml. The difference between these proportions, tested using a chi-square test, was statistically significant (p <0.0001). In our sample, the positive predictive value for previous infection with IgG levels above 1000 AU/ml 6–8 months after dose 2 was 93.3% and the negative predictive value of IgG levels below 1000 AU/ml for infection in the last 3 months was 99.5%, but the overall negative predictive value was 84.7%. In other words, high IgG levels are predictive of a previous infection when tested 6–8 months after dose 2, and lower IgG levels is highly predictive of no recent (<3 months) infection but cannot reliably exclude earlier infection.

These findings suggest that anti-S IgG levels could be used as markers of a previous infection. This could prove useful where anti-N IgG assays and/or PCR testing is not available. Because different assays report different arbitrary levels, this approach would need to be repeated with each of the commonly used serological assays. In addition, the type of vaccine used, time from vaccination, patient age, and SARS-CoV-2 variant are also likely to influ-

ence the optimal discriminatory thresholds, which would be time-specific and would need to be calculated separately following the third dose. Our sample is too small to determine optimal thresholds using receiver operating characteristic curves. Our objective in this letter is not to propose definitive diagnostic thresholds but to demonstrate through proof-of-concept that anti-S IgG levels can be used to discriminate between natural infection and vaccination and to encourage others teams, in particular those with large immunogenicity datasets, to analyze their data in this way and possibly, to pool data in order to build a reference library of IgG levels that includes different timings, vaccines, and assays. A similar approach can be applied after booster. Such an approach will enable future studies to retrospectively identify previously infected individuals at a time in the pandemic where PCR tests are not always available and countries are moving away from mass testing and will otherwise never be able to determine whether or not individuals were infected. This could have implications for vaccine policy to determine who requires further doses and potentially down the line for long COVID-19 claims, where providing evidence of previous infection could be crucial.

Disclosures

Funding source

Ethics statement

Informed consent was obtained for all participants. The study was approved by Ziv Medical Centre's ethics committee (0133–20-ZIV).

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