**Protective effectiveness of SARS-CoV-2 infection risk between hybrid and vaccine-induced immunity against the omicron variant**

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**Abstract (344 words)**

**Background.** Immunogenicity research indicates that the effectiveness of vaccines might decline over time, although it is not clear how this reduction translates to protective effectiveness against breakthrough infections. We examined the antibody decay trend and associated infection risks across age groups between hybrid and vaccine-induced immune populations based on community-based nationwide surveillance after the Omicron wave in South Korea.

**Methods:** We categorized the population's immune status into hybrid and vaccine groups based on serology (S and N status) information. To address potential imbalances in the case-control data and to control for confounding variables, we implemented the nested case-control method between cases and controls. We then performed Kaplan-Meier survival analyses and Cox proportional hazards regression to compare the protective efficacy of the two primary immunity groups, focusing on the probability of remaining free from infection after the initial surveillance period. We used mixed-effects logistic regression models to quantify the protective efficacy, defined as the probability of remaining infection-free, associated with antibody levels across age subgroups by the immunity groups.

**Results:** We showed that the antibody waning and infection risk trends are highly correlated. While the antibody decay was clearly observed after 6th month from the last immunological events in both groups, the hybrid group maintained about 2 times higher antibody levels at 12 months compared to the vaccine group. In terms of age subgroups, the <20 age group shows the greatest infection risk, following the>60, 40-60, and 20-40 age groups in the vaccine group. Although the antibody decay trend was similar across age groups, the protection effectiveness against infection was more quickly decreased for the>60 age group but in a greater magnitude for the <20 group over time in the vaccine group. Our results show that S antibody levels greater than 250,000, 20,000, and 15,000 for <20, >60, and 40-60 age groups are associated with >80% probability of remaining infection-free.

**Conclusion**: The different infection risks across age groups reflect heterogeneous individual immune responses and levels of exposure. The vaccination campaigns should be tailored according to individual immune status, and characteristics of the population risk factors to achieve optimal protection across individuals.

**Introduction**

Both natural SARS-CoV-2 infection and COVID-19 vaccination play crucial roles in developing population-level immunity. [REF] While immune responses are complex and multifaceted, epidemiological studies often rely on measurable indicators like antibodies to assess population immunity and the durability of antibody responses following infection and/or vaccination. [REF] Immunogenicity research indicates that the effectiveness of vaccines might decline over time, as measured by primarily decreasing antibody titers and vaccine effectiveness (breakthrough infections) [1], [2]. However, it is not clear how this reduction translates to protective effectiveness against infection. [REF] Moreover, estimating the magnitude and durability of this protection in the population has become a challenge because of the surge in the omicron (B.1.1.529) variant, which has resulted in many individuals with hybrid immunity (immunity developed through a combination of SARS-CoV-2 infection and vaccination), varying rates and timings of past infection and vaccination, multiple types of vaccination and numbers of doses, and variants of concern that can escape pre-existing immunity [3]. Moreover, most existing seroepidemiological studies have focused on specific groups (e.g., healthcare workers or specific high-risk immune-suppressed patients), and the durability of antibody responses after infection and/or vaccination is less well explored within the general population [Ref].

 Epidemiological studies show a benefit of hybrid immunity over vaccine- or infection-induced immunity[9](https://www.nature.com/articles/s41598-023-45718-8#ref-CR9),[16](https://www.nature.com/articles/s41598-023-45718-8#ref-CR16),[17](https://www.nature.com/articles/s41598-023-45718-8#ref-CR17).

These findings are substantiated by immunological data showing high levels of antibodies and neutralization in vaccinated persons with a history of infection prior to vaccination[15](https://www.nature.com/articles/s41598-023-45718-8#ref-CR15). The level and duration of boosting by infections in vaccinated persons is largely unknown, as well as the factors influencing the response.

Gaps-- Waning of hybrid immunity, particularly due to Omicron infections is not yet characterized in magnitude or duration. More data are needed for a precise quantification of the immune protection from hybrid immunity compared with vaccine-induced immunity normalised for the same antigen exposure.

We argue that, rather than trying to tease apart the contributions of factors such as age, viral variants and time since vaccination, the rates of breakthrough infection are best seen as a consequence of the level of immunity at any moment in an individual, the variant to which that individual is exposed and the severity of disease being considered. We also address key open questions concerning the transition to endemicity, the potential need for altered vaccine formulations to track viral variants, the need to identify immune correlates of protection, and the public health challenges of using various tools to counter breakthrough infections, including boosters in an era of global vaccine shortages.

Systematic reviews of SARS-CoV-2 vaccine effectiveness studies estimated the durability of protection conferred by previous infection combined with previous vaccination (i.e., hybrid immunity) and previous infection alone against multiple clinical outcomes of SARS-CoV-2 infection caused by the omicron variant [4] [4]. They found that both previous infection alone and hybrid immunity conferred rapidly waning protection against SARS-CoV-2 infection with the omicron variant but high and sustained protection against hospital admission or severe disease due to the omicron variant. The study also determined that previous infection was found to provide higher protection against reinfection and more sustained protection against hospital admission or severe disease than vaccination alone. Such a pattern might be explained by natural infection invoking a more diverse immune response to multiple antigenic sites on the virus compared to the immunity developed through vaccines that target only spike antigens. Overall, individuals with hybrid immunity had the highest magnitude and durability of protection against all outcomes, emphasizing the importance of providing vaccination to previously infected individuals. Infection-induced protection against reinfection wanes rapidly, and vaccination increases durability. [REF]

In South Korea, a large majority of the population has either hybrid or vaccine-induced immunity, with only 8% having previous infection alone and 10% with no immunity. In this study, we directly compared anti-S levels and the relative incidence of breakthrough infections between groups with hybrid immunity and those with vaccine-induced immunity, given that neutralizing antibodies are a subset of spike (S) antibodies. We utilized data from a nationwide community-based cohort, allowing for large-scale analysis across various age subgroups. This cohort enabled us to conduct follow-up assessments with repeated antibody testing in August and December 2022, alongside detailed demographic and clinical characterization. The objectives of this research are threefold: First, we assessed the variation in anti-S antibody waning following the last immunological event across different age subgroups, comparing hybrid immunity with vaccine-induced immunity. Second, we evaluated the protective efficacy of these two groups over time, specifically focusing on the probability of remaining free from COVID-19 infection. Third, we examined the overall impact of anti-S antibody levels on the probability of remaining infection-free, comparing the effects across age groups between hybrid and vaccine-induced immunity cohorts.

Here we assess serological immune response after SARS-CoV-2 breakthrough infection in persons with primary and booster vaccination with and without previous infection. First, we determine the sensitivity of N-antibodies as a tool to identify breakthrough infection. Subsequently, we investigate the boosting of Spike S1-specific responses after breakthrough infection and the influence of time since vaccination and N-seroconversion on the S1-specific antibody levels. The study was performed during the transition period of the Delta variant to Omicron[9](https://www.nature.com/articles/s41598-023-45718-8#ref-CR9), which provided a unique basis to relate immune activation to the virus strain involved. Therefore, lastly, we investigate the change in the response towards the variant of infection as an indication for the development of broader hybrid immunity.

# **Methods**

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## **Sample Collection and Definitions.** A nationwide community-based surveillance study was conducted from August to December 2022, during the 6th and 7th epidemic peaks of COVID-19 in South Korea. This study involved 258 public health centers and 113 private medical facilities across 259 cities and counties. A representative sample of 10,000 individuals aged 5 and older was randomly selected from 5,000 households. Data collection was conducted through one-on-one interviews by trained surveyors using tablets to ensure accuracy. More detailed descriptions of the cohort study design and data collection methods are published elsewhere (REF).

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Figure 1: Flow Chart Illustrating the Cohort Study Design

**Inclusion/Exclusion and matching criteria.** In this analysis, we defined the cohort by the following four distinct immunity types: hybrid-induced immunity (*S*+*N* +), vaccine-induced immunity (*S*+*N−*), infection-induced immunity (*S−N* +), and naive (*S−N−*) [5], [6]. Notably, the hybrid-induced and vaccine-induced immunity groups comprised over 98% of the cohort population (Figure 1). Consequently, subsequent research focused primarily on these two groups. The demographic and clinical characteristics of the two groups are described in Table 1.

We analyzed two primary outcomes: S antibody levels at the first and second surveillance points (Figure 2) and the occurrence of infection events between these points (Figure 3). To ensure the validity of our assessment of S antibody levels on COVID-19 protection (Figure 3), we implemented rigorous exclusion criteria. Individuals not participating in the second survey and those vaccinated between surveillance periods were excluded to maintain the relevance of initial S antibody levels. We employed a nested case-control (NCC) design [7], [8], matching cases and controls by age and S antibody levels while treating other characteristics as potential confounders. This approach ensured robust comparisons and controlled for confounding variables in the logistic regression model.

**Statistical analyses**. We began by conducting descriptive analyses to generate trajectory plots that track the average changes in S antibody levels across different immunity groups and age subgroups following immunological events. This allowed us to visualize the dynamic changes in antibody levels over time. To address potential imbalances in the case-control data and to control for confounding variables, we implemented the nested case-control (NCC) method using the R package ‘Epi’ [9], adjusting the matching ratio from 1:6 to 1:3 between cases and controls. This adjustment was made based on the recommendations of Pearce [8] to optimize the balance between statistical power and practical feasibility. Following this, we performed Kaplan-Meier survival analyses to compare the protective efficacy of the two primary immunity groups, focusing on the probability of remaining free from COVID-19 infection after the initial surveillance period. The outcome within each group was defined as the infection status during the first and second surveillance periods. Furthermore, we employed Cox proportional hazards regression to investigate the effects of various variables on survival time, adjusting for potential confounders to provide a more comprehensive understanding of the associated risk factors. Finally, mixed-effects logistic regression models were used to evaluate the protective efficacy, defined as the probability of remaining infection-free, associated with S antibody levels across different immunity and age subgroups. This comprehensive approach allowed us to rigorously assess the impact of S antibody levels on COVID-19 protection across diverse populations.

Determinant of infection : logistic regression

Risk over time : survival curve and cox proportional hazard

Probability of protective effectiveness: mixed effect regression model

**Results****.**

In terms of population characteristics, the hybrid group has a younger age distribution than the vaccine group. Most hybrid (78%) and vaccine (86%) groups completed at least 3rd booster vaccines. The hybrid group had the latest event (infection/vaccine) more recently than the vaccine group. (77% had the latest immunological event within 6 months for the hybrid group vs 42% for the vaccine group). The hybrid group likely took the 4th booster less than the vaccine group (21% vs 36% -- perhaps due to the recent infection that they had).

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Table 1: Summary table of cohort study design

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Figure 2: Trajectory plot for different age groups

The trajectory plots of S antibody levels indicate that individuals in the hybrid-induced immunity group exhibit higher overall S antibody levels compared to those in the vaccine-induced group. Specifically, the hybrid group is able to maintain elevated S antibody levels (exceeding 10,000) for up to one year, whereas the S antibody levels in the vaccine-induced group decline below 5,000 within six months post-vaccination. Within the hybrid group, S antibody levels are slightly lower in individuals under 20 and over 60 compared to those aged 20-60. In contrast, there is no significant difference across age groups within the vaccine-induced group.

The S antibody decay after 6 months is about 2 times greater for the hybrid group compared to the vaccine group and remains relatively constant by and after 1 year; this translated into the infection events survival reduced by about 10% by 6 months, but 40-50% by 12 months. Together, this translated into the risk probability by S antibody for vaccine group is 10-45% lower than hybrid group across age subgroups.

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Figure 3: Survival Plot for Hybrid-induced and Vaccine-induced Groups

The Kaplan-Meier survival analysis (Figure 3) demonstrates the survival probabilities, interpreted as the likelihood of remaining free from COVID-19 infection, over time for populations with vaccine-induced and hybrid-induced immunity. The analysis reveals substantial differences in survival probabilities between these two types of immunity across various age groups. Individuals with hybrid-induced immunity consistently exhibit higher survival probabilities, maintaining a rate of 98% over one year, compared to those with vaccine-induced immunity, whose probabilities decrease to 50-60% over the same period. Antibody decay is notably observed after the sixth month following the last immunological event for the vaccine group, with a more rapid decline in those over 60 and a greater magnitude of decline in those under 20 compared to the hybrid group.

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Table 2: Cox Proportional Hazards Regression Results

The hazard ratio (exp(coef)) for hybrid group is significantly low as 0.09, indicating hybrid-induced immunity reduces the risk to approximately 9% of that for vaccine-induced immunity. For the, a hazard ratio of age groups>20 show 42-69% lower risk of infection than age <20 group with increasing risk trends with older age groups.

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Figure 4: Regression line comparison for vaccine-induced (left) and hybrid-induced (right) groups

Our results show that S antibody levels greater than 250,000, 20,000, and 15,000 for <20, >60, and 40-60 age groups are associated with >80% probability of remaining infection-free in the vaccine group. However, the hybrid group maintains over 95% of remaining infection-free regardless of the S antibody levels and age groups, similar to the results of the survival analyses.

# **Discussion**

Our study examined the protective effectiveness of COVID-19 infection across age groups between hybrid and vaccine-induced immunity in the general population based on community-based nationwide surveillance. In this study, we utilized nationwide community-based surveillance data (large sample sizes and wide age spectrums) from South Ko- rea, involving 10,000 participants, with detailed demographic information and serological assessment twice after an epidemic wave of omicron variants.

Children without previous infection is the high risk and priority group for booster vaccine.

Our study highlights the younger and older vaccine immunity groups are the potential risk groups especially after 6 months, while these number may decrease over time. While our study shows the hybrid group maintains high level of immunity across age more than a year, understanding the potential risk factor of this group is increasingly important as this group is increasing with ongoing epidemics.

In the hybrid group, except for gender, no clear and significant risk factors for infection (may be due to small events) Interestingly, we found that the hybrid 3rd or 4th booster showed a higher risk than the hybrid 2nd primary group; the counterintuitive result may be due to the different types of latest events between those with the 4th booster and 2nd primary vaccines (as vaccine vs infection). 🡪 Within the hybrid group, the protection level may significantly differ by the type of latest event and time since the latest event as well.  The ability of older adults to mount comparable responses, and the importance of the interplay between natural infection and vaccination in this group, remain less clear. While we recognise these associations, the small number of cases precludes any further statistical analysis on these particular observations but highlights key areas for investigation going forward.

It is vital that we better understand the functional immune response to natural infection and vaccination in this high-risk population in order to inform targeted public health policy and vaccine strategy, including identification of priority groups for vaccine booster(s).

Where breakthrough infections did occur, this was more likely to have occurred in individuals with significantly reduced neutralising activity (Fig. [4](https://www.nature.com/articles/s43856-023-00303-y#Fig4)). Higher antibody levels alongside less frequent and less severe downstream SARS-CoV-2 infections have also been observed with younger adults[13](https://www.nature.com/articles/s43856-023-00303-y#ref-CR13),[28](https://www.nature.com/articles/s43856-023-00303-y#ref-CR28), and it is encouraging to note similar responses in this vulnerable group especially as recent systematic reviews have commented on inability to reliably control for age[13](https://www.nature.com/articles/s43856-023-00303-y#ref-CR13). Indeed, Bobrowitz and colleagues comment directly on the lack of data specific to older adults and caution extrapolation of findings in younger adults to guide booster vaccine policy for this group until more data is available[13](https://www.nature.com/articles/s43856-023-00303-y#ref-CR13),[14](https://www.nature.com/articles/s43856-023-00303-y#ref-CR14). How emerging variants of concern, with potential capacity for immune escape, may affect these findings is yet unclear. In the meantime, these findings highlight the vital importance of delivering vaccines to older adults, including those with prior infection, and particularly in communities challenged by limited access.

In our study, the immunity of hybrid is very high across all ages due in part to the latest infection during the omicron and majority of them had at least 3rd vaccine, resulting very small infection cases in this groups. Compared to other study, the hybrid groups show more heterogeneous patterns depending on

Our study shows that children and adolescent who have never been infected before but only vaccinated can be the highest risk. However,

Omicron is very transmissive to those who are only vaccinated with lower S ab titer (young and old age groups).

Hybrid group—by vaccine type, timing, infection sequence,

The study time period is after omicron wave so the event numbers are small.

Our result also shows that with high young age infection, hybrid group become more younger distribution than vaccine group.

Fourth, because no deaths and few hospitalisations due to COVID-19 were reported during the study period, we could not evaluate hybrid immunity against severe disease.

Increasing heterogeneity within the hybrid and vaccine groups. – in terms of vaccine types, does, infection sequence etc.

The hybrid effectiveness estimates were derived without considering the order of immunological events, specifically whether vaccination occurred before or after previous infection. This distinction could not be made due to small group sizes, as there were small number of cases of individuals being infected after vaccination during the pre-Omicron era. The investigation of factors such as the order of immunological events is being undertaken in other studies that encompass both the pre-Omicron and Omicron eras, with a focus on exploring immune imprinting effects.[15](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10404859/#bib15),[49](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10404859/#bib49),[50](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10404859/" \l "bib50)

Seroprevalence rates also varied by age, with lower rates reported for the youngest (0-9) and oldest age groups (60+) compared to adults aged 20-29 years in studies up to end of 20211.  In most low- and middle-income countries, the rise in seroprevalence in this period has been driven by an increase in infections rather than vaccination, given the very low vaccination rates through the end of 2021.

Especially Omicron variants have shown their potential to escape vaccine-induced humoral immunity, resulting in many vaccine breakthrough infections and the development of hybrid immunity[2](https://www.nature.com/articles/s41598-023-45718-8#ref-CR2),[3](https://www.nature.com/articles/s41598-023-45718-8#ref-CR3),[4](https://www.nature.com/articles/s41598-023-45718-8#ref-CR4),[5](https://www.nature.com/articles/s41598-023-45718-8#ref-CR5),[6](https://www.nature.com/articles/s41598-023-45718-8#ref-CR6),[7](https://www.nature.com/articles/s41598-023-45718-8#ref-CR7). revious infection with Omicron protects against subsequent infections by other Omicron variants, and this protection may be better than hybrid immunity induced by SARS-CoV-2 variants preceding Omicron[8](https://www.nature.com/articles/s41598-023-45718-8#ref-CR8),[9](https://www.nature.com/articles/s41598-023-45718-8#ref-CR9). How Omicron-induced hybrid immunity protects against future variants remains to be seen.

Limitation—how to account the breakthrough infection based on the serologic data , not just infection report. We show that SARS-CoV-2 breakthrough infections can be identified by N-specific IgG antibodies and boosting of vaccine-induced immunity by infection, leading to hybrid immunity. We show that up to 82% of the individuals that experienced their first infection with SARS-CoV-2 after vaccination developed antibodies to N of SARS-CoV-2, regardless of the virus variant and independent from COVID-19 vaccination. Following breakthrough infection, N-seroconversion was associated with increased S1 antibody levels. N seroconversion might therefore be a more reliable proxy for the development of hybrid immunity rather than a positive PCR or antigen test only confirming breakthrough infection. In a recent population serosurvey among individuals without a history of COVID-19 vaccination, 79% of the participants that had reported a PCR-confirmed infection and clinical symptoms were N seropositive between 2 and 6 weeks after infection.

Assessment of the development of hybrid immunity in the population requires a clear identification of a passed SARS-CoV-2 infection. Such information has often been obtained from testing registries based on diagnostic SARS-CoV-2 RT-PCR and rapid antigen testing. However, testing behavior and policy varies over time and since April 2022 community testing has been scaled down. Serological testing for virus-induced antibodies could be an alternative to detect SARS-CoV-2 infections and can usually be detected many months after virus exposure.

Immunogenic SARS-CoV-2 proteins that are absent in most vaccines, such as Nucleoprotein (N), can be regarded as a potential tool to identify the development of hybrid immunity through breakthrough infections in a vaccinated population[11](https://www.nature.com/articles/s41598-023-45718-8#ref-CR11). Both the development of Spike-antibody mediated hybrid immunity and induction of detectable N-specific antibodies require immune activation through replication of SARS-CoV-2 after breakthrough infection. Sufficient immune activation after breakthrough infection may be limited in a proportion of the vaccinated population, due to e.g. presence of vaccine-induced S-specific antibodies that may reduce viral replication, thereby also reducing de novo induced N-specific antibodies following breakthrough infection[12](https://www.nature.com/articles/s41598-023-45718-8#ref-CR12),[13](https://www.nature.com/articles/s41598-023-45718-8#ref-CR13). Identification of breakthrough infections by antibodies to non-vaccine viral antigens would allow for research further elucidating of characteristics of the development of hybrid immunity and also shed light on risk factors for breakthrough infection, e.g. pre-infection antibody levels, virus variants, comorbidities or vaccination status[14](https://www.nature.com/articles/s41598-023-45718-8#ref-CR14),[15](https://www.nature.com/articles/s41598-023-45718-8#ref-CR15).

 First, infections not directly adjacent to a retrospective antibody measurement to determine the infection, may be missed, e.g. due to antibody waning as described for N[11](https://www.nature.com/articles/s41598-023-45718-8#ref-CR11). Therefore, this leaves the possibility for an earlier infection to have occurred unnoticed. – vaccinated group may be those who got infection long time ago.

econdly, variant of infection changes with calendar time like vaccinations were administered at given time periods resulting in a correlation between protection by vaccination and the virus variant causing the breakthrough infection. Also, previous infection differs between the Delta and Omicron variants. This leads to differences in vaccination and previous infection status between Delta and Omicron infections, where Omicron cases more often had received their COVID-19 booster vaccination.—calender time with variant and vaccination. Event after omicron – most recent infection provide high immunity to subsequent omicron variatns.. small events for hybrid.

As evidence supporting the protective role of hybrid immunity over either natural or vaccine-acquired immunity alone continues to arise ([18](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref18)–[21](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref21)), the complexity of this phenomenon becomes more evident. A series of different factors, such as the number of vaccine doses received, vaccine platform used, the severity of the first COVID-19 episode, SARS-CoV-2 variants, and subvariants responsible for both first infection and reinfection and time-dependent waning protection seem to have a significant effect on the level of protection conferred by hybrid immunity ([22](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref22)–[28](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref28)).

Furthermore, since immune imprinting, a phenomenon in which B-cell immune response from first exposure to antigens related to an infectious agent (either through vaccination or previous infection) was demonstrated to condition the host response toward SARS-CoV-2 reinfections ([10](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref10), [29](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref29), [30](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref30)), studies have started to include the order in which immunity-generating events (vaccination and infection) occur as an additional factor to be considered in order to understand hybrid immunity better ([31](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref31)–[33](https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2023.1146059/full#ref33)). Even though real-world evidence of hybrid-immunity protection continues to emerge, studies integrating the order of immunity-generating events are still scarce.

We propose that the observed association of N seroconversion with stronger boosting of S1 antibodies makes the N response a better predictor for the development of hybrid immunity than a positive PCR test, since positive tests in some cases are accompanied with a weak humoral immune response. Although breakthrough infections by distinct virus variants are equally detected by the induction of N antibodies, the breakthrough infections also result in variant-specific antibody levels during the development of hybrid immunity. The generation of de novo responses, the boosting of vaccine-target antibody levels, and broadening of humoral immunity by breakthrough infections likely enhances immunity to current Omicron and future variants.

Our study highlights significant differences in the S antibody decay trends and protective effectiveness against breakthrough infection of the Omicron variant across various age groups between hybrid-and vaccine induced immunity; Our result shows that although the antibody decay trend was similar across age groups, the infection risk was more quickly increased for the>60 age group but in a greater magnitude for the <20 group over time in the vaccine group, following >60, 40-60 and 20-40 age groups. When the Omicron variant emerged, the presumed population immunity was between 80% and 85%, although nearly half of the children were estimated to have no immunity [10]. As the younger (<20) age group was less vaccinated or previously exposed due to shielding guidance, they may become more at risk with the lift of the social distancing policy. The older (>60) age group showed the second highest infection risks and the highest infection hospitalization risk among all age groups, although the hospitalization events were quite small (which limited sample size precludes more detailed statistical analysis: Appendix Table S1). This can be explained by the decreased immune system efficiency with age (as known as immunosenescence). Previous studies showed individuals 60 years or older had lower antibody levels after vaccination compared to younger participants and increased age has been associated with reduced vaccine responses in a number of studies irrespective of vaccine type [11]. A similar effect has been seen with antibody responses after infection. According to another study based on cross-sectional samples from Apr 2021-Jan 2022 before the Omicron wave, on the other hand, there were no consistent associations of lower antibody levels with age, but a significantly lower incidence with older age of post-vaccination infection. Overall, these results are consistent with the risk of infection being a complex combination of SARS-CoV-2 variants characteristics, individual immune response to vaccination, and individual level of exposure [12].

Our findings indicate that the need for and timing of booster doses may differ for individuals with hybrid immunity compared to those with vaccine-induced immunity, with the possibility of delaying booster doses by six months for those with hybrid immunity while still maintaining strong protection against severe disease. On a broader scale, the optimal number of vaccine doses and intervals may vary depending on the prevalence of vaccine versus infection-induced immunity within a population, suggesting that booster strategies should account for both vaccination history and previous infections, alongside the clinical characteristics of those infections. Further research is needed to better understand the interaction between natural and vaccine-induced immunity, the effects of variant exposure, and the timing between exposures. These insights can help policymakers project population-level protection using local vaccination and seroprevalence data, thus informing the strategic use and timing of COVID-19 vaccinations as a vital public health tool.

Age specific consideration-- Whereas approximately 98%–99% of the U.S. population has measurable antibody titers against SARS-CoV-2 from infection, vaccination, or both (hybrid immunity), adults aged ≥65 years are less likely to have immunity resulting from infection (including immunity from infection only or hybrid immunity), compared with adults aged 30–49 years and 50–64 years (*7*). In addition, immunosenescence, the age-related decline in the functioning of the immune system, results in a less complete immune response to novel antigens and a reduced ability to develop robust immunity after infections or vaccination (*8*). The pool of naive T-cells diminishes with age, and this insufficient naive T-cell pool affects the ability to generate neutralizing antibody responses and cytotoxic T-cells in response to SARS-CoV-2 (*9*).

Thus, adults aged ≥65 years are more likely than are younger adults, adolescents, and children to rely upon vaccination to increase immunity that might have waned and might need more frequent vaccine doses to maintain protection. Coverage with the updated COVID-19 vaccine among adults aged ≥65 years was 42% as of February 3, 2024 (*10*,*11*).

The need for, and optimal timing of, the primary vaccination series and booster doses may differ according to whether individuals have had a SARS-CoV-2 infection. At a population level, the number of doses and inter-dose interval, including for booster doses, may differ between settings with high and lower seroprevalence resulting from infection-induced immunity16.  These considerations may be particularly important in identifying simplified vaccination schedule in lower-priority use groups identified in the WHO Roadmap prioritizing use of COVID-19 vaccines16.

Any policy regarding the vaccination of lower priority use groups should involve clear specification of the value of vaccination under consideration and multiple trade-offs, with seroprevalence in these groups representing one of the factors to be considered in the future. For instance, a modelling study in Kenya estimated that vaccination of young adults may not be cost-effective in a high seroprevalence setting17. At the same time, evidence from observational studies suggests that vaccination can reduce incidence of post COVID condition18.  Prevention of mild disease, indirect impact on transmission, and reduction of incidence of post COVID condition can provide a strong rationale for vaccinating low priority use groups irrespective of previous exposure.

Our results show that hybrid immunity confers higher protection by almost 5-fold compared to vaccination alone for COVID-19 re-infection with the Omicron variant, regardless of whether immunization was full or boosted.

Despite the fact that the COVID-19 vaccine-induced protection against infection appears to wane overtime, the protective effect against hospitalization and death remains robust, with no evidence of waning for several months after the additional vaccine dose [[15]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9482842/#b0075), [[16]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9482842/#b0080), [[17]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9482842/#b0085), [[18]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9482842/#b0090).

Our study has some limitations. Firstly, our analysis did not account for the sequence permutations and timing between vaccination and prior infection in individuals with hybrid immunity. Our results in terms of the relative protection levels of S antibodies in the hybrid vs. vaccine-only groups may thus differ by the subgroup distributions and calendar time such as variant types and the epidemic stage. However, previous studies show the hybrid immunity show the highest immunity regardless the xxxx and our focus was to compare the age specific pattern within the group.

Second, we measured binding antibodies rather than neutralizing antibodies, which are arguably a better surrogate for immune protection. The antibody assays used in our study were developed before the emergence of Variants of Concern, raising questions about whether these assays, based on the original Wuhan-Hu-1 strain, accurately capture infections with other variants—a matter that should be evaluated in ongoing seroepidemiological studies. Potential sources of misclassification bias include the decay of neutralizing antibodies, the sensitivity and specificity of antibody testing, and the timing of testing relative to infection and vaccination. Furthermore, unmeasured bias in exposure may stem from the observational nature of the study, where individuals who chose to get vaccinated or had previous infections did so at different times, without controlling for possible differences in healthcare-seeking behavior or risk aversion among participants.

Limitations include the observational nature of the study, which carries a risk of bias by underidentification of infection among those who did not interact with the medical system for vaccination or testing due to lack of access to care or other socioeconomic barriers. We were able to assess infection only but not hospitalization or other complications. Asymptomatic infections may have gone undetected, as well as infections detected by unreported home antigen testing.

Heterogeneity in hybrid and vaccine—we could not address because it was very heterogeneous in terms of vaccine types and doses and infection sequence, which reduce the sample size. Sine events was small, the future segregation may limit statistical power so we rather focused on different risk across age groups for this analyses.

Also, as this study was implemented after Omicron, there was not many cases. The study was performed during the transition period of the Delta variant to Omicron[9](https://www.nature.com/articles/s41598-023-45718-8#ref-CR9), which provided a unique basis to relate immune activation to the virus strain involved.

The strengths of our study include being population-based, the large sample size, evaluation of multiple Omicron sublineage-predominant periods, stratification by the latest vaccine dose and longer observation durations, and adjustment for available confounding factors. Our study has several limitations. We could only capture prior infections confirmed by PCR tests performed in healthcare settings in Ontario; results of rapid antigen tests were unavailable. Canada-wide data indicate that infection-acquired seropositivity has risen steadily during the Omicron waves among older adults (from <10% to about 62% by February 2023) [[26](javascript:;)], thus undocumented infections are probable. – discuss the infection definition—we exlucded those who increased S ab level in the 2nd surveillance. To avoid potential undetected cases. – this may underestimated our outcome?

 It is unclear if pauci-symptomatic or asymptomatic infections (untested or diagnosed by antigen tests in community settings) generate less durable protection, which deserve further study [[27](javascript:;), [28](javascript:;)]. – there was no clear difference between symptomatic vs asymptomatic infection with S ab. – data not shown.

First, we determine the sensitivity of N-antibodies as a tool to identify breakthrough infection. Subsequently, we investigate the boosting of Spike S1-specific responses after breakthrough infection and the influence of time since vaccination and N-seroconversion on the S1-specific antibody levels.—we took more conservative approach and did not account the N/S andibodies to determine the breakthrough infection with the limitation of the testing kit. Futher study may investigate how the N/S antibody titier changes with hybrid based on stratified by vaccine types, infection sequence.

Finally, we did not perform individual analyses for each vaccine and booster combination, provided that not all combinations had a large enough number of outcomes to allow for adequate comparisons. The role of vaccination and boosters’ interaction with predominant circulating variants remains an area of opportunity for future research. Given that most reinfections occurred during periods of Omicron predominance and that immunity-boosting by infection with Omicron seems to be low, real-world studies on hybrid immunity that consider the effects of immune imprinting from SARS-CoV-2 on protection against reinfection will be fundamental for determining the need of booster shots and its frequency in future vaccination waves.

First, there is misclassification. Our infected cohort and new infection cohort did not include those who might have had a SARS-CoV-2 infection but were not tested. The effect of this bias is conservative, leading to underestimation of the true effects. Importantly, the analysis of severe COVID-19 is unlikely to be affected by this bias, as people with symptoms indicative of severe acute respiratory disease would be hospitalized (and tested). Some patients might be admitted to the hospital with, but not because of, SARS-CoV-2 infection. We tried to minimize this by limiting hospitalized COVID-19 cases with ICD-10 diagnoses indicative of respiratory (infection) disease.

Confounding is expected to arise due to the lack of randomization, given substantial differences in the clinical backgrounds and sociodemographic characteristics of the comparison groups. Even though in the analysis, we accounted for sex, education, comorbidities, SARS-CoV-2 testing probability and SARS-CoV-2 environmental exposure risk, residual confounding is possible. Our results might be affected by differences between the groups in terms of health behaviours (such as social distancing and mask wearing), a possible confounder that was not assessed. While these biases might influence our estimates, they seem unlikely to have caused the clear patterns observed in this study.

 We would like to highlight that our use of a large, population-based sample size increases the generalizability to other countries with similar population structures (and public/health care provisions).

Number of factors on breakthrough infection..

**Conclusions**

In summary, in this large cohort study, we compared the antibody responses, and their infection risks induced by different COVID-19 immunity statuses (hybrid and vaccine) across age groups. We showed that S antibody waning and risk infection trends are highly correlated; hybrid group resulted in higher and longer-lasting antibody responses compared to vaccination alone, in the long term as well. In terms of age groups in vaccine group, <20 group show the greatest infection risk following >60, 40-60 and 20-40. All these differences accounted for heterogeneous individual vaccine responses and individual levels of exposure. Importantly, antibody decay was clearly observed after 6th month from the last immunological events for the vaccine group (more quickly for the>60 age group but greater magnitude for the <20 group) compared to hybrid groups. In the hybrid group, however, 4th booster vaccines have a significantly higher infection risk than the primary vaccine group, highlighting the continuous vaccine compliance in this group). Moreover, in addition to the S ab level and infection risk trend over time by age group, we quantified the probability of free from infection risk by age group so it can guide the specific vaccine prioritization and interval for age groups. Taken together, our results indicate that vaccination campaigns should be tailored according to individual immune status, previous history of SARS-CoV-2 infection, and characteristics of the population risk factors (age, gender) to achieve optimal responses and protection across individuals.

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Based on the key demographic characteristics (Table 1), we conducted logistic regression to determine specific risk factors on infection events for hybrid and vaccine groups, respectively. All available characteristics were converted to categorical variables, with the first subgroup designated as the reference category. This al- lowed for the calculation of log odds ratios, where a higher log odds ratio indicates a greater infection risk compared to the reference group. A log odds ratio of zero signifies equal risk with the reference group.

Among all characteristics, age and S antibody level demonstrated the most significant association with individual infection status, exhibiting clear patterns and non-overlapping con- fidence intervals. (Figure S1) Notably, the youngest group exhibited the highest individual infection risks. Except for the youngest group, there was a positive correlation between age and infection risk, indicating that risk increases with age. In order to determine the risk probability of remaining infection-free by S antibody levels across age groups, we constructed logistic regression including age only based on the finding. (Figure 4 and S2)

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Figure S1: Forest plot displaying characteristic patterns: Top - Vaccine-induced group; Bottom - Hybrid-induced group

Cox proportional hazard model

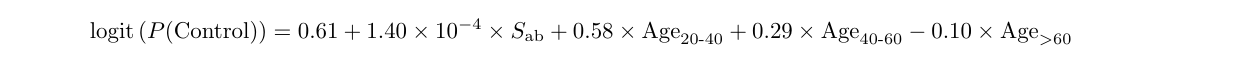
Mixed effect model

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Figure S2: ROC curves for logistic regression models

The ROC curve analysis demonstrates that the mixed-effects logistic regression model (red curve) exhibits superior discriminative capability compared to the simple logistic regression model (blue curve). This conclusion is supported by the consistently higher position of the mixed-effects model’s ROC curve across all threshold levels. Moreover, the mixed-effects model achieves a higher Area Under the Curve (AUC) value of 0.82, in contrast to 0.77 for the simple logistic model. This indicates a marked improvement in the mixed-effects model’s performance in predicting the binary outcome, thereby suggesting its enhanced utility in applications requiring robust classification accuracy. The equation for a simple logistic regression is provided below. On the left side of each equation is the logit transformation of the probability of being in the control group, which represents the likelihood of being free from COVID-19 infection.



And the equations of mixed effects logistic regression are as follows:

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|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | <20 | 20-40 | 40-60 | >60 | Total |
| Hybrid | Infections | 4 | 6 | 20 | 17 | 48 |
| Hospitalization | 0 | 0 | 0 | 2 | 2 |
| Infection hospitalization risk | **0.00** | **0.00** | **0.00** | **0.14** | **0.04** |
| Vaccine | Infections | 43 | 83 | 178 | 281 | 585 |
| Hospitalization | 1 | 1 | 3 | 11 | 16 |
| Infection hospitalization risk | **0.02** | **0.01** | **0.02** | **0.04** | **0.03** |

Table S1. Infection hospitalization risks by immunity status and age groups.