Correlation Between Postvaccination Anti-Spike Antibody Titers and Protection Against Breakthrough Severe Acute Respiratory Syndrome Coronavirus 2 Infection: A Population-Based Longitudinal Study

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In this population-based cohort of 7538 adults, combined immunoglobulin (Ig) G, IgA, and IgM (IgG/A/M) anti-spike titers measured after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination were predictive of protection against breakthrough SARS-CoV-2 infection. Discrimination was significantly improved by adjustment for factors influencing risk of SARS-CoV-2 exposure, including household overcrowding, public transport use, and visits to indoor public places. Anti-spike IgG/A/M titers showed positive correlation with neutralizing antibody titers ($r_s = 0.80$ [95% confidence interval, .72–.86]; $P < .001$) and S peptide–stimulated interferon-γ concentrations ($r_s = 0.31$ [.13–.47]; $P < .001$).

Keywords. SARS-CoV-2; breakthrough infection; combined antibody response; vaccination.

Identifying a robust correlate of vaccine-induced protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could provide insights into mechanisms of protective immune responses and accelerate licensure for candidate SARS-CoV-2 vaccines [1]. Neutralizing antibody titers have been shown to be correlated with vaccine efficacy and risk of postvaccination SARS-CoV-2 infection [2, 3], but their

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determination is both resource and time intensive. Studies evaluating neutralizing antibody titers as potential correlates of protection have largely been conducted in clinical trial populations [2, 4, 5], limiting generalizability of their findings to the general population. Moreover, such trials do not routinely collect data on factors influencing postvaccination SARS-CoV-2 exposure, which precludes adjustment for these factors in statistical analyses.

In the current study, we explore the predictive strength of a new marker—combined immunoglobulin (Ig) G, IgA, and IgM responses to the SARS-CoV-2 trimeric spike glycoprotein (anti-spike IgG/A/M)—which can be assayed in dried blood spots (DBSs) at low cost. We hypothesized that anti-spike IgG/A/M titers would be positively correlated with authentic-virus neutralizing antibody titers, and that higher titers would therefore associate with reduced risk of breakthrough infection in vaccinated individuals.

METHODS

COVIDENCE UK is a prospective, longitudinal, population-based observational study of COVID-19 in the UK general population, launched on 1 May 2020 (https://www.qmul.ac.uk/covidence). Inclusion criteria were age ≥16 years and UK residence at enrollment, with no exclusion criteria. Participants were asked to complete online baseline and monthly follow-up questionnaires to capture information on SARS-CoV-2 vaccinations, incident SARS-CoV-2 infection, and potential determinants of vaccine response and SARS-CoV-2 exposure. Further details on COVIDENCE UK have been published elsewhere [6]. The study was approved by Leicester South Research Ethics Committee (ref 20/EM/0117) and is registered with ClinicalTrials.gov (NCT04330599). All participants provided informed consent.

Participants who had received a primary course of SARS-CoV-2 vaccination (ie, 2 vaccine doses or 1 dose of a single-dose regimen) were eligible to take part in a postvaccination antibody study. They were sent a kit containing instructions, lancets, and DBS collection cards, which were returned and analyzed at the Clinical Immunology Service, Institute of Immunology and Immunotherapy of the University of Birmingham (Birmingham, UK). Antibody titers in DBS eluates were determined using a commercially available enzyme-linked immunosorbent assay that detects anti-spike IgG/A/M with 98.3% specificity and 98.6% sensitivity (product code MK654; The Binding Site) [6–8]. A subset of participants also attended an in-person visit, providing blood samples that permitted the measurement of neutralizing antibody titers in serum using an authentic virus (Wuhan Hu-1 strain)

neutralization assay and determination of S peptide–stimulated interferon (IFN) γ concentrations [9] (see Supplementary Methods). Correlations between these responses were evaluated using the Spearman rank test.

Analysis of breakthrough infections included data from participants with a postvaccination anti-spike IgG/A/M titer, who had received either a ChAdOx1 nCoV-19 (Oxford-AstraZeneca; ChAdOx1) or BNT162b2 messenger RNA (Pfizer-BioNTech) primary vaccination course but not a booster dose. Breakthrough infections were defined as a reported positive result on a reverse-transcription polymerase chain reaction or lateral flow test for SARS-CoV-2. Participants were followed up from the date of their blood sample. Because duration of follow-up is a strong predictor of infection, we truncated follow-up at 18 weeks after sample provision; this duration was chosen to maximize the number of participants and infections included in the analysis.

We estimated the mean difference in titers between participants with or without breakthrough infection using the Student t test. Titers were log-transformed for all analyses, and results were converted to World Health Organization international standard units (see Supplementary Methods). After conversion, the lower limit of detection corresponded to 41 binding antibody units (BAU)/mL.

We used receiver operating characteristic (ROC) curve analysis to assess anti-spike IgG/A/M as a predictor of breakthrough infection, overall and stratified by vaccine type. We first included anti-spike IgG/A/M titer as a continuous predictor in a logistic regression model, adjusting for time between full vaccination and blood sample provision. We then adjusted for variables reflecting risk of SARS-CoV-2 exposure: sharing a home with schoolchildren or working-age adults, number of people per bedroom, and monthly behaviors recorded through breakthrough infection or the end of follow-up (number of weekly visits to the shops, to other indoor public spaces, on public transport, or to/from other households). Finally, we compared the area under the ROC curve between minimally and fully adjusted models and estimated a titer threshold associated with 80% protection against breakthrough infection.

We did 2 sensitivity analyses: first, excluding immunocompromised participants, and second, limiting inclusion to participants whose blood samples were provided 4–16 weeks after vaccination, to avoid periods of rapid change in anti-spike IgG/A/M. Statistical analyses were performed using Stata/MP (version 17.0) and GraphPad Prism (version 9.1.2) software.

RESULTS

The correlation analysis included 113 vaccinated participants with data on anti-spike IgG/A/M, neutralizing antibodies, and IFN-γ responses, of whom 71 (62.8%) were female and 107 (94.7%) were white; 79 participants (69.9%) had received ChAdOx1 and 34 (30.1%) had received BNT162b2. Samples were obtained a median of 57 days (interquartile range [IQR], 47–71 days) after full vaccination. Correlation was high between anti-spike IgG/A/M and neutralizing antibody titers but low between anti-spike IgG/A/M titers and IFN-γ concentrations (Figure 1). Results were similar when stratified by vaccine type (Supplementary Table 1).

The breakthrough infection analysis included 7538 vaccinated participants, 5348 (70.9%) of whom were female, with a median (IQR) age of 64.1 (57.4–69.7) years. Among these, 7296 participants (96.8%) were white, 73 (1.0%) were Asian, 25 (0.3%) were black, and 144 (1.9%) were of mixed or other ethnic groups, and 387 participants (5.1%) were immunocompromised. Baseline characteristics were similar to those reported previously for the COVIDENCE UK cohort [6]. Of the participants, 5039 (67.0%) had received ChAdOx1 and 2499 (33.0%) had received BNT162b2. Blood samples were provided a median (IQR) of 56 (43–68) days after full vaccination, and the subsequent 18-week follow-up ended a median of 22 (20–24) weeks after full vaccination. Anti-spike IgG/A/M titers were fairly stable until 18 weeks after vaccination, declining thereafter (Figure 1C).

Between 12 January 2021 and 14 March 2022, 291 participants (3.9%) reported a breakthrough SARS-CoV-2 infection during their 18-week follow-up (215 ChAdOx1 recipients [4.3%] and 76 BNT162b2 recipients [3.0%]), a median (IQR) of 86 (57–111) days after their blood sample and 142 (111–168) days after vaccination. Most infections occurred between July and November 2021, when the Delta variant was dominant (Supplementary Figure 1), and they were generally mild, with 6 participants (2%) requiring hospitalization. Participants reporting breakthrough infection had lower titers than those without breakthrough infection (mean [standard deviation], 177 [102] vs 222 [113] BAU/mL; mean difference, 55 BAU/mL [95% confidence interval (CI), 50–61 BAU/mL]; $P < .001$).

In minimally adjusted models, anti-spike IgG/A/M was modestly predictive of breakthrough infection (Figure 2). Adjustment for factors influencing SARS-CoV-2 exposure significantly improved discrimination of the models overall ($P < .001$) and by vaccine type (ChAdOx1, $P < .001$; BNT162b2, $P = .02$; Figure 2).

Overall, anti-spike IgG/A/M titers >103 BAU/mL were associated with protection against breakthrough infection over 18 weeks, with 80% (95% CI, 79%–81%) specificity and 24% (20%–30%) sensitivity and with performance differing by vaccine type (Supplementary Table 2). For ChAdOx1, titers >85 BAU/mL were associated with protection with 80% (95% CI, 79%–81%) specificity and 23% (17%–29%) sensitivity, whereas for BNT162b2, titers >250 BAU/mL were associated with protection with 80% (79%–82%) specificity and 28% (18%–39%) sensitivity. Exclusion of immunocompromised participants or restriction to those whose blood samples were obtained 4–16 weeks after vaccination did not substantially change our results (see Supplementary Results).
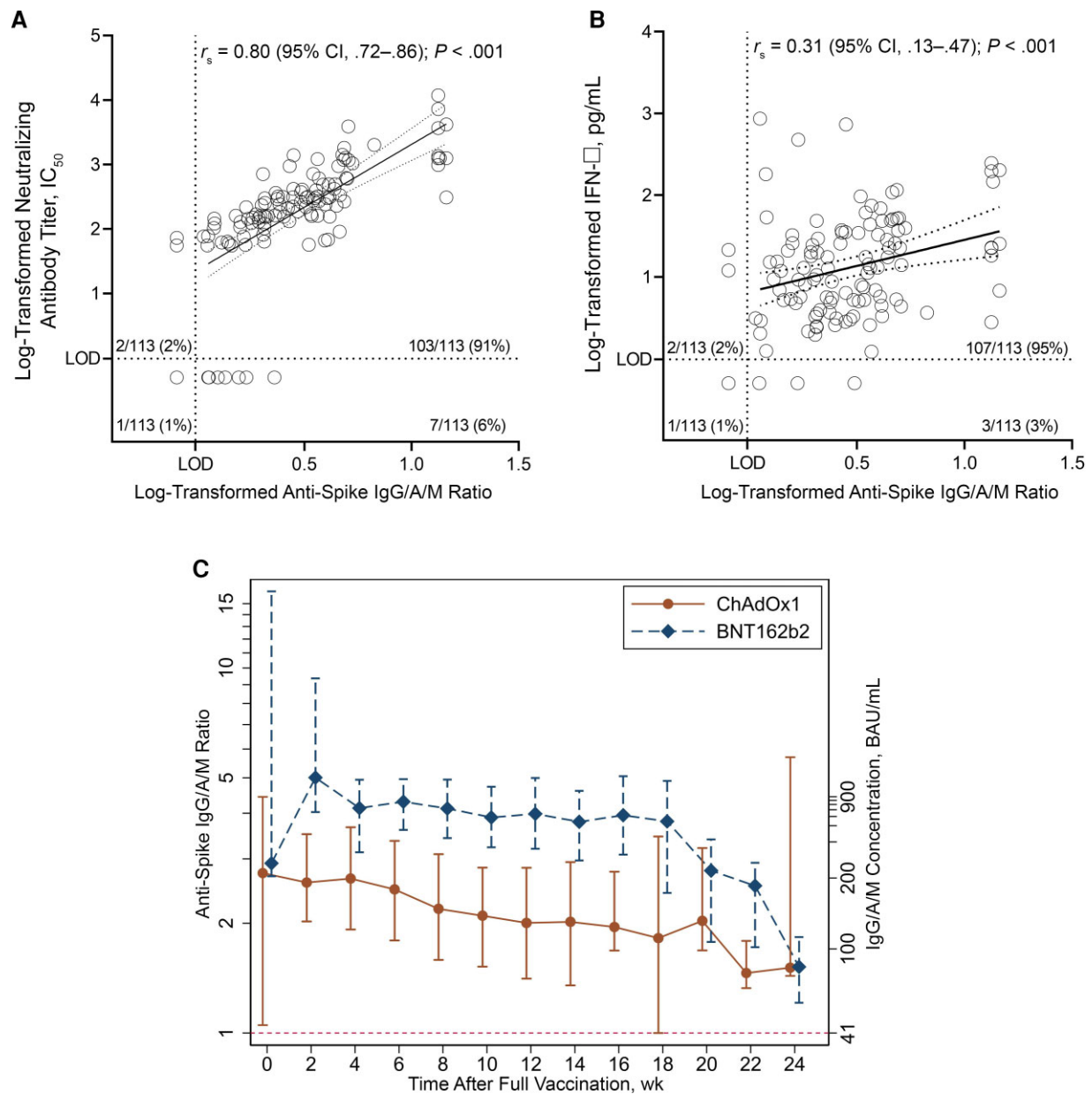
Figure 1. Correlation between combined anti-spike immunoglobulin (Ig) G, IgA, and IgM (IgG/A/M) and neutralizing antibody (half-maximal inhibitory concentration [IC_{50}]) titers (*A*) and anti-spike IgG/A/M and interferon (IFN) γ (*B*), and distribution of anti-spike IgG/A/M titers across the sampling period (*C*). *A*, *B*, Correlation analyses were performed in 113 participants who were fully vaccinated for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); distributions are shown with linear regression lines and 95% confidence intervals (CIs). *C*, Median anti-spike IgG/A/M titers measured at different time points after SAR-CoV-2 vaccination, with interquartile ranges. Horizontal dashed line presents the assay's limit of detection (LOD). Abbreviations: BAU, binding antibody units; ChAdOx1, ChAdOx1 nCoV-19.

DISCUSSION

In this analysis of a large population-based study, we show strong correlation between anti-spike IgG/A/M and neutralizing antibody titers and a weaker correlation between anti-spike IgG/A/M titers and IFN-γ responses. Anti-spike IgG/A/M was modestly predictive of breakthrough SARS-CoV-2 infection in the 5 months after full vaccination, with discrimination significantly improved by including key exposure variables, such as sharing a home with schoolchildren. Estimated thresholds of protection differed markedly by vaccine type, possibly reflecting differences in antibody dynamics [10] or distinct profiles of immunological response [1, 11], which may be differently correlated with anti-spike IgG/A/M titers.

The modest discrimination of anti-spike IgG/A/M for predicting infection, alongside the low sensitivity of the estimated thresholds of protection, casts into doubt their effectiveness as a stand-alone correlate of protection. Previous studies have shown strong correlations between postvaccination antibody
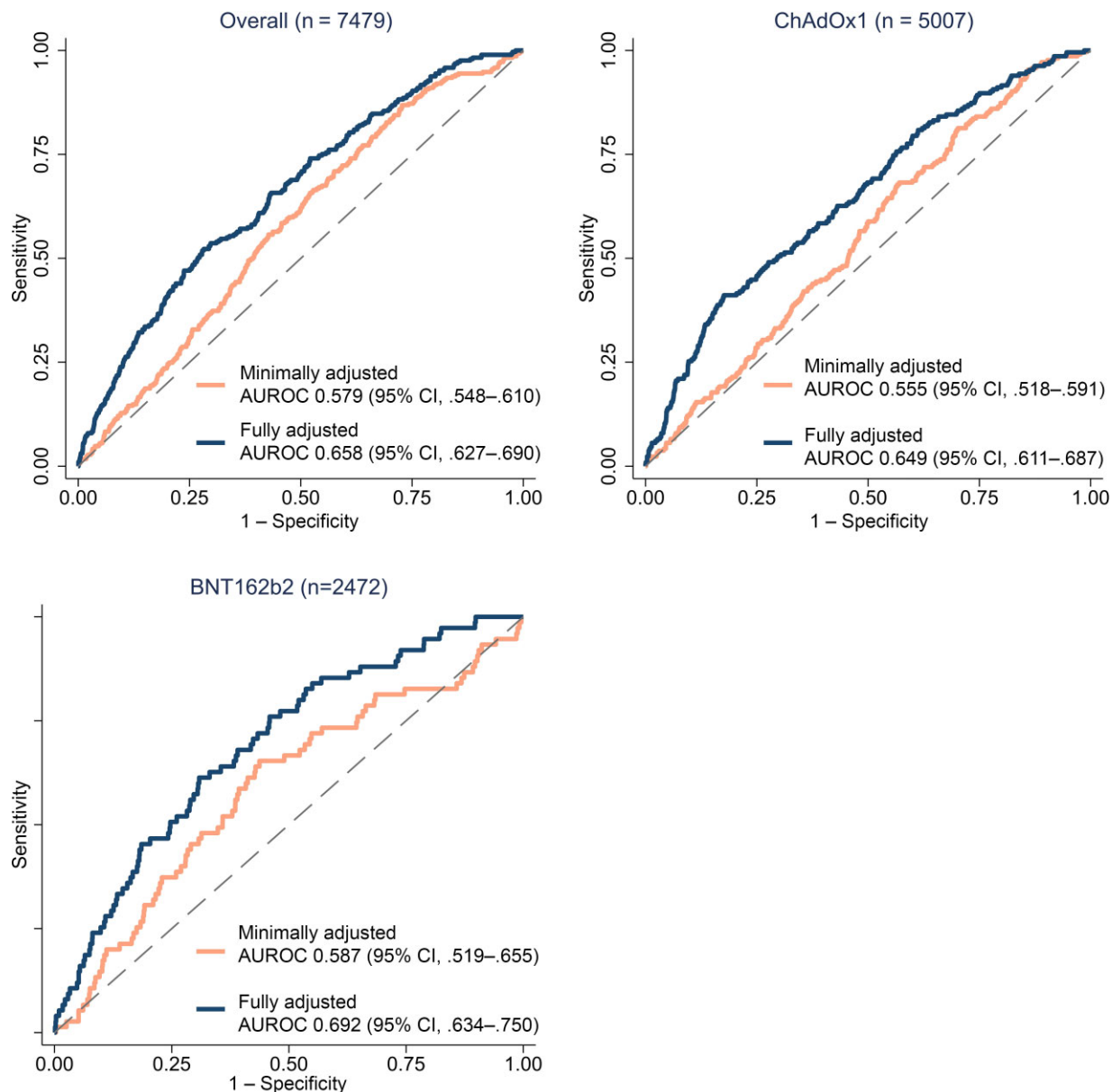
Figure 2. Receiver operating characteristic (ROC) curve analysis for breakthrough severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection after SARS-CoV-2 vaccination. ROC curves and area under the ROC curve (AUROC) for combined anti-spike immunoglobulin (Ig) G, IgA, and IgM (IgG/A/M) ratios as a predictor of breakthrough infection, alone or adjusted for variables reflecting SARS-CoV-2 exposure, for all participants with data for included covariates and by vaccine type. Abbreviations: ChAdOx1, ChAdOx1 nCoV-19; CI, confidence interval.

titers and vaccine efficacy [1, 2, 5], but anti-spike IgG–based thresholds of protection have varied substantially [1, 5, 10]. In addition, results are conflicting when stratified by vaccine type, with individual thresholds for ChAdOx1 and BNT162b2 found to be similar [10] or to differ markedly [1]. While our IgG/A/M-based estimates cannot be directly compared with those derived from IgG alone, the large variation observed between ChAdOx1 and BNT162b2 recipients supports different thresholds by vaccine type.

While previous studies have focused on IgG [1, 4, 5, 10], our study also captures the contributions of IgA and IgM to long-

term protection by using an assay that measures combined titers of all 3 antibody isotypes, thus accounting for variations in antibody distribution [8] and providing enhanced detection of immune response [7]. Although typically considered a short-term response, adaptive IgM has been shown to contribute to long-term protection against pathogen infections in mice [12]. Of specific relevance to our findings, higher IgM titers after SARS-CoV-2 vaccination have been found to associate with higher neutralizing activity [13], supporting the concept that IgM titers are a correlate of protection. However, the modest predictive ability of anti-spike IgG/A/M suggests that it misses

a key element of protection, such as cellular responses. This is supported by the weak correlation found between anti-spike IgG/A/M titers and IFN-γ concentrations, strengthening existing evidence [14] by including ChAdOx1 recipients. Although humoral and cellular responses are sometimes correlated, they can be discordant [15], suggesting that anti-spike IgG/A/M alone will not be sufficient to predict risk of postvaccination SARS-CoV-2 infection.

Among the strengths of this analysis is the population-based nature of the cohort. Previous studies on correlates of protection have largely relied on post hoc analyses of vaccine trials [2, 4, 5], with a wider range of inclusion and exclusion criteria. Our cohort provides a more general population, including participants with poor health, comorbid conditions, and immunosuppression. In addition, our monthly questionnaires permit detailed evaluation of exposure risks, capturing time-varying behaviours such as interactions with other households. Finally, we used a sensitive CE-marked, validated assay of combined IgG/A/M antibody responses, with results translated into World Health Organization international standard units.

Our study has several limitations. Our findings are restricted to ChAdOx1 and BNT162b2 recipients, although these vaccines are widely used and operate via different mechanisms. Small numbers of hospitalizations meant we lacked statistical power to evaluate anti-spike IgG/A/M as a predictor of protection from severe disease. The infections reported mostly occurred when the Delta variant was dominant, preventing us from evaluating the performance of the assay against the Omicron variant. However, we showed strong correlation with neutralizing antibodies against the ancestral strain, which can be used to predict neutralization against other variants [3]; in addition, the observed correlation with IFN-γ responses, while weak, suggests that the assay can serve as a marker of global immune response, which has continued relevance for protection against severe disease caused by new variants [4]. Our model relies on a single antibody measurement per individual, preventing incorporation of individual antibody dynamics. However, most measurements were made in a similar period (75% obtained 5–10 weeks after full vaccination), during which median antibody levels remained fairly stable; in addition, restricting analyses to samples provided 4–16 weeks after vaccination did not affect our results. As another limitation, our model does not include functional antibody measurements, which may have limited its predictive performance. However, the strong correlation with neutralizing antibody suggests that anti-spike IgG/A/M can indirectly capture functional antibody activity. Moreover, neutralizing antibodies are difficult and expensive to measure, and our aim was to evaluate the performance of a practical, cost-effective tool. Finally, the age and ethnicity distribution of our cohort may limit the generalizability of our results to younger people or populations of nonwhite ethnicity.

In conclusion, our combined measure of anti-spike IgG/A/M was only modestly predictive of breakthrough infection in vaccinated individuals, with performance significantly improved by incorporating factors reflecting postvaccination SARS-CoV-2 exposure. Combined with the poor correlation observed between anti-spike IgG/A/M and IFN-γ responses, these findings suggest that the limited predictive value of anti-spike IgG/A/M titers for protection may stem from their inability to reflect protective vaccine-induced cellular responses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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