의학박사 학위논문

Analysis of humoral immune responses as correlates of protection against SARS-CoV-2 infection in the Omicron era

오미크론 시대의 SARS-CoV-2 감염에 대한 면역학적 방어지표로서 체액면역반응 분석

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이 논문을 의학박사 학위 논문으로 제출함

2023 년 8 월

울산대학교 대학원
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임 소 윤

임소윤의 의학박사학위 논문을 인준함

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2023 년 8 월

감사의 글

전공의 1년차 9월, 감염내과학을 전공하고자 여러 교수님들께 처음 인사를 드렸던 때가 엊그제 같은데 어느덧 박사학위 논문을 제출하게 되었습니다. 그동안 많은 가르침과 도움을 주신 분들께 감사드립니다.

우선, 부족한 저에게 늘 좋은 기회를 주시고 진지한 과학 연구의 길로 이끌어 주신 김성한 교수님께 가장 진심 어린 깊은 감사를 드립니다. 그리고 늘 정확한 가르침을 주시고 감염내과 의사로 살아감에 있어 아낌없는 조언을 해 주신 김양수 교수님께 깊은 감사를 드립니다.

다음으로, 인생에 대한 많은 가르침을 주신 이상오 교수님, 늘 따뜻한 마음과 환자에 대한 명료한 통찰력을 주신 최상호 교수님, 바쁘신 중에도 세심하고 애정어린 가르침으로 저를 더욱 발전시켜 주신 정용필 교수님, 학문에 대해 새로운 접근법과 독창적 사고를 제시해 주신 김민재 교수님께 감사드립니다. 철저한 지식과 논리적 사고로 의대생 시절부터 저를 더욱 열심히 노력하게 해 주신 정지원 교수님, 끊임없는 긍정적 지지와 격려를 주신 배성만 교수님, 좋은 의사가 되기 위한 올바른 방향을 보여주신 장의진 교수님께 깊은 감사를 드립니다. 바쁜 와중에도 논문 심사를 위해 조언과 격려를 해 주신 박성연 교수님께도 감사드립니다.

전임의 시절 임상연구를 하면서 동고동락한 실험실 김지연 박사님, 권지수 박사님, 이현정 선생님, 장채영 선생님, 차혜희 선생님에게도 감사의 말씀을 드립니다.

많은 시간을 저와 함께하면서 덕분에 즐거운 전임의 생활을 할 수 있게 해준 이윤우 선생님, 그리고 박혜진, 진솔 선생님 감사합니다.

마지막으로 저를 잘 키워주고 지지해준 부모님과 언니, 남동생, 그리고 우리 가족이 된 든든한 희민에게 고마움을 전합니다. 지금까지의 배움을 바탕으로 하여 앞으로도 지치지 않고 부단히 노력하여 훌륭한 연구자로 성장해가도록 하겠습니다. 감사합니다.

2023년 8월

임 소 윤 올림

Summary

Introduction: Serum anti-spike IgG and neutralizing antibody against SARS-CoV-2 are accepted as immune correlates of protection (ICP) against COVID-19. However, there are limited data available on the ICP after the Omicron variant wave and the introduction of the BA.4/5 bivalent vaccine. Thus, this study aimed to analyze the antibody responses as correlates of protection in the Omicron era and to investigate the protective factor against COVID-19 amid complex immunologic status of the population.

Methods: A prospective cohort of healthcare workers was enrolled at Asan Medical Center, the largest tertiary care hospital in South Korea between December 2022 and January 2023, and followed up for 5 months. Study participants had previously received a 3- or 4-dose of COVID-19 vaccine and either planned to receive a bivalent BA.4/5 vaccine or not. Blood and saliva samples were collected at 0, 1, and 3 months from the time of study enrollment, while for individuals who did not receive the bivalent vaccine, samples were collected only at 0 and 3 months. Immunological assessments were conducted using ELISA to measure variant-specific serum S1-IgG and saliva S1-IgA, and virus reduction neutralization test to measure neutralizing antibody levels.

Results: A total of 482 participants were enrolled, of whom 69 (14.3%) underwent subsequent infection after study enrollment during 5-month follow-up, while 413 (85.7%) did not. Of these 482 participants, 381 (79.0%) experienced previous SARS-CoV-2 infection before, and 166 (34.4%) received the BA.4/5 bivalent booster vaccination at the study enrollment. There was a significant difference in baseline antibody levels of Wuhan-Hu-1 (Wuhan) S1-IgG, BA.5 S1-IgG, and neutralizing antibody (nAb) against BA.5 between individuals with and without subsequent infection (all P <0.001), whereas baseline saliva Wuhan S1-IgA and BA.5 S1-IgA

did not show significant difference according to the subsequent infection. The infection rate showed a decreasing trend as baseline serum antibody levels increased (all *P* for trend <0.001), but no significant trend was observed with baseline saliva antibody levels. The optimal cutoff value for distinguishing subsequent infection was determined from receiver operative characteristic (ROC) curves as 1.36 log OD ratio for Wuhan-Hu-1 S1-IgG, 1.43 log OD ratio for BA.5 S1-IgG and 7.00 $\log_2$50% neutralization dose [ND50] for neutralizing antibody against BA.5. Baseline serum Wuhan S1-IgG (adjusted hazard ratio [aHR] 0.43; *P*=0.02), BA.5 S1-IgG (aHR 0.32; *P*=0.005) and neutralizing antibody against BA.5 (aHR 0.26, *P*=0.045) exceeding the cutoff value, were found to be independent protective factors against subsequent SARS-CoV-2 infection in multivariable Cox regression analysis, and also hybrid immunity was a robust protective factor against subsequent infection in all analysis based on each immune marker (all *P* <0.01). However, saliva S1-IgA did not show a protective role against SARS-CoV-2 infection. The high antibody levels exceeding cutoff values, Wuhan S1-IgG (aHR 0.40; *P*=0.007), BA.5 S1-IgG (aHR 0.30; *P* <0.001), as well as neutralizing antibodies against BA.5 (aHR 0.15; *P*=0.02) and hybrid immunity (all *P* <0.05), were also significantly protective against subsequent infection in the 1-month subgroup analysis that reflected the antibody levels after the bivalent booster vaccination in those vaccinated. Furthermore, combining high baseline antibody levels that exceed optimal cutoff values and hybrid immunity, the sensitivity and specificity for protection from subsequent SARS-CoV-2 infection were 89.0% and 42.0% for Wuhan S1-IgG, 88.5% and 47.8% for BA.5 S1-IgG, and 87.2% and 56.3% for neutralizing antibody against BA.5, respectively, with similar positive predictive value (90.2%, 91.0% and 92.3%, respectively).

Conclusions: This 5-month observational cohort study reconfirms that humoral immune responses are immune correlates of protection against SARS-CoV-2 infection in the Omicron era with the complex immune status of the population, and hybrid immunity is an independent

protective factor against subsequent infection. Also, high serum antibody levels exceeding the cutoff value when combined with hybrid immunity demonstrate a significant predictive performance for protection against subsequent SARS-CoV-2 infection.

Keywords: antibody response, correlates of protection, hybrid immunity, mucosal immunity, SARS-CoV-2

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Introduction

The measurement of particular immune responses as correlates of protection against infection or disease is essential to evaluate the protective efficacy of vaccines[1]. After the rollout of Coronavirus Disease-19 (COVID-19) vaccines, serum anti-spike protein IgG antibody and neutralizing antibody against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been accepted as correlates of protection, serving as key immune markers of vaccine efficacy[2]. The level of spike-specific binding antibody was demonstrated as mechanistic correlates of protection in a study assessing immunogenicity and protection of mRNA-1273 vaccine in nonhuman primates[3]. Also, the titer of neutralizing antibodies induced by multiple COVID-19 vaccines in humans[4-7] has been recognized as immunological correlates of protection. Meanwhile, considering that these correlates of protection were determined based on studies involving individuals who had not previously been infected, it appears crucial to reevaluate the correlates of protection within the context of the complex immunity of the population established during the Omicron wave that made a significant portion of the population to acquire hybrid immunity after booster vaccination.

Hybrid immunity made by exposure to both natural SARS-CoV-2 infection and COVID-19 vaccine has been known to provide more durable and broader cross-variant protection against COVID-19 than vaccination or infection alone[8-10]. One of the possible contributing factors to hybrid immunity could be mucosal immunity that develops during natural infection. Mucosal immunity is also considered an important immune correlates of protection against COVID-19[11,12], and secretory IgA, a key component of mucosal immunity, plays a critical role in protecting against respiratory viral infections including SARS-CoV-2[13]. Furthermore, it appears that overall mucosal immunity induced by vaccination is not as robust as that generated by natural SARS-CoV-2 infection, despite mucosal IgA responses being reported variably according to the natural infection or vaccination in previous studies[14-18]. Thus, it could be assumed that hybrid immunity may have a strong protective effect due to mucosal

immunity induced by natural infection. Consistent with this, robust epidemiological data suggest that hybrid immunity provides substantial protection against subsequent infection[19,20], particularly in the context of the Omicron variant[21,22]. However, there are limited immunological data on the landscape of immune correlates of protection in the Omicron era where a significant portion of the population has hybrid immunity. Also, as the BA.4/5 bivalent vaccine was introduced in August 2022 in response to the need for an updated COVID-19 vaccine due to the waning antibody responses and immune escape of variants from neutralizing antibodies, this study aimed to investigate the immune correlates of protection against COVID-19 focusing on the role of hybrid immunity and the effects of BA.4/5 bivalent vaccine.

Methods

1. Study design and participants

South Korea introduced its COVID-19 vaccine in February 2021, with the AZD1222 (AstraZeneca, Cambridge, UK) and BNT162b2 (Pfizer-BioNTech, New York City, NY, USA, and Mainz, Germany). In the hospital setting, BNT162b2 was assigned to healthcare workers who were in close contact with COVID-19 patients and AZD1222 to those not directly involved with COVID-19 patient care in accordance with the South Korean government policy. Booster vaccination was conducted using mRNA vaccines in fall, 2021. After the booster vaccination, there have been three waves of COVID-19 caused by the Omicron SARS-CoV-2 variant in South Korea: the first wave from February to May 2022 was dominated by BA.1 subvariant at the beginning, but after a few weeks, BA.2 subvariant predominated, followed by BA.5 subvariant from July to October 2022, and finally BA.5 again from November 2022 to January 2023 with a slow overlap of the BN.1 subvariant (Figure 1)[23]. As a result, a substantial number of healthcare workers in South Korea who previously received booster vaccination and remained naïve to infection acquired hybrid immunity against COVID-19 during this Omicron wave.

In this context, a cohort of healthcare workers was enrolled at Asan Medical Center, the largest tertiary medical center in Seoul, South Korea, with 2,700 beds, between December 2022 and January 2023, who had completed a booster vaccination and had either planned to receive the bivalent booster vaccination or not. Individuals with a history of SARS-CoV-2 infection twice or more were excluded to ensure clarity in evaluating the concept of hybrid immunity. This study was approved by the institutional review board of Asan Medical Center (IRB No. 2022-1269).

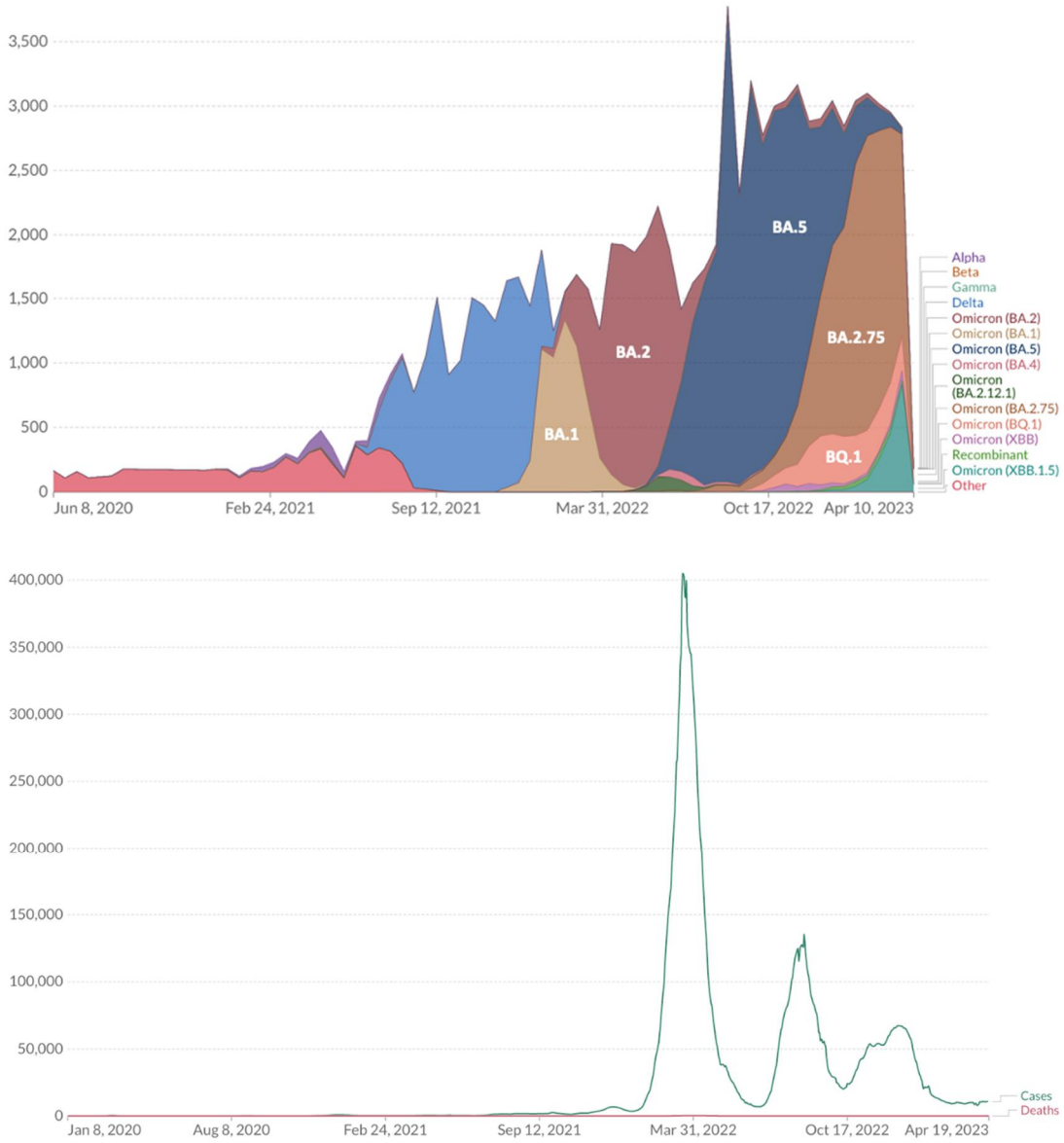


Figure 1. SARS-CoV-2 variants in analyzed sequence and epidemic curve during the entire period of the COVID-19 pandemic, South Korea

2. Definitions

Demographic characteristics, underlying diseases, medications, previous SASR-CoV-2 infection, and COVID-19 vaccination history were reviewed. Hybrid immunity was defined as a combination of a 3- or 4-dose COVID-19 vaccine and previous SARS-CoV-2 infection which was identified as having a positive nasopharyngeal polymerase chain reaction (PCR) prior to the study as well as a positive nucleocapsid protein (NP) antibody detected in the baseline sampling of the participants. The type of previous vaccination was classified as heterologous and homologous vaccination: the former involved one or more doses of AZD1222 followed by mRNA vaccines while the latter consisted of consecutive mRNA vaccinations. The BA.4/5 bivalent vaccine that the study participants received was all BNT162b5 (Pfizer-BioNTech, New York City, NY, USA, and Mainz, Germany). Subsequent infection was defined as a SARS-CoV-2 infection during the 5 months following study enrollment. For infection-naïve individuals at the time of enrollment, those whose follow-up nucleocapsid protein antibody changed from negative to positive were presumed to have experienced a subsequent infection even if they did not self-report any infection. However, for participants with previous infections at the start of the study, subsequent infections could not be determined based on seroconversion of the nucleocapsid protein antibody. Thus, if an antibody titer increase was observed in individuals with hybrid immunity, greater than the 25^{th} percentile of the increase in those who had a subsequent infection without bivalent booster vaccination, it was assumed that these individuals likely experienced a subsequent infection despite reporting no infection.

3. Specimen collection

The baseline blood and saliva sampling were done on the day of enrollment in participants without bivalent vaccination and on the same day as the bivalent vaccination or before the vaccination if not feasible. Following the study enrollment, information on subsequent SARS-

CoV-2 infection was obtained regularly, and specimen collection was done at 1 month and 3 months for all participants, except those who did not receive the bivalent vaccine; these individuals underwent follow-up sampling only after 3 months.

4. Estimation of SARS-CoV-2 variants of the previous infection

The SARS-CoV-2 variants of previous infection in those with hybrid immunity were estimated by considering the most prevalent variants during the time of infection, with the information provided by the Korea Disease Control and Prevention Agency from January 20, 2020, to the present[24].

5. Immunological evaluation of the study cohort

SARS-CoV-2 S1-specific serum IgG antibody and saliva IgA were determined by in-house ELISA that used Wuhan-Hu-1 (Wuhan) and Omicron BA.5 subvariant spike protein S1 as antigen. The in-house ELISA began with careful preparation of the primary antigen, the SARS-CoV-2 S1 protein, which I diluted to a concentration of 2ug/mL in phosphate-buffered saline (PBS). I gently pipetted 100uL of this solution into each well of the immunoplate and left it to incubate undisturbed overnight at a chilly 4°C. After this incubation period, I carried out 3 times of washes, each with 200uL of PBS infused with 0.05% Tween 20, ensuring the removal of any unbound S1 protein. Subsequently, I applied a blocking solution consisting of PBS plus 1% bovine serum albumin (BSA), using it to fill each well with 200uL of the mixture. The immunoplate was then allowed to incubate for at least an hour, providing ample time for the BSA to bind any remaining protein-free sites and prevent nonspecific binding. The standard and samples required a series of dilutions in PBS plus 1% BSA, beginning at 1/100 and extending to 1/1,000, 1/10,000, and beyond. Following this, I added 100uL of these diluted entities (sample, positive control, or negative control) into the immunoplate and left it at room temperature, patiently waiting for 2 hours. The horseradish peroxidase (HRP) conjugated anti-

human Fc gamma antibody was next in line, which I diluted in PBS plus 1% BSA to a precise working concentration of 40ng/mL (1:20000 dilution from the stock antibody of 0.8mg/mL). After ensuring this mixture was filtered through a 0.2 um filter, I proceeded with a more rigorous washing protocol, involving five washes with 200uL of PBS plus 0.05% Tween 20. A 100uL aliquot of the meticulously prepared detection antibody was added to each well, followed by an hour-long incubation at room temperature in the dark, ensuring the antibody had sufficient time to bind to the antigen without any interference from light. After this crucial step, I engaged in an even more rigorous washing regimen, flushing the wells seven times with 200uL of PBS plus 0.05% Tween 20. Subsequently, I dispensed 100uL of tetramethylbenzidine (TMB) substrate into each well, which was incubated again at room temperature in the dark for 30 minutes, allowing for the enzymatic reaction to occur. To halt the reaction, I swiftly added 50uL of stop solution to each well. The final act was to read the optical density (OD) at a wavelength of 450 nm, which, in essence, is a measure of the binding between the primary antigen and the detection antibody. In-house ELISA for Wuhan S1-specific serum IgG was standardized with reference pooled sera from the International Vaccine Institute (Seoul, South Korea). However, since no reference pool was provided for the BA.5 variant, antibody units were not normalized to international units and were kept as OD for statistical comparison.

In-house ELISA for S1-specific saliva IgA and the commercially available SARS-CoV-2 IgA ELISA kit (EuroImmun, Lübeck, Germany) showed a high correlation coefficient of 0.74 (P <0.001) with 100% (95% CI, 83.2-100) of sensitivity and 100% (95% CI, 85.8-100) of specificity when the commercial kit was used as the standard.

The 50% neutralizing dose (ND50) was determined using virus reduction neutralization test (VRNT) as previously described[25]. Briefly, viruses were directly detected with fluorescent antibodies in a 96-well plate, and neutralizing antibody titers were determined by analyzing the number of infected cells. There was a high correlation between the results of the plaque reduction neutralization test (PRNT) and VRNT in a laboratory (R^2=0.95). Also, both Wuhan-

S1 IgG and BA.5 S1-IgG showed a good correlation with the VRNT assay (R=0.73 and R=0.80, respectively; both P <0.001) (Figure 2).

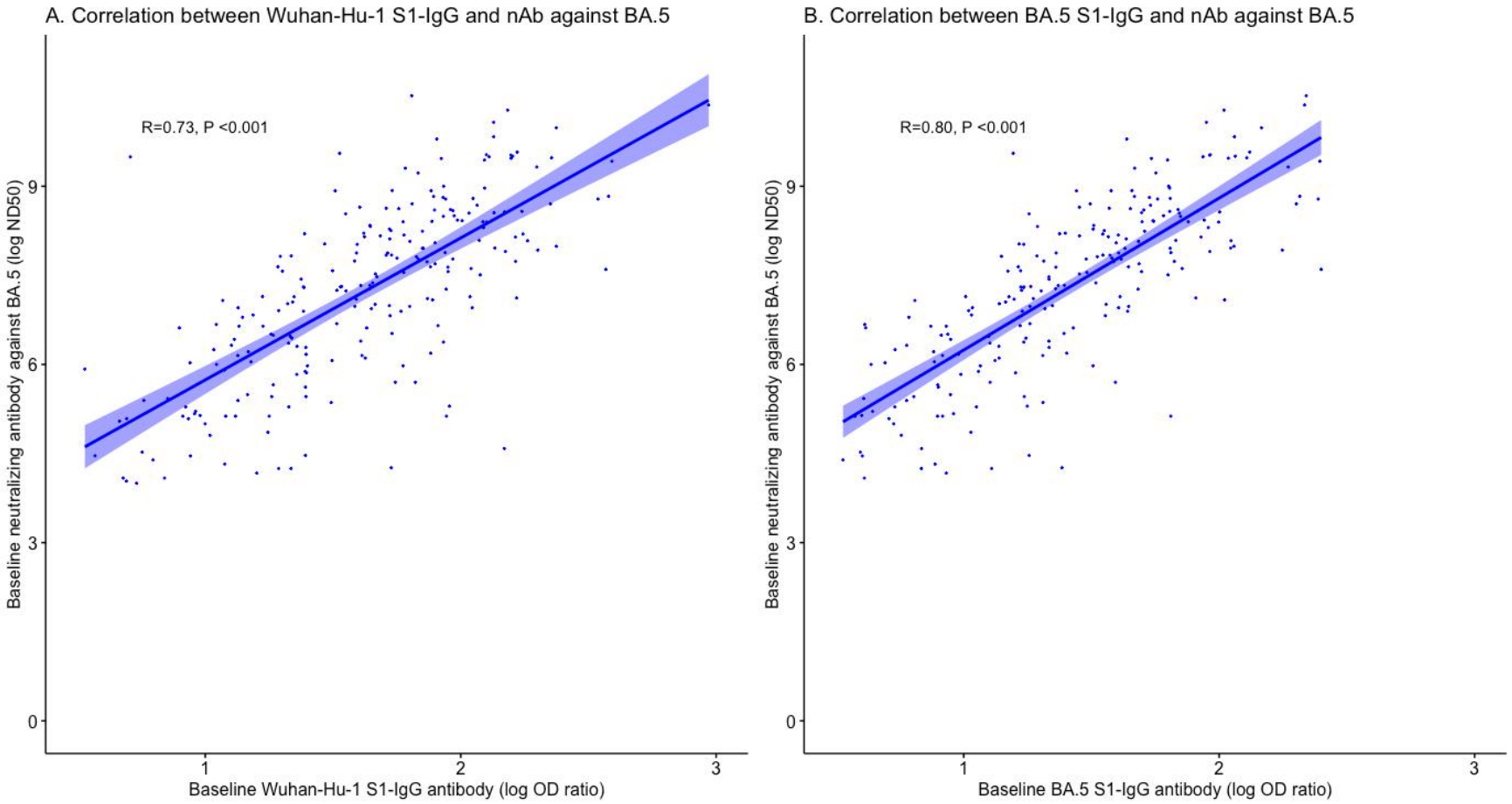


Figure 2. Correlation between serum binding antibody and neutralizing antibody

6. Statistical analysis

The titer of the S1-specific IgG and IgA antibody was log-transformed using base 10, and the ND50 was transformed using base 2. The $\chi 2$ or Fisher's exact test was used to compare categorical variables and the Wilcoxon rank sum was used to compare continuous variables, as appropriate. The correlation between the two variables was analyzed using Spearman's rank correlation analysis. The *P* for trend was calculated using the Cochran-Armitage test. The area under the receiver operating characteristic (AUROC) curve was calculated for each immune marker to measure the ability to discriminate subsequent SARS-CoV-2 infection, and DeLong test was used to compare ROC curves. A cutoff value of each immune marker was determined using the Youden index[26]. The sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals (CI) were computed under the optimal cutoff values for each immune marker to evaluate the predictive performance for protection from subsequent SARS-Cov-2 infection. A Cox-proportional hazard model was used to estimate the protective factors from subsequent SARS-CoV-2 infection after study enrollment. Variables with *P* values < 0.10 in the univariate analysis, were included in the multivariable analysis. Kaplan-Meier methods were used to draw cumulative incidence curves for subsequent infection. All tests of significance were two-tailed, and *P* values of <0.05 were considered to indicate statistical significance. Data analysis and graph plotting were conducted using R software version 4.1.3 (R Project for Statistical Computing, Vienna, Austria).

Results

1. Baseline characteristics

A total of 482 participants were enrolled in this cohort. Of these participants, 381 (78.8%) had previous SARS-CoV-2 infection (hybrid immunity), yet 34 (9%) were unaware of their past infection and were identified as having a previous infection based on a positive nucleocapsid protein antibody result. The variants of previous infection were estimated as follows: 4 (1.0%) with Delta, 222 (58.3%) with BA.1/2, and 121 (31.8%) with BA.4/5. Among the 381 with hybrid immunity, 119 (31.2%) received the bivalent vaccine, while 262 (68.8%) did not; in the 102 infection-naïve individuals, 47 (46.1%) received the bivalent vaccine and 55 (53.9%) did not. A schematic diagram of the study participants is shown in Figure 3. Of the participants, 15.4% were male and the median age was 33 years (interquartile range [IQR], 28-41). In this study cohort, 34.4% of the participants received bivalent booster vaccination. During the 5-month observation, 69 (14.3%) experienced subsequent SARS-CoV-2 infection. Previous SARS-CoV-2 infection was significantly more common in participants who did not experience subsequent infection. Also, the time since the last exposure to the SARS-CoV-2 antigen, whether through vaccination or infection, was significantly shorter in individuals without subsequent infection. Demographic and baseline clinical characteristics of the study participants according to the subsequent infection are presented in Table 1.

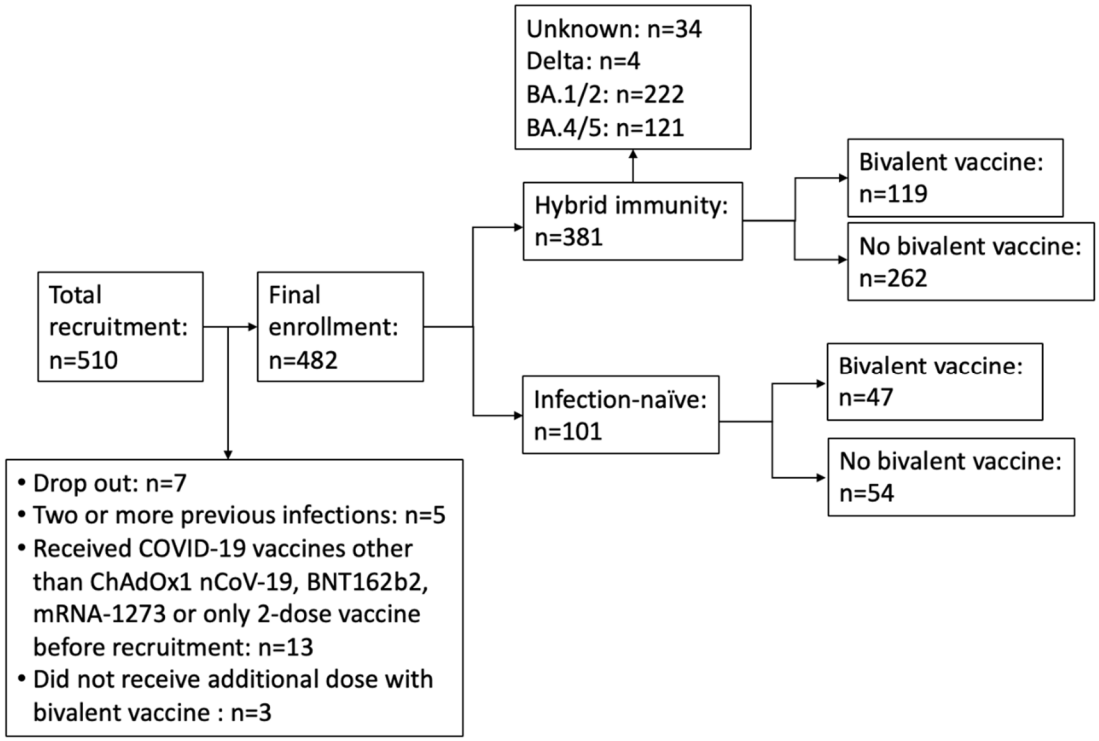


Figure 3. Schematic diagram of the study participants

Table 1. Demographics and baseline characteristics of study participants according to the subsequent SARS-CoV-2 infection

Variable	Total (n=482)	Subsequent infection (n=69)	No subsequent infection (n=413)	*P* value
Age, years, median (IQR)	33 (28–41)	35 (28–42)	33 (28–41)	0.68
Male	74 (15.4)	13 (18.8)	61 (14.8)	0.39
Underlying comorbidity	55 (11.4)	9 (13.0)	46 (11.1)	0.64
Number of previous vaccinations				0.77
3-dose	457 (94.8)	65 (94.2)	392 (94.9)	
4-dose	25 (5.2)	4 (5.8)	21 (5.1)	
Type of previous vaccination				0.66
Heterologous	400 (83.0)	56 (81.2)	344 (83.3)	
Homologous	82 (17.0)	13 (18.8)	69 (16.7)	
Time from last vaccination to study enroll, days, median (IQR)	375 (365–383)	376 (366, 383)	375 (365, 383)	0.96
Previous SARS-CoV-2 infection	381 (79.0)	31 (44.9)	350 (84.7)	<0.001
Time from the previous infection, days, median (IQR)[a]	251 (127–275)	269 (116–286)	250 (127–272)	0.20
Bivalent booster vaccination	166 (34.4)	23 (33.3)	143 (34.6)	0.83
Time from last SARS-CoV-2 antigen exposure, days, median (IQR)[a]	260 (128–296)	338 (269–378)	254 (127–284)	<0.001

Data represent n (%) unless otherwise indicated.
IQR, interquartile range
[a]Calculated without 34 individuals whose infection date was not identified.

2. Baseline antibody responses and subsequent SARS-CoV-2 infection

Participants with a subsequent SARS-CoV-2 infection showed significantly lower baseline serum antibody levels of Wuhan S1-IgG, BA.5 S1-IgG, and neutralizing antibodies against BA.5 compared to those who did not experience a subsequent infection (all P <0.001). These differences were statistically significant across both the hybrid immunity and infection-naive groups, except for neutralizing antibodies against BA.5 in the infection-naive participants (Figure 4). However, baseline saliva SARS-CoV-2 Wuhan S1-IgA (P=0.42) and BA.5 S1-IgA (P=0.18) antibody levels did not show significant differences between those with and without subsequent infection (Figure 5).

Baseline serum and saliva antibody levels were significantly higher in participants with hybrid immunity (all P <0.01, Figure 6), regardless of immune markers. Also, there was a significant inverse correlation between the interval from the previous infection to baseline antibody measurement and the level of each immune marker (all P <0.01, Figure 7).

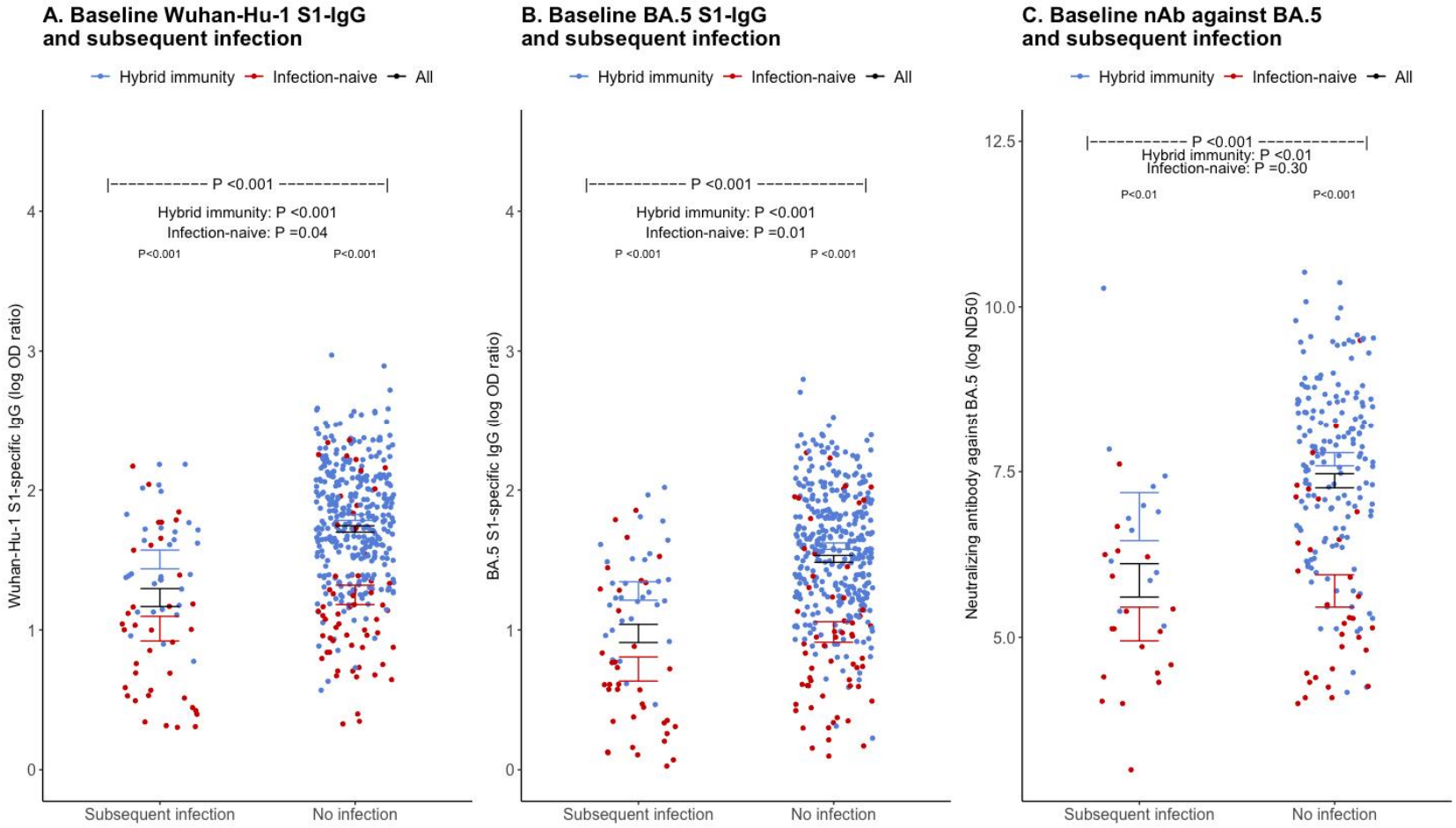


Figure 4. Baseline serum SARS-CoV-2 S1-IgG and neutralizing antibody levels according to the subsequent SARS-CoV-2 infection. *A*. Wuhan-Hu-1 S1-specific IgG. *B*. BA.5 S1-specific IgG. *C*. Neutralizing antibody against BA.5. Horizontal lines indicate mean ± standard error.

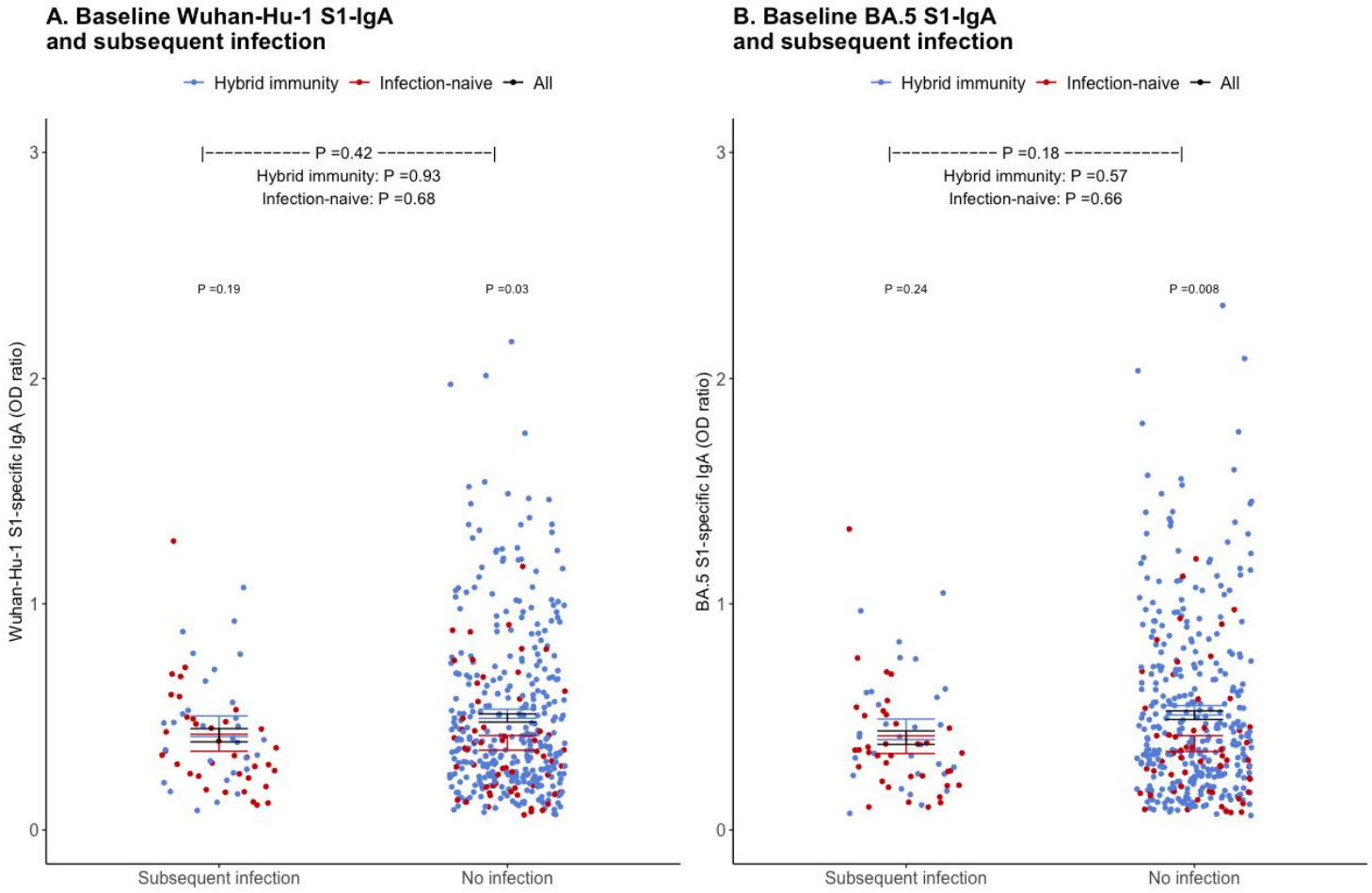
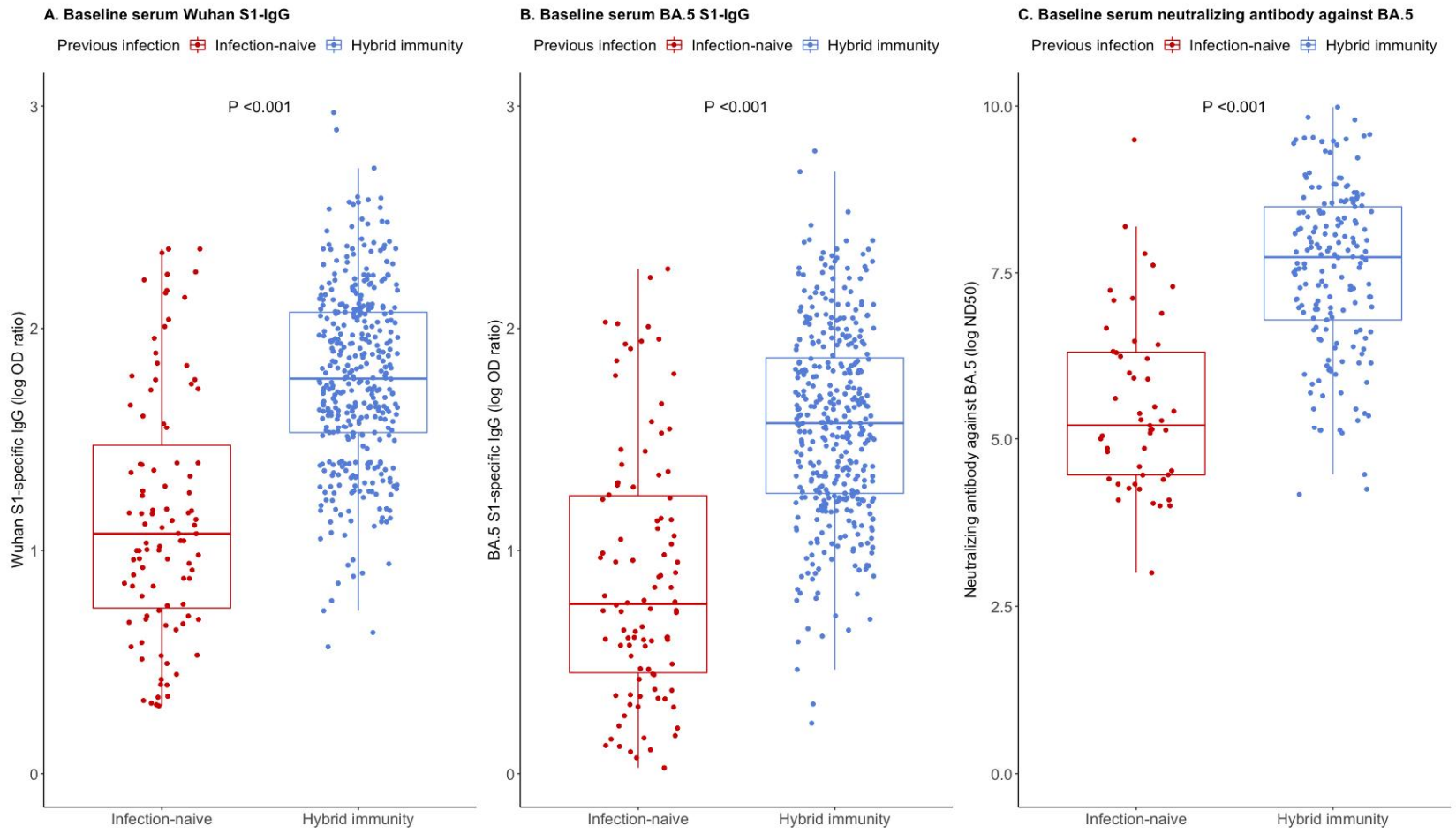


Figure 5. Baseline saliva SARS-CoV-2 S1-IgA levels according to the subsequent SARS-CoV-2 infection. *A*. Wuhan-Hu-1 S1-specific IgA. *B*. BA.5 S1-specific IgA. Horizontal lines indicate the mean ± standard error.


A. Baseline serum Wuhan S1-IgG
Previous infection Infection-naive Hybrid immunity
P <0.001
Wuhan S1-specific IgG (log OD ratio)
0
1
2
3
Infection-naive
Hybrid immunity
B. Baseline serum BA.5 S1-IgG
Previous infection Infection-naive Hybrid immunity
P <0.001
BA.5 S1-specific IgG (log OD ratio)
0
1
2
3
Infection-naive
Hybrid immunity
C. Baseline serum neutralizing antibody against BA.5
Previous infection Infection-naive Hybrid immunity
P <0.001
Neutralizing antibody against BA.5 (log ND50)
0.0
2.5
5.0
7.5
10.0
Infection-naive
Hybrid immunity

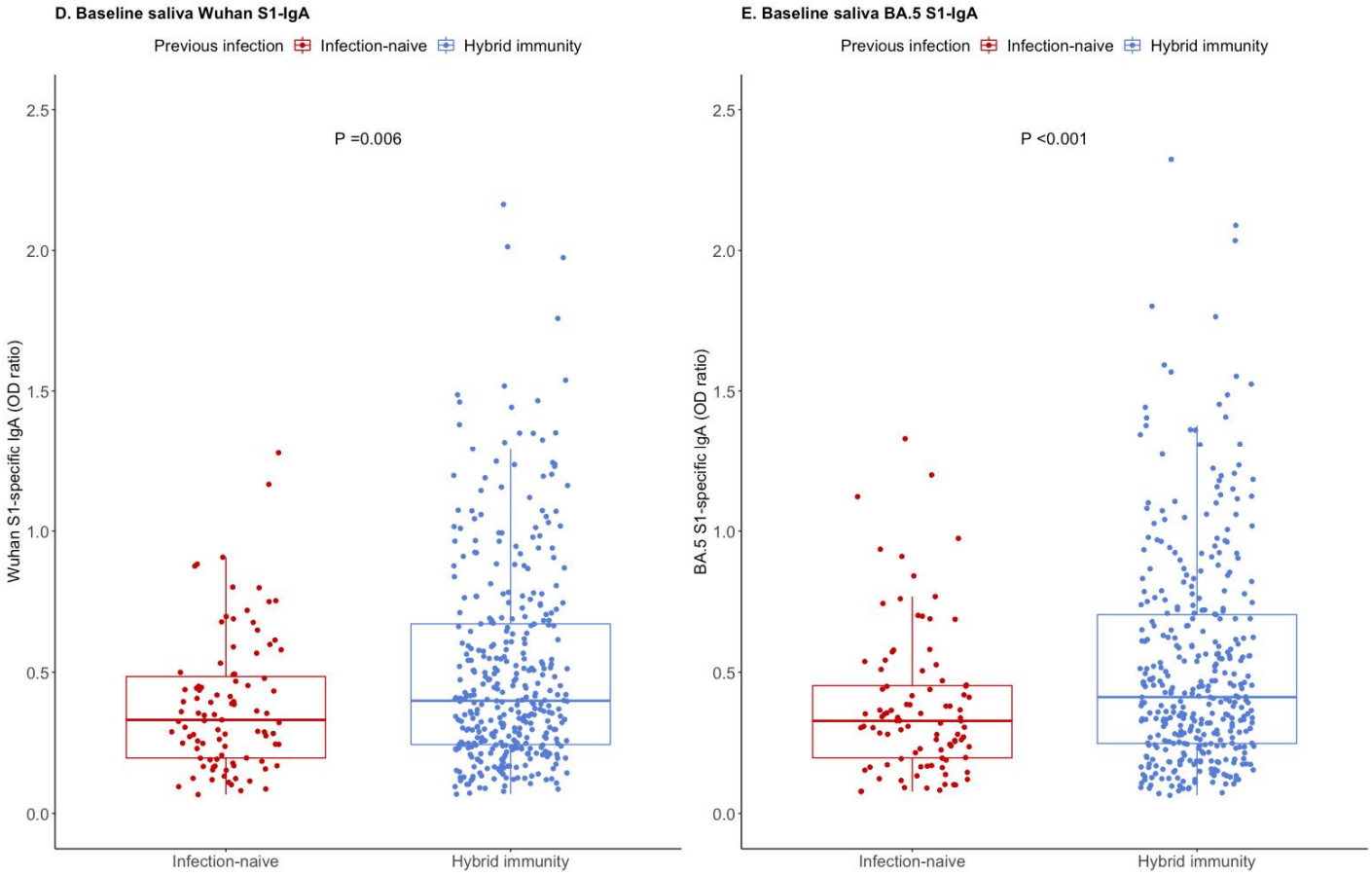
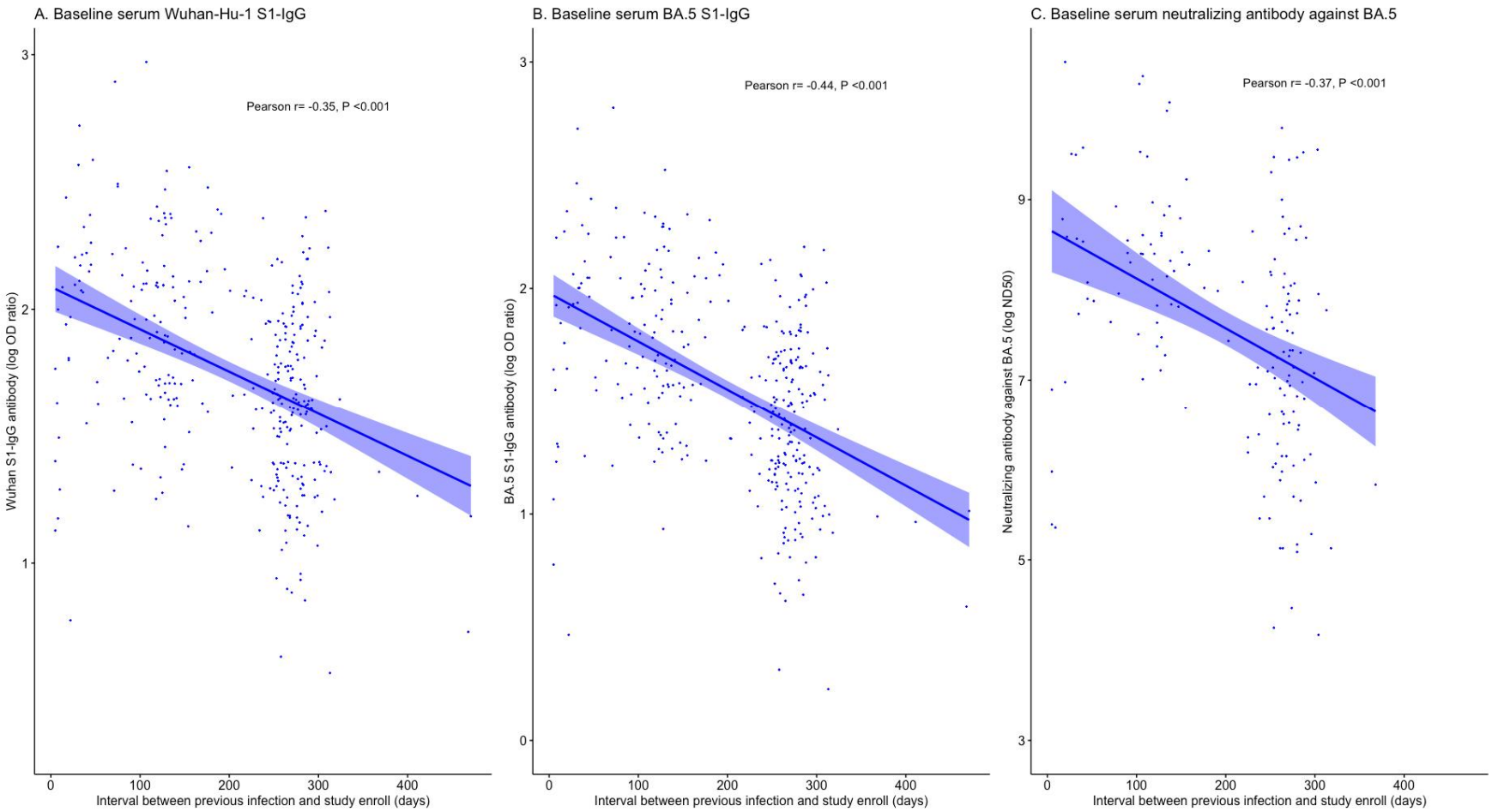


Figure 6. The baseline antibody levels according to the previous infection history. Each box denotes the interquartile range (IQR), where the lower boundary of the box indicates the 25th percentile and the upper boundary indicates the 75th percentile. The line inside the box represents the median value. Whiskers extend from the box to the minimum and maximum values within 1.5 times the IQR.


A. Baseline serum Wuhan-Hu-1 S1-IgG
Pearson r= -0.35, P <0.001
Wuhan S1-IgG antibody (log OD ratio)
Interval between previous infection and study enroll (days)
B. Baseline serum BA.5 S1-IgG
Pearson r= -0.44, P <0.001
BA.5 S1-IgG antibody (log OD ratio)
Interval between previous infection and study enroll (days)
C. Baseline serum neutralizing antibody against BA.5
Pearson r= -0.37, P <0.001
Neutralizing antibody against BA.5 (log ND50)
Interval between previous infection and study enroll (days)

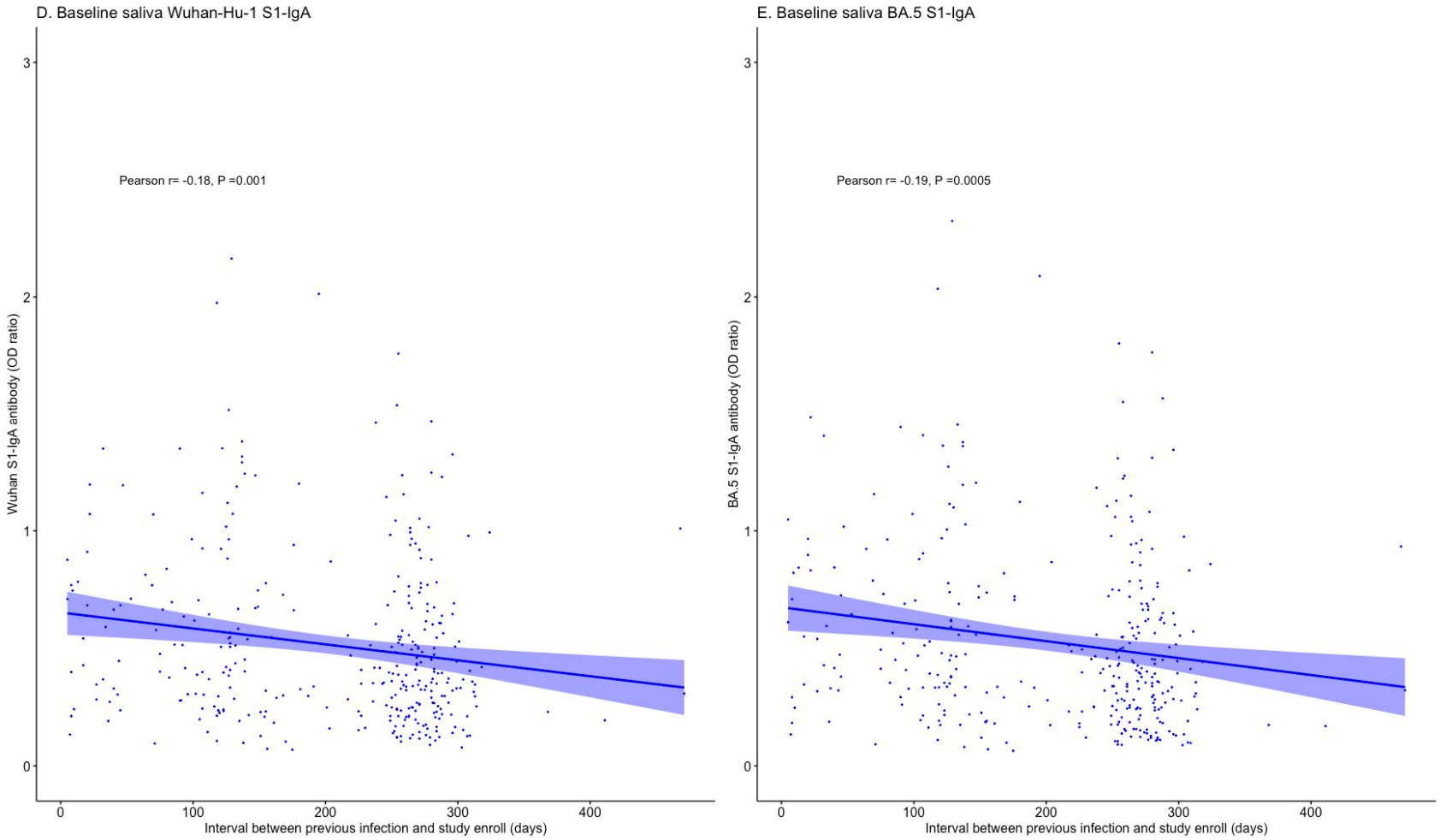


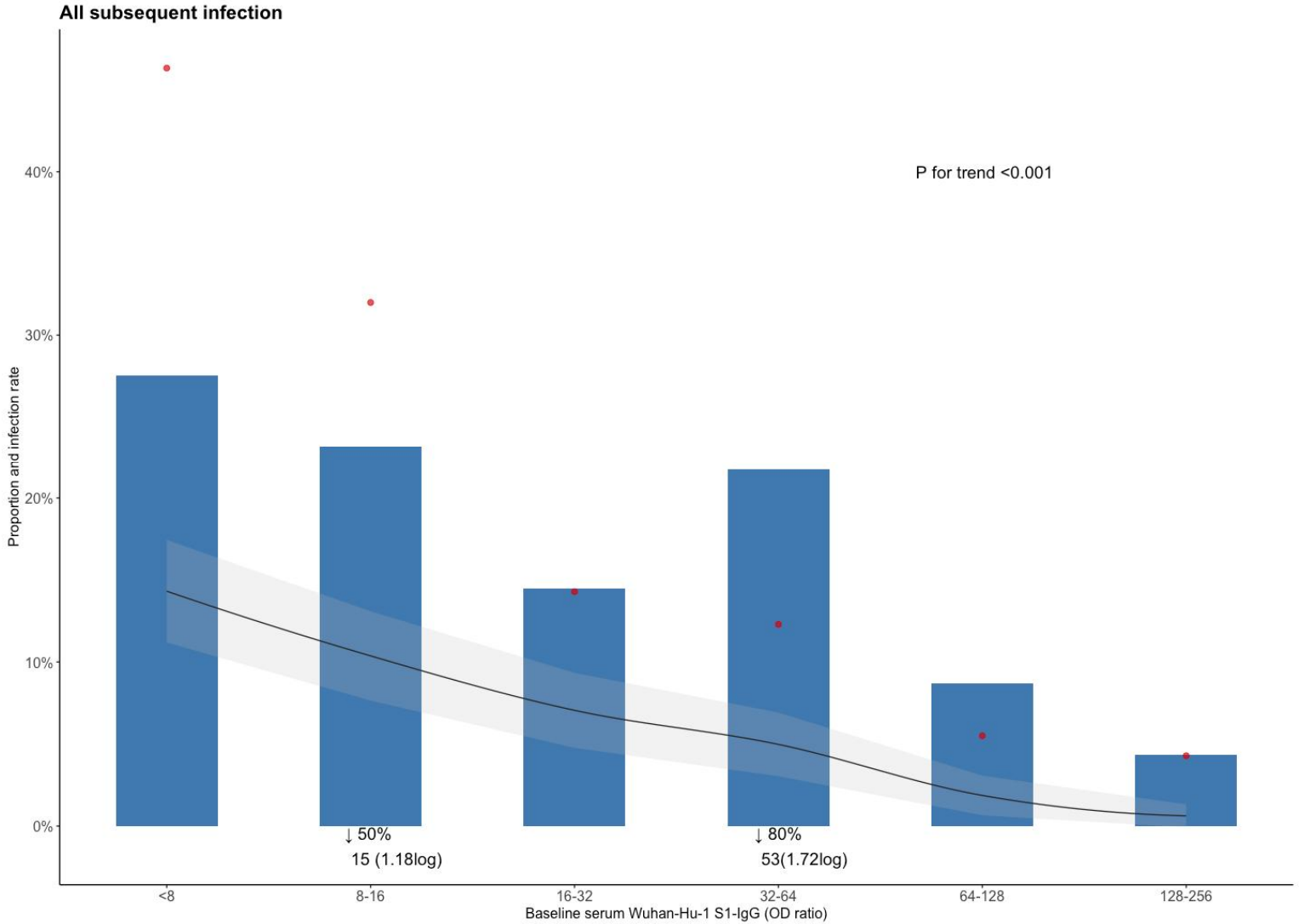
Figure 7. Correlation between time from previous SARS-CoV-2 infection and baseline antibody levels

3. Participants with subsequent infection stratified by baseline SARS-CoV-2 antibody titer

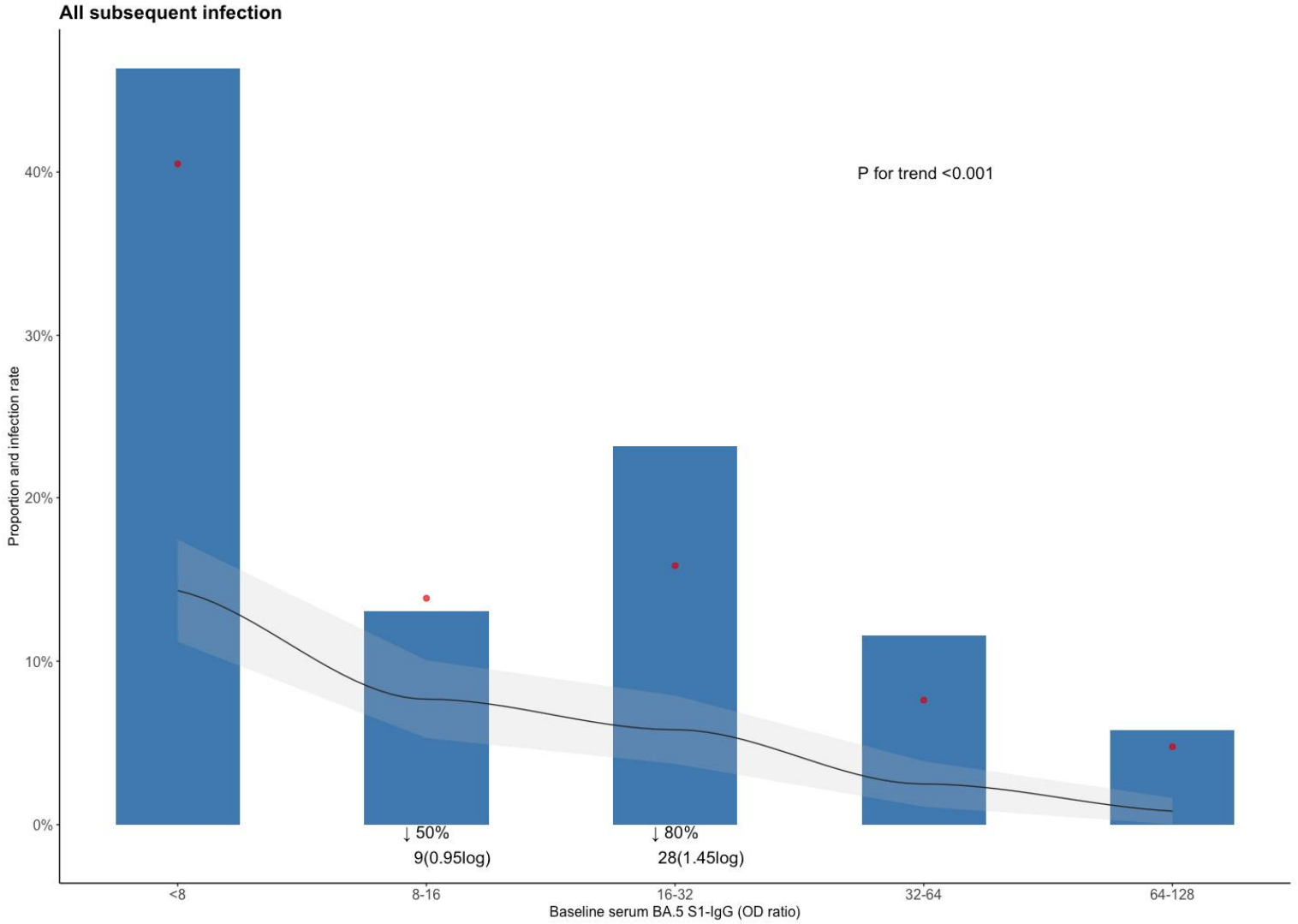
We analyzed the distribution of participants with subsequent infection by antibody level. The 50 percent of infected participants during the study period had antibody levels lower than 1.18 log OD ratio for Wuhan S1-IgG, 0.95 log OD ratio for BA.5 S1-IgG and 5.86 log_2ND50 for neutralizing antibody against BA.5, respectively, and the 80 percent had lower than 1.72 log OD ratio for Wuhan S1-IgG, 1.45 log OD ratio for BA.5 and 6.79 log_2ND50 for neutralizing antibody against BA.5, respectively (Figure 8). The infection rate showed a decreasing trend as serum antibody levels increased (Figure 8A, 8B, and 8C, all *P* for trend <0.001), but no significant trend was observed with saliva antibody levels (Figure 8D, 8E).

For the Wuhan S1-IgG antibody, the probability of having subsequent infection decreased from 14.3% (69/482, all subsequent infection) to 7.1% (34/482) with baseline IgG 1.20 log OD ratio. If Wuhan S1-IgG was higher than 1.81 log OD ratio, the probability of becoming infected among all participants dropped to 1.9% (9/482) (Figure 8A). Similarly, the probability of having subsequent infection decreased as BA.5 S1-IgG increased, reducing to 7.7% (37/482) with baseline IgG 0.9 log OD ratio and 0.8% (4/482) with baseline IgG 1.81 log OD ratio (Figure 8B). Similarly, for baseline neutralizing antibody titers were up to 7.00 log_2ND50, the probability of having subsequent infection was 6.1% (14/228), and among participants with neutralizing antibody titer higher or equal to 7.00 log_2ND50 the probability of subsequent infection dropped to 2.2% (5/228) (Figure 8C).

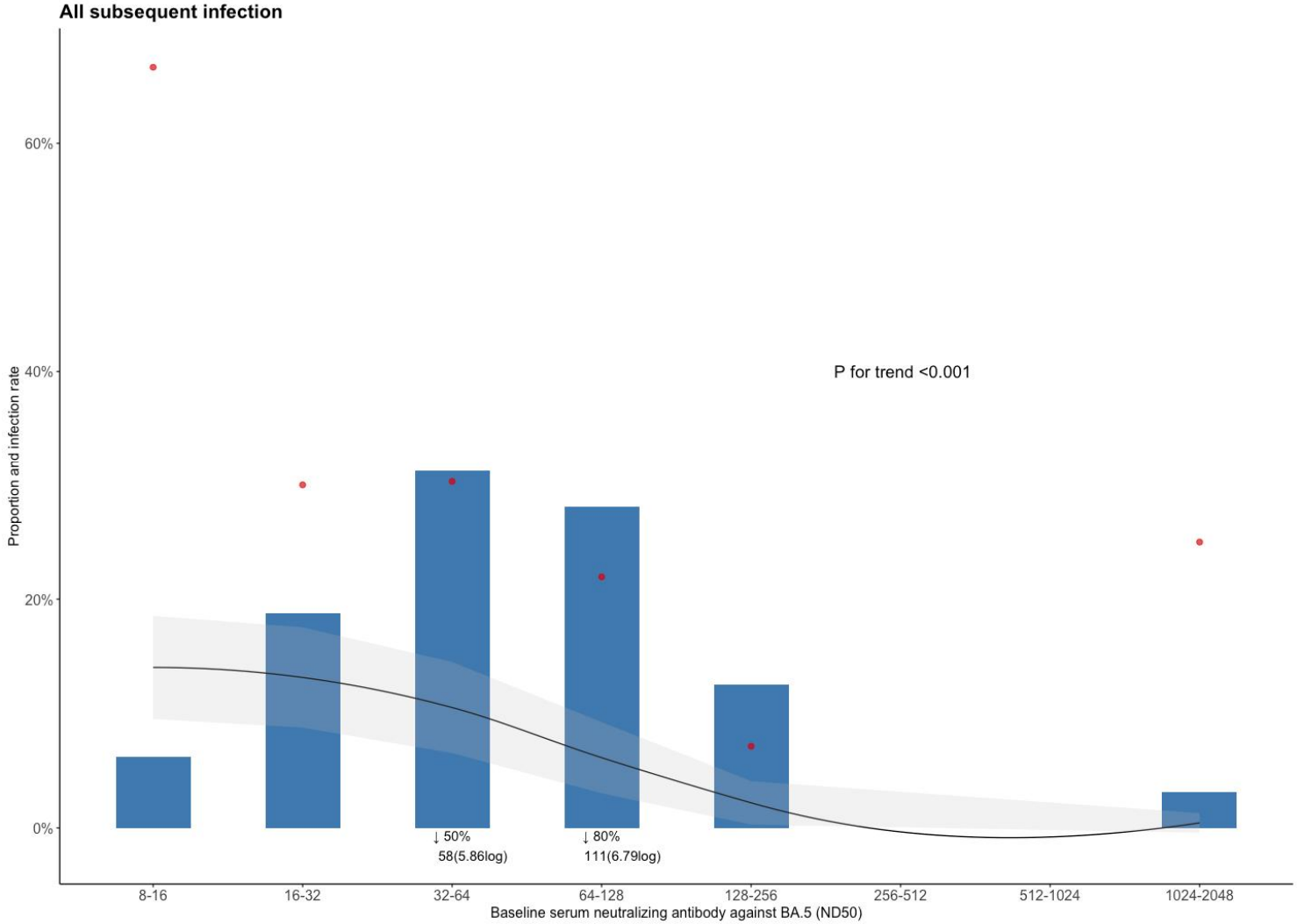
A. Serum Wuhan-Hu-1 S1-IgG



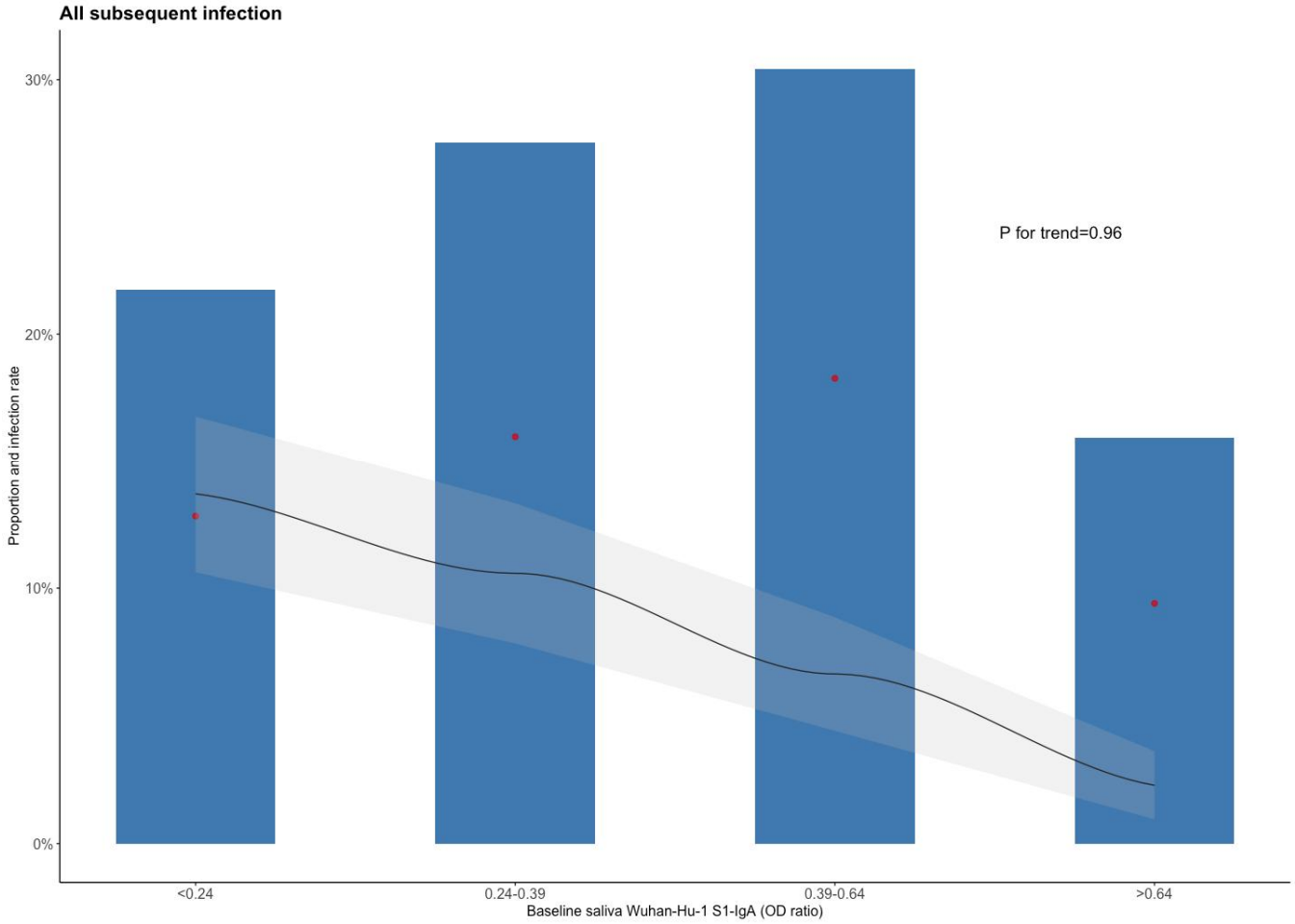
B. Serum BA.5 S1-IgG



C. Serum neutralizing antibody against BA.5



D. Saliva Wuhan-Hu-1 S1-IgA



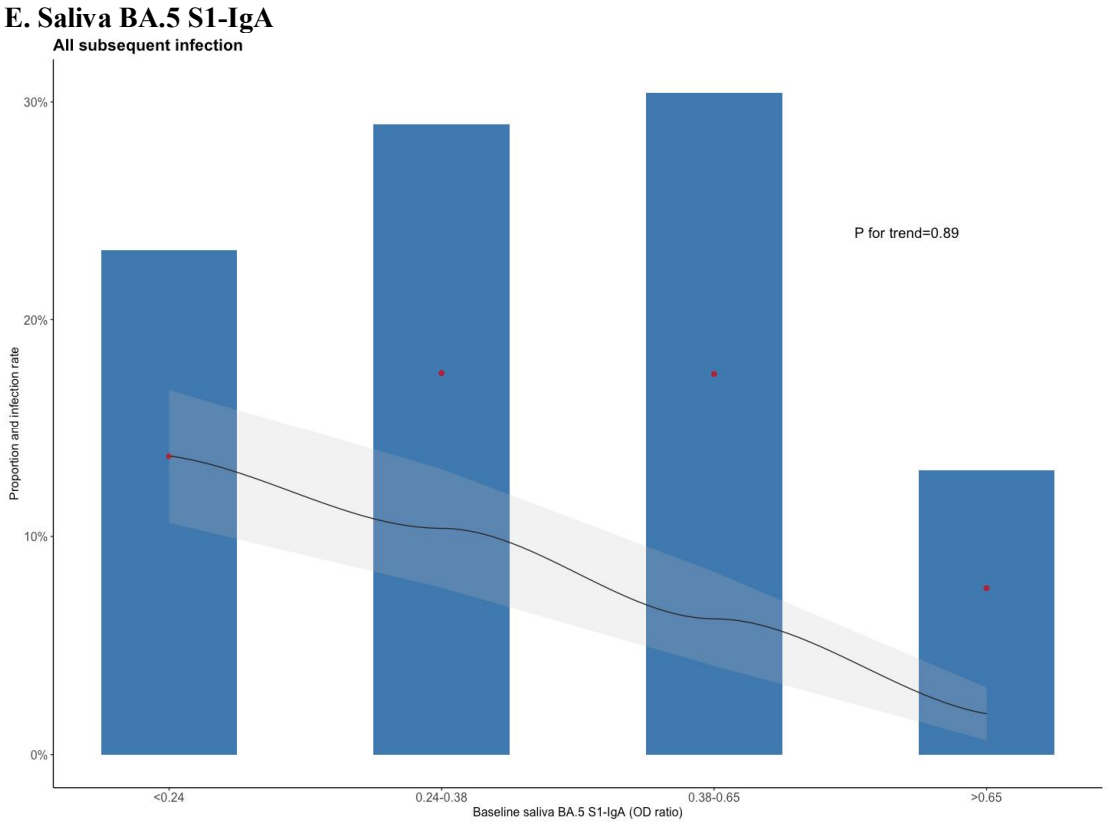


Figure 8. Participants with subsequent infection stratified by baseline SARS-CoV-2 antibody levels. *A*. By serum Wuhan-Hu-1 S1-IgG. *B*. By serum BA.5 S1-IgG. *C*. By serum neutralizing antibody against BA.5. *D*. By saliva Wuhan-Hu-1 S1-IgA. *E*. By saliva BA.5 S1-IgA. The blue bars represent the distribution of infected individuals attributed to each specific antibody titer. The red dots indicate the infection rate at each specific antibody titer. Black lines indicate the probability of getting infected with antibody titers exceeding the given values, providing cumulative incidence, and the grey shaded area denotes the 95% confidential interval. The *P* value for trend of the infection rate was calculated using the Cochran-Armitage test. The cutoff levels corresponding to 50% and 80% of the infected individuals were indicated for the serum antibody responses. The x-axis of saliva antibody levels was divided into quartiles.

4. Receiver operating characteristic curves of each immune marker

We estimated the receiver operating characteristic (ROC) curves of each immune marker to estimate the optimal cutoff value to discriminate subsequent infection in this study. The optimal cutoff values for distinguishing subsequent infection were 1.36 log OD ratio for baseline serum Wuhan-Hu-1 S1-IgG, 1.43 log OD ratio for baseline serum BA.5 S1-IgG and 7.00 log_2ND50 for baseline serum neutralizing antibody against BA.5, respectively.

At the study enrollment, the area under the receiver operating characteristic curve (AUROC) was the highest for neutralizing antibody against BA.5 (0.78; 95% CI, 0.70–0.86) followed by BA.5 S1-IgG (0.77; 95% CI, 0.71–0.82) and Wuhan S1-IgG (0.75; 95% CI, 0.69–0.81). The AUROCs were not significantly different between the three immune markers (Figure 9). Meanwhile, AUROC of baseline saliva Wuhan S1-specific IgA was 0.53 (95% CI, 0.46–0.60), and that of baseline saliva BA.5 S1-IgA was 0.55 (95% CI, 0.48–0.62) (Figure 10).

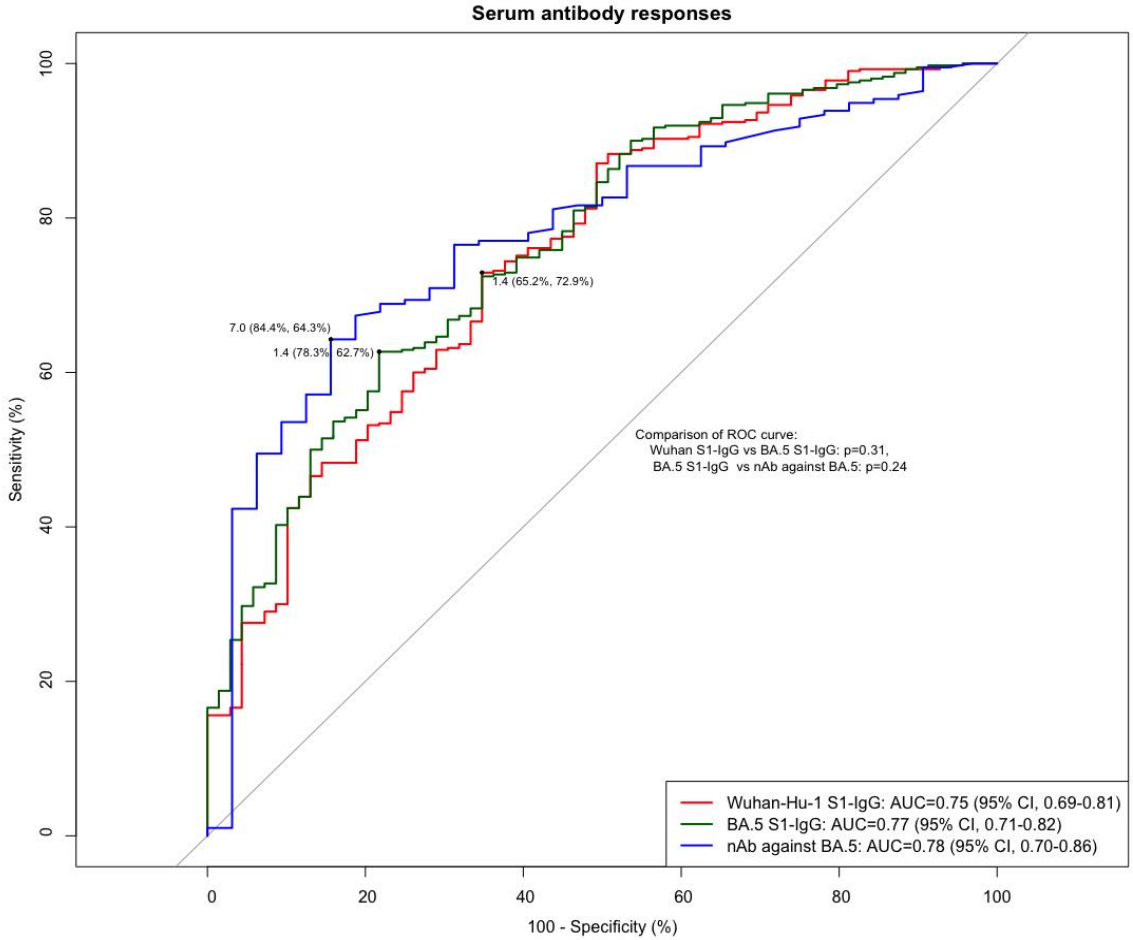


Figure 9. Receiver operating characteristic curves of baseline serum Wuhan-Hu-1 S1-IgG, BA.5 S1-IgG, and neutralizing antibody against BA.5 for distinguishing subsequent infection. Black dots indicate the optimal cutoff value by the Youden index method and the two numbers in parentheses refer to specificity and sensitivity, sequentially.

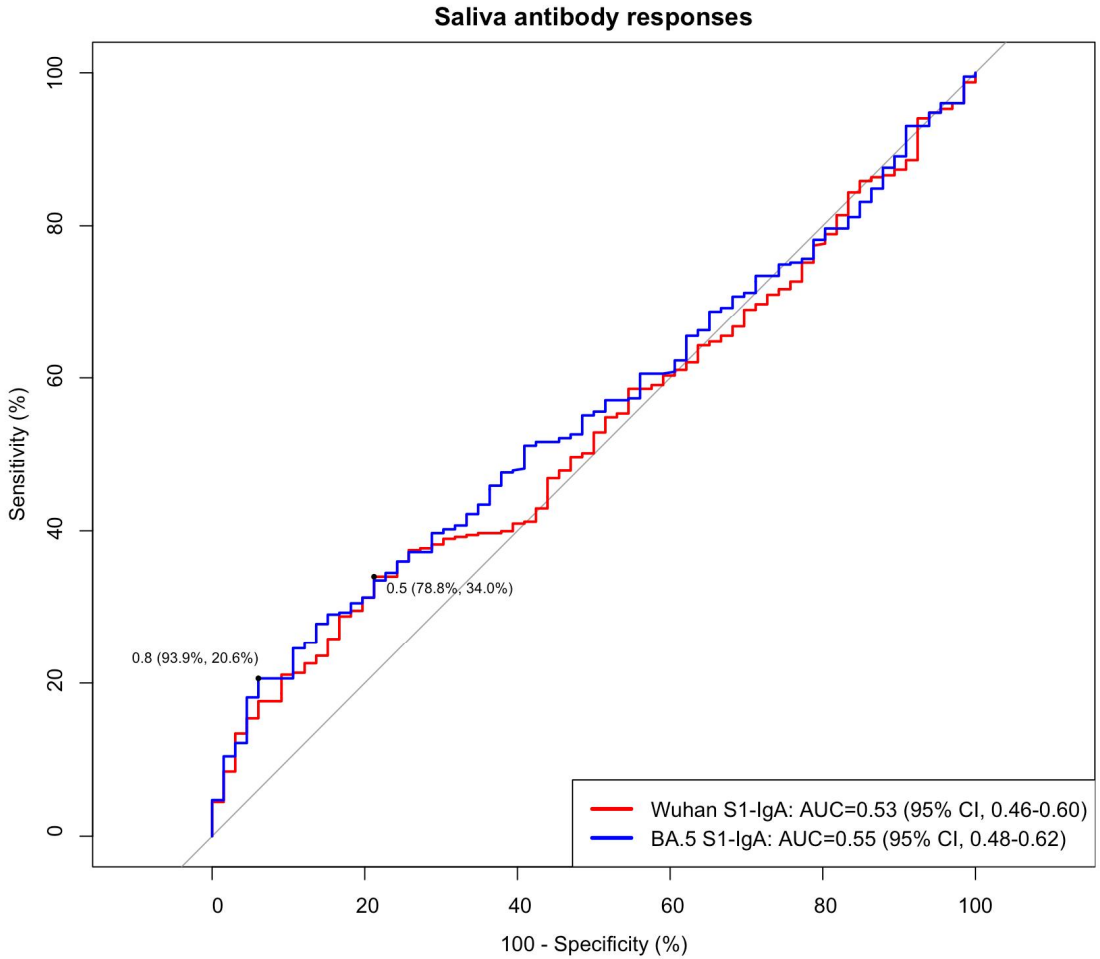


Figure 10. Receiver operating characteristic curves of saliva Wuhan-Hu-1 S1-IgA and BA.5 S1-IgA for distinguishing subsequent infection. Black dots indicate the optimal cutoff value by the Youden index method and the two numbers in parentheses refer to specificity and sensitivity, sequentially.

5. Protective factors against subsequent SARS-CoV-2 infection

We fitted a Cox-proportional hazard model to estimate the hazard ratio against SARS-CoV-2 infection. Baseline antibody levels of each immune marker higher than optimal cutoff values determined by ROC curves were used as predictive variables. In univariate analysis, hybrid immunity (P <0.001) and high baseline serum antibody level of Wuhan S1-IgG (P <0.001), BA.5 S1-IgG (P <0.001), neutralizing antibody against BA.5 (P <0.001), Wuhan S1-IgA (P=0.045), BA.5 S1-IgA (P=0.02) were found to be protective factors against subsequent SARS-CoV-2 infection. In multivariable Cox regression analysis, hybrid immunity (adjusted hazard ratio [aHR] 0.20 in analysis with Wuhan S1-IgG, aHR 0.20 in analysis with BA.5 S1-IgG, and aHR 0.19 in analysis with neutralizing antibody against BA.5, respectively; all P <0.01) and baseline serum antibody level higher than cutoff value (serum Wuhan S1-IgG, aHR=0.43, P=0.02; BA.5 S1-IgG, aHR=0.32, P=0.005; neutralizing antibody against BA.5, aHR=0.26, P=0.045) were independent protective factor against subsequent SARS-CoV-2 infection during the 5-month follow-up period, whereas saliva IgA antibody levels did not show protective effect (Table 2).

Table 2. Cox-proportional hazard model for prediction of subsequent infection using baseline antibody levels

	Univariate analysis		Multivariable analysis	
Variable (prediction by baseline serum Wuhan S1-IgG) (n=466)	**Hazard ratio (95% confidence interval)**	***P* value**	**Adjusted odds ratio (95% confidence interval)**	***P* value**
Homologous previous vaccination	1.30 (0.67–2.53)	0.44		
Hybrid immunity	0.13 (0.07–0.23)	<0.001	0.20 (0.10–0.39)	<0.001
Bivalent BA.4/5 booster vaccination	1.40 (0.81–2.41)	0.23		
Time since last exposure to SARS-CoV-2 antigen[a] before study enroll (≤ 90 days)	0.16 (0.02–1.17)	0.07	0.46 (0.06–3.53)	0.46
Baseline serum Wuhan S1-specific IgG ≥1.36[b]	0.20 (0.11–0.35)	<0.001	0.43 (0.21–0.87)	0.02
Variable (prediction by baseline serum BA.5 S1-IgG) (n=466)				
Homologous previous vaccination	1.30 (0.67–2.53)	0.44		
Hybrid immunity	0.13 (0.07–0.23)	<0.001	0.20 (0.10–0.37)	<0.001
Bivalent BA.4/5 booster vaccination	1.40 (0.81–2.41)	0.23		
Time since last exposure to SARS-CoV-2 antigen[a] before study enroll (≤ 90 days)	0.16 (0.02–1.17)	0.07	0.55 (0.07–4.25)	0.57
Baseline serum BA.5 S1-specific IgG level ≥1.43[b]	0.15 (0.08–0.31)	<0.001	0.32 (0.15–0.71)	0.005
Variable (prediction by baseline serum neutralizing antibody against BA.5) (n=219)				
Homologous previous vaccination	1.30 (0.67–2.53)	0.44		
Hybrid immunity	0.13 (0.07–0.23)	<0.001	0.19 (0.07–0.51)	0.001
Bivalent BA.4/5 booster vaccination	1.40 (0.81–2.41)	0.23		
Time since last exposure to SARS-CoV-2 antigen[a] before study enroll (≤ 90 days)	0.16 (0.02–1.17)	0.07	0.00 (0.00–NA)	>0.99

Baseline serum neutralizing antibody against BA.5 ≥7.00[b]	0.09 (0.03–0.32)	<0.001	0.26 (0.07–0.97)	0.045
Variable (prediction by baseline saliva Wuhan S1-IgA) (n=454)				
Homologous previous vaccination	1.30 (0.67–2.53)	0.44		
Hybrid immunity	0.13 (0.07–0.23)	<0.001	0.14 (0.08–0.26)	<0.001
Bivalent BA.4/5 booster vaccination	1.40 (0.81–2.41)	0.23		
Time since last exposure to SARS-CoV-2 antigen[a] before study enroll (≤ 90 days)	0.16 (0.02–1.17)	0.07	0.43 (0.06–3.26)	0.42
Baseline saliva Wuhan S1-specific IgA ≥0.53[b]	0.49 (0.25–0.99)	0.045	0.73 (0.36–1.47)	0.38
Variable (prediction by baseline saliva BA.5 S1-IgA) (n=454)				
Homologous previous vaccination	1.30 (0.67–2.53)	0.44		
Hybrid immunity	0.13 (0.07–0.23)	<0.001	0.14 (0.08–0.27)	<0.001
Bivalent BA.4/5 booster vaccination	1.40 (0.81–2.41)	0.23		
Time since last exposure to SARS-CoV-2 antigen[a] before study enroll (≤ 90 days)	0.16 (0.02–1.17)	0.07	0.45 (0.06–3.36)	0.43
Baseline saliva BA.5 S1-specific IgA level ≥0.76[b]	0.25 (0.08–0.81)	0.02	0.40 (0.12–1.29)	0.12

NA, Not available

[a]Either vaccination or infection

[b]Optimal cutoff value determined from ROC curve (values are presented as the $\log_{10}$ of the OD ratio for IgG and $\log_2$ of the ND50 for neutralizing antibody)

6. Stratified analysis according to the baseline serum antibody level

The cumulative incidence of subsequent infection according to the baseline serum antibody levels of each immune marker was estimated using the Kaplan-Meier method (Figure 11). The high and low antibody levels were divided based on the cutoff value derived from the ROC curves, and the cumulative SARS-CoV-2 infection rate was significantly higher among participants with low baseline serum antibody levels (all P <0.001).

Based on baseline antibody level at study enrollment, a stratified analysis was also performed. In participants with low baseline serum antibody levels of Wuhan S1-IgG, BA.5 S1-IgG and neutralizing antibody against BA.5, those with hybrid immunity showed a significantly low cumulative incidence of subsequent infection compared with infection-naïve participants (all P <0.001) (Figure 12A). Among participants with high baseline antibody levels of Wuhan S1-IgG and BA.5 S1-IgG antibody, there was a significant difference in cumulative incidence of subsequent infection between those with hybrid immunity and infection-naïve (P <0.001 and P=0.001, respectively), but this difference was not observed among those with high levels of neutralizing antibody against BA.5 (P=0.68) (Figure 12B).

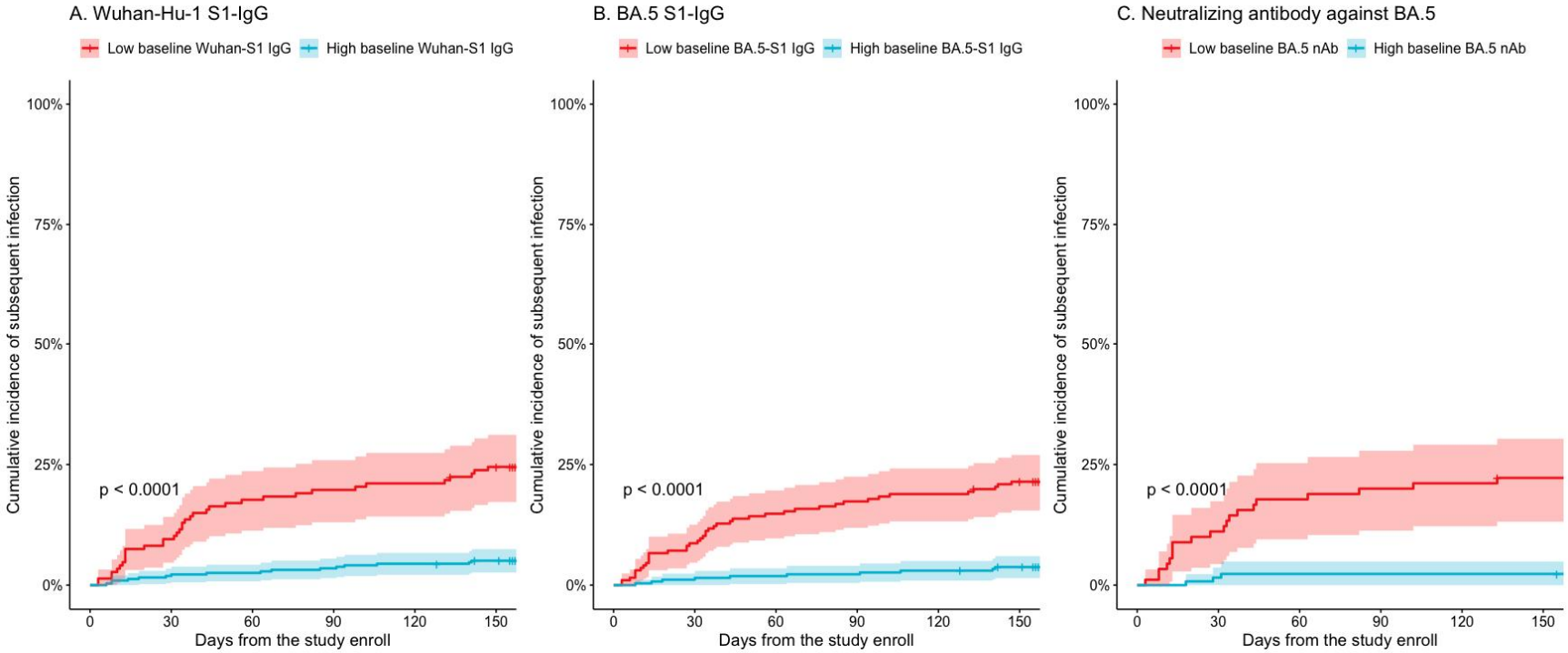
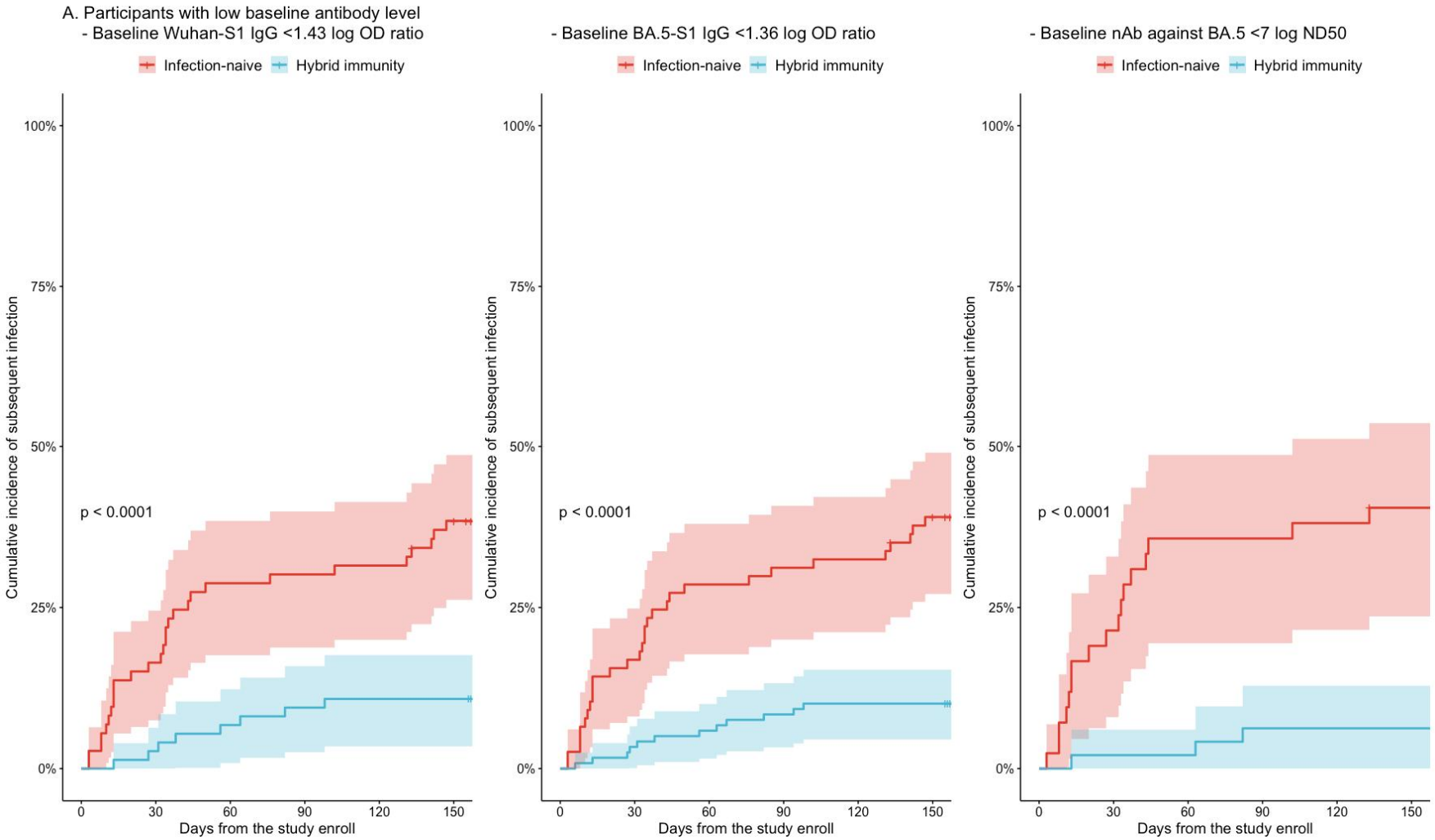


Figure 11. Cumulative incidence of subsequent infection by baseline serum antibody levels higher and lower than cutoff values determined by ROC curves. *A*. Wuhan-Hu-1 S1-IgG. *B*. BA.5 S1-IgG. *C*. Neutralizing antibody against BA.5.


A. Participants with low baseline antibody level
- Baseline Wuhan-S1 IgG <1.43 log OD ratio
Infection-naive
Hybrid immunity
Cumulative incidence of subsequent infection
100%
75%
50%
25%
0%
p < 0.0001
0
30
60
90
120
150
Days from the study enroll
- Baseline BA.5-S1 IgG <1.36 log OD ratio
Infection-naive
Hybrid immunity
Cumulative incidence of subsequent infection
100%
75%
50%
25%
0%
p < 0.0001
0
30
60
90
120
150
Days from the study enroll
- Baseline nAb against BA.5 <7 log ND50
Infection-naive
Hybrid immunity
Cumulative incidence of subsequent infection
100%
75%
50%
25%
0%
p < 0.0001
0
30
60
90
120
150
Days from the study enroll

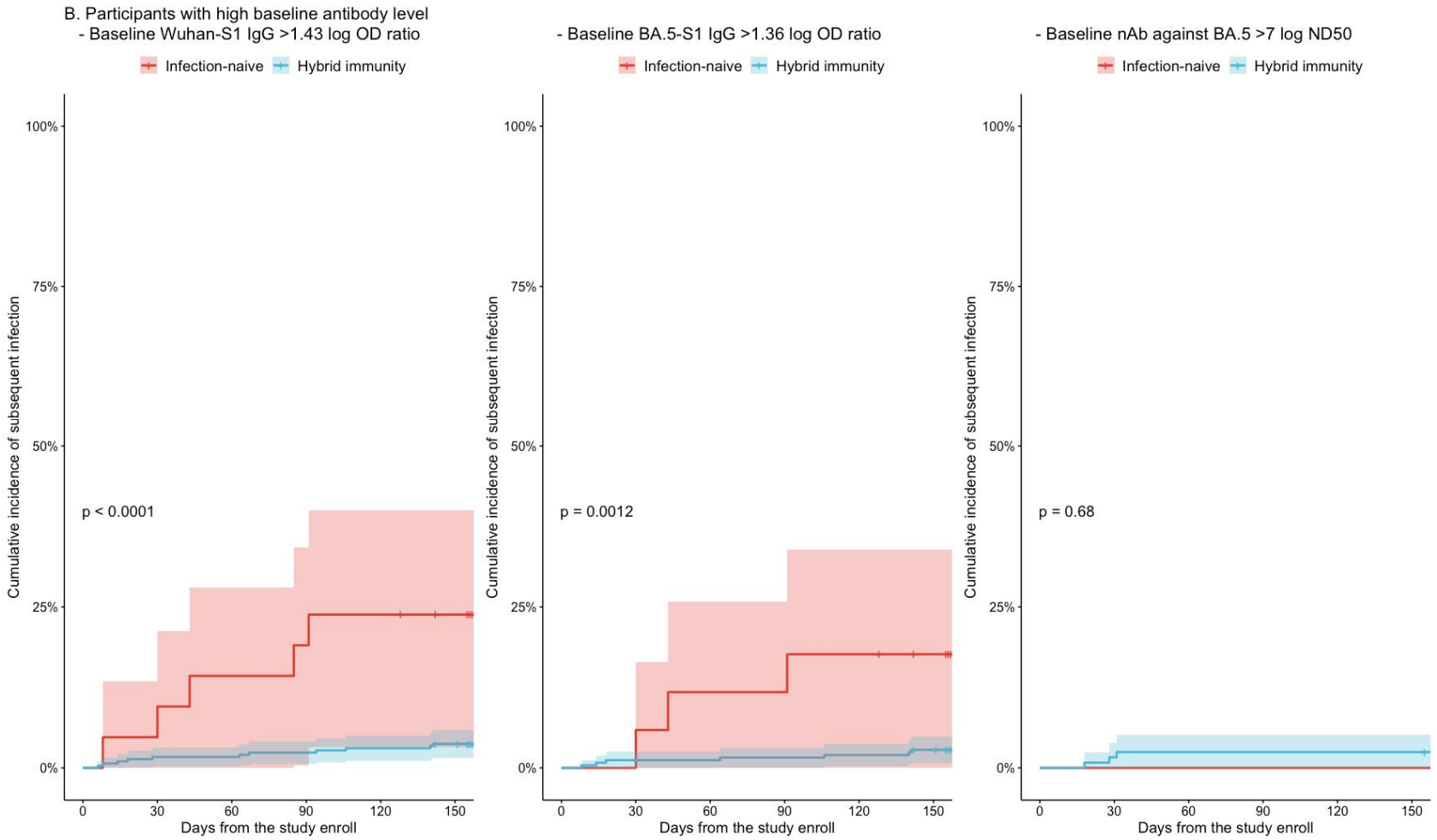


Figure 12. Comparison of cumulative incidence of subsequent infection according to the previous SARS-CoV-2 infection stratified by baseline serum antibody level. *A*. Participants with low baseline antibody levels. *B*. Participants with high baseline antibody levels.

7. Subgroup analysis 1-month after bivalent vaccine administration

Subgroup analysis was also conducted to reflect the effect of bivalent vaccine on antibody responses after excluding participants who were infected within 1 month after vaccination. A total of 470 participants were included in the subgroup analysis with 57 (12.1%) subsequently infected participants.

In the 1-month subgroup analysis, the participants who received the bivalent vaccine were assessed based on their antibody levels 1 month after vaccination, while those who did not receive the bivalent vaccine were evaluated using their baseline antibody levels. The optimal cutoff values for distinguishing subsequent infection in the 1-month subgroup analysis were determined to be 1.85 log OD ratio for serum Wuhan-Hu-1 S1-IgG, 1.55 log OD ratio for serum BA.5 S1-IgG and 7.86 $\log_2$ND50 for serum neutralizing antibody against BA.5, respectively (Figure 13).

Hybrid immunity and antibody levels exceeding optimal cutoff value were also significant protective factors against subsequent infection in the 1-month subgroup analysis (Table 3). However, there was no significant difference in the cumulative incidence of subsequent infection according to the bivalent vaccine administration in the 1-month subgroup analysis (Figure 14).

The differences in demographics and baseline characteristics according to the bivalent vaccine administration are shown in Table 4. There were significantly more participants who experienced previous SARS-CoV-2 infection in those who did not receive the bivalent vaccine while those who received the bivalent vaccine were significantly older.

Additionally, the baseline Wuhan S1-IgG and BA.5 S1-IgG antibody levels were significantly higher in participants who did not receive the bivalent vaccine (all P <0.05) regardless of the previous infection history, despite only observing a numerical trend in terms of neutralizing antibody levels (hybrid immunity, P=0.20; infection-naïve, P=0.77) (Figure 15).

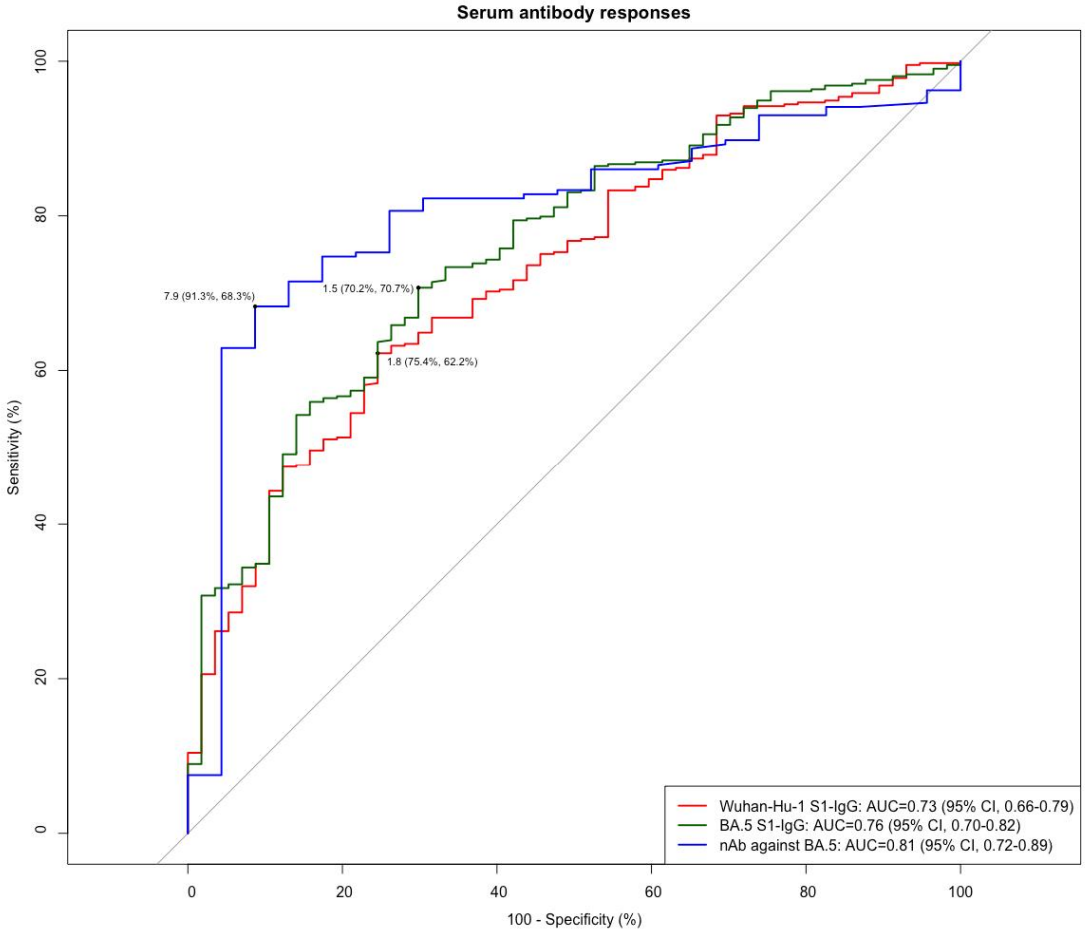


Figure 13. Receiver operating characteristic curves for 1-month subgroup analysis with serum Wuhan-Hu-1 S1-IgG, BA.5 S1-IgG, and neutralizing antibody against BA.5 for distinguishing subsequent infection. Black dots indicate the optimal cutoff value by the Youden index method and the two numbers in parentheses refer to specificity and sensitivity, sequentially.

Table 3. Cox-proportional hazard model for the 1-month subgroup analysis for prediction of subsequent infection.

	Univariate analysis		Multivariable analysis	
Variable (prediction by Wuhan S1-IgG) (n=454[a])	**Hazard ratio (95% confidence interval)**	***P* value**	**Adjusted odds ratio (95% confidence interval)**	***P* value**
Homologous previous vaccination	1.39 (0.66–2.91)	0.38		
Hybrid immunity	0.14 (0.08–0.27)	<0.001	0.17 (0.09–0.31)	<0.001
Bivalent BA.4/5 booster vaccination	0.76 (0.38–1.53)	0.44		
Time since last exposure to SARS-CoV-2 antigen[b] before study enroll (≤ 90 days)	0.20 (0.03–1.49)	0.12		
Wuhan S1-specific IgG ≥1.85[c]	0.31 (0.16–0.59)	<0.001	0.40 (0.21–0.78)	0.007
Variable (prediction by BA.5 S1-IgG) (n=454[a])				
Homologous previous vaccination	1.39 (0.66–2.91)	0.38		
Hybrid immunity	0.14 (0.08–0.27)	<0.001	0.19 (0.10–0.36)	<0.001
Bivalent BA.4/5 booster vaccination	0.76 (0.38–1.53)	0.44		
Time since last exposure to SARS-CoV-2 antigen[b] before study enroll (≤ 90 days)	0.20 (0.03–1.49)	0.12		
BA.5 S1-specific IgG level≥1.55[c]	0.21 (0.11–0.41)	<0.001	0.30 (0.15–0.59)	<0.001
Variable (prediction by neutralizing antibody) (n=200[a])				
Homologous previous vaccination	1.39 (0.66–2.91)	0.38		
Hybrid immunity	0.14 (0.08–0.27)	<0.001	0.22 (0.07–0.72)	0.01
Bivalent BA.4/5 booster vaccination	0.76 (0.38–1.53)	0.44		
Time since last exposure to SARS-CoV-2 antigen[b] before study enroll (≤ 90 days)	0.20 (0.03–1.49)	0.12		

Neutralizing antibody against BA.5≥7.63[c]	0.09 (0.02–0.39)	0.001	0.15 (0.03–0.75)	0.02

[a]The 1-month subgroup analysis excluded those with the occurrence of subsequent infection within 1-month after bivalent booster vaccination.
[b]Either vaccination or infection
[c]Optimal cutoff value determined from Youden index (values are presented as the $\log_{10}$ of the OD ratio for IgG and $\log_2$ of the ND50 for neutralizing antibody). Antibody titers were used as the baseline antibody level for individuals who did not receive the bivalent vaccine and as the antibody level one month after vaccination for those who did receive the bivalent vaccine.

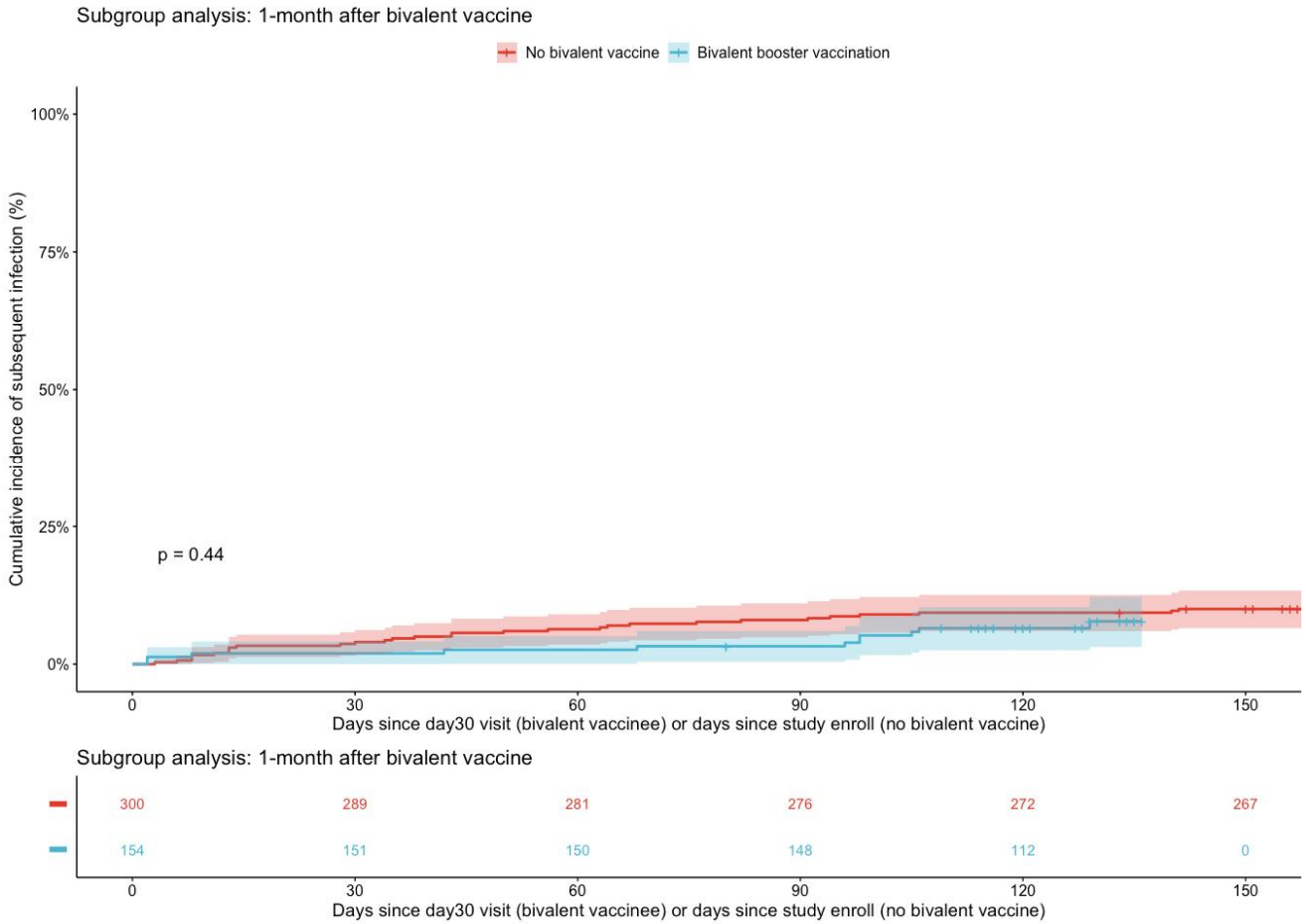
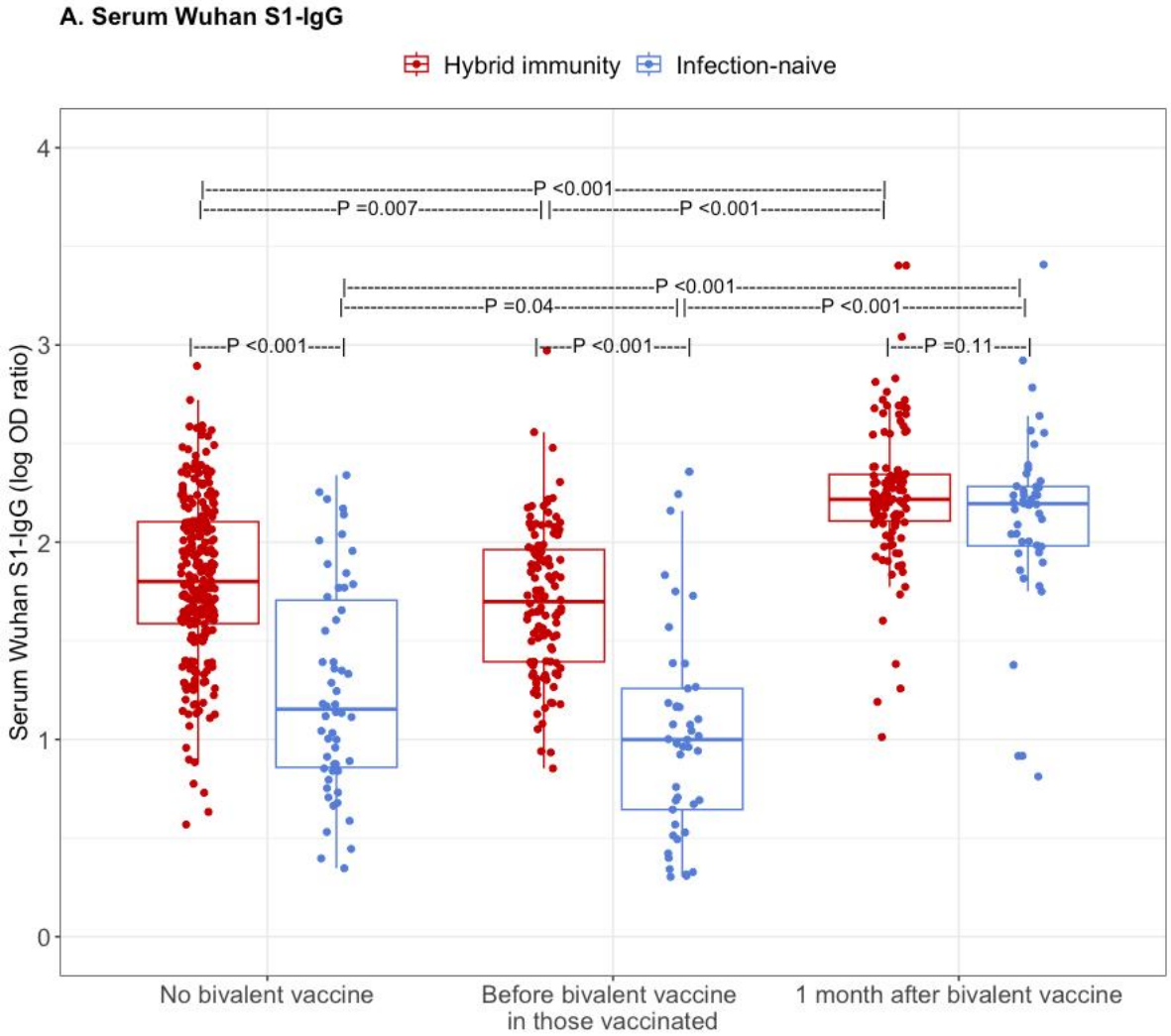


Figure 14. Cumulative incidence of subsequent SARS-CoV-2 infection according to the bivalent vaccine administration in the 1-month subgroup analysis. The 1-month subgroup analysis excluded those with the occurrence of subsequent infection within 1-month after bivalent booster vaccination.

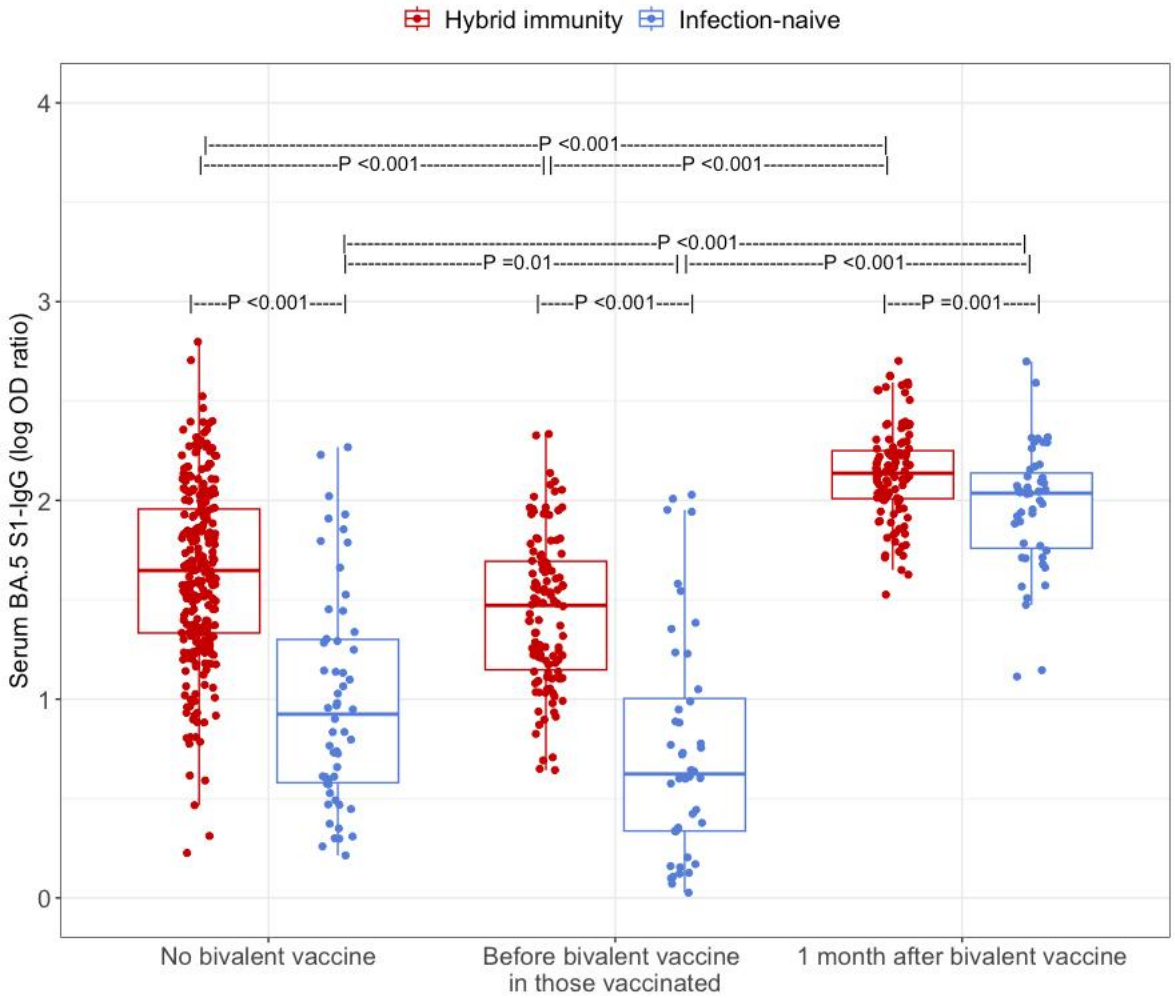
Table 4. Demographics and baseline characteristics of study participants according to the bivalent vaccine administration

Variable	Bivalent vaccine (n=166)	No bivalent vaccine (n=316)	*P* value
Age, years, median (IQR)	39 (32–46)	31 (27–38)	<0.001
Male	33 (19.9)	41 (13.0)	0.046
Underlying comorbidity	25 (15.1)	30 (9.5)	0.07
Number of previous vaccinations			0.02
3-dose	152 (91.6)	305 (96.5)	
4-dose	14 (8.4)	11 (3.5)	
Type of previous vaccination			0.41
Heterologous	141 (84.9)	259 (82.0)	
Homologous	25 (15.1)	57 (18.0)	
Time from last vaccination to study enroll, days, median (IQR)	375 (364–383)	375 (366–383)	0.99
Previous SARS-CoV-2 infection	119 (71.7)	262 (82.9)	0.004
Time from the previous infection, days, median (IQR)[a]	255 (150–271)	242 (120–277)	0.13
Time from last SARS-CoV-2 antigen exposure, days, median (IQR)[a]	264 (149–330)	256 (125–290)	0.03

Data represent n (%) unless otherwise indicated.
IQR, interquartile range
[a]Calculated without 34 individuals whose infection date was not identified.


A. Serum Wuhan S1-IgG
Hybrid immunity
Infection-naive
Serum Wuhan S1-IgG (log OD ratio)
0
1
2
3
4
P <0.001
P =0.007
P <0.001
P <0.001
P =0.04
P <0.001
P <0.001
P <0.001
P =0.11
No bivalent vaccine
Before bivalent vaccine in those vaccinated
1 month after bivalent vaccine

B. Serum BA.5 S1-IgG



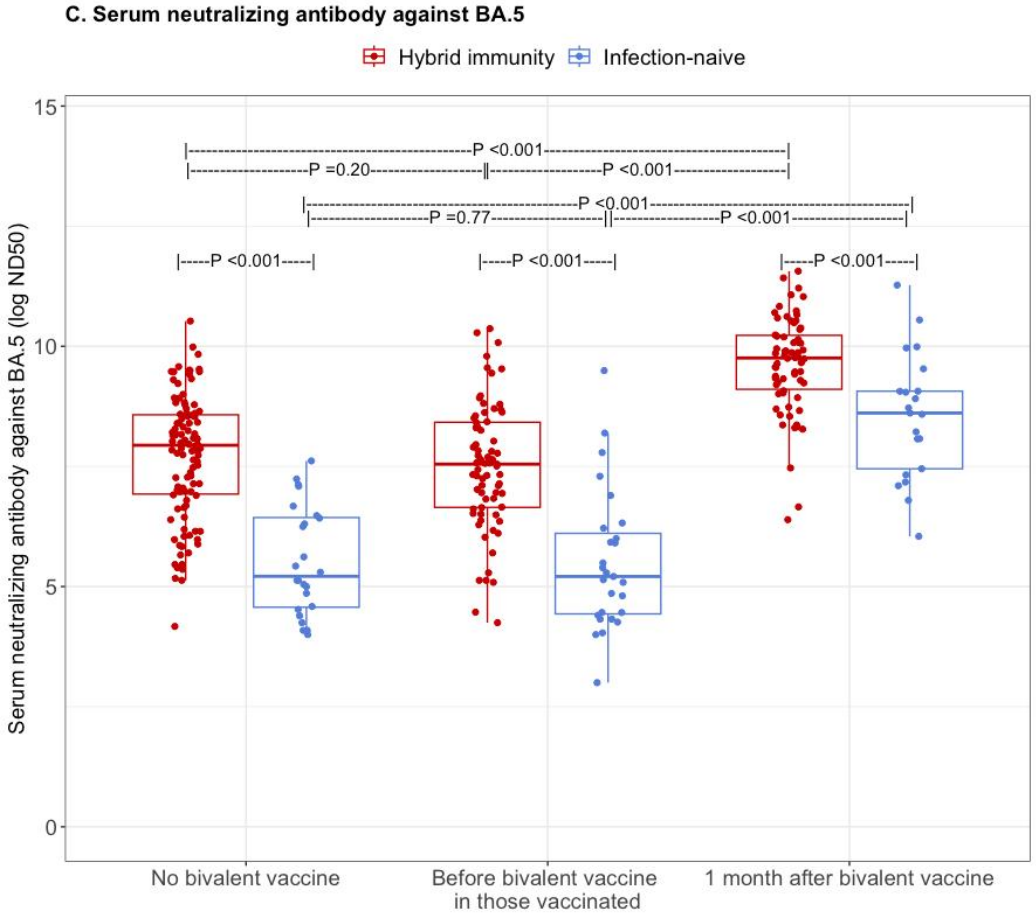


Figure 15. Serum antibody levels before and after bivalent booster vaccine. *A*. Serum Wuhan-Hu-1 IgG. *B*. Serum BA.5 S1-IgG. *C*. Serum neutralizing antibody against BA.5. Each box denotes the interquartile range (IQR), where the lower boundary of the box indicates the 25th percentile and the upper boundary indicates the 75th percentile. The line inside the box represents the median value. Whiskers extend from the box to the minimum and maximum values within 1.5 times the IQR.

8. Predictive performance of baseline serum immune markers

With the optimal cutoff values determined from ROC curves (1.43 log OD ratio for Wuhan S1-IgG, 1.36 log OD ratio for BA.5 S1-IgG and 7.00 log_2ND50 for neutralizing antibody, respectively), Wuhan S1-IgG showed the highest sensitivity (72.9%) compared with neutralizing antibody against BA.5 (64.3%) and BA.5 S1-IgG (62.7%), with highest specificity of neutralizing antibody against BA.5 (84.4%) followed by BA.5 S1-IgG (78.3%) and Wuhan S1-IgG (65.2%). Wuhan S1-IgG, BA.5 S1-IgG, and neutralizing antibody against BA.5 showed similar positive predictive values (92.6%, 94.5%, and 96.1%, respectively) and negative predictive values (28.7%, 26.0%, and 28.3% respectively) (Table 5).

We also assessed the predictive performance of antibody levels for protection from subsequent SARS-CoV-2 infection when combined with hybrid immunity (previous SARS-CoV-2 infection). Among the 111 cases that did not experience subsequent infection despite baseline Wuhan S1-IgG level lower than the cutoff value, 66 (59.5%) had hybrid immunity. Adding hybrid immunity increased the sensitivity of Wuhan S1-IgG to 89.0%, whereas decreased the specificity of Wuhan S1-IgG to 42.0%. A similar pattern was observed with BA.5 S1-IgG when combined with hybrid immunity, showing an increased sensitivity of 88.5%, but a decreased specificity of 47.8%. Likewise, the integration of hybrid immunity enhanced the sensitivity of neutralizing antibody against BA.5 reaching 87.2%, while concurrently reducing its specificity to 56.3%.

Table 5. Predictive performance of baseline serum Wuhan S1-IgG, BA.5 S1-IgG, and neutralizing antibody against BA.5 at optimal cutoff values for protection against subsequent SARS-CoV-2 infection

	Sensitivity (%) (n/N, 95% CI)	Specificity (%) (n/N, 95% CI)	PPV (%) (n/N, 95% CI)	NPV (%) (95% CI)
Baseline Wuhan S1-IgG[a]	72.9 (299/410, 68.4–77.2)	65.2 (45/69, 52.8–76.3)	92.6 (89.2–95.2)	28.7 (21.8–36.5)
Baseline BA.5 S1- IgG[a]	62.7 (257/410, 57.8–67.4)	78.3 (54/69, 66.7–87.3)	94.5 (91.1–96.9)	26.0 (20.1–32.5)
Baseline nAb against BA.5[a]	64.3 (126/196, 57.2–71.0)	84.4 (27/32, 67.2–94.7)	96.1 (91.2–98.7)	28.3 (19.6–38.4)
Hybrid immunity	84.8 (350/413, 80.9–88.1)	55.1 (38/69, 42.6–67.1)	91.9 (88.7–94.4)	37.6 (28.2–47.8)
Baseline Wuhan S1-IgG + hybrid immunity	89.0 (365/410, 85.6–91.9)	42.0 (29/69, 30.2–54.5)	90.2 (86.9–92.9)	39.0 (27.9–51.0)
Baseline BA.5 S1-IgG + hybrid immunity	88.5 (363/410, 85.1–91.5)	47.8 (33/69, 35.7–60.2)	91.0 (87.8–93.7)	41.1 (30.2–52.7)
Baseline nAb S1-IgG + hybrid immunity	87.2 (171/196, 81.8–91.6)	56.3 (18/32, 37.7–73.6)	92.3 (87.4–95.7)	42.4 (27.5–58.4)

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; Wuhan, Wuhan-Hu-1; nAb, neutralizing antibody

[a]cutoff value: Wuhan S1-IgG, 1.43 log OD ratio; BA.5 S1-IgG, 1.36 log OD ratio; nAb against BA.5, 7.00 $\log_2$ND50

Discussion

In this prospective cohort study of 5-month follow-up, we demonstrated that antibody responses still provide the immune correlates of protection against SARS-CoV-2 infection during the period dominated by the Omicron variant and when most people have hybrid immunity. Also, hybrid immunity was an independent predictive factor for protection against subsequent infection. Moreover, the combination of antibody levels exceeding the optimal cutoff value and the presence of hybrid immunity demonstrated a predictive performance for protection against subsequent SARS-CoV-2 infection, exhibiting approximately 90% sensitivity and positive predictive value.

This study has three unique findings. First, this study reaffirmed that antibody responses continue to serve as immunologic correlates of protection against COVID-19 during the Omicron predominance and in population with hybrid immunity. There was a significant difference in the serum S1-IgG antibody and neutralizing antibody level between those who had subsequent infection and those who did not in this study. Also, higher baseline serum antibody levels were consistently protective against subsequent SARS-CoV-2 infection regardless of immune markers in multivariable analysis. There has been a recent study reporting the association of baseline antibody level before 4th dose COVID-19 vaccine and subsequent SARS-CoV-2 infection rate[27], but only infection-naïve individuals were included, and all participants received the booster vaccine. Thus, the effect of hybrid immunity and booster vaccination could not be considered in that analysis. In this study, almost all participants with hybrid immunity had prior exposure to the Omicron variant (except the unknown variant) and the cohort included both those who received the bivalent vaccine and those who did not. Therefore, we could collectively draw a comprehensive analysis of immunologic correlates of protection against subsequent SARS-CoV-2 infection reflecting the effect of hybrid immunity and booster vaccination in the Omicron era. Moreover, the serum antibody responses and hybrid immunity were also significant protective factors in the 1-

month subgroup analysis that reflected antibody responses 1-month after vaccination in those vaccinated. Interestingly, the participants who did not receive the bivalent vaccine had a significantly higher proportion of previous SARS-CoV-2 infection and significantly higher baseline antibody levels compared to those who received the bivalent vaccine. Additionally, the individuals who received the bivalent vaccine were significantly older than those who did not. Considering these unfavorable conditions among recipients of the bivalent vaccine along with the robust impact of hybrid immunity, it is plausible that these factors might have contributed to the lack of difference in the cumulative incidence of subsequent infection following the administration of the bivalent booster vaccination.

Second, hybrid immunity emerged as an independent protective factor against subsequent infection in this study. Hybrid immunity exposes the immune system not only to spike-derived epitopes provided by COVID-19 vaccines but also to a wide range of viral epitopes stimulation by natural SARS-CoV-2 infection, resulting in enhanced immune breadth to both spike and non-spike epitopes. This is also important to the breadth of T-cell responses[28]. Also, whereas the antibody responses decline over time and can be compromised by evolving mutations of neutralizing antibody epitopes, T cell epitopes exhibited substantial conservation both in spike protein and non-spike protein across different variants including Omicron[29] and thus T cell responses showed limited escape by variants[30]. Moreover, hybrid immunity acquired from natural SARS-CoV-2 infection is known to lead to the acquisition of mucosal immunity in the nasal cavity and tissue-specific immunity (specifically lung) exclusively detected in individuals with hybrid immunity[17,31] and might contribute to protection against the initial acquisition of SARS-CoV-2 Omicron infection consequently[11,12]. In contrast, the baseline saliva IgA antibody in this study did not demonstrate a protective role. This discrepancy might be attributed to possible explanations: Firstly, the participants' past infections were predominantly mild potentially leading to an insufficient acquisition of mucosal immunity; Secondly, a substantial time interval had elapsed between the previous

infection and study enrollment, which might have resulted in the waning of mucosal IgA levels to an extent that precluded a measurable protective effect against subsequent infection in this study. Given the reported rapid waning of airway IgA, as early as 3 months post-infection[14], the protective effect of mucosal IgA in this study may have been influenced by the median interval of 7 months between previous infection and study enrollment. Taken together, my study suggests that hybrid immunity could be a protective factor against SARS-CoV-2 infection, independently of the antibody responses despite the exact mechanism may require further immunologic studies.

Finally, antibody responses in combination with hybrid immunity demonstrated high sensitivity and positive predictive value for protection from subsequent infection. In other words, those who have high antibody titers along with a history of previous SARS-CoV-2 infection (hybrid immunity) demonstrated a high likelihood of avoiding a subsequent infection, although it should be noted that the prevalence of subsequent infection in this study was relatively low, at around 14.3%. However, there was a substantial overlapping distribution of antibody responses in those with and without subsequent infection which made it difficult to identify a protective serum antibody threshold level that could clearly distinguish between those with and without SARS-CoV-2 infection, unlike what has been observed in the previous study on measles[32]. It could be due to the pathogenesis of SARS-CoV-2, which is largely driven by invasive mucosal infection through the respiratory tract, rather than viremia.

There are some limitations to this study. First, because all participants with subsequent infections had mild COVID-19, we could not estimate correlates of protection against severe COVID-19 infection or death. Second, there are lack of data on T-cell responses or memory immune responses in this study participants. Memory immune responses may have some roles to confer protection against infection despite the waning serum antibody level and prevent the progression of severe infections. Lastly, we estimated the occurrence of subsequent infection in participants with hybrid immunity by evaluating the degree of antibody titer increase even

in the absence of self-reported subsequent infection as described in the method section. In the context of a relatively low incidence of subsequent infections observed in this study, such estimation could have potentially influenced the results.

Conclusions

In conclusion, this 5-month observational cohort study suggests that serum humoral immune responses, both serum binding antibody and neutralizing antibody provide immunological correlates of protection against subsequent SARS-CoV-2 infection whereas saliva IgA does not. Also, hybrid immunity was an independent protective factor against subsequent SARS-CoV-2 infection. High baseline serum antibody levels exceeding optimal cutoff levels in combination with hybrid immunity showed substantial predictive performance for protection from subsequent SARS-CoV-2 infection.

References

1. Correlates of vaccine-induced protection: methods and implications. In: Innitiative for Vaccine Research (IVR) of the Department of Immunization VaB, editor.: World Health Organization; 2013.

2. Gilbert PB, Donis RO, Koup RA, Fong Y, Plotkin SA, Follmann D. A Covid-19 Milestone Attained - A Correlate of Protection for Vaccines. *N Engl J Med* 2022; **387**(24): 2203-6.

3. Corbett KS, Nason MC, Flach B, et al. Immune correlates of protection by mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *Science* 2021; **373**(6561): eabj0299.

4. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27**(7): 1205-11.

5. Goldblatt D, Fiore-Gartland A, Johnson M, et al. Towards a population-based threshold of protection for COVID-19 vaccines. *Vaccine* 2022; **40**(2): 306-15.

6. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27**(11): 2032-40.

7. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 2022; **375**(6576): 43-50.

8. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* 2021; **595**(7867): 426-31.

9. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *N Engl J Med* 2022; **386**(13): 1207-20.

10. Suryawanshi RK, Chen IP, Ma T, et al. Limited cross-variant immunity from SARS-CoV-2 Omicron without vaccination. *Nature* 2022; **607**(7918): 351-5.

11. Havervall S, Marking U, Svensson J, et al. Anti-Spike Mucosal IgA Protection against SARS-CoV-2 Omicron Infection. *N Engl J Med* 2022; **387**(14): 1333-6.

12. Zuo F, Marcotte H, Hammarstrom L, Pan-Hammarstrom Q. Mucosal IgA against SARS-CoV-2 Omicron Infection. *N Engl J Med* 2022; **387**(21): e55.

13. Sterlin D, Mathian A, Miyara M, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med* 2021; **13**(577).

14. Cagigi A, Yu M, Osterberg B, et al. Airway antibodies emerge according to COVID-19 severity and wane rapidly but reappear after SARS-CoV-2 vaccination. *JCI Insight* 2021; **6**(22).

15. Liew F, Talwar S, Cross A, et al. SARS-CoV-2-specific nasal IgA wanes 9 months after hospitalisation with COVID-19 and is not induced by subsequent vaccination. *EBioMedicine* 2023; **87**: 104402.

16. Sheikh-Mohamed S, Isho B, Chao GYC, et al. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. *Mucosal Immunol* 2022; **15**(5): 799-808.

17. Tang J, Zeng C, Cox TM, et al. Respiratory mucosal immunity against SARS-CoV-2 after mRNA vaccination. *Sci Immunol* 2022; **7**(76): eadd4853.

18. Azzi L, Dalla Gasperina D, Veronesi G, et al. Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. *EBioMedicine* 2022; **75**: 103788.

19. Goldberg Y, Mandel M, Bar-On YM, et al. Protection and Waning of Natural and Hybrid Immunity to SARS-CoV-2. *N Engl J Med* 2022; **386**(23): 2201-12.

20. Bobrovitz N, Ware H, Ma X, et al. Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. *Lancet Infect Dis* 2023; **23**(5): 556-67.

21. Altarawneh HN, Chemaitelly H, Ayoub HH, et al. Protective Effect of Previous SARS-CoV-2 Infection against Omicron BA.4 and BA.5 Subvariants. *N Engl J Med* 2022; **387**(17): 1620-2.

22. Malato J, Ribeiro RM, Leite PP, et al. Risk of BA.5 Infection among Persons Exposed

to Previous SARS-CoV-2 Variants. *N Engl J Med* 2022; **387**(10): 953-4.

23. Edouard Mathieu HR, Lucas Rodés-Guirao, Cameron Appel, Charlie Giattino, Joe Hasell, Bobbie Macdonald, Saloni Dattani, Diana Beltekian, Esteban Ortiz-Ospina and Max Roser. "Coronavirus Pandemic (COVID-19)". May 1 2023 2020. https://ourworldindata.org/coronavirus (accessed May 1 2023).

24. SARS-CoV-2 variants in analyzed sequences, South Korea. 2023-03-22. https://www.kdca.go.kr/contents.es?mid=a20107030000 (accessed April 26 2023).

25. Whiteman MC, Bogardus L, Giacone DG, et al. Virus Reduction Neutralization Test: A Single-Cell Imaging High-Throughput Virus Neutralization Assay for Dengue. *Am J Trop Med Hyg* 2018; **99**(6): 1430-9.

26. Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. *Epidemiology* 2005; **16**(1): 73-81.

27. Barda N, Canetti M, Gilboa M, et al. The Association Between Prebooster Vaccination Antibody Levels and the Risk of Severe Acute Respiratory Syndrome Coronavirus 2 Infection. *Clin Infect Dis* 2023; **76**(7): 1315-7.

28. Minervina AA, Pogorelyy MV, Kirk AM, et al. SARS-CoV-2 antigen exposure history shapes phenotypes and specificity of memory CD8(+) T cells. *Nat Immunol* 2022; **23**(5): 781-90.

29. Choi SJ, Kim DU, Noh JY, et al. T cell epitopes in SARS-CoV-2 proteins are substantially conserved in the Omicron variant. *Cell Mol Immunol* 2022; **19**(3): 447-8.

30. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature* 2022; **603**(7901): 488-92.

31. Lim JME, Tan AT, Le Bert N, Hang SK, Low JGH, Bertoletti A. SARS-CoV-2 breakthrough infection in vaccinees induces virus-specific nasal-resident CD8+ and CD4+ T cells of broad specificity. *J Exp Med* 2022; **219**(10).

32. Chen RT, Markowitz LE, Albrecht P, et al. Measles antibody: reevaluation of protective titers. *J Infect Dis* 1990; **162**(5): 1036-42.

국문요약

오미크론 시대의 SARS-CoV-2 감염에 대한 면역학적 방어지표로서 체액면역반응 분석

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서론: SARS-CoV-2 의 혈청 항 스파이크 IgG 와 중화 항체는 COVID-19 의 면역학적 방어지표로 인정되어 왔다. 그러나 Omicron 변이주 유행과 BA.4/5 2 가 백신의 도입 이후 COVID-19 의 면역학적 방어지표에 대한 데이터는 제한적이다. 따라서 본 연구는 오미크론 시대에 COVID-19 에 대한 면역학적 방어지표로서 항체 반응을 분석하고, 오미크론 유행 및 여러 차례의 부스터 백신 접종 이후 복잡한 면역상태를 가지게 된 인구집단에서 COVID-19 에 대한 보호인자를 조사하는 것을 목적으로 하였다.

방법: 2022 년 12 월부터 2023 년 1 월까지 한국의 2,700 병상 규모의 3 차 진료 병원인 서울아산병원의 의료 종사자로 구성된 코호트를 등록하여, 5 개월간 추적 관찰하였다. 연구 참가자들은 이전에 코로나 19 백신을 3 회 또는 4 회 접종한 적이 있으며, BA.4/5 2 가 백신을 접종하거나 하지 않는 참여자들이었다. 연구 등록 시점부터 0, 1, 3 개월째에 혈액 및 타액 샘플을 채취하였고, 2 가 백신을 접종하지 않는 경우는 0, 3 개월째에만 채취하였다. 면역학적 평가는 Wuhan-Hu-1 및 BA.5 스파이크 단백질 (S1)에 특이적인 혈청 IgG 및 타액의 IgA 를 측정하는 ELISA 와 중화 항체 수준을 측정하는 virus reduction neutralization test 를 사용하여 수행되었다.

결과: 총 482 명의 참가자가 코호트로 등록되었고, 이 중 69 명 (14.3%)이 5 개월 간의 추

적 관찰 기간 동안 후속 감염이 발생했고 413 명 (85.7%)은 후속감염이 없었다. 총 482 명의 참가자 중 381 명 (79.0%)은 이전에 SARS-CoV-2 감염 경험이 있었고, 166 명 (34.4%)은 연구 등록 시 BA.4/5 2 가 추가 백신을 접종 받았다. 후속 감염이 있는 사람과 없는 사람 간에 baseline 혈청 Wuhan-Hu-1 (Wuhan) S1-IgG, BA.5 S1-IgG 및 BA.5 에 대한 중화항체 기저 수치에 유의미한 차이가 있었지만 (모든 P <0.001), baseline 타액 Wuhan S1-IgA 및 BA.5 S1-IgA 의 기저수치는 후속감염에 따른 차이가 확인되지 않았다. Baseline 혈청 항체 수치가 올라감에 따라 해당 항체 구간의 감염률은 감소하는 경향성을 보였지만 (모든 P <0.001), 타액 항체 수치에 따라서는 이러한 경향성이 관찰되지 않았다. ROC 곡선에서 결정된 최적의 항체 cutoff 값은 Wuhan-Hu-1 S1-IgG 의 경우 1.36 log OD ratio, BA.5 S1-IgG 의 경우 1.43 log OD ratio, BA.5 에 대한 중화항체의 경우 7.00 $\log_2$50% neutralizing dose [ND50]이었다. 다변량 콕스 회귀분석을 시행하였을 때, 이 연구에 사용된 세 가지 면역 마커인 Wuhan S1-IgG (조정 위험비 [aHR] 0.43; P=0.02), BA.5 S1-IgG (aHR 0.32; P=0.005), BA.5 에 대한 중화항체 (aHR 0.26, P=0.045)는 ROC 곡선에서 결정된 최적의 cutoff 값보다 높을 경우 후속 SARS-CoV-2 감염에 대한 독립적인 보호 인자로 밝혀졌으며 , 각 면역 마커를 기준으로 한 분석에서 하이브리드 면역은 후속 감염에 대한 강력한 보호 인자였다 (모든 P <0.01). 그러나 타액의 S1-IgA 는 후속 SARS-CoV-2 감염에 대한 보호 효과를 보여주지 못하였다. 2 가 백신 접종 1 달 뒤의 항체수치를 반영하여 진행한 1-month subgroup 분석에서도 최적의 cutoff 값보다 높은 Wuhan S1-IgG (aHR 0.40; P=0.007), BA.5 S1-IgG (aHR 0.30; P <0.001) 그리고 BA.5 에 대한 중화항체 (aHR 0.15; P=0.02) 및 하이브리드 면역(모든 P <0.05)은 후속감염에 대한 유의미한 보호 인자로 확인되었다. ROC 곡선에서 결정된 최적의 기준값 (Wuhan S1-IgG, 1.43 로그 OD ratio; BA.5 S1-IgG, 1.36 로그 OD ratio, BA.5 에 대한 중화항체, 7.00 $\log_2$ND50)을 초과하는 높은 baseline 항체값과 하이브리드 면역을 결합하였을 때, SARS-CoV-2 후속 감염으로부터 보호하는 민감도와 특이도는 Wuhan S1-IgG 의 경우 89.0%와 42.0%, BA.5 의 경우 88.5%와 47.8%, BA.5 에 대한 중화항체의 경우 87.2%와 56.3%로 나타났고, 양성 예측값은 각 항체 종류에 대해 90.2%, 91.0%, 92.3%로 비슷하였다.

결론: 결론적으로, 5 개월간 의료 종사자를 추적한 전향적 코호트로 수행한 이 연구에서 체액성 면역 반응은 인구의 면역 상태가 복잡한 오미크론 시대에도 SARS-CoV-2 감염에 대한 가장 중요한 면역학적 방어지표임을 재확인하였고, 하이브리드 면역은 후속 감염으로부터 독립적인 보호 인자로 나타났다. 또한, 높은 혈청 항체 수치를 하이브리

드 면역과 결합하였을 때, SARS-CoV-2 후속 감염으로부터 보호에 대해 유의미한 예측력을 보여주었다.

중심단어: 항체 반응, 방어지표, 하이브리드 면역, 점막면역, SARS-CoV-2