The relationship between antibodies to wild-type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens and the risk of breakthrough infections is unclear, especially during circulation of the Omicron strain. We investigated the association of anti-spike and anti-receptor binding domain antibody levels and the risk of subsequent breakthrough coronavirus disease 2019 (COVID-19). We included adult paramedics from an observational cohort study who received ≥ 2 mRNA vaccines but did not have COVID-19 before the blood collection. Higher postvaccination antibody levels to wild-type SARS-CoV-2 antigens were associated with a reduced risk of COVID-19. Further research into clinical utility of antibody levels, to inform a threshold for protection and timing of boosters, should be prioritized.

**Keywords.** COVID-19; SARS-CoV-2; antibody levels; mRNA vaccine; Omicron.

Over 5.2 billion people (68% of the world’s population, as of 19 June 2022) have received at least 1 dose of a coronavirus disease 2019 (COVID-19) vaccine [1]. Although COVID-19 vaccines have been shown to be effective against the disease severity [2], questions remain regarding waning immunity, long-term durability of vaccine protection, and the need and frequency of booster doses.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody levels have been used extensively in the study of COVID-19 as a surrogate marker of immune protection. Although investigations of the mRNA-1273 [3], ChAdOx1 [4], and other [5] vaccines have demonstrated that antibody levels to wild-type antigens are associated with risk of subsequent wild-type COVID-19, further research is required to define the relationship between antibody levels and immunity, including any potential roles in clinical care (such as individualized booster vaccination schedules).

Further complicating questions of long-term vaccine protection is the emergence of immune-evasive SARS-CoV-2 variants of concern, particularly the Omicron strain, the rise of which has been associated with an increase in breakthrough infections. The relative contribution of waning immunity versus changing SARS-CoV-2 phenotypes to breakthrough infections is unclear. Multiple studies have demonstrated waning of SARS-CoV-2 antibodies after vaccination [6, 7]. However, whether these waning antibody levels, which were developed to bind to wild-type antigens, are indicative of declining immune protection in an era of Omicron or other SARS-CoV-2 variants is uncertain.

For these reasons, we sought to determine whether quantitative antibody levels against wild-type spike antigens are associated with the risk of SARS-CoV-2 breakthrough infections. As the SARS-CoV-2 virus underwent a formative evolution with the Omicron variant, we separately examined this relationship in the pre-Omicron and Omicron eras, to determine if this relationship differed between the time periods.

**METHODS**

**Study Setting and Design**

We used samples from the observational prospective cohort study, COVID-19 Occupational Risks, Seroprevalence and Immunity among Paramedics in Canada (CORSIP; approved by the universities of British Columbia and Toronto), which enrolled adult paramedics (starting in January 2021) in Canada. Participants provided blood samples and questionnaires every 6 months and documented positive COVID-19 tests (polymerase chain reaction [PCR] and rapid antigen tests) and vaccinations through an online portal [8]. Omicron-variant SARS-CoV-2 was identified in Canada on 28 November 2021; by late December 2021, the Omicron variant accounted for >95% of Canadian COVID-19 cases [9].

**Selection of Participants**

We separately selected samples to examine 2 groups: pre-Omicron (examining COVID-19 cases diagnosed up to 30 November 2021) and Omicron-era (examining COVID-19 cases diagnosed 26 December 2021–31 March 2022, inclusive).
Table 1. Baseline Characteristics of Study Participants

| Variables                        | Pre-Omicron era | Omicron era |
|----------------------------------|-----------------|-------------|
|                                 | n = 523         | n = 579     |
| Male sex at birth, n (%)         | 282 (54)        | 313 (54)    |
| Age, y, mean (SD)                | 41 (10)         | 40 (10)     |
| Vaccine type, n (%)              |                 |             |
| BNT162b2                         | 405 (77)        | 461 (80)    |
| mRNA-1273                        | 118 (23)        | 118 (20)    |
| Doses, n (%)                     |                 |             |
| 1st and 2nd dose BNT162b2        | 405 (77)        | 461 (80)    |
| 1st and 2nd doses mRNA-1273      | 118 (23)        | 118 (20)    |
| 3rd dose BNT162b2                | ...             | 404 (70)    |
| 3rd dose mRNA-1273               | ...             | 100 (17)    |
| Influenza vaccine, n (%)         | 417 (80)        | 474 (82)    |
| V1-to-V2 dosing interval, d, median (IQR) | 36 (28-42) | 36 (28-42) |
| V2-to-V3 dosing interval, d, median (IQR) | —               | 272 (256-291) |
| V1-to-BC, d, median (IQR)        | 109 (78-187)    | 367 (358-375) |
| Last vaccination-to-BC date, d, median (IQR) | 74 (38-139) | 64 (45-82) |
| Actual BC date, median (IQR)     | 5 May 2021      | 14 Jan 2022 |
|                                 | (1 Mar 2021–20 Jul 2021) | (5 Jan 2022–25 Jan 2022) |
| Medical history, n (%)           |                 |             |
| Hypertension                     | 50 (10)         | 52 (9.0)    |
| Diabetes                         | 11 (2.1)        | 11 (1.9)    |
| Asthma                           | 78 (15)         | 78 (13.5)   |
| Chronic lung disease             | 4 (0.8)         | 4.0 (0.7)   |
| Heart disease                    | 3 (0.6)         | 4.0 (0.7)   |
| Kidney disease                   | 3 (0.6)         | 3.0 (0.50)  |
| Liver disease                    | 9 (1.7)         | 9.0 (1.6)   |
| Cancer                           | 12 (2.3)        | 17 (2.9)    |
| COVID-19 infection, n (%)        | 8 (1.5)         | 73 (13)     |
| Antibody concentration, gM (gSD) |                 |             |
| V-PLEX spike IgGa*               | 74 034 (3.6)    | 123 217 (4.2) |
| V-PLEX RBD IgGa*                 | 47 888 (4.2)    | 74 992 (4.7) |
| Elecsys total spike antibodyb    | 2515 (4.2)      | 15 409 (4.0) |
| BC to COVID-19 interval, d (IQR) | 240 (163–270)   | 76 (65–85)  |

Abbreviations: BC, blood sampling date after 2 vaccinations; gM, geometric mean; gSD, geometric standard deviation; IgG, immunoglobulin G; IQR, interquartile range; V1, first vaccination; V2, second vaccination; V3, booster (third) vaccination.

aMeasured in arbitrary units per mL (AU/mL).
bMeasured in units per mL (IU/mL).

Serological Testing

All samples were tested with (1) Elecsys Anti-SARS-CoV-2 nucleocapsid assay (Roche) to confirm eligibility; (2) the quantitative Roche Elecsys Anti-SARS-CoV-2 assay (Roche) for measuring spike total antibody concentrations; and (3) the Meso Scale Discovery V-PLEX COVID-19 Coronavirus Panel 2 IgG assay for measuring immunoglobulin G (IgG) to spike and receptor-binding domain (RBD) antigens.

Outcome Variable

The primary outcome was the interval (measured in days) from the blood sample collection date to COVID-19 diagnosis (identified by a positive PCR or rapid antigen test) date. For the pre-Omicron group we only considered COVID-19 diagnoses up to 30 November 2021, and for the Omicron-era group we only considered COVID-19 diagnoses between 26 December 2021 and 31 March 2022, inclusive.

Statistical Analysis

We described patient characteristics using counts (with percentages) for categorical variables and means (with standard deviations), median (with interquartile range [IQR]), and geometric mean (with geometric standard deviations) for the continuous variables. We plotted Kaplan-Meier curves, showing the accrual of COVID-19 infections over time in the 2 cohorts. We also plotted adjusted Kaplan-Meier curves adjusted for sex at birth, age, type of mRNA vaccine administered, the vaccine dosing interval (the number of days between the first and second vaccination), influenza vaccination, and medical history of participants (diabetes, hypertension, cancer, kidney diseases, chronic liver disease, asthma, and chronic lung disease).

Multiple previous studies have demonstrated that SARS-CoV-2 antibody levels decline over time after vaccination [6, 7], and thus antibody level decay needs to be accounted for when examining the relationship between levels (measured at a single time-point) and future outcomes. For this reason, we fit a multivariate Cox proportional hazard model to investigate the association between antibody levels and the primary outcome (time to COVID-19 diagnosis) based on the estimated adjusted hazard ratios (aHR). The estimated aHR ranged from 0 to infinity, where aHR < 1 signifies that higher levels
of antibodies were associated with reduced risk of COVID-19. Also, aHR > 1 signifies that increasing antibody levels increases the risk of COVID-19, while aHR = 1 indicates that there is no association between antibody concentration and risk of COVID-19. We adjusted for important covariates in the Cox proportional hazard model as well as the plotted Kaplan-Meier curves.

RESULTS

A total of 523 adult participants (mean age 41 years; 282 [54%] reporting male sex at birth) were included in the pre-Omicron cohort, and 579 participants (mean age 40 years; 313 [54%] reporting male sex at birth) were included in the Omicron-era cohort. The majority of participants (77% pre-Omicron, 80% Omicron era) received 2 doses of BNT162b2 vaccine, while the remaining were vaccinated with the mRNA-1273 vaccine; 404 (70%) of the Omicron-era cohort also received a booster dose. Antibody concentrations in the pre-Omicron and Omicron-era groups are shown in Table 1.

Of participants in the pre-Omicron group, 8 (1.5%) were diagnosed with COVID-19. The median observation period from the blood sample collection to COVID-19 or end of observation was 240 days (IQR, 163–270 days).

For participants in the Omicron-era cohort, 73 (13%) were diagnosed with COVID-19. The median observation period from blood sample collection to COVID-19 or end of observation, was 76 days (IQR, 65–85 days).

The Kaplan-Meier curves in Supplementary Figures 1–4 demonstrate the time from the blood sample collection until time of COVID-19 diagnosis in the pre-Omicron and Omicron-era groups. Results of the multivariate Cox proportional hazard model are shown in Table 2. In the pre-Omicron cohort, higher total anti-spike antibody concentrations were associated with a 34% (adjusted hazard ratio [aHR], 0.66; 95% confidence interval [95% CI], .46–.93) reduced risk of COVID-19 diagnosis. Similarly, higher anti-spike IgG and anti-RBD IgG concentrations were associated with a 35% (aHR, 0.65; 95% CI, .44–.97) and 37% (aHR, 0.63; 95% CI, .41–.98) reduced risk of COVID-19, respectively.

In the Omicron-era cohort, higher total anti-spike antibody concentrations were associated with a 17% (aHR, 0.83; 95% CI, .68–.99) reduced risk of COVID-19 diagnosis. Similarly, higher anti-spike IgG and anti-RBD IgG concentrations were associated with a 20% (aHR 0.80; 95% CI, .65–.98) and 21% (aHR, 0.79; 95% CI, .65–.96) reduced risk of COVID-19, respectively.

DISCUSSION

We investigated the association between quantitative antibody levels among over 1000 adult paramedics who were vaccinated with at least 2 doses of mRNA vaccine during the pre-Omicron and Omicron eras of the COVID-19 pandemic. We found that, in both time periods, lower antibody levels to wild-type strain SARS-CoV-2 antigens were associated with increased risk of breakthrough SARS-CoV-2 infections. Although confidence intervals of these associations overlapped, results suggest that the association was stronger in the pre-Omicron era.

We compared samples collected in the pre-Omicron and Omicron eras. While the last vaccination-to-blood sample collection interval was similar between groups, antibody levels appeared higher in Omicron-era samples. This may be explained by the third vaccine dose that the majority of the Omicron-era participants had received before the blood sample collection. Despite higher antibody levels in the Omicron-era cohort, there was a higher COVID-19 incidence during the Omicron period. Furthermore, while the Cox proportional hazard model showed a significant association between increasing postvaccination antibody concentration levels and reduced risk of COVID-19 infection across participants in both cohorts, the association of antibody levels and COVID-19 risk appeared mitigated during the Omicron wave. Hypotheses for increased breakthrough infections against the Omicron variant could include the highly infectious nature and changing phenotype of the omicron variant, or antibody waning after vaccination; however, our data indicating higher antibody levels in the Omicron cohort suggests against the latter. We cannot also disregard that a lack of sterilizing immunity after vaccination and COVID-19 exhaustion did not create an environment enabling higher rates of infection. Our data are consistent with other data indicating decreased effectiveness of vaccines developed against the wild-type strain [10].

Antibody levels have been used as surrogate measures of immune protection in numerous SARS-CoV-2 investigations. However, questions remain regarding the clinical utility of these measurements. Thus, determining an easily calculated

Table 2. Multivariate Analysis of Association Between Antibody Levels and Risk of COVID-19 Infection

| Era            | Model | Exposure Variables | aHR (95% CI) | P Value |
|----------------|-------|--------------------|--------------|---------|
| Pre-Omicron era | Model 1 | Elecsys total spike | 0.66 (.46–.93) | .017    |
|                | Model 2 | VPLEX spike IgG    | 0.65 (.44–.97) | .035    |
|                | Model 3 | VPLEX RBD IgG      | 0.63 (.41–.98) | .041    |
| Omicron era    | Model 1 | Elecsys total spike | 0.83 (.68–.99) | .045    |
|                | Model 2 | VPLEX spike IgG    | 0.80 (.65–.98) | .037    |
|                | Model 3 | VPLEX RBD IgG      | 0.79 (.65–.96) | .020    |

Adjusted for male sex, current age, vaccine type (BNT162b2 vs mRNA-1273), dosing interval (days) between 1st and 2nd vaccination, influenza vaccination, and medical history in all the 3 models for participants in pre-Omicron era cohort. Adjusted for male sex, current age, vaccine type (BNT162b2 vs mRNA-1273), dosing intervals (days) between 1st and 2nd vaccination and 2nd vaccination to 3rd vaccination, influenza vaccination, and medical history in all the 3 models for participants in Omicron era cohort. Participants medical history included hypertension, diabetes, asthma, chronic lung diseases, heart diseases, chronic kidney diseases, liver related diseases, and cancer.

Abbreviations: aHR, adjusted hazard ratio, which measures the effect of the antibody levels (continuous variable) on risk of time to COVID-19 diagnosis; 95% CI, 95% confidence interval of aHR, outcome variable = COVID-19 infection.
threshold value for antibody levels indicating adequate protection would be helpful for patient care. Our data demonstrate a relationship between antibody levels and future COVID-19 risk, which provides a rationale for using antibody levels. However, this is complicated by 2 factors: (1) postvaccination exponential waning of antibody levels, requiring complex predictive modeling of future levels to assess protection; and (2) high variability between patients with regards to antibody response postvaccination (even after the same number of vaccinations and time since last vaccination). If models could be developed and threshold values for protection established, these may assist with determining the optimal timing of subsequent booster doses. Evidence shows that lack of vaccination contributes to increased risk of infection and reinfection with COVID-19 compared to individuals with previous SARS-CoV-2 infections [11]. Thus, achieving full mRNA vaccination against COVID-19 is important to prevent COVID-19 infections and reinfections. Several jurisdictions have administered booster doses to health care workers and the general population before and during the emergence of omicron variant to provide maximum protection against disease severity. However, evidence of waning immunity within 6 months against Omicron has been predicted [12]. Therefore, scientist and researchers should prioritize identifying the minimum antibody level that increases risk of COVID-19 infections to recommend for a booster vaccination.

Our study corroborated with some existing studies that investigated the association between antibody levels and COVID-19 risk. Bergwerk et al reported that higher peri-infection neutralizing antibody titers were associated with lower infectivity [13]. Khoury and colleagues showed that neutralizing antibody levels are highly predictive of immune protection from the wild-type strain COVID-19 [14].

This was an observational study, which limits conclusions on associations. Our study benefited from a relatively homogenous group of middle-aged paramedics. Participant characteristics, in terms of age, comorbidities, sex distribution, and vaccine types, were similar between cohorts. In addition, whereas there is likely wide variation in COVID-19 exposure between individuals in society, paramedics have routine and frequent occupational exposure with sick individuals. However, we could not account for personal activities of participants, which may have affected risk of COVID-19. There may have been unmeasured confounders that we did not account for. Also, the nonavailability of data on isolation, mask mandate, and lockdown could have affected the study to some extent, resulting in a possible residual confounding. We may have missed asymptomatic COVID-19 or infections that were not diagnosed. Although the Omicron variant became the dominant strain in Canada in a short time frame, our assumption that all cases in the Omicron time period were Omicron, and vice versa, may have caused misclassification in our study.

**CONCLUSION**

Higher postvaccination SARS-CoV-2 antibody titers were associated with a significantly reduced risk of COVID-19. However, this association tended to decrease during the Omicron variant era.

**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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