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Development of antibody levels and subsequent decline in individuals with vaccine induced and hybrid immunity to SARS-CoV-2

Joanne Reekie[1,*], Henrik Stovring[2], Henrik Nielsen[3,4], Isik S. Johansen[5,6], Thomas Benfield[7,8], Lothar Wiese[9], Nina Breinholt Stærke[10,11], Kasper Iversen[12], Ahmed Basim Mustafa[7,8], Kristine Toft Petersen[3], Maria Ruwald Juhl[3], Lene Surland Knudsen[9], Mette Brouw Iversen[9], Sidsel Dahl Andersen[10], Fredrikke Dam Larsen[10,11], Eva Anna Marianne Baerends[10], Susan Olaf Lindvig[6], Line Dahlerup Rasmussen[5], Lone Wulff Madsen[5,13], Wendy Bannister[1], Tomas Oestergaard Jensen[1], Lisa Loksø Dietz[10,11], Sisse Rye Ostrowski[8,14], Lars Østergaard[10,11], Martin Tolstrup[10,11], Jens D. Lundgren[1,8], Ole Schmeltz Søgaard[10,11], on behalf of the ENFORCE study group

[1] *Center of Excellence for Health, Immunity and Infections, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark*
[2] *Clinical Pharmacology, Pharmacy and Environmental Medicine, University of Southern Denmark, Odense, Denmark*
[3] *Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark*
[4] *Department of Clinical Medicine, Aalborg University, Aalborg, Denmark*
[5] *Department of Infectious Diseases, Odense University Hospital, Odense, Denmark*
[6] *Department of Clinical Research, University of Southern Denmark, Odense, Denmark*
[7] *Department of Infectious Diseases, Copenhagen University Hospital - Amager and Hvidovre, Hvidovre, Denmark*
[8] *Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*
[9] *Department of Medicine, Zealand University Hospital, Roskilde, Denmark*
[10] *Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark*
[11] *Department of Clinical Medicine, Aarhus University, Aarhus, Denmark*
[12] *Department of Cardiology and Department of Emergency Medicine, Herlev, Denmark*
[13] *Department of Regional Health Research, University of Southern Denmark, Odense, Denmark*
[14] *Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark*

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ABSTRACT

Objectives: This study aimed to compare antibody trajectories among individuals with SARS-CoV-2 hybrid and vaccine-induced immunity.

Methods: Danish adults receiving three doses of BTN162b2 or mRNA-1237 were included prior to first vaccination (Day 0). SARS-CoV-2 anti-spike IgG levels were assessed before each vaccine dose, at Day 90, Day 180, 28 days after 3rd vaccination (Day 251), Day 365, and prior to 4th vaccination (Day 535). SARS-CoV-2 PCR results were extracted from the national microbiology database. Mixed-effect multivariable linear regression investigated the impact of hybrid-immunity (stratified into 4 groups: no hybrid immunity, PCR+ prior to 3rd dose, PCR+ after 3rd dose and before Day 365, PCR+ after Day 365) on anti-spike IgG trajectories.

Results: A total of 4,936 individuals were included, 47% developed hybrid-immunity. Anti-spike IgG increases were observed in all groups at Day 251, with the highest levels in those PCR+ prior to 3rd dose (Geometric Mean; 535,647AU/mL vs. 374,665AU/mL with no hybrid-immunity, $P<0.0001$). Further increases were observed in participants who developed hybrid immunity after their 3rd dose. Anti-spike

* Corresponding author.
E-mail address: joanne.reekie@regionh.dk (J. Reekie).

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IgG levels declined from Day 251-535 in individuals without hybrid-immunity and in those who developed hybrid-immunity prior to their 3rd dose, with lower rate of decline in those with hybrid-immunity. *Conclusion:* Hybrid-immunity results in higher and more durable antibody trajectories in vaccinated individuals.

Introduction

National vaccine campaigns were instrumental in transitioning to long-term management of COVID-19. In addition, the dominance of viral escape variants, such as Omicron (B.1.1.529), with increased transmissibility [1,2] resulted in a large proportion of the global vaccinated population being infected with SARS-CoV-2 and a significant proportion of vaccinated individuals have thus acquired hybrid immunity.

Following completion of the two-dose primary vaccine schedules, several studies reported waning immunity [3-6] or only observed a sustained response in those with previous infection [7]. This led to the introduction of 3rd vaccine doses (booster), and subsequently bivalent vaccines targeted at the circulating dominant strain [8]. However, while it is widely accepted that the current vaccines against SARS-CoV-2 significantly reduce morbidity and mortality, the durability of vaccine protection and the impact of hybrid immunity is still not fully understood.

Early studies suggested that natural immunity could be longer lasting than vaccine-induced immunity alone [9,10]. Higher immune response has previously been reported with hybrid immunity, either based on SARS-CoV-2 infection prior to receiving the 1st dose of vaccine [11,12], or after vaccination [13,14]. More recently a systematic review and meta-analysis looking at protective effectiveness of hybrid immunity against the Omicron variant found rapidly waning immunity against infection but persisting protection against severe disease and hospitalisation [15]. The review suggested that people with hybrid immunity may not require booster vaccines as frequently as those with only vaccine-induced immunity [16]. However, subgroups at increased risk of severe disease, such as older individuals, those with multiple comorbidities or immunosuppression were not the focus of this study [17].

The Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 Vaccines (ENFORCE) is a phase 4 observational study of adults who received a SARS-CoV-2 vaccine. Participants were followed for two years with antibody measurements collected at regular intervals. Enrolment for ENFORCE was specifically targeted to include a high proportion of older individuals and those at increased risk of severe SARS-CoV-2 [18] and so provides an opportunity to study hybrid immunity in target-populations for future vaccine strategies. We therefore aimed to compare antibody-response in individuals with hybrid and vaccine-induced immunity in ENFORCE.

Methods

ENFORCE is a non-randomized, parallel group, open label, observational study of people who received a SARS-CoV-2 vaccine as part of the Danish Vaccination program [5,18]. Adults (aged>18 years) with a scheduled appointment for SARS-CoV-2 vaccination were invited to participate in the study and enrolled prior to receiving their 1st vaccination. Enrolment into the study began in February 2021 and was completed in August 2021.

Study visit

Included in this analysis were the first five main scheduled study visits, 0 to 14 days prior to 1st vaccination, 0 to 5 days prior to 2nd vaccination, and then at Day 90 (+/- 14 days), Day 180 (+/- 14 days), and Day 365 (+/- 14 days) after the 1st vaccination. Participants were also invited for two additional study visits each time they received an additional SARS-CoV-2 vaccine, one immediately (0 to 8 days) prior to the booster vaccination and one 28 days (+/- 8 days) after.

Data sources

At enrolment information was collected including age, sex, and whether they belonged to one of the Danish Health Authorities targeted vaccination priority groups. Additionally, the Danish Vaccine Registry (DDV) provided information on the dates and type of each SARS-CoV-2 vaccine received, dates and results of all SARS-CoV-2 antigen and PCR tests were collected from the Danish Microbiology Database (MiBa), and the Danish National Patient Registry provided information on all hospital contacts, including dates of diagnoses.

Serology

Serology samples were collected at each study visit and analysed through two assays. Antibody levels against the total Spike IgG and the Nucleocapsid for the wild-type strain were assessed using a multiantigen serological assay (Meso Scale Diagnostics LLC, Maryland, USA) at the Department of Infectious Diseases, Aarhus University Hospital. The presence of SARS-CoV-2 spike antibodies was also measured using an ELISA-based assay (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) by Statens Serum Institut in Copenhagen.

Statistical analysis

ENFORCE participants were included who had received three doses of either BTN162b2 or mRNA-1237 prior to their Day 365 study visit, since this was the minimum recommended number of doses in Denmark.

Each participant was followed until either their last study visit before the 4th vaccine dose or administrative censoring date (23rd February 2023).

To investigate the effect of hybrid immunity on participants' total spike IgG trajectories, the cohort was stratified into four independent groups based on the timing of their first positive SARS-CoV-2 PCR test and the scheduled ENFORCE study visits:

1. No positive PCR test during follow-up (PCR-). No hybrid immunity.
2. At least one positive PCR test prior to the 3rd dose. This included both breakthrough infections and those who had tested positive prior to receiving any SARS-CoV-2 vaccinations (PCR+ before 3rd Vax).

3. At least one positive PCR test after the 3rd vaccine dose and prior to the sample collection on Day 365 (PCR+ after 3rd Vax & before Day 365).
4. At least one positive PCR test after the Day 365 study visit and prior to receiving a 4th vaccine dose at Day 535 (PCR+ >3rd Vax & Day 365).

Data on PCR testing was extracted from the national database and participants were not required to undergo any additional PCR testing as part for the study. Participants with a positive PCR test both before and after their 3rd dose, and participants who met our definition of seroconversion but had no record of a positive PCR test during the study period were excluded from the analysis. Seroconversion was assessed at each study visit and defined as an anti-nucleocapsid IgG>3000 AU/mL and >2 fold higher than the pre-vaccination level [19]. SARS-CoV-2 PCR testing was freely available in Denmark with individuals testing positive via antigen test recommended to confirm results via PCR.

Participant demographics at study enrolment (prior to 1st vaccine dose) were summarized overall and by hybrid immunity status.

Mixed effect multivariable linear regression modelling was used to investigate the changes in anti-spike IgG levels over time and the impact of hybrid immunity. Models were adjusted for potential confounding variables selected a priori. These included age (natural cubic spline 3 knots, years), sex, vaccine type (BTN162b2, mRNA-1237), Charlson Comorbidity Index (CCI score), vaccine priority group (increased risk, general population), study time (linear spline with knots at 30, 91, 182, 251, 365 and 535 days corresponding to the planned time of study visits). The CCI score was calculated at enrolment based on ICD10 codes reported in the Danish National Patient Registry [20,21]. The definition of increased risk vaccine priority group is provided in the Supplementary Material.

The predicted geometric mean antibody levels at each study visit for the four immunity groups were then plotted graphically together with the 95% confidence intervals at the median time of each study visit (calculated as the median number of days from the 1st vaccine dose). Having a linear spline in the model as a covariate allowed us to estimate the average change per day for all periods between knots, which is approximately the change between subsequent study visits. As the average change is estimated as linear for logarithmic antibody level, this corresponds to a constant relative change per day in median antibody level. The trends were compared using a t-test with small-sample degrees of freedom adjustment by expressing their differences as a parameter on the log-scale and testing whether it was zero using Rubin's formula for computing the overall estimate across imputed datasets and its standard error [22].

All analysis was performed using STATA 17.0 [23].

Results

A total of 4,936 (70.8%) participants were included in the analysis out of 6,972 participants enrolled in ENFORCE (Supplementary Figure 1).

The majority (n=2,966, 60.1%) received 3 doses of BTN162b2 vaccine with the remainder (n=1,970, 39.9%) receiving 3 doses of mRNA-1237. The median age was 64 years (IQR 54-75) and 54.8% (n=2,706) were female (Table 1). Almost half of the cohort (n=2,320, 47.0%) developed hybrid immunity (groups 2-4). The majority (n=1,821, 78.5%) first tested positive for SARS-CoV-2 after the 3rd dose and prior to their Day 365 study visit.

Table 2 gives an overview of the number of participants who completed each study visit and the timing of visits. The 4,936 participants provided a total of 28,945 anti-spike IgG measurements (median 6 measurements (IQR: 5-7) per participant). The median time from 1st vaccine dose to last study visit was 369 days (IQR: 357-515), as only 37% of participants had a Day 535 visit.

Antibody trajectories in the whole cohort

Figure 1 shows the geometric means for the total spike IgG at each study visit for the cohort overall. An increase in total spike IgG level following the first two vaccine doses was observed, with the first peak in antibody levels measured at Day 90. A decline in antibodies was then observed from Day 90-180. This decline was estimated to be 23% per 30 days (95% CI 22-24%).

Figure 1 also clearly shows the effects of the 3rd dose, where a large increase in total spike IgG levels was observed from Day 222 (immediately prior to the 3rd dose) to Day 251 (28 days after the 3rd dose) to a level almost 2.5 times higher than at Day 90 (after receiving 2 vaccine doses) (Geometric Mean Ratio [GMR] 2.5 Day 251 vs Day 90 95% CI: 2.37-2.61). Following the Day 251 study visit, a decline in total spike IgG levels was then observed to Day 535 (immediately prior to the 4th dose), but this remained higher than the peak observed at Day 90, when individuals had received only two doses (GM 293,003 AU/mL (95% CI 276,651-310,320) Day 535 vs GM 163,995 AU/mL (95% CI 157,381-170,886) Day 90).

Antibody trajectories considering hybrid immunity

Considering the impact of hybrid immunity on the antibody trajectories, Figure 2 shows the predicted total spike IgG levels from the multivariable mixed linear regression model, stratified by the four different hybrid immunity groups. A similar figure using the raw data instead of the predicted geometric mean levels is provided in the supplementary material (Figure S2) with consistent results.

From Day 0-251, each group followed similar trajectories after two vaccine doses. However, participants who developed hybrid immunity in this early period had the highest antibody levels and while a large increase in anti-spike IgG was observed in all groups following the 3rd dose (Day 251), the highest antibodies were still observed in those with hybrid immunity prior to their 3rd dose (GM 535,647AU/mL, 95% CI 447,367-641,347).

Following Day 251 the antibody trajectories started to diverge. For those with no hybrid immunity and those who developed hybrid immunity in the early period prior to their 3rd dose a decline in anti-spike IgG was observed from Day 251-535. However, even at Day 535, anti-spike IgG levels for those who developed hybrid immunity early remained higher than for those with no hybrid immunity (GM 341,858 AU/mL (95% CI 272,268-429,235) vs. GM 138,569AU/mL (95% CI 126,805-151,425)). In contrast, an initial increase in anti-spike IgG was observed from Day 251-365 for individuals who developed hybrid immunity during this same period, followed by a decline from Day 365-535. Finally, for those who developed hybrid immunity after Day 365, an initial decline in total spike IgG was observed from Day 251-365, followed by a subsequent increase in the period when hybrid immunity occurred (Day 365-535).

To compare these changes across time periods and hybrid immunity groups, we calculated the relative change in antibody levels between each of the study visits as a percentage change per 30 days (Table 3). The first decline in antibodies during Day 90-180 after the initial peak following the 2nd dose was estimated to be similar for those with no hybrid immunity (23% per 30 days) and those who later developed hybrid immunity either during Day 251-365 (24%) or after Day 365 (24%), whereas a lower rate of decline was observed in those who already had hybrid immunity during this period (13%), P=0.0001. We also estimated a slightly lower rate of decline from Day 251-365 (3% vs. 8% per 30 days, P=0.07) and from Day 365-535 (5% vs. 11% per 30 days, P=0.003) in those

Table 1
Characteristics of participants at enrolment stratified by their hybrid immunity status at their last study visit.

		Total	Never PCR positive	Positive prior to 3rd vaccine dose	Positive after 3rd dose, prior to 1 year visit	Positive after 1 year visit
Total (*n*, % of total)		4,936 (100)	2,616 (53.0)	288 (5.8)	1,821 (36.9)	211 (4.3)
Age group	**<50**	818 (16.6)	369 (14.1)	78 (27.1)	361 (19.8)	10 (4.7)
	50-64	1,737 (35.2)	819 (31.3)	124 (43.1)	717 (39.4)	77 (36.5)
	≥65	2,381 (48.2)	1,428 (54.6)	86 (29.9)	743 (40.8)	124 (58.8)
Sex	**Female**	2,706 (54.8)	1,393 (53.3)	165 (57.3)	1,028 (56.5)	120 (56.9)
	Male	2,230 (45.2)	1,223 (46.8)	123 (42.7)	793 (43.6)	91 (43.1)
Vaccine received	**BTN162b2**	2,966 (60.1)	1,699 (65.0)	158 (54.9)	992 (54.5)	117 (55.5)
	mRNA-1237	1,970 (39.9)	917 (35.1)	130 (45.1)	829 (45.5)	94 (44.6)
Danish vaccine priority group	**Increased risk**	1,247 (25.3)	767 (29.3)	50 (17.4)	377 (20.7)	53 (25.1)
	General population	3,689 (74.7)	1,849 (70.7)	238 (82.6)	1,444 (79.3)	158 (74.9)
Charlson comorbidity index	**None**	3,503 (71.0)	1,720 (65.8)	235 (81.6)	1,395 (76.6)	153 (72.5)
	Mild (1-2)	1,192 (24.2)	722 (27.6)	45 (15.6)	373 (20.5)	52 (24.9)
	Moderate (3+)	241 (4.9)	174 (6.7)	8 (2.8)	53 (2.9)	6 (2.8)
Date first PCR positive	**(median, range)**	-	-	July 2021 (March 2020-March 2022)	February 2022 (November 2021- June 2022)	July 2022 (March 2022-October 2022)

IQR: Interquartile range.

Table 2
Overview of the study visits completed during follow-up.

Study visit number	Day	Study visit description	Number contributing total Spike IgG results (*N*, %)	Days from 1st vaccination to study visit (median, IQR)	Date of study visit (median, range)
1	0	Enrolment (prior to 1st vaccine dose)	4,936 (100)	0 (-2 to 0)	April 2021 (February 2021-August 2021)
2	28	Prior to 2nd vaccine dose	4,630 (93.80)	28 (21 to 35)	May 2021 (March 2021-August 2021)
3	90	Three months after 1st vaccine dose	4,462 (90.40)	92 (89 to 96)	July 2021 (May 2021-December 2021)
4	180	6 months after 1st vaccine dose	4,108 (83.23)	182 (179 to 186)	October 2021 (August 2021-March 2022)
3X	222	Prior to 3rd vaccine dose	1,794 (36.35)	222 (208 to 232)	December 2021 (October 2021-April 2022)
3Xc	251	28 days after 3rd vaccine dose	3,257 (66.00)	251 (237 to 262)	January 2022 (October 2021-September 2022)
5	365	1 year after 1st vaccine dose	3,936 (79.74)	364 (360 to 370)	April 2022 (February 2022-September 2022)
4X	535	Prior to 4th vaccine dose	1,822 (36.91)	532 (508 to 554)	October 2022 (February 2022-February 2023)

with hybrid immunity prior to their 3rd vaccine compared to those with no hybrid immunity. Among those with no hybrid immunity, the rate of decline following the 3rd dose was lower than the decline observed after only two doses (23% per 30 days during Day 90-180, 8% during Day 251-365, $P<0.0001$).

The full results of the mixed linear model are given in supplementary Table S2. In addition to hybrid immunity status, older participants, and males were found to have lower predicted mean total spike IgG levels. Furthermore, individuals with moderate/severe Charlson Comorbidity Index had 32% lower mean total spike IgG levels compared to those with no comorbidities (95% CI 0.60-0.77, $P<0.0001$), and those in the increased risk vaccine priority group had 40% lower mean total spike IgG levels (95% CI 0.56-0.64, $P<0.0001$) compared to the general population group and independent of hybrid immunity status. Participants who received 3 doses of mRNA-1237 were predicted to have higher mean total spike IgG levels than those receiving BTN162b2.

Discussion

ENFORCE is uniquely positioned to investigate hybrid immunity due to both pre-vaccination infection and breakthrough infections in a large cohort with a significant proportion of older and high-risk individuals. Among individuals receiving three doses of mRNA vaccine against SARS-CoV-2, hybrid immunity was found to have an important role in antibody trajectories. Those with hybrid immunity were observed to have significantly higher antibody levels compared to those with only vaccine-induced immunity. Further, the observed decline in antibody levels following a peak, either from a vaccine booster dose or from a new infection, was lower in those with hybrid immunity.

The relative higher antibody levels after hybrid immunity remained up to 535 days after the 1st vaccine dose and were seen even in the group who were infected with SARS-CoV-2 prior to their 3rd vaccine dose. The reasons for this are not fully understood but others have speculated that prior SARS-CoV-2 infection enhances and reshapes the spike protein specific memory induced by vaccination [24,25]. The type of antigen exposure [26], particularly during the first infection [27], may also be important for the hybrid immune response. The majority of infections in our cohort occurred in early 2022 and after the 3rd vaccine dose, at a time when the Omicron variants were dominant in Demark [28] which has been shown to result in a higher immune response than earlier variants [26,29].

Despite a decline in antibody levels following the 3rd vaccine dose, antibody levels remained high in those without hybrid immunity and the rate of decline was lower than following the first two doses. This supports findings from previous studies that the

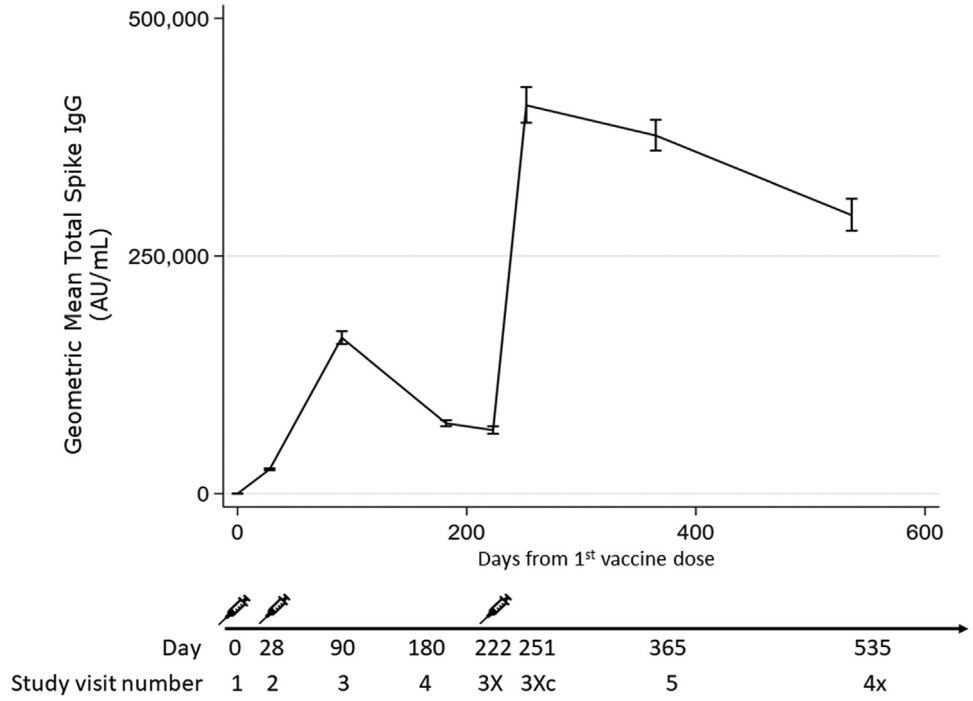

Figure 1. Antibody trajectories in adults receiving three doses of mRNA vaccine. Plotted as the geometric means (GM) for the total spike IgG measured at each study visit together with 95% confidence intervals (CI).

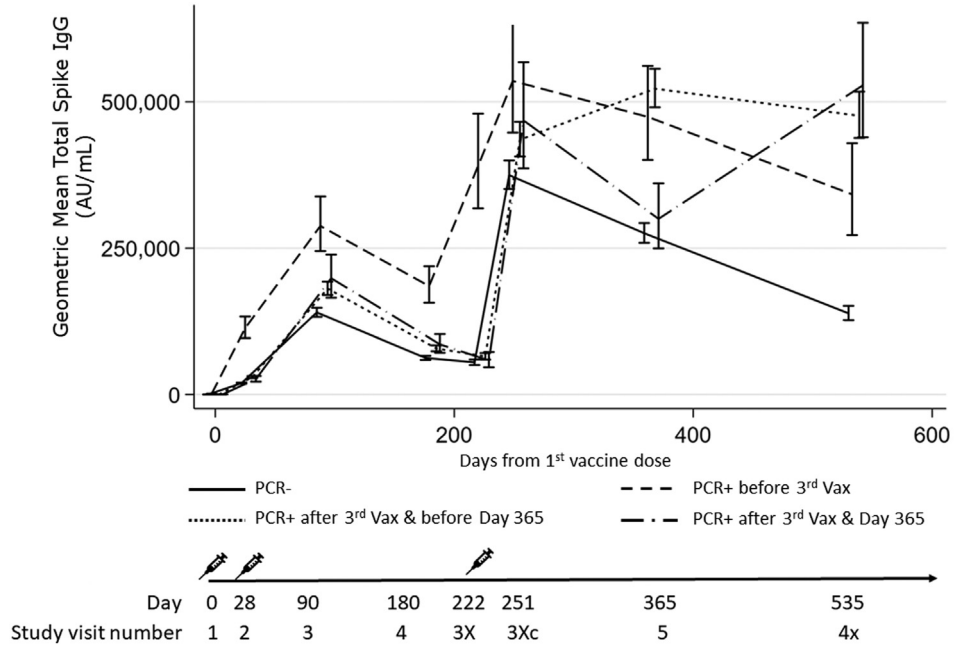

Figure 2. Antibody trajectories in adults receiving three doses of mRNA vaccine stratified by hybrid immunity status. Plotted as the predicted geometric means (GM) for the total spike IgG measured at each study visit together with 95% confidence intervals (CI) from the mixed linear regression model, also adjusted for age, sex, Charlson Comorbidity Index, and Danish vaccine priority group. PCR-: No positive PCR test during follow-up (*N*=2620); PCR+ before 3rd Vax: At least one positive PCR test prior to receipt of a 3rd vaccine (*N*=287); PCR+ after 3rd Vax and before Day 365: At least one positive PCR test after receiving a 3rd vaccine dose and prior to Day 365 (*N*=1820, including 160 (8.8%) individuals who tested positive after their 3rd dose but prior to their Day 251 study visit); PCR+ after 3rd Vax & Day 365: At least one positive PCR test after the Day 365 and prior to receiving a 4th vaccine dose at Day 535 (*N*=209).

durability of response following the 3rd vaccine dose is greater than after only two doses [30,31]. With high-risk individuals now receiving more than three vaccine doses, it will be important to investigate if durability continues to increase with each subsequent dose or new infection. However, the scaling back of national testing strategies will make it challenging for future studies to determine the timing of newer SARS-CoV-2 infections to the same degree as in this study which was conducted when testing was freely available and widespread across Denmark.

This study adds to the findings from a recent systematic review looking at the protective effectiveness of hybrid immunity against the Omicron variant [15] which suggested that people with hybrid immunity may not need booster vaccines as frequently as those with only vaccine-induced immunity [16]. Our study contains a

Table 3
Estimated change in total Spike IgG between each study visit.

Observation period description	Number of doses received	Estimated percentage change per 30 days (95% CI)			
		Never PCR positive	Positive prior to 3rd dose	Positive after 3rd dose and before Day 365	Positive after Day 365
Day 0 (immediately to prior 1st dose) to Day 28 (immediately prior to 2nd dose)	1	33,644 (31,633, 35,783)	12,040 (10,006 14,483)	53,688 (49,960, 57,693)	47,170 (38,224, 58,203)
Day 28 (immediately prior to 2nd dose) to Day 90	2	156 (149, 163)	56 (43, 69)	137 (129, 144)	162 (138, 187)
Day 90 to Day 180	2	-23 (-25, -22)	-13 (-18, -8)	-24 (-25, -22)	-24 (-29, -19)
Day 180 to Day 222 (immediately prior to 3rd dose)	2	-10 (-16, -4)	70 (45, 100)	-14 (-20, -8)	-25 (-37, -11)
Day 222 (immediately prior to 3rd dose) to Day 251 (28 days after 3rd dose)	3	634 (567, 708)	39 (10-76)	618 (554, 689)	771 (582, 1,013)
Day 251 (28 days after 3rd dose) to Day 365	3	-8 (-9, -6)	-3 (8, 2)	5 (3, 7)	-11 (-16, -6)
Day 365 to Day 535 (immediately prior to 4th dose)	3	-11 (-13, -10)	-5 (-9, 1)	-1 (-3, 0)	11 (7, 14)

high proportion of high-risk individuals, which were not the focus of the systematic review. Data from our cohort indicates that a lower portion of these individuals at higher risk of severe COVID-19 had developed hybrid immunity. In part, this may be due to different behaviour patterns with less exposure because of increased concern over developing severe disease. Furthermore, our model predicted individuals with a higher number of comorbidities to have lower levels of antibodies independent of hybrid immunity status. This is consistent with what has previously been observed in both the ENFORCE cohort [5] and other studies [6], with studies also reporting faster waning of antibodies among older individuals [30]. Thus, the findings from this study support vaccine strategies targeting subsequent booster doses to these older and high-risk individuals, that are less likely to have hybrid immunity.

Our study has several strengths. We had nationwide data collection for all SARS-CoV-2 PCR tests conducted in Denmark, a country which had one of the highest rates of PCR testing globally during the pandemic [28]. Additionally, unlike some other studies, the inclusion of anti-nucleocapsid data allowed us to separate those who may have had an asymptomatic infection without being tested for SARS-CoV-2 and those who may have tested positive only via SARS-CoV-2 antigen testing from the no hybrid immunity group (PCR-). As there is no consensus on the definition for seroconversion based on anti-nucleocapsid measurements we opted to exclude these individuals from our analysis. This strengthened our classifications of hybrid and vaccine-induced immunity and reduced the risk of detection bias or misclassifications.

Some limitations should also be considered. The figures presented in this study show the geometric mean levels of antibody response across each of the immunity groups. However, within these groups there remains a small proportion of individuals with poor vaccine-response (hypo-responders). Additionally, some individuals with hybrid immunity may have been given monoclonal antibodies as part of their treatment, possibly leading to higher antibody levels. Whilst this treatment was initially offered to all risk groups it was later restricted to only high-risk individuals and therefore, we expect this to have had little impact on the mean antibody levels. Further, this study focused on the total spike IgG measured against the wild-type strain and assessed waning immunity based on the changes in total spike IgG over time, and we did not investigate vaccine effectiveness for subsequent risk of reinfection or severe disease. We also noted the peak antibody response among all three hybrid immunity groups was very similar and maybe due to the levels reaching the maximum detectable limit of the assay.

In conclusion, this study found that hybrid immunity has an important impact on the antibody trajectories in vaccinated individuals and thus recent infection should also be considered when evaluating the need for additional vaccine doses. The current approach adopted by many countries of targeting booster vaccine programs at older and other high-risk individuals seems well justified. However, there may be other individuals with no prior infection who could also benefit from additional vaccine doses.

Data availability

The data reported in this study cannot be deposited in a public repository. Data is restricted to protect the privacy of the study participants in this cohort. Data from the ENFORCE cohort may be made available to researchers upon approval of an application to the ENFORCE scientific steering committee and further approval by relevant authorities. Applications for data must be sent to enforce.rigshospitalet@regionh.dk. Detailed information about data access may be found here: https://chip.dk/Research/Studies/ENFORCE/Study-Governance. Public study reports are available at https://chip.dk/Research/Studies/ENFORCE/Study-Reports. This paper does not report original code. Requests for code should be directed to the corresponding author.

Ethics

ENFORCE was approved by the Danish Medicines Agency (# 2020-006003-42) and the National Committee of Health Research Ethics (# 1-10-72-337-20).

Author contributions

JR, HS, LØ, MT, JL and OS conceptualized the design of the study. HN, ISJ, TB, LW, NBS, KI, ABM, KTP, MRJ, LSK, MBI, FDL, SOL, LDR and LM collected the data. SDA, EB, LLD and MT performed the laboratory analysis. HS and JR performed data curation, statistical analysis and visualization. JR, HS, WB, TOJ, SRO, MT, JL and OS contributed to the interpretation of the results. JR drafted the initial manuscript. All authors reviewed and approved the manuscript.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: HN declares participation on advisory board meeting with G.S.K. and M.S.D. TB declares receipt of unrestricted research or travel grants from GSK, Pfizer, Gilead Sciences, MSD; being principal investigator on trials conducted by Boehringer Ingelheim, Roche, Novartis, Kancera, Pfizer, MSD and Gilead; being a board member on Pentabase, and advisory board member for MSD, Gilead, Pfizer, GSK, Janssen and AstraZeneca; consulting fees from GSK and Pfizer; receiving donation of study drug from Eli Lilly; and receiving honorarium for lectures from GSK, Pfizer, Gilead Sciences, Boehringer Ingelheim, Abbvie and AstraZeneca. NS declares being principal investigator on studies conducted by Pfizer, Gilead and Bavarian Nordic. All other authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107111.

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